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ORIGINAL ARTICLE

Effect of topically applied hyaluronic acid on pain and palatal epithelial wound healing: An examiner-masked, randomized, controlled clinical trial

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Abstract

Background: This study aims to evaluate the effects of two different concentrations of topical hyaluronic acid (HA) on postoperative patient discomfort and wound healing of palatal donor sites after free gingival graft (FGG) surgery.

Methods: Thirty-six patients requiring FGG were randomly assigned into three groups in an examiner-masked, randomized, controlled clinical trial. After harvesting palatal grafts, 0.2% and 0.8% HA gels were used in test groups 1 and 2, respectively. Gels were applied on donor sites and protected with periodontal dressing in the test groups, whereas the wound was covered only with periodontal dressing in the control group. On days 3, 7, 14, and 21, pain and burning sensation were recorded using a visual analog scale (VAS) as well as other parameters such as complete epithelization (CE) and color match on days 3, 7, 14, 21, and 42.

Results: Test groups experienced less pain than the control group on days 3 and 7 ($P < 0.001$ and $P < 0.001$, respectively). Mean VAS score for burning sensation was higher in the control group on day 3 compared with test groups 1 and 2 ($P = 0.03$ and $P = 0.02$, respectively). CE in all patients was achieved on day 21 in both test groups, whereas it was achieved on day 42 in the control group. The test groups showed higher color match scores than the control group on days 21 ($P < 0.001$ and $P < 0.001$, respectively) and 42 ($P = 0.004$ and $P = 0.002$, respectively).

Conclusion: Topical application of HA exhibits positive impact on postoperative pain and burning sensation, and accelerates palatal wound healing in terms of epithelization and color match.

KEYWORDS

operative, pain, postoperative complications, surgical procedures, transplants, wound healing

Free gingival graft (FGG) procedure is one of the surgical approaches used to create a wide zone of attached gingiva around the teeth and implants.[1,2] As part of this procedure, FGG is harvested each time from the palatal donor area that heals by secondary intention.[1] The most common postoperative complications are pain, burning sensation, and delayed wound healing on the donor site.[1–3] To reduce postoperative complications and promote wound healing, various hemostatic agents,[4,5] dressing materials,[6] platelet-rich fibrin,[7] and analgesics[8] have been used over the years. However, in the relevant literature no ideal agent has been emphasized.[7,8]

Recently, prominence has been given to use of hyaluronic acid (HA) for cosmetic and medical reasons.[20–22] HA is a high molecular weight, non-sulfated polysaccharide

component of the family of glycosaminoglycans, present in various body fluids such as synovial fluid, serum, saliva, and gingival crevicular fluid. Furthermore, it represents a major component of the extracellular matrix (ECM) of skin, connective tissue, synovial joints, and other tissues.[9] It has been identified in all periodontal tissues, being particularly prominent in non-mineralized tissues such as gingiva and periodontal ligament, and only in low quantities in mineralized tissues such as cementum and alveolar bone.[9–12] Primarily mesenchymal cells, such as fibroblasts, chondrocytes, and osteoblasts, synthesize HA in the cell membrane.[13]

One of the major features of HA is hygroscopicity that allows it to maintain conformational stiffness and to retain water. Another major feature is viscoelasticity that provides stability and elasticity to tissues and delays penetration of viruses and bacteria.[14] The highly biocompatible[15] and non-immunogenic[15] nature of HA has bacteriostatic,[16] fungistatic,[17] anti-inflammatory,[15] antiedematous,[12] osteoinductive,[18] and proangiogenetic[19] properties, leading to promotion of wound healing in a variety of tissues. Its properties have made HA of interest for treatment of various diseases in medical areas such as orthopedics,[20] ophthalmology,[21] and dermatology.[22] In dentistry, HA has been used for acceleration of the healing process in tooth sockets,[23,24] treatment of temporomandibular joint osteoarthritis,[25] maintaining space in non-grafted sinus lifting,[26] and management of soreness of recurrent aphthous ulceration.[27] In periodontology, HA has been advocated as monotherapy[28] or as an adjunct to non-surgical[29] and/or surgical[29,30] periodontal treatment to reduce inflammation and promote wound healing.

To the best of the authors' knowledge, despite all previously mentioned unique properties, no study has examined the effects of HA on palatal wound healing after FGG surgery. The aim of this study is to evaluate the effects of topical HA on postoperative patient discomfort and wound healing of palatal donor sites after FGG surgery in terms of pain, burning sensation, complete epithelization (CE), and color match (CM).

1 | MATERIALS AND METHODS

The present study was a prospective, examiner-masked, randomized, controlled, clinical superiority trial with a parallel-group design.

1.1 | Sample size and randomization

Postoperative pain was chosen as the primary outcome variable and size estimation was performed based on a previous study.[8] Power calculation was performed at $\alpha = 0.05$ and at $\beta = 0.20$, equal to 80% of power.* Under this assumption, at least 11 patients were needed for each group. Twelve patients per treatment group were included for any possible dropouts. Burning sensation, CE, and CM were evaluated as secondary outcomes.

Patients were randomly assigned into three groups according to a computer-assisted randomization table by a clinician (SY). In test groups 1 and 2, 0.2%† and 0.8%‡ HA gels were applied on the palatal donor wound sites together with periodontal dressing,§ respectively. In the control group, the wound was left to spontaneous healing and covered only with periodontal dressing (Fig. 1).

1.2 | Study population

Sixty-three patients were examined for study eligibility from July 2013 to August 2015 in the Department of Periodontology, Marmara University, Istanbul, Turkey. Of 63 patients, 36 (Fig. 1) (9 males and 27 females, aged 21 to 62 years; mean age: 32.58 ± 7.81 years) met the inclusion criteria. Patients >18 years requiring FGG surgery with ≤1 mm width of gingiva in the mandibular anterior and premolar region were included. Exclusion criteria were: 1) systemic diseases; 2) history of periodontal surgical treatment; 3) loss of maxillary premolars and molars; 4) medications or antibiotics used in the previous 6 months; 5) pregnancy or lactation; and 6) smoking.

After being informed about the procedures and possible occurrence of pain to be tolerated without any analgesics, volunteering patients provided written informed consent. The study protocol was approved by the Ethics Committee, Yeditepe University, Istanbul, Turkey (dated June 18, 2013; number 315). This study was registered at Clinical Trials.gov as NCT02534415.

1.3 | Surgical procedures

Initial periodontal therapy was applied to all patients. An individual acrylic stent containing a horizontally placed 15-mm wire was made to sit on the second premolar and first molar teeth adjacent to the donor site, to guide harvesting of the palatal graft to fit in a similar mesiodistal length. Initial periodontal therapy, surgeries, and gel applications were performed by a single experienced periodontist (SY).

After local infiltrative anesthesia,¶ an adequate size of supraperiosteal recipient bed was prepared to receive the

* GraphPad Software, InStat, La Jolla, CA.

† Gengigel, Ricerfarma SRL, Milano, Italy.

‡ Gengigel Prof, Ricerfarma SRL.

§ Peripac, DENTSPLY DeTrey GmbH, Konstanz, Germany.

¶ Ultracain DS Forte, Sanofi-Aventis, Frankfurt, Germany.

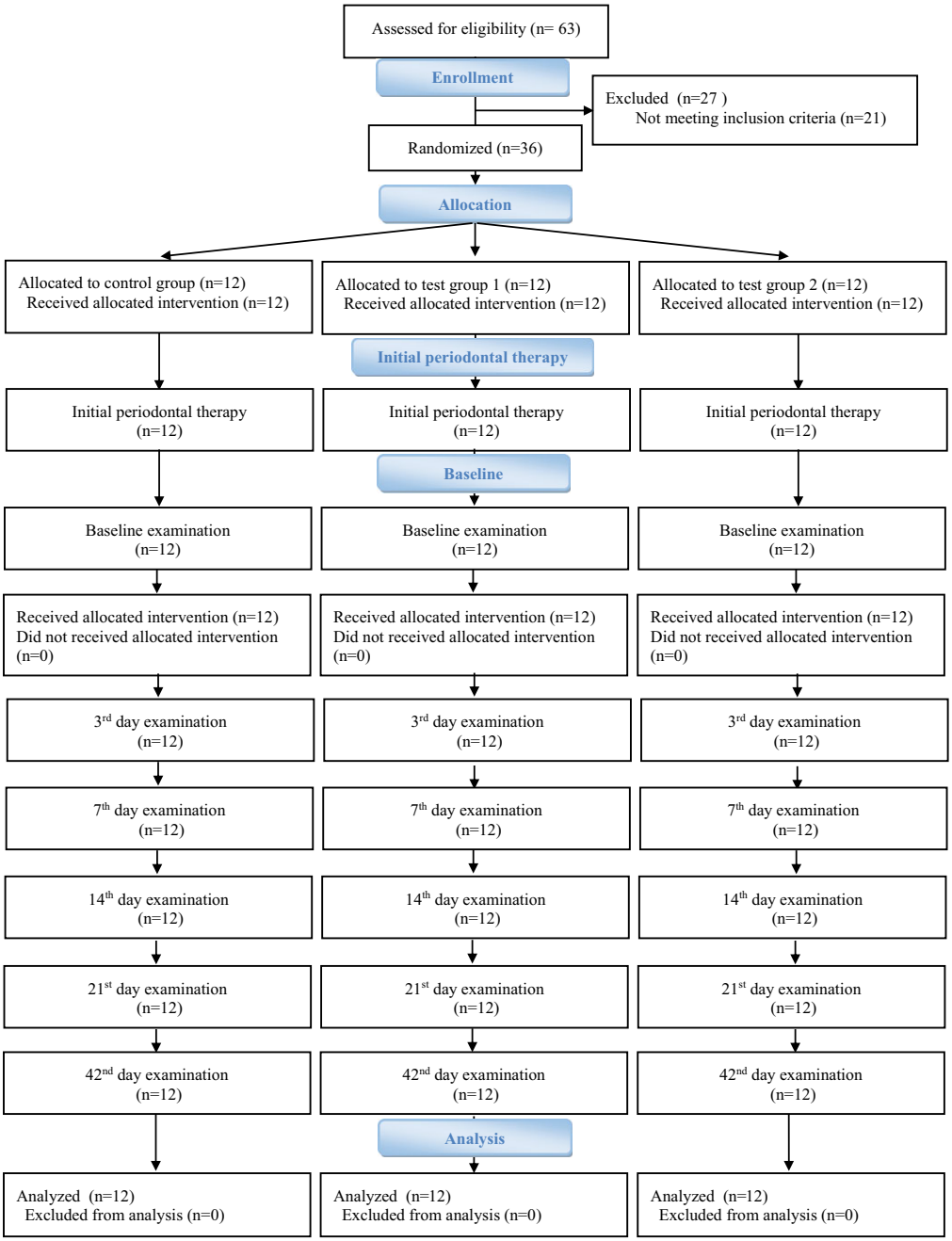

FIGURE 1 Study layout and follow-up

palatal graft of 15-mm mesiodistal length with 8-mm apico-coronal extension. Partial-thickness flap was sutured apically using 4-0 silk suture* to finalize bed preparation.

For the donor palatal graft, the area between mesial line angle of maxillary second premolar and distal finish line of the first molar was used.[31] The most coronal horizontal incision, 15 mm long (individual stent guidance), was made at least 2 mm apical from the gingival margin. A second crescent-shaped horizontal incision joining with mesial and distal ends of the first incision was drawn 8 mm away (with periodontal probe† guidance) from the midpoint of the first, in a more apical position. Care was taken to obtain a graft thickness of ≈1 to 1.5 mm. Thickness was measured at the center of the graft during surgery using an endodontic reamer, silicon stopper, and a caliper.‡[32] Grafts were positioned, firmly adapted to the bed, and stabilized with simple periosteal sutures.

* Doğsan, Istanbul, Turkey.

† PCP-UNC 15, Hu-Friedy, Chicago, IL.

‡ Absolute Solar Capiler Series 500, Mitutoyo, Aurora, IL.

On the donor site, 1-minute manual compression with a wet gauze was used to achieve hemostasis before application of HA gels in the test groups. The wound was covered with periodontal dressing. The control group donor site was protected only with periodontal dressing.

1.4 | Postoperative protocol

Postoperative care was aimed at maintaining wound stability. Postoperative instructions included discontinuing toothbrushing and flossing around the surgical sites until the day of periodontal dressing removal (day 7). Patients were directed to consume only soft foods during the first week and to avoid any mechanical trauma. No medication was prescribed. Patients were cared for and followed professionally by the same periodontist (SY), scheduled to be seen on days 3, 7, 14, 21, and 42.

Periodontal dressings were first removed on day 3, and donor sites were evaluated using selected parameters in all groups. After evaluation, HA gels together with periodontal dressing were reapplied in the test groups, whereas only periodontal dressing was used in the control group. On day 7, the periodontal dressings were removed for reevaluation of the wound area in all groups. Thereafter, all patients were followed up on days 14, 21, and 42 for further evaluation.

1.5 | Parameters

Patients were asked to assess pain and burning sensation on days 3, 7, 14, and 21 using a visual analog scale (VAS)[6] with a range of 0 (no pain/burning sensation) to 10 (severe pain/burning sensation).

CE was assessed clinically after direct visualization via inspection supported by means of clinical photographs taken at each post-surgical visit to provide insight into the differences among patients across all groups.[3] CE was recorded on days 3, 7, 14, 21, and 42 as dichotomous scoring by a clinical investigator (HÖÖ), masked to all groups. CE was recorded as "yes" or "no" according to inspectional evaluation of surface characteristics and reflection as well as clarity and distinctness of wound outline.[33] If the epithelial barrier was intact and if distinction of a demarcation line was unclear when assessing the edges, CE was scored as "yes." CM was simultaneously evaluated alongside CE by comparing color of the adjacent and opposite sides again using VAS scores from 0 (no CM) to 10 (excellent CM).[5]

1.6 | Statistical analyses

Statistical analyses were performed using statistical software.* The patient was considered the statistical unit. Descriptive statistics for all parameters were expressed as mean ± SD, median, or frequency (%).

Multiple comparisons among groups at different time points were performed using Kruskal-Wallis test for parameters of pain, burning sensation, and CM. When significant difference occurred, Mann-Whitney U test with Bonferroni correction was used for paired comparisons. Because three groups were compared, α level was divided by three ($0.05/3 = 0.017$). $P < 0.017$ for Mann-Whitney U test was regarded as statistically significant.

Multiple comparisons within groups at different time points were performed using Friedman test for parameters of pain, burning sensation, and CM. χ^2 test was used to compare number of patients with CE among the groups. $P < 0.05$ was considered statistically significant.

2 | RESULTS

There was no significant difference among the groups for sex, age, and mean graft thickness ($P > 0.05$). Mean values of graft thickness in test groups 1 and 2 and the control group were 1.13 ± 0.18, 1.18 ± 0.16, 1.17 ± 0.15, respectively ($P = 0.67$) (see supplementary Table 1 in online *Journal of Periodontology*). None of the patients dropped out (Fig. 1). None of them experienced sensation loss/bleeding complications or declared intake of analgesics after surgery. Moreover, no side effect regarding HA usage was reported. Preoperative and postoperative clinical appearances representing each study group are shown in Fig. 2.

Table 1 shows results of pain scores. Pain was observed in all groups on day 3. The control group revealed the highest mean pain score of 6.42 ± 1.83, whereas in test groups 1 and 2 the pain scores were 1.67 ± 1.55 and 1.92 ± 1.83, respectively. Pain gradually decreased in all groups in the subsequent healing period ($P < 0.001$). Both test groups showed significantly less pain than the control group on day 7 ($P < 0.001$), but no significant differences were observed between the test groups ($P > 0.05$). Test group 1 declared no pain starting from day 14, and test group 2 and the control group from day 21. However, on day 14, no statistical differences were detected in all groups ($P > 0.05$).

Mean VAS scores for burning sensation were significantly higher in the control group (3.50 ± 3.42) on day 3 compared with test groups 1 and 2 (0.67 ± 1.30 and 0.50 ± 1.24, respectively) ($P = 0.01$) (Table 2). No burning sensation was observed on day 14 in the test groups and in the control group on day 21. Intragroup comparison of burning sensation revealed a significant difference ($P = 0.005$) only in the control group, observed between days 3 and 21.

Data about CE are shown in Table 3. None of the patients showed CE in the first week. In test group 1, 50% of patients showed CE on day 14; in test group 2 and the control group,

* SPSS v.15.0, SPSS, Chicago, IL.

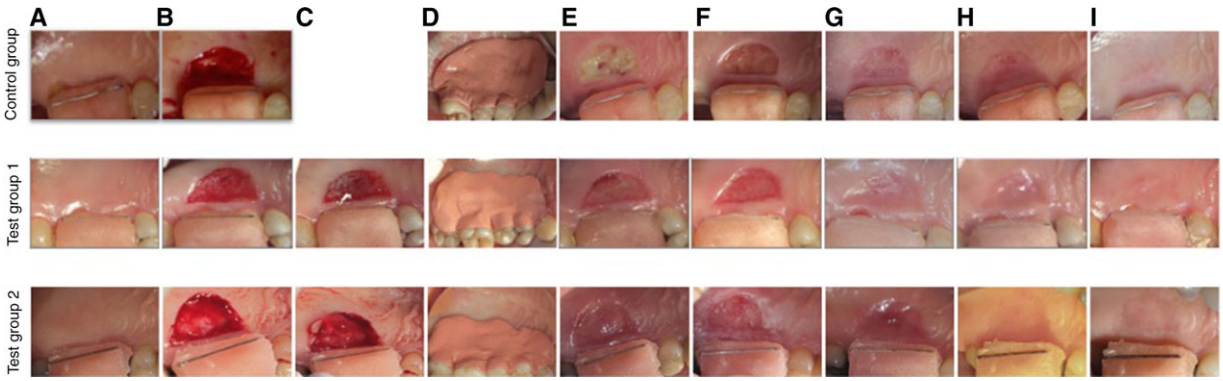

FIGURE 2 Comparison of control and test groups. **A)** Presurgical appearance. **B)** Appearance of palatal site after graft is harvested. **C)** Application of HA (control group panel blank). **D)** Application of periodontal dressing in all groups. Clinical appearance after surgery on **(E)** day 3 (control = CE "no"/Pain "8;" test group 1 = CE "no"/Pain "2;" test group 2 = CE "no"/Pain "3"); **(F)** day 7 (control = CE "no"/Pain "6;" test group 1 = CE "no"/Pain "0;" test group 2 = CE "no"/Pain "1"); **(G)** day 14 (control = CE "no"/Pain "5;" test group 1 = CE "yes"/Pain "0;" test group 2 = CE "no"/Pain "0"); **(H)** day 21 (control = CE "no"/Pain "0;" test group 1 = CE "yes"/Pain "0;" test group 2 = CE "yes"/Pain "0"); and **(I)** day 42 (control = CE "yes"/Pain "0;" test group 1 = CE "yes"/Pain "0;" test group 2 = CE "yes"/Pain "0")

TABLE 1 VAS pain scores by group

	Control group (n = 12)	Test group 1 (n = 12)	Test group 2 (n = 12)	*P* Value[a]	*P* Value[b]	*P* Value[c]	*P* Value[d]
Day 3							
Median (min to max)	7 (3 to 8)	1.5 (0 to 4)	2 (0 to 5)				
Mean ± SD	6.42 ± 1.83	1.67 ± 1.55	1.92 ± 1.83	**<0.001**	**<0.001**	**<0.001**	0.93
+\− Pain (n)	12\0	8\4	8\4				
Day 7							
Median (min to max)	4.5 (1 to 9)	0.5 (0 to 4)	0 (0 to 5)				
Mean ± SD	4.50 ± 2.27	1.25 ± 1.54	0.83 ± 1.52	**<0.001**	**<0.001**	**<0.001**	0.55
+\− Pain (n)	12\0	6\6	4\8				
Day 14							
Median (min to max)	0 (0 to 5)	0 (0 to 0)	0 (0 to 1)				
Mean ± SD	1.25 ± 1.91	0.00 ± 0.00	0.17 ± 0.38	0.08	0.17	0.38	0.54
+\− Pain (n)	4\8	0\12	2\10				
Day 21							
Median (min to max)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)				
Mean ± SD	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	>0.99	>0.99	>0.99	>0.99
+\− Pain (n)	0\12	0\12	0\12				
P value[e]	**<0.001**	**<0.001**	**<0.001**				

min = minimum; max = maximum.
[a]Kruskal-Wallis test.
[b]Mann-Whitney *U* test; control versus test group 1.
[c]Mann-Whitney *U* test; control versus test group 2.
[d]Mann-Whitney *U* test; test group 1 versus test group 2.
[e]Friedman test; $P < 0.05$.

this ratio was found to be 8.3% ($P = 0.02$). All donor sites in the test groups demonstrated CE on day 21. However, CE occurred on day 42 in the control group.

