Evaluation of the Effect of Hyaluronic Acid Application on the Vascularization of Free Gingival Graft for Both Donor and Recipient Sites with Laser Doppler Flowmetry: A Randomized, Examiner-Blinded, Controlled Clinical Trial

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This study aimed to evaluate with laser Doppler flowmetry (LDF) the effect of topical hyaluronic acid (HA) application on the vascularization of free gingival graft (FGG) donor and recipient sites during the early wound healing period and to investigate the effect of HA application on the dimensional change of the FGG. Forty systemically healthy, nonsmoking patients who required FGG due to insufficient amount of attached gingiva in a partial edentulism were randomly assigned to a study group: test (FGG+HA) or control (FGG alone). The LDF values of the donor and recipient sites were measured in both groups before the operation and at 4, 7, 10, 14, and 30 postoperative days. LDF measurement of the graft was performed as soon as the graft was taken from the palatal site. FGG dimensions (width, height, and thickness) were assessed and recorded at baseline and on day 30, as well as the percentage of the changes in these values. LDF values of the recipient site in the FGG+HA group were found to be statistically higher than those in the control group on days 4 and 7 (P = .013 and P = .020, respectively); however, no differences were found for days 10, 14, and 30. Additionally, no differences were found for the LDF values of the palatal site between the FGG+HA and control groups (P > .05) at all examined time points. The height of the graft measured on day 30 was statistically higher in the FGG+HA than the control group (P < .001). The percentage change in thickness and height of the FGG was statistically lower in the FGG+HA than control group (P = .028 and P < .001, respectively). Application of HA on the recipient bed under the FGG at the first week of healing allows the formation of a well-vascularized layer, which acts as a barrier against tissue tensions by functioning as a scaffold between the recipient bed and FGG, thus reducing the shrinkage of the graft, especially in the vertical direction. This study further showed that the graft taken from the donor site had a remaining blood perfusion value of its own. Int J Periodontics Restorative Dent 2020;40:233–243. doi: 10.11607/prd.4494

Following tooth extraction, tissue loss causes a decrease in the keratinized tissue (KT). Although free gingival graft (FGG) is a technique that was described almost 40 years ago, it is still being successfully used to increase KT dimensions. Studies conducted to date have mostly focused on the reduction of postoperative complications in the donor site, while there are only a few studies1,2 that have evaluated postoperative healing of both the donor and recipient sites.

Hyaluronic acid (HA) is a biomaterial that induces proliferation of capillaries and angiogenesis with its components increasing vascularization after their formation via biodegradation.3 It has been previously reported that application of the acellular dermal matrix with exogenous HA prevents shrinkage of skin graft and increases vascularization.4 A previous study investigating the effects of sodium hyaluronate on angiogenesis showed that HA accelerates wound healing by promoting vascular endothelial growth factor release from fibroblasts and angiogenesis.5 HA has been used for periodontal purposes, but there is only one study6 wherein HA has been used to accelerate wound healing of a donor site. Laser Doppler Flowmetry (LDF) technique is used to investigate the effect of periodontal disease,7 smoking,8 and

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periosteal stimulation on gingival blood flow.

There has been no comparison in the literature between the use of HA to induce recipient-site wound healing as an alternative to the use of conventional FGG surgery. To the best of the authors’ knowledge, the present study is the first to evaluate the effect of HA on microcirculation of both the donor and recipient sites after FGG. Aims of the present study were to investigate the effect of HA applied to both the donor and recipient sites on vascularization following FGG surgery with LDF and to compare the final dimensional changes of FGG between the test group (with application of HA) and control group (conventional FGG surgery) at both donor and recipient sites.

Materials and Methods

A randomized, examiner-masked, controlled, prospective clinical study was approved by the Ethics Committee at Ankara University (April 19, 2017; number 08/14) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. This study was performed on 40 patients (20 male, 20 female) with a mean age of 45 years who applied to Gazi University Faculty of Dentistry, Department of Periodontology, due to inadequate attached gingiva in a region of partial edentulism. Inclusion criteria were as follows: absence of local or systemic diseases that may constitute a contraindication for periodontal surgery; no previous history of mucogingival surgery in the same partial edentulism region; and at least 1.2 mm in gingival thickness on the labial side of the recipient site. Smoking and use of medications or antibiotics in the previous 6 months were defined as exclusion criteria. Participants included in the study read and signed an informed consent form. Following the phase-1 therapy, all participants were randomly assigned to two groups according to the computer-generated randomization list. At baseline, plaque index (PI), gingival index (GI), and probing pocket depth (PPD) were recorded.

