Effects of Ozone Therapy on the Early Healing Period of Deepithelialized Gingival Grafts: A Randomized Placebo-Controlled Clinical Trial

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Background: This study evaluates the effects of ozone therapy (OT) on the early healing period of deepithelialized gingival grafts (DGG) placed for non-root coverage gingival augmentation by laser Doppler flowmetry (LDF).

Methods: Thirty-three patients were assigned to study groups: 1) test: DGG + OT; or 2) control group: DGG alone. Thirty patients completed the study. Ozone was applied on DGGs placed in the recipient bed and donor site immediately after surgery and at days 1 and 3 post-surgery. Blood perfusion in the recipient site was measured by LDF on the day of surgery and at 1, 2, 3, 6, 8, 10, and 13 days after surgery. Quality of life (assessed by the Oral Health Impact Profile-14) and pain at donor/recipient sites (assessed by visual analog scale) were also investigated.

Results: Increase in blood perfusion units in the test group was significantly higher than control group at 1, 2, 3, 6, and 8 days post-surgery (P <0.001). Significant differences occurred between test and control groups in terms of visual analog scale values during the first week post-surgery for both donor and recipient sites (P <0.05). The ozone-treated group showed significantly higher quality of life than control group on postoperative day 6 (P = 0.002).

Conclusions: OT enhanced blood perfusion units in the first postoperative week. This outcome is also consistent with improvement in wound healing, accompanied by an increase in quality of life and decrease in postoperative pain in the test group. *J Periodontol 2016;87:663-671.*

KEY WORDS

Autografts; pain; transplants; wound healing.

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Inadequate attached gingiva, which prevents effective plaque control, is a major mucogingival problem in many individuals.[1] The amount of the "adequate" width of the keratinized gingiva to preserve periodontal/peri-implant health is not clear.[2] Although it is believed that the presence of a certain width of keratinized gingiva is important for maintenance of periodontal health and prevention of soft tissue recession, the need for keratinized tissue at all has been discussed in the literature.[3-6] Recently, a consensus report from the American Academy of Periodontology regeneration workshop indicated that a minimum amount of keratinized tissue is not needed to prevent attachment loss when optimal plaque control is present. However, if plaque control is suboptimal, a minimum of 2 mm of keratinized tissue is needed.[7] Kim and Neiva[8] stated that autogenous gingival grafts are still considered to be the gold standard procedure, with unmatched success rates when gingival augmentation procedures are required.

Among the various gingival grafting techniques described for keratinized tissue augmentation, the most commonly used are free grafts (free connective tissue graft/free gingival graft [FGG])[9] and connective tissue grafts combined, with different flap procedures.[10] Studies have shown that these procedures are predictable and effective for providing newly

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created keratinized tissue.[11,12] The initial healing phase (0 to 3 days) of free grafts, which consist of avascular plasmic circulation from the recipient site, is a vital period for the survival of the graft. Ischemia resulting from inadequate blood perfusion during this period may be a serious complication.[13] In addition, a patch-like, unesthetic appearance is another unfavorable outcome encountered in free graft–treated patients.[14] Free connective tissue grafts may be an alternative to FGGs in this situation, since complete color integration with the surrounding gingival tissue has been reported in a previous study.[15]

Ozone is a natural gaseous molecule made up of three oxygen atoms that can be used in gaseous and liquid (dissolved in water and oil) forms.[16] Because of the proven advantages of ozone therapy (OT), many fields in dentistry, including periodontology, have used ozone for various benefits.[17] OT works with the principle of transformation of the environmental diatomic oxygen molecules into ozone on contact with a special probe tip on the treated area.[18] Ozone can react with blood components (erythrocytes, leukocytes, platelets, endothelial cells, and the vascular system) and positively affect oxygen metabolism, cell energy, the antioxidant defense system, and microcirculation.[19] Additionally, ozone leads to a high expression of cytokines and interferons that are important for wound healing.[20] Furthermore, it has antibacterial, antifungal, and antiviral effects.[19] Patel et al.[20] have reported that ozonated oil can significantly improve epithelial healing and gingival health after topical application to surgical sites.