For the control group and test groups 1 and 2, VAS scores exhibiting CM were 0.00 ± 0.00, 1.17 ± 1.85, and 0.33 ± 0.78, respectively, on day 3 ($P = 0.08$), revealing significant increase toward the end of the experimental period as expected ($P < 0.001$) (Table 4). Test groups 1 and 2 showed a similar course of CM at all follow-up time periods, with significantly higher scores than the control group observed on

TABLE 2 VAS burning sensation scores by group

	Control group (n = 12)	Test group 1 (n = 12)	Test group 2 (n = 12)	*P* Value[a]	*P* Value[b]	*P* Value[c]	*P* Value[d]
Day 3							
Median (min to max)	2.5 (0 to 9)	0 (0 to 4)	0 (0 to 4)				
Mean ± SD	3.50 ± 3.42	0.67 ± 1.30	0.50 ± 1.24	**0.01**	**0.03**	**0.02**	0.76
+\− Burning (n)	8\4	3\9	2\10				
Day 7							
Median (min to max)	3.5 (0 to 9)	0 (0 to 5)	0 (0 to 4)				
Mean ± SD	3.33 ± 3.28	0.92 ± 1.73	0.58 ± 1.24	**0.04**	0.08	0.06	0.89
+\− Burning (n)	7\5	3\9	3\9				
Day 14							
Median (min to max)	0 (0 to 6)	0 (0 to 0)	0 (0 to 0)				
Mean ± SD	1.33 ± 2.10	0.00 ± 0.00	0.00 ± 0.00	**0.01**	0.18	0.18	>0.99
+\− Burning (n)	4\8	0\12	0\12				
Day 21							
Median (min to max)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)				
Mean ± SD	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	>0.99	>0.99	>0.99	>0.99
+\− Burning (n)	0\12	0\12	0\12				
P value[e]	**0.005**	0.07	0.08				

min = minimum; max = maximum.
[a]Kruskal-Wallis test.
[b]Mann-Whitney *U* test; control group versus test group 1.
[c]Mann-Whitney *U* test; control group versus test group 2.
[d]Mann-Whitney *U* test; test group 1 versus test group 2.
[e]Friedman test; $P < 0.05$.

TABLE 3 CE (%) in the study groups

	Day 3	Day 7	Day 14	Day 21	Day 42
Control group	0	0	8.3	66.7	100
Test group 1	0	0	50	100	100
Test group 2	0	0	8.3	100	100
P value	>0.99	>0.99	**0.02**	**0.01**	>0.99
P value (control versus test group 1)	>0.99	>0.99	0.07	**0.03**	>0.99
P value (control versus test group 2)	>0.99	>0.99	>0.99	**0.03**	>0.99
P value (test group 1 versus test group 2)	>0.99	>0.99	0.07	>0.99	>0.99

χ^2 test; $P < 0.05$.

days 14 ($P = 0.01$ and $P = 0.05$), 21 ($P < 0.001$ and $P < 0.001$), and 42 ($P = 0.004$ and $P = 0.002$). At last visit (day 42), 25% of patients in the control group, 83% in test group 1, and 92% in test group 2 revealed excellent CM.

3 | DISCUSSION

The improvement of postoperative comfort together with successful treatment outcomes has been a dynamic periodontal research area favoring patients' well-being.[34] Many studies have focused on various minimally invasive surgical techniques and protection of wound sites, especially when left to healing by secondary intention.[4–7] In the literature, healing of donor sites of FGG surgery was reported as one of the most painful.[2,3,5,35,36] In this study, palatal donor sites were treated with the application of two different concentrations of HA to evaluate relief of postoperative pain and acceleration of the wound-healing process leading to an end result of CE.

The wound-healing pattern of a palatal donor site of FGG surgery has not been specifically evaluated in the related literature. Studies usually focus on healing of the connective tissue

TABLE 4 Vas CM scores by group

	Control group (n = 12)	Test group 1 (n = 12)	Test group 2 (n = 12)	*P* Value[a]	*P* Value[b]	*P* Value[c]	*P* Value[d]
Day 3							
Median (min to max)	0 (0 to 0)	0 (0 to 5)	0 (0 to 2)				
Mean ± SD	0.00 ± 0.00	1.17 ± 1.85	0.33 ± 0.78	0.08	0.18	0.51	0.41
Day 7							
Median (min to max)	0 (0 to 2)	3 (0 to 5)	2.5 (0 to 4)				
Mean ± SD	0.17 ± 0.58	3.08 ± 1.24	1.92 ± 1.78	**<0.001**	**<0.001**	**0.02**	0.16
Day 14							
Median (min to max)	4 (0 to 6)	5.5 (3 to 7)	4.5 (3 to 7)				
Mean ± SD	3.75 ± 1.54	5.50 ± 1.24	5.17 ± 1.40	**0.02**	**0.01**	**0.05**	0.55
Day 21							
Median (min to max)	6 (4 to 7)	8(6 to 10)	8 (6 to 10)				
Mean ± SD	5.83 ± 0.84	8.33 ± 0.99	7.75 ± 1.21	**<0.001**	**<0.001**	**<0.001**	0.20
Day 42							
Median (min to max)	8 (7 to 10)	10 (9 to 10)	10 (9 to 10)				
Mean ± SD	8.33 ± 1.23	9.83 ± 0.39	9.92 ± 0.29	**<0.001**	**0.004**	**0.002**	0.76
P value[e]	**<0.001**	**<0.001**	**<0.001**				