Surgical Procedures

All surgical operations were performed under local infiltrative anesthesia (Ultracaine D-S Forte, Sanofi-Aventis) as described by Yıldırım et al. After a submarginal horizontal incision was made along the mucogingival line, the recipient bed was prepared by lifting a partial-thickness flap toward the mucogingival line using 5-0 polypropylene suture (Prolene, Ethicon) such that a thin immobilized layer of connective tissue and periosteum was left on the bone. Measurements of FGG dimensions were performed as described by Silva et al. The change in the dimensions of the graft on day 30 was measured by calculating the percentage change compared with the baseline values. Baseline periodontal treatment, surgeries, and HA applications were performed by a single experienced periodontist (Z.T.Ç.).

HA Application for FGG+HA Group

The chemically cross-linked HA gel package containing 20 mg/ml N-hyaluronate (Hyadent, Regedent), with a 12.130-mPas viscosity, stored at room temperature, was opened. The 12-mm-long disposable needle with 0.3-mm diameter that came within the HA package was attached to the syringe. In the FGG+HA group, HA was applied on to the entire recipient bed before suturing, and the FGG was firmly sutured to the recipient site by 5-0 polypropylene suture. After the FGG was taken from the palatal site, sterile gauze was applied with moderate finger pressure during 60s. After the bleeding stopped, HA was topically applied (1 to 2 mm thick) to the recipient site (Fig 1). The patients used an individual acrylic stent in the maxilla during the first postsurgical week. In the control group, HA was not applied to the recipient or the donor sites.

LDF Measurement Time Points and Calibration

PeriSoft software (version 2.10, Perimed) was used to gather, record, and analyze the data collected by the laser doppler device (5010 Periflux, Perimed), and the data were shown as perfusion units (PU) (Fig 2). Baseline LDF measurements were made in both the recipient and donor sites, before local anesthesia was performed prior to the FGG operation. The obtained LDF values were recorded as preoperative
values for the donor (-D) and recipient (-R) sites, respectively. The second LDF measurement was made after the graft was removed from the palatal site, and the obtained value was recorded as the remaining blood perfusion (RBP) of the harvested graft. Other LDF measurements were performed on postoperative days 4, 7, 10, 14, and 30. Perfusion of both the donor and recipient sites was determined by an LDF device that transmits low-power laser light (wavelength 780 nm) to the tissue by means of a flexible fiber optic probe (PROBE 407 Small Straight Probe, Perimed). The back-reflected light was processed,
and the relative speed and number of blood cells in the tissue were calculated as blood perfusion (BP). The same blinded periodontist (S.G.) performed measurements under the supervision of the technical staff responsible for the device. Real-time microvascular perfusion measurements were seen on the monitor, which was connected to the main unit computer software in accordance with the manufacturer’s instructions, and the datum point was recorded after the BP line on the screen continued in the same pattern for 5 seconds (Fig 2). Measurements were performed by fitting the probe tip to the disposable probe holders (PH 07-4 Tape Fixed Probe Holder, Perimed) with a radius of 0.5 mm. To ensure immobility of the probe holders, 0.25 mL N-butyl-2-cyanoacrylate of tissue adhesive (TopoCryl, Heal & Care) was dropped circumferentially on the farthest parts of the holders. The reproducibility of LDF measurements was tested at the beginning of the study on 40 periodontally healthy volunteers on whom two sets of LDF measurements were performed at the palatal sites at a 1-day interval. A high degree of reliability was found between repeated LDF measurements where the intraclass correlation coefficient was 0.974 with a confidence interval from 0.952 to 0.986 (P < .001).

Postoperative Protocol

Study participants were informed about consuming soft foods and preventing mechanical trauma during the first week. The patients were prescribed a 0.12% chlorhexidine mouth rinse (two times daily). Patients were advised to refrain from toothbrushing and flossing for 10 days. In both the FGG+HA and control groups, the recipient site was covered with aluminum foil, and a eugenol-free periodontal dressing (COE-PAK, GC America) was applied throughout the first 3 days.

Statistical Analyses

All statistical analyses were performed using SPSS for Windows 11.5 software program (IBM). The compatibility of data with normal distribution was examined graphically with the Kolmogorov-Smirnov test. Quantitative variables were stated as mean ± standard deviation (SD), with a confidence interval at 95% (95% CI) for mean, median, minimum, and maximum values, whereas categorical variables were shown as number and percentage. In the examination of a statistically significant difference between the categories of a qualitative variable with two categories in terms of a quantitative variable, Student t test was used if the normal distribution assumption was met, otherwise Mann-Whitney U test was used. Chi-square test was applied to compare the relationship between categorical outcomes. In the examination of a statistically significant difference between baseline and follow-up days of quantitative variables, paired t test was used if the normal distribution assumption was met, otherwise Wilcoxon signed rank test was used. A value of P < .05 was considered statistically significant. When the median values of the recipient site LDF (PU) in FGG+HA and control groups for day 4 were 165.88 (range: 113.32 to 272.43) and 134.00 (range: 86.06 to 198.66), respectively, the power calculation was made by using Mann Whitney U test with 0.05 significance and 20 samples per group; the power of the study was found to be 0.91.

Results

All patients completed the healing period without any complication. Of the 44 subjects enrolled, 4 were dropped from the study: 2 patients in the FGG+HA group moved to another city, and 2 patients in control group did not attend postoperative visits. There was no difference between the two groups in terms of distribution of the localization of the operation sites in the maxilla and mandible. No statistical difference was found between the FGG+HA and control groups in terms of preoperative LDF-D and LDF-R values (Table 1). The comparison of BP in the recipient site between the FGG+HA and control groups on day 4 was considered the primary outcome variable, and the vertical dimensional change of the graft on day 30 was considered the secondary outcome variable. LDF-R values on postoperative days 4 and 7 were statistically significantly higher in the FGG+HA group than the control group (P = .013 and P = .020, respectively). No difference was found on day 10 LDF-R values between the
FGG+HA and control groups. On day 14, LDF-R values were found to be higher in the control group than the FGG+HA group ($P = .028$), whereas no difference was found between the two groups on day 30. Although the LDF-D values were higher in the FGG+HA group than the control group on all postoperative
measurement days, there was no statistically significant difference ($P > .05$). There was no difference in RBP values of the graft between the FGG+HA and control groups. There was a statistically significant difference in RBP and LDF-R values on days 4, 7, 10, 14, and 30 between the two groups. In the FGG+HA group, LDF values of the graft were higher than those of the control group until day 10, at which point the values became equal, and after which BP decreased in both groups until day 30. A statistically significant difference was found between the preoperative palatal LDF values and the LDF values on days 4, 7, 10, 14, and 30 in the FGG+HA and control groups (Table 2). The LDF values peaked on day 10 in both the donor and recipient sites (Fig 3). Table 3 shows the initial and day-30 values for the dimensions of the graft and the percentage change of these values. There was no difference between the FGG+HA and control groups in terms of the initial dimensions. On day 30, the height of the graft was found to be higher in

| Table 1 Demographic and Periodontal Parameters of the Study Groups |
|--------------------------|--------------------------|--------------------------|----------|
| Variables                | FGG+HA (test) group       | FGG (control) group       | $P$      |
| Age, y                   | 35.40 ± 5.23 (32.95–37.85) | 34.70 ± 6.10 (31.85–37.55) | .699$^b$ |
| Gender, n (%)            |                          |                          | .736$^a$ |
| Female                   | 13 (65)                  | 14 (70)                  |          |
| Male                     | 7 (35)                   | 6 (30)                   |          |
| Localization, n (%)      |                          |                          | 1.000$^a$|
| Maxilla                  | 8 (40)                   | 8 (40)                   |          |
| Mandible                 | 12 (60)                  | 12 (60)                  |          |
| PI                       | 0.34 ± 0.15 (0.27–0.41)  | 0.32 ± 0.12 (0.26–0.38)  | .355$^c$ |
| GI                       | 0.31 ± 0.09 (0.27–0.35)  | 0.30 ± 0.08 (0.26–0.34)  | .661$^b$ |
| PPD, mm                  | 1.48 ± 0.14 (1.42–1.54)  | 1.47 ± 0.13 (1.41–1.53)  | .817$^b$ |
| Systolic blood pressure, mmHg | 124.50 ± 9.30 (120.15–128.85) | 124.00 ± 9.68 (119.47–128.53) | .869$^b$ |
| Diastolic blood pressure, mmHg | 70.25 ± 6.78 (67.08–73.42) | 72.75 ± 6.78 (69.58–75.92) | .196$^c$ |
| Heart rate, bpm          | 67.30 ± 6.49 (64.26–70.34) | 66.35 ± 7.28 (62.94–69.76) | .666$^b$ |
| Body temperature, ºC     | 36.43 ± 0.28 (36.30–36.55) | 36.34 ± 0.21 (36.24–36.44) | .282$^b$ |

FGG = free gingival graft; HA = hyaluronic acid; SD = standard deviation; CI = confidence interval; PI = Plaque Index; GI = Gingival Index; PPD = probing pocket depth.

Each group was comprised of 20 patients.

$^a$Chi-square test.

$^b$Student $t$ test.

$^c$Mann Whitney $U$ test.
the FGG+HA group (P < .001). The percentage change in thickness and height of the graft was found to be lower in the FGG+HA group than the control group (P = .028 and P < .001, respectively).

### Discussion
The present study is the first research to evaluate the effect of HA on both the donor and recipient sites using LDF-based data. In the present study, the hypothesis was that HA applied to the recipient bed would facilitate the rapid reestablishment of blood supply, creation of the microvascular network, and the revascularization of the graft.
by participating in vascular mechanisms related to the viability of the FGG. The present study differs from other studies assessing wound healing after FGG in that it (1) evaluates recipient- and donor-site healing through noninvasive LDF measurements that provide objective quantitative data independently from the patient self-assessments, (2) measures the remaining BP of the FGG itself, and (3) takes baseline gingival thickness of the recipient site into consideration among its inclusion criteria. In addition, the present study also differs from other studies in terms of how the probe tip was fixed during LDF measurement. When an LDF measurement is made from any area including teeth and gingiva, it is highly important to differentiate whether the measured perfusion value belongs to the teeth or the gingiva, so as to obtain the true perfusion value of the targeted tissue. In this study, both the depth reached by the laser and the circumferential area were taken into consideration to obtain the targeted perfusion value. Regions with partial edentulism were included in the study for exclusion of the perfusion values of teeth and tooth roots. To avoid measuring the perfusion values of the alveolar bone and periosteum, measurements were made on gingiva with a certain gingival thickness. Probe holders were used to prevent measurement of the untargeted adjacent gingiva. Attaching the probe holders to the probe tip also enabled standardized positioning of the probe tip on the tissue and provided stabilization during measurement by limiting the area to be measured. In the present study, the probe tip was considered the center of the circle with the probe holder as the circumference, which ensured that measurements were confined to the circumferential area of the probe holder. Another important point of the present study was to demonstrate RBP of the FGG immediately after harvesting from the donor site (a laser Doppler value of 8 to 20 PU), which was attempted for the first time within the scope of this study. These data can be interpreted as the FGGs containing an internal microvascular system by maintaining their RBP before suturing to the recipient bed. These findings are consistent with those of another study examining the effect of smoking on free and pedicular flaps monitored by laser Doppler in rats, where all the alive flaps had a laser Doppler value of 20 PU or more, whereas this value was < 20 PU in those that had lost their vitality. Although these FGGs preserve their vitality while having a relatively low flow value, the RBP value will decrease or drop to zero after being sutured to the recipient bed.

Fig 3 LDF measurement values (with a 95% confidence interval) of the (a) donor and (b) recipient sites in perfusion units (PU). RBP = remaining blood perfusion of free gingival graft.
bed as a result of total thrombosis of vascular anastomosis. Therefore, supporting the blood supply via HA will facilitate and improve healing and minimize the expected graft shrinkage rate. In the present study, it was observed that covering the recipient bed with HA before suturing increases the vascularization with the mechanism described above. In addition, diminishing the shrinkage of the FGG reduces irregularities in the superficial appearance of the graft. In a study wherein changes in gingival blood flow after periodontal flap surgery were measured by LDF, it was reported that the LDF beam penetrates into the tissues with a depth of approximately 0.6 mm. According to a systematic review including the penetration depths of beams with different wavelengths, it was reported that a 700-nm–wavelength beam penetrates to a depth of 750 µm and an 800-nm–wavelength beam penetrates to a depth of 1,200 µm. It has been previously reported that the laser Doppler device used in the present study transmits low-power laser beams at 780-nm wavelength to tissues, and the measurement depth of gingiva, the target tissue in the present study, was reported as 0.75 to 1.2 mm. Therefore, in cases where gingival thickness is < 0.75 mm, the measurements will also include the surrounding tissues such as the root of the tooth and the periosteum underneath the gingiva. To avoid this inconvenience, gingival thickness must be 0.75 to 1.2 mm thick or more. For this reason, a gingival thickness of ≥ 1.2 mm was determined as an inclusion criterion to ensure that the baseline measurements at the recipient site only measured the perfusion value of the gingiva.