Laser doppler flowmetry (LDF), based on the Doppler shift, is a non-invasive method that evaluates microcirculation.[21] Investigators have used LDF in determining the vitality of dental pulp and gingival blood flow.[22] Studies have demonstrated that LDF may be useful in depicting the dynamic nature of periodontal flap blood perfusion and that it can be used in clinical practice to determine marginal gingival health.[21,23]

The present study aims to evaluate effects of OT on early healing of deepithelialized gingival grafts (DGGs) placed for gingival augmentation by LDF. The effects of OT on postoperative pain and quality of life were also investigated.

MATERIALS AND METHODS

Study Population and Randomization

This randomized, placebo-controlled clinical study was approved by the Erciyes University, Faculty of Medicine Ethics Committee (meeting date: July 16, 2013; decision number: 2013/477). Thirty-three participants (nine males and 24 females, aged 22 to 49 years; mean age: 34.6 years) with inadequate or no attached gingiva in the lower incisor region were recruited at the Department of Periodontology, Faculty of Dentistry, Erciyes University, Kayseri, Turkey, from November 2013 to August 2014. Patients were assigned to two study groups (test, DGG + OT; or control, DGG alone) using an electronically generated list of random numbers by a statistician (Dr. Ferhan Elmali, Department of Biostatistics, Erciyes University, Kayseri, Turkey). Allocation concealment was done by well-sealed opaque envelopes, each representing a patient in the study group, before surgery. Demographic data of the patients are shown in Table 1.

All patients met the following inclusion criteria: 1) 18 to 65 years of age; 2) systemically healthy; 3) inadequate amount or absence of keratinized gingiva on the labial side of the lower incisor region (<2 mm keratinized tissue width or <1 mm attached gingiva that interferes with effective oral hygiene procedures); 4) no restorations on the lower incisor region; 5) no mucogingival surgical history in the lower incisor region; 6) no periodontal treatment within the previous year; 7) no systemic antibiotics taken for ≥6 months before the study; 8) not pregnant or lactating; 9) nonsmoking; and 10) no contraindications for periodontal surgery and ozone application.

The study details were explained to the participants, and all patients signed a written informed consent form.

Clinical Periodontal Examinations

The following clinical parameters were assessed before surgery, upon completion of the initial periodontal

Table 1.

Demographic and Periodontal Parameters of the Study Groups

Parameter	Control Group	Test Group	*P*
Age (years)	34.6 ± 7.9	34.2 ± 7.5	0.970*
Sex, n			0.390†
Females	10	13	
Males	5	2	
PI			0.888*
Median	0.33	0.16	
Maximum, minimum	0.5, 0.0	0.5, 0.0	
GI	0.29 ± 0.2	0.24 ± 0.2	0.508‡
PD (mm)	1.54 ± 0.2	1.52 ± 0.2	0.911‡
BOP (%)			0.567*
Mean ± SD	14.3 ± 18.6	9.9 ± 17.4	
Maximum, minimum	50, 0	50, 0	
WKT (mm)	1.74 ± 0.2	1.79 ± 0.2	0.456‡
WKT2 (mm)	6.34 ± 0.5	6.12 ± 0.4	0.270‡

Data are mean ± SD unless noted otherwise. WKT = width of keratinized tissue (before surgery; WKT2 = width of keratinized tissue (13 days post-surgery). $P < 0.05$ considered statistically significant.
* Mann-Whitney *U* test.
† Chi-square test.
‡ Student *t* test.

therapy of scaling and root planing and oral hygiene motivation: 1) plaque index (PI);[24] 2) gingival index (GI);[25] 3) bleeding on probing (BOP); and 4) probing depth ([PD] distance between the free gingival margin and the base of gingival sulcus), and were recorded at three sites (mesio-buccal, mid-buccal, and disto-buccal) of the teeth confined in the operation region by periodontal probes with markings.‡ Width of keratinized tissue (distance between free gingival margin and the mucogingival junction) measurements were taken before and 13 days after surgery. All clinically measured distances were rounded to the nearest millimeter. All periodontal clinical examinations were performed by one calibrated examiner (ZT). Intraclass correlation test showed scores >0.90.

Measurements of Parameters Related to Blood Perfusion

Each patient was examined for temperature (digital temperature measuring device), pulse, and blood pressure (cuff kit), which may be related to blood perfusion, on the days of blood perfusion measurement by the same nurse (Tülay Kahraman Akçakoyunlu, Department of Periodontology, Erciyes University, Kayseri, Turkey). Additionally, the wake-up times of the patients were recorded.

Evaluation of Surgery-Related Parameters

Parameters evaluated were: 1) length of operation; 2) graft waiting period elapsed from harvesting to suturing in the recipient site; 3) graft length (horizontal); 4) graft width (vertical); 5) graft thickness (average of recordings performed at each corner and middle of the graft); and 6) number of analgesic tablets taken daily by each patient. A digital caliper§ was used for measurements.

Examination of Blood Perfusion

Blood perfusions in the recipient site were measured by LDF‖ on the day of surgery and 1, 2, 3, 6, 8, 10, and 13 days post-surgery, in the following order: 1 hour post-surgery after recovery from local anesthesia; day 1 post-surgery (before OT, just after OT, and 2 hours after OT); day 2 post-surgery; day 3 post-surgery (before OT, just after OT, and 2 hours after OT), and days 6, 8, 10, and 13 post-surgery.

Calibration and Reproducibility of LDF Measurements

LDF measurements were completed between 8:00 am and 11:00 am and were repeated five times (average values were recorded). Caution was taken performing measurements of the same unit¶ (using memory of seating position of unit), with the same patient position (mandible parallel to the floor). Plastic stents,# which were constructed before surgery with a prosthodontist (HA), were used for standard placement and stabilization of the caries probes** during measurements. The prosthodontist removed the amount of plaster coinciding with the borders of recipient bed, taking into consideration the guidance of the periodontist (ZT), who planned the localization and borders of the recipient bed. The precise geometric center of the graft was determined immediately after completion of the surgery, and the spot on the stent was created by using a drill compatible with the diameter of the LDF head. All LDF measurements were performed by a calibrated examiner (ZT). The reproducibility of the LDF measurements was tested before the onset of the study on five periodontally healthy volunteers. LDF measurements were recorded at 1-day intervals at two gingival areas on keratinized gingiva in the mandibular incisor region. A paired-samples t test revealed that no significant differences existed between the measurements ($P = 0.454$).

Evaluation of Postoperative Pain

Postoperative pain in donor and recipient sites was assessed at 1, 2, 3, 6, 8, 10, and 13 days after surgery with a visual analog scale (VAS) of 10 units, in combination with a graphic rating scale.[26] On the VAS, the left and right end of the graphic represented the absence of pain (score 0) and the most severe pain (score 10), respectively. Patients were warned to complete the VAS taking into consideration the intensity of their pain in the previous 24 hours on all recall days.

Evaluation of Surgery-Related Quality of Life

A self-administered questionnaire, the Turkish version of the Oral Health Impact Profile (OHIP)-14,[27] was used to measure the impact of deepithelialized gingival grafting surgery on quality of life presurgery and 6 and 13 days post-surgery. It was adapted and validated for the Turkish population[27] and encompassed seven conceptual domains of impact: 1) functional limitation (e.g., difficulty in chewing foods); 2) physical pain (e.g., toothache); 3) psychologic discomfort (e.g., self-consciousness); 4) physical disability (e.g., avoiding foods); 5) psychologic disability (e.g., embarrassment); 6) social disability (e.g., difficulty in performing one's job); and 7) handicap (e.g., total inability to function). Each item of the OHIP-14[27] was scored as: "never" (0), "hardly ever" (1), "occasionally" (2), "fairly often" (3), and "very often" (4).

Surgical Procedure

The surgical procedures were performed under local anesthesia.†† Recipient site was prepared with a submarginal horizontal incision with a #15c‡‡ blade to the

‡ Williams Probe, Hu-Friedy, Chicago, IL.
§ Shahe 150 mm, Wenzhou Sanhe, Zhejiang, China.
‖ PeriFlux 4001 Master, Perimed, Jarfalla, Sweden.
¶ Planmeca, Helsinki, Finland.
# Raintree, DENTSPLY, York, PA.
** CA Probe, Ozonytron, Bionix, München, Germany.
†† Ultracaine DS Forte, Sanofi-Aventis, Frankfurt, Germany.
‡‡ Unaldi Medical, Istanbul, Turkey.

periosteum. Incisions were extended approximately twice the desired width of the keratinized tissue. Before harvesting the graft in the appropriate size, the donor gingiva on the palatal region of the premolar teeth was extensively deepithelialized via back-action chisel[§§] as described by McLeod et al.[28] After deepithelialization, the bevel of the #15c surgical blade was used as a guide to establish a connective tissue thickness of ≈1 mm. The procedure was performed in the same manner as harvesting an FGG, taking care to keep a consistent 1-mm thickness. The DGG was sutured in the recipient site with a resorbable 4-0 round suture.[‖‖] The patients wore a prefabricated plastic stent in the maxilla during the first week post-surgery to control postoperative bleeding and preserve the donor site from trauma.

Postoperative Management

Study participants were informed about postoperative problems (bleeding, pain, and discomfort) and were prescribed 100 mg flurbiprofen to be taken in case of pain and a 0.12% chlorhexidine mouth rinse (two times daily). Patients were warned to avoid toothbrushing until 10 days after surgery because of possible effects on blood perfusion units.

Ozone Application

An ozone generator[¶¶] with a probe[##] was used for OT. Ozone was applied on donor and recipient sites immediately after surgery and at days 1 and 3 post-surgery in the test group. Ozone application in the control group was simulated without starting the ozone generator. The first and second ozone applications were at 75% power for 30 seconds (75 µg/mL), and the third was at 30% power for 30 seconds (30 µg/mL), based on manufacturer's instructions.

Statistical Analyses

A 10-unit blood perfusion difference, based on the Retzepi et al.[23] study and confirmed in a pilot study with different patients, was used for sample size calculation. Preliminary statistical analysis revealed that ≥12 participants in each study group would be necessary to provide 80% power to detect a difference at the 0.05 significance level. Thirty patients were included in the final analysis of this study. The Kolmogorov-Smirnov test was used to test the normality of the data. Mann-Whitney *U* and Student *t* tests were used to analyze non-parametric and parametric data, respectively. The categorical variable samples' intergroup comparisons were performed by χ^2 analysis. Repeated-measures two-way analysis of variance (Bonferroni post hoc) test was used for the test and control groups to analyze the repeated blood perfusion measurements. Correlations among the different parameters were analyzed using Pearson correlation coefficients. All analyses were conducted using statistical software*** with the significance level set as 0.05.

RESULTS

Thirty-three patients were randomized, and 30 completed the study. Three patients (two from the control group, one from the test group) did not attend the control visits for blood perfusion measurements. The post-surgical healing phase was uneventful for all patients.

There were no significant differences between the groups with respect to age or sex ($P > 0.05$); there were also no significant differences in terms of clinical parameters between the test and control groups before surgery ($P > 0.05$). Width of keratinized tissue–related findings showed that there was a significant increase in the amount of keratinized tissue at day 13 post-surgery compared with presurgical values ($P < 0.05$). However, no significant difference existed between the two study groups in terms of gain in width of keratinized tissue at 13 days ($P > 0.05$).

Parameters Related to Blood Perfusion

No statistically significant differences existed in temperature, pulse, blood pressure values, wake-up times, or blood perfusion measurements between the control and test groups for all recall days ($P > 0.05$).

Surgery-Related Parameters

There were no statistically significant differences in graft size or length of surgery between the test and control groups ($P > 0.05$) (Table 2).

Blood Perfusion Values

Intragroup analysis showed that the blood perfusion units statistically increased in the first week in both test and control groups ($P < 0.001$). However, the increase in blood perfusion units in the test group was significantly higher than in the control group. Significant differences in blood perfusion units between the two groups were seen for all recall days ($P < 0.001$) (Table 3).

Quality of Life and VAS

Significant differences were observed in the quality of life of the test and control patients on day 6 after surgery, but this difference disappeared by day 13 (Fig. 1).

VAS values showed significant differences between test and control groups during the first week post-surgery in both donor and recipient sites (Fig. 2) ($P < 0.05$). Parallel to these findings, significant differences also existed in terms of analgesic consumption on the same days post-surgery (i.e., control patients took more analgesics than test patients) ($P < 0.05$).

§§ Hu-Friedy.
‖‖ Dogsan, Trabzon, Turkey.
¶¶ Ozonytron, Bionix.
CA Probe, Ozonytron, Bionix.
*** SPSS Statistics, v.21, IBM, Chicago, IL.

Table 2.

Surgery-Related Data and Analgesic Consumption of Study Groups

Factor	Control Group	Test Group	*P*
Length of operation (minutes)	22.9 ± 2.9	23.3 ± 3.5	0.740*
Graft waiting period (minutes)			0.653†
Median	5.0	5.0	
Maximum, minimum	6.0, 4.0	6.0, 4.0	
Graft dimensions (mm)			
Thickness	0.95 ± 0.6	0.97 ± 0.6	0.320†
Height	8.6 ± 0.8	9.0 ± 1.0	0.208†
Width	6.8 ± 0.2	6.9 ± 0.2	0.663†
Analgesic consumption (number of total tablets)	8.08 ± 1.0	5.1 ± 0.9	<0.001†

Data are mean ± SD unless noted otherwise. $P < 0.05$ considered statistically significant.
* Mann-Whitney *U* test.
† Student *t* test.

Table 3.

Changes in Blood Perfusion Units During the Study Period

Time Post-surgery	Control Group	Test Group	*P**
Day of surgery			
1 hour	97.6 ± 5.19^{aA}	97.7 ± 7.12^{aA}	0.98
Before OT	110.9 ± 5.53^{bA}	129.5 ± 5.2^{bB}	<0.001
Just after OT	110.9 ± 6.7^{bA}	129.4 ± 5.5^{bB}	<0.001
2 hours after OT	111.3 ± 5.8^{bA}	132.2 ± 5.1^{cB}	<0.001
Day 2	134.9 ± 4.8^{cA}	154.8 ± 6.7^{dB}	<0.001
Day 3			
Before OT	150 ± 2.6^{dA}	177.9 ± 6.3^{eB}	<0.001
Just after OT	149.7 ± 3.3^{dA}	178 ± 6.0^{eB}	<0.001
2 hours after OT	149.8 ± 3.2^{dA}	179.4 ± 6.9^{eB}	<0.001
Day 6	175.8 ± 9.6^{eA}	206.4 ± 7.9^{fgB}	<0.001
Day 8	200.7 ± 7.1^{fA}	210.7 ± 7.7^{fB}	<0.001
Day 10	212.4 ± 6.0^{gA}	198.6 ± 4.8^{gB}	<0.001
Day 13	204.7 ± 4.9^{fA}	191.1 ± 3.9^{hB}	<0.001
*P**	<0.001	<0.001	

Data are mean ± SD unless noted otherwise. Different lowercase letters (a through h) within columns differ significantly. Different uppercase letters (A or B) across row differ significantly.
* Repeat-measures two-way analysis of variance.

DISCUSSION

OT, which has often been used in medical science, first attracted attention in the field of dentistry in 1930[16] and has become more popular in recent years for its antimicrobial, disinfectant, biocompatibility, and healing properties.[17] The effects of OT on the non-surgical treatment of periodontal disease and peri-implantitis have been investigated.[29,30] To the best of the authors' knowledge, no study exists on the use of OT in periodontal surgical procedures to improve wound healing. The acceleration of vascularization after grafting procedures may play an important role in graft survival[31] and the quality of life and postoperative comfort of the patients.

Demographic specifications and periodontal status were found to affect blood flow such that women showed lower blood flow than age-matched men, and cerebral blood flow decreased with age.[32,33] Gingival inflammation and severity of disease also affected the blood flow directly.[24] Because no difference was observed in this study between the test and control groups in terms of demographic values and disease severity, their effects on blood flow were discounted.

LDF is a useful tool for examining changes in microvascular blood flow, which may be affected by many factors, including temperature, pulse, blood pressure, and the physical position of the patient.[34-36] The effects of these factors on the LDF measurements could not be determined; the patients had similar temperature, pulse, and blood pressure values because of the strict inclusion criteria. Furthermore, the same dental unit with the same seating position was used for all patients.

Surgery-related parameters, such as length of operation and graft waiting period, may affect the healing process, and indirectly, the postoperative comfort of the patient. Studies have shown that shorter surgery time may be related to less inflammation, edema, and pain during the postoperative period.[37,38] Although these factors were also major concerns in this study, the absence of statistical differences between the test and control groups for these factors enabled us to dismiss these issues.

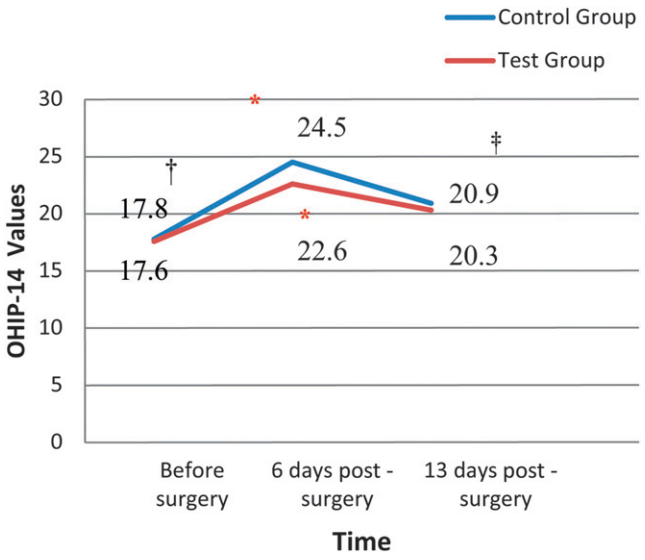

Figure 1.
*Changes in quality of life with time. * Significant difference between test and control group at day 6 post-surgery (P = 0.002). † No significant difference between test and control group before surgery (P = 0.345). ‡ No significant difference between test and control group at day 13 post-surgery (P = 0.465).*

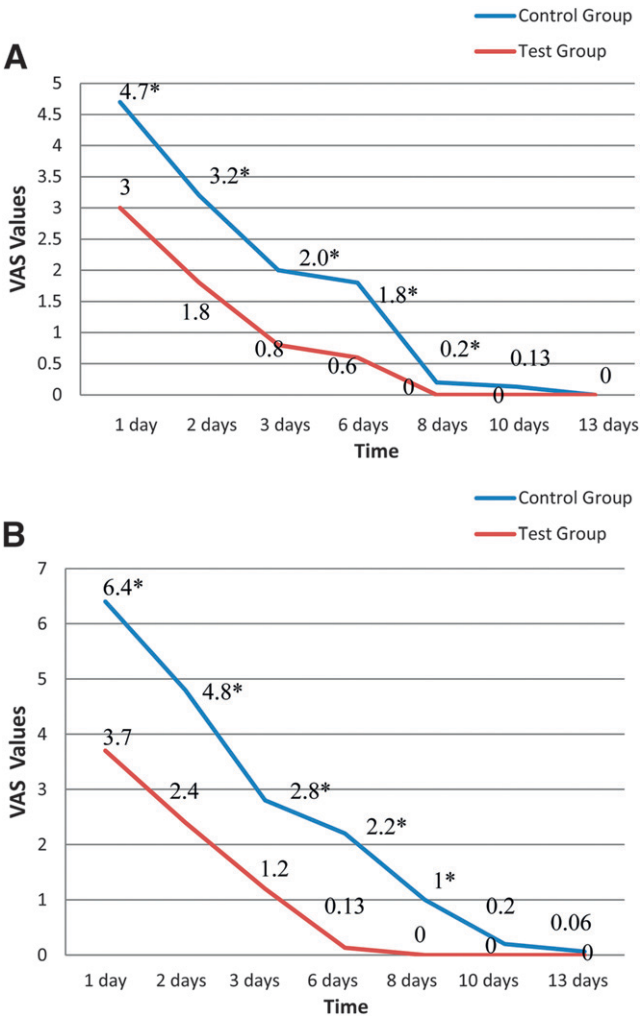

Figure 2.
A) *Changes in VAS (recipient site) values with time.* ***B)*** *Changes in VAS (donor site) values with time. * Significant difference between test and control group.*

Researchers have compared the blood flow in healthy and inflamed,[39] stimulated and unstimulated,[40] and free and attached gingiva.[23] The effects of periodontal surgical procedures on gingival blood flow values have also been examined.[21,23] To date, no study exists that has investigated blood perfusion in gingival grafts placed in the recipient bed for gingival augmentation.

OT was performed on the day of surgery and days 1 and 3 post-surgery. Because the critical healing period in FGGs is the first 48 hours, there is no vascularization.[41] The findings of the test group demonstrated that on the first postoperative day, blood perfusion was not influenced by OT immediately after application, but it showed a significant increase 2 hours after application. In the last application of OT (postoperative day 3) no significant change was observed in blood perfusion. This may be explained by the reduced power of the ozone application according to the manufacturer's instructions.

Various studies exist regarding the passive effects of OT on wound healing.[20,42] However, no study has investigated its effects in terms of blood perfusion changes. Intergroup comparisons have revealed that OT has significant positive effects on blood perfusion measurements during the early healing period. However, it should be noted that this significant increase in blood perfusion units in the first postoperative week may not represent a significant difference in the final short- and medium-term clinical results. In this study, it was clear that while the blood perfusion values (units) decreased after 8 days in the test group, they decreased after 10 days in the control group. This may be interpreted as the test group grafts showing more rapid healing than the control group grafts (Fig. 3). Increased vascularization due to angiogenesis reestablishes the microvascular network in the connective tissue and supplies nutrients and oxygen to the wound area,[43] resulting in accelerated wound healing. In this sense, the present findings parallel those of Patel et al.,[20] who applied ozonized oil to FGG surgical sites (grafted site and palatal donor site). The authors reported that the regenerative changes observed after the topical application of the ozonized oil in the epithelial cells of the healing tissue were significant, ultimately resulting in rapid keratinization and better healing of the gingival tissue.[20] Although the exact mechanisms of OT that played a role in the

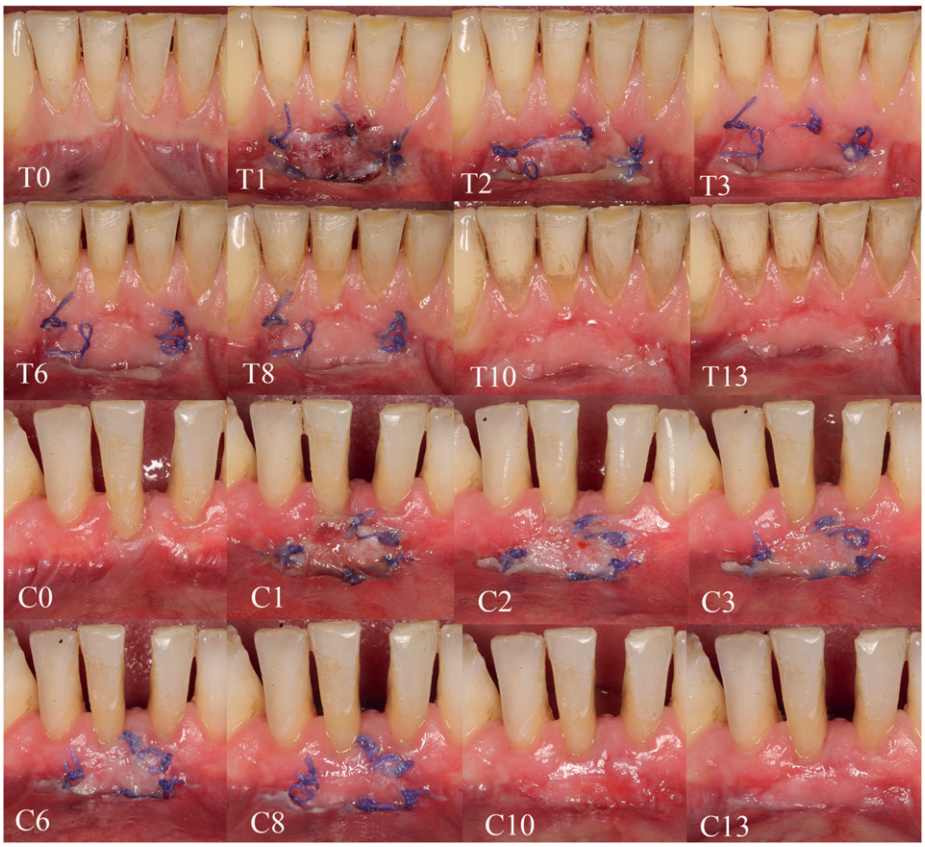

Figure 3.
Healing of graft in ozonated (test: T) and non-ozonated (control: C) groups during study period. T0/C0 = before surgery, T1/C1 = day 1 post-surgery, T2/C2 = day 2 post-surgery, T3/C3 = day 3 post-surgery, T6/C6 = day 6 post-surgery, T8/C8 = day 8 post-surgery, T10/C10 = day 10 post-surgery, and T13/C13 = day 13 post-surgery.

acceleration of healing are still unclear, the antimicrobial property of ozone, and its effect on the immune response in terms of the increased release of transforming growth factor-1β, interferons (β, α, γ), interleukins (IL-1, IL-2, IL-6, IL-8), and tumor necrosis factor-α,[20] should not be ruled out; these are important factors in human wound healing.

The determination of the changes in postoperative pain is subjective and varies among individuals based on physical and physiologic features.[44] The VAS values in the control group were significantly higher than those in the test group during the first week post-surgery, and so was analgesic consumption. These findings corroborate those of Kazancioglu et al.[18] who reported that patients in the ozonated group experienced a significantly lower degree of pain and took fewer analgesic tablets than the control group. Wessel and Tatakis[45] compared patient-based outcomes (pain, number of analgesic pills taken, and number of days pills were taken) between FGG and connective tissue grafts. The investigators stated that all FGG group patients experienced pain at 3 days post-surgery, and ≈50% of patients still experienced pain 3 weeks post-surgery. Because of great differences in methodology between the current study and Wessel and Tatakis[45] a proper comparison of the findings could not be performed. First, differences exist in the way VAS values were assessed (total VAS values at 3 days and 3 weeks versus recipient and donor site VAS values at 1, 2, 3, 6, 8, 10, and 13 days). Second, unlike the present study, Wessel and Tatakis[45] did not consider the effects of graft size/thickness and operation time on VAS values. As the graft size and thickness increase and the operation time is prolonged, an increase in pain may be expected.

Wessel and Tatakis[45] reported greater VAS values throughout the study period compared with the present study. The above factors may explain this discrepancy between the two studies.

Bleeding, pain, swelling, infection, and a feeling of weakness are some of the complications that arise in the first postoperative week.[46] The present findings indicated that the DGG surgery affected patients' quality of life in the early healing period in both groups, descending in the next week. However, it was a striking finding that the ozone-treated test group showed a better quality of life than the control group at postoperative day 6, paralleling the VAS values ($r = 0.512$). Similarly, Kazancioglu et al.[18] demonstrated the positive effects of OT on patients' quality of life after third molar extractions. Studies have reported that periodontal flap surgery affects the quality of life negatively.[47,48] The findings of Ozcelik et al.[48] revealed that quality of life values have a tendency to return to baseline at the postoperative first week. However, it should be kept in mind that different periodontal surgical procedures may affect the quality of life in different patterns, depending on the nature of wound healing, i.e., secondary versus primary wound healing.

The present study has some limitations. First, this study may be considered to have a small sample size. However, as many clinicians would appreciate, frequent monitoring of the study population precluded extension of the study population because patient

attendance might have become the major problem encountered during the study period. Second, analgesic consumption was not standardized in terms of dosage and length of consumption, because of the different pain thresholds among the study participants, and one may suspect that this affects both blood perfusion units and VAS values. Third, although the bias should not be expected to be large, the surgeon not being blinded to the treatments may be considered a limitation. Only a few studies[18,49,50] have investigated the effects of ozone generation used in this study. Moreover, each study followed different protocols according to the instructions of the manufacturer while applying ozone. Therefore, the timing and power of ozone application was based on the manufacturer's proposal rather than on scientific evidence.

CONCLUSIONS

Within the limits of the present study, it was concluded that OT enhanced blood perfusion units in the first postoperative week. The increase in blood perfusion may be interpreted as improvement in wound healing. This outcome is also consistent with the increase in quality of life and decrease in postoperative pain in the ozone-treated group. Further studies may be performed examining the effects of OT on clinical outcomes of long-term wound healing in different surgical procedures.

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