min = minimum; max = maximum.
[a]Kruskal-Wallis test.
[b]Mann-Whitney *U* test; control versus test group 1.
[c]Mann-Whitney *U* test; control versus test group 2.
[d]Mann-Whitney *U* test; test group 1 versus test group 2.
[e]Friedman test; $P < 0.05$.

recipient bed.[37,38] However, wound healing of the donor site can happen in three phases as in the recipient area.[36] During the initial phase (0 to 3 days), a layer of exudate or a blood clot occurs. In the second phase (4 to 10 days), capillaries establish vascularization and perfusion of blood clot,[36,37] and epithelial cells proliferate from adjacent tissues. In the third phase (11 to 42 days), a newly formed vascular system in the connective tissue appears and an epithelial layer covers the area, maturing into a keratin layer. As shown in animal and human studies, pain and burning sensation start during the initial phase, decrease throughout the second phase, and disappear in the third phase.[36–38] This disappearance comes with the concomitant surface CE.[36] During the healing period of the current study, postoperative discomfort was scored by patient self-assessment using two aforementioned parameters, pain and burning sensation after exogenous use of HA, introduced as a practical means to enhance wound healing with unique physiochemical and biologic properties[15–19] and, thus, quality of life.[39]

Pain was evaluated as the primary outcome. No analgesics were prescribed. Subsequent relief was assessed by patient perception alone. However, there are studies in the literature evaluating postoperative pain indirectly on the basis of mean consumption of analgesics.[40,41] Indirect assessment of pain in terms of consumption of analgesics in milligrams is considered a more quantitative method, and prescription of analgesics certainly favors postoperative comfort by decreasing pain and edema. However, with implementation of the present study protocol, postoperative morbidity was treated by means of periodontal dressings.[42] The palatal donor wound sites were covered with periodontal dressings as a necessity for non-problematic flow of the study. Although wound healing can be traumatized during removal and renewing of periodontal dressing (day 3), this was done on purpose for a variety of reasons: on the one hand, to evaluate the wound at least at one measurement point as early as suggested and applied in the relevant literature;[4,5,8,35,36] on the other hand, to use periodontal dressing benefits on surgical wound care, wound protection, pain reduction,[42] as well as HA retention and renewal as recommended for the product. Because periodontal dressings were applied to all groups, all patients encountered the same degree of trauma without jeopardizing results of this study.

Pain scores in both test groups were significantly lower than those in the control group on days 3 and 7, and totally disappeared on day 14 only in test group 1 in which 0.2% HA

gel was used. Pain improvement in 76% of patients at 2 weeks was also reported in another study, in which 0.2% HA gel was applied to recurrent aphthous ulcers and oral ulcers of patients with Behçet disease.[43] Regarding the burning sensation in patients, lower scores in the test groups were significantly different compared with the control group on day 3, at the first evaluation after the first application of HA gel. On day 14, none of the patients had burning sensation in the test groups, whereas four had burning sensation in the control group. These results are consistent with properties of HA.[39] Its application may act as a barrier for protection against irritating stimuli arising in the oral cavity which promotes disappearance of pain and burning sensation as stated in a previous study.[27]

CE is an important phase of secondary wound healing and can be used as a parameter for assessing effects of a tested method or application on epithelization.[3] In this study, 15 × 8–mm palatal grafts were harvested in a standardized manner. As one of the major objectives was evaluation and comparison of the donor area treated with/without HA, creation of standardized palatal wounds in all patients was crucial; fortunately, palatal donor area permits creation of a standard wound site and in many studies has been used as a healing assessment model.[28,35,36] Supreme care was taken to ensure minimal dimensional differences for the graft and donor wound areas among patients. CE was evaluated with direct visualization through inspection. Moreover, evaluation was supported by means of a color slide taken at each postoperative visit.[3] Dichotomous scoring was conducted according to surface characteristics and reflection of the area as well as clarity and distinctness of the wound outline.[33] CE on day 14 was present for 8.3% of patients in the control group and test group 2 and for 50% of patients in test group 1. On day 21, CE was observed in all patients of the test groups but only in eight of the control group. Therefore, it may be speculated that epithelization of donor sites in the test groups is completed faster than that in the control. However, as a result of 0.2% HA gel demonstrating better CE results on day 14 compared with 0.8% HA gel, efficacy of different concentrations of high molecular HA[12] remains to be evaluated in detailed future studies. Findings of CE, pain, and burning sensation taken together support previous assumptions of the effects of HA on wound healing due to impact on early granulation tissue formation,[44] its anti-inflammatory and antimicrobial effects, as well as its proangiogenetic potential.[44,45] As a natural component of ECM, presence of HA may also provide a structural framework, hydration, lubrication, non-immunogenic environment, and consequent assistance for regeneration.[44]

VAS CM scores showed sequential improvement toward day 21 in all groups. However, at all follow-ups except day 3, significant differences were found among the study groups in favor of the test groups. This may be interpreted as a sign of better wound healing, which enables a faster course of CM. The authors of the present study believe that the effects of HA gel leading to tissue maturation and rapid epithelization ensure good CM.

To the best of the authors' knowledge, no human study for direct comparison of results of this study is available in the literature. This is the only human study investigating the effects of topical HA applied on palatal donor site after FGG surgery. However, Keceli et al.[5] evaluated the effectiveness of a medicinal plant extract in achieving hemostasis and early wound healing of the palatal donor site. The only similarity between the current study and that study is the control group in which a wet gauze was applied alone on the wound after graft harvesting. CE was detected in 19% of patients on day 21, which is the only matching post-surgical evaluation time point. It was 66.7% in the control group of the present study. This difference may be attributed to different wound coverage approaches as well as their evaluation method by means of H_2O_2 application once a week during a 4-week follow-up. Although H_2O_2 as an indicator is a well-defined, semi-objective, and practical method to distinguish areas of CE, it was not used in the present study. Direct visualization without any chemicals was preferred as the evaluation method, especially for a biocompatible and non-toxic agent (HA) with wound healing properties. Furthermore, H_2O_2 may slow down the healing process with its toxic reactions and with negative effects on fibroblasts.[46]

Findings of this study must be interpreted in the context of the following points. First, recent wound healing studies have used clinical, biochemical, and histologic parameters to have standardized and reproducible models to better understand the pathology of healing and improve medical technologies.[47] Therefore, it is important to evaluate and confirm effects of a biomaterial or agent to promote the healing process using multidisciplinary parameters. Furthermore, parameters measured in the mathematic model[48] are of concern in histologic evaluation of the wound-healing process. Although this study was primarily designed to evaluate clinical parameters, multidisciplinary analysis may have been able to detect changes that occur in depth or length of the wound as well as more information and details about angiogenesis of the area, cell characteristics, and activity during the healing process. Second, patient-based evaluation of pain and burning sensation through perception is rather subjective compared with any objective parameter. VAS provides a mathematic scale for human judgments to be used in a wide range of statistical methods.[36,49] However, scores may vary from one person to another.[35,36] Furthermore, although direct visualization via inspection was supported by clinical photographs to provide valuable insight into differences among all patients,[3] the method used could be considered conflicting because of technical difficulties of the standardization of photographic shots. Combining the current method with some staining agents or

with paper benchmarks on graphic software could have provided a more reliable evaluation for CE.[5,50] A specifically tailored paper benchmark used in computer software measurements of the lesion can be used in future investigations.[50]

4 | CONCLUSIONS

Within the limitations of this study, it is shown that topical HA applied alongside periodontal dressing is a beneficial and supportive method for reducing postoperative discomfort and accelerating epithelization of the palatal donor wound sites compared with coverage with periodontal dressing only. To the best of the authors' knowledge, this is the first examiner-masked, randomized, controlled clinical study describing the relieving and healing effects of two different concentrations of HA. Significantly faster CE was observed in patients treated with 0.2% HA gel, and this is open to further investigations.

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