When the FGG+HA and control groups were compared in terms of the change in graft dimensions, it was found that change in the apico-coronal direction was 26.37%, which was significantly lower than the

<table>
<thead>
<tr>
<th>Graft variables</th>
<th>FGG+HA test group</th>
<th>FGG control group</th>
<th>P</th>
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<tr>
<td></td>
<td>Mean ± SD (95% CI)</td>
<td>Median (min–max)</td>
<td>Mean ± SD (95% CI)</td>
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<tr>
<td>Initial width, mm</td>
<td>13.68 ± 2.47</td>
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<td>30-d width, mm</td>
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<tr>
<td>Initial height, mm</td>
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<td>6.71</td>
<td>6.09 ± 1.35</td>
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<td>30-d height, mm</td>
<td>4.82 ± 0.96</td>
<td>4.94</td>
<td>3.46 ± 0.94</td>
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<tr>
<td>Initial thickness, mm</td>
<td>1.29 ± 0.27</td>
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<td>30-d thickness, mm</td>
<td>1.13 ± 0.29</td>
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<td>Height change, %</td>
<td>26.37 ± 9.39</td>
<td>24.88</td>
<td>42.69 ± 12.49</td>
</tr>
</tbody>
</table>

FGG = free gingival graft; HA = hyaluronic acid; SD = standard deviation; CI = confidence interval.

Each group was comprised of 20 patients.

*Statistically significant differences between groups.

a Student t test.

bMann Whitney U test.

Table 3 Initial and 30-Day Graft Width, Height, and Thickness and Percentage of Change
percentage of the control group (42.69%). This percentage value on day 30 that is in favor of the FGG+HA can be explained by the ability of HA to accelerate early recovery. This finding showed that HA reduced graft shrinkage in an apico-coronal direction. In studies where graft shrinkage was evaluated by changes in the surface area of the graft, the graft area was obtained by multiplying the length and width of the graft with rectangular area formula.\textsuperscript{16} Since graft healing does not occur as an actual rectangle with 90-degree angles in all corners, changes in length and width were evaluated separately in the present study instead of calculating the area. In the FGG+HA group, the higher LDF values obtained in the recipient site on days 4 and 7 compared with the control can be interpreted as the neovascularization and revascularization provided by HA significantly increasing the quality of healing. The lack of difference in LDF values between the FGG+HA and control groups on day 14 indicates that HA is effective in the early period, and the higher LDF values in the control group on day 14 indicates that healing is still ongoing in this group. It can be argued that through the provision of sufficient perfusion to the entire graft, HA’s mechanism of action is an imitation of microvascularization according to the inosculation phenomenon. In the present study, when the FGG+HA and control groups were compared, it was observed that HA in the FGG+HA group significantly affected the LDF values in the recipient site, and the effect in the donor site was not at a statistically significant level. This difference in the effect of HA between the recipient and donor sites may be due to the fact that HA covers the recipient bed like a top dressing before grafting, and once the graft is sutured, it acts as a wound dressing that keeps HA between the recipient bed and the graft. In the donor site, because it is not possible to maintain HA on the palatal wound surface, HA may have been biodegraded in the donor site without having enough time to exert its physiologic functions. Therefore, to maintain the effectiveness of HA application in the donor site, the use of HA-releasing, HA-based membranes may be preferred. Another approach may be to utilize a variety of manufacturing processes wherein the biodegradation resistance is increased through methods in which natural polymers are chemically modified, thereby increasing the residence time.

Conclusions

According to the results of the present study, the use of HA enables the formation of a well-vascularized layer, and when HA is applied topically on the recipient bed, it functions as a barrier against graft shrinkage between the recipient bed and the graft. Within the limits of the present study, it may be concluded that HA application on to the recipient bed as a thin layer accelerates vascularization by providing extra reperfusion on its entire surface along with the graft center. It was shown herein for the first time that FGG had its own perfusion value, which is named as “RBP” within the scope of this study. Further studies may be performed to evaluate the effects of different HA concentrations on recipient sites. In addition, future studies may consider the effects of HA-based membranes on donor-site palatal wound healing in different surgical procedures.

Acknowledgments

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