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Original Article

The effect of Ozone therapy on pain perception after free gingival graft surgery in patients with mucogingival defects. A Randomized Controlled Clinical Trial

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Abstract

Aim: The aim of this clinical study is to evaluate the effect of Ozone therapy versus the natural healing process on pain perception and the healing at the palatal wound in patients with mucogingival defects after harvesting of free gingival graft (FGG). **Methodology:** The study included 20 patients in need for FGG surgery for correction of mucogingival defects. Patients were randomly assigned into two equal groups; ozone therapy group (group I; OT) or control group (group II), periodontal dressing group. Evaluation of the postoperative pain after FGG harvest for both groups was recorded using the visual analogue scale (1-10) for a period of 7 days as primary outcome. While the post-surgical evaluation; the number of analgesic tablets, the palatal wound healing and complete re-epithelialization were defined as secondary outcomes. **Results:** Group I (OT); showed significantly lower VAS score at day 3 and day 7 compared to control group. While group II; significant reduction in VAS score at day 21 (SD= 0.3) and 28. Wound healing was evaluated using Landry's healing index, where group I; showed significant higher healing index scores at days 7 ($p<0.001$) and 14 ($p<0.001$) compared to the control group. **Conclusion:** Ozone therapy produces more significant pain reduction at day 3 and day 7, compared to control sites. Gaseous ozone application to the palatal donor wound induced complete epithelialization after two weeks and accelerated the healing process compared to control sites.

Keywords: Ozone application, free gingival grafts, pain, wound re-epithelialization, palatal donor site

INTRODUCTION

There is an increasing need for gingival grafts that represent an important component of surgeries for root coverage or gingival augmentation. FGGs are employed in gingival augmentation procedures either around natural teeth or implants and for ridge augmentation procedures while the subepithelial connective tissue graft. (SCTG) and de-epithelialized gingival graft. (DGG) are often used with bilaminar technique for root coverage often for sites with thin gingival phenotype [1].

The drawbacks of the free gingival autografts were suggested to be the surgical technique used to harvest the FGG as the palatal wound is more invasive and prone to heal by secondary intention with significant postoperative pain and the proximity of the wound to the greater palatine artery with possibility of bleeding. Several materials have been applied for protection of palatal wounds after harvesting of free gingival grafts including, collagen matrix alone or protected by cyanoacrylate adhesive [2], platelet

rich fibrin (PRF) [3], hyaluronic acid gel [4], simvastatin gel [5], phenytoin gel [6] and low-level laser therapy [7].

Ozone is an allotropic form of oxygen that naturally occurs as a gaseous molecule [8]. It is an assembly of three oxygen atoms with a short half-life (40 min at 20ºC) and is highly unstable. Ozone is denser than oxygen (1.6-fold) and is 10-fold more soluble. Ozone used for medical purposes is administered as a balanced mixture of oxygen and ozone has to be differentiated from toxic ozone considered as a product of air pollution especially when present in combination with NO_2 and organic matter. Ozone acts by inducing moderate and controlled oxidative stress that can generate second messenger molecules that activate different transcription factors that regulate different biological and physiological cellular responses that trigger the antimicrobial, antioxidant, anti-inflammatory, immune modulating and anti-hypoxic mechanisms of action of ozone [9].

MATERIALS AND METHODS

Study settings: The present randomized, controlled, parallel-grouped clinical trial included 20 patients (2 males and 18 females, aged 20 to 46 years) with mucogingival defects that required gingival augmentation and harvesting of free gingival graft (FGG). Patients were randomly assigned into two equal groups; group I (OT); where the palatal donor site was treated by ozone gas, administered at Day 0, day 3 and day 7. Group II (control group); where the palatal donor site was only covered with a periodontal dressing (periodontal pack) on the day of the surgery.

Patients were selected from the outpatient clinic of the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Cairo University between June/2022 and December/2022. Screening and recruiting patients were continued till achieving the target sample through patients' database. This clinical trial was registered in U.S. National Institutes of Health Clinical Trials Registry, Clinical Trials.gov Identifier ID: NCT05291715.

Ethical Procedure

The study protocol was approved by the Ethics Committee of Scientific Research, Faculty of Dentistry, Cairo University (12/2021).

Eligibility criteria

Inclusion Criteria

• Patients older than 18 years who are able to tolerate surgical periodontal procedures and ready to perform oral hygiene instructions with compliance to the maintenance program.

• Patients with mucogingival defects requiring augmentation with free gingival grafts harvested from the palate.

• No history of periodontal surgery in the area to be treated.

• Full mouth plaque score and full mouth bleeding score; less than 20% (Burkhardt & Lang 2014).

Exclusion criteria

• Smoking [10].

• Pregnancy and lactating women.

• History of systemic diseases or medications that may interfere with periodontal wound healing or contraindicate ozone treatment.

Power & Sample Size Calculation

Based on a previous study by Yildrim et al, 2017 the difference in the pain scores on day 7 between the test groups 1 and 2; was 1.25 ± 1.54 and 0.83 ± 1.52 respectively. Power calculation was performed at $\alpha = 0.05$, equal to 80% of power. Under this assumption, at least 10 patients were needed for each group. Then increased by 15% to compensate for dropouts; 12 patients per treatment group were included. Sample size calculation was achieved using PS program.

Randomization

The participants included in this trial were randomly assigned to either group I (OT) or group II (control group), a computer-generated randomization list (http://www.randomizer.org) was executed by a faculty member who was not involved in the recruitment with 1:1 allocation ratio.

Allocation concealment mechanism

Allocation concealment was achieved by sequentially numbered opaque sealed envelopes that contained the treatment to be performed to the enrolled subjects based on the randomized numbers in the randomization list. The sealed envelope was opened at the time of the surgery and the number was picked by another person other than the operator.

Blinding

The outcome assessor and the statistician were blinded. It was impossible for both the investigator or the participants to be blinded as the interventions were completely different.

Clinical photographs

Clinical photographs were taken for the palatal donor site at baseline, day 0, day 3, day 7, Day 14, day 21 and day 28 after harvesting the FGG.

Treatment protocol

Presurgical phase

All patients received phase I therapy comprising supragingival scaling and subgingival debridement. Full mouth supra and subgingival debridement were performed using ultrasonic scalers[1] followed by universal and Gracey's curettes. Patient preparation was completed in a single visit.

The patients were given oral hygiene instructions that included toothbrushing two times daily by soft toothbrush applying modified Stillman technique and mouth rinsing with 0.12 % chlorhexidine[2] was prescribed twice daily for two weeks. After completion of phase I therapy, patients were re-examined to ensure meeting all inclusion criteria.

Then they were allocated to one of the 2 groups:

• Group I (OT): received ozone therapy for the palatal wound after harvesting FGG.

• Group II (control group): received a periodontal dressing to the palatal wound after harvesting of the FGG.

Surgical phase

The procedure for gingival augmentation of keratinized tissue using FGG was outlined and developed by Sullivan and Atkins [11,12]. After preparing the recipient site, a FGG was harvested from the palate, between the first premolar & first molar.

The FGG was sutured to the periosteal bed. The palatal wound was treated differently in the two groups. Gaseous ozone (OT) was used according to the manufacturer's instructions at the donor sites immediately after surgery, which was left to heal without the addition of any periodontal dressings or stents. While the control group, the palatal donor site was then covered by a non-eugenol periodontal dressing.

Post-Operative Care

The patients were advised to use an antimicrobial rinse (0.12% chlorhexidine, 15 ml for 60 seconds twice daily) for 4 weeks. They were also given a prescription of Ibuprofen (600 mg every 12 hours) during the first week to be taken, if necessary, the patients were asked to record the number of tablets taken, to report if there were any bleeding from the donor site and the severity of pain giving a score from (0-10).

[1] (EMS- Piezon S Ultrasonic Dental Scaler)

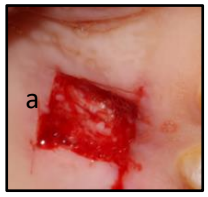
[2] (Hexitol antiseptic mouthwash; chlorhexidine hydrochloride 125 mg/ 100 ml)

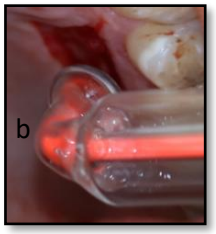
Outcomes

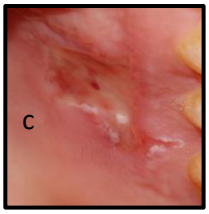
Patient-reported postoperative pain was evaluated by visual analog scale (VAS) [13] in form of a score between 0 (no pain) and 10 (the most pain the patient has ever experienced) for a follow up period of 4 weeks. The patients were asked to report the number of analgesic tablets ingested within the first week post-surgically.

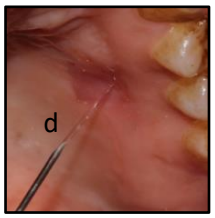
Complete re-epithelialization rate of the palatal donor sites was evaluated with hydrogen peroxide test. H_2O_2 was dripped over the wound to assess the quality of epithelial bridging and presence of observable bubbles, that was monitored by visual inspection (Yes/ No) [14]. The soft tissue healing at the donor sites was evaluated by Landry et al, healing index (1988) [15], which characterizes the healing process according to the palatal mucosal color, bleeding on palpation, presence of granulation tissue, epithelialization of the incision margin, and suppuration.

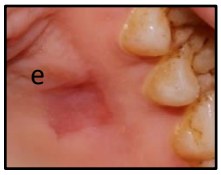
Group (OT):

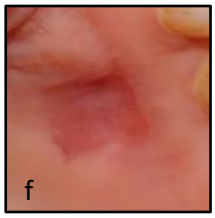




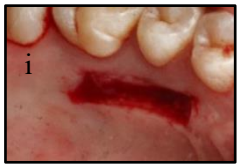


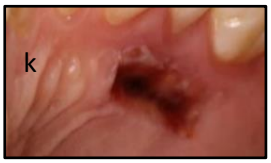


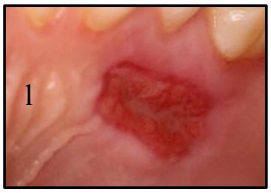


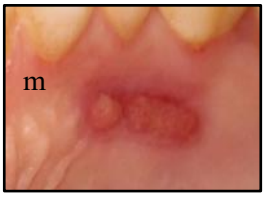


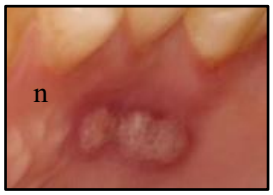
The Control Group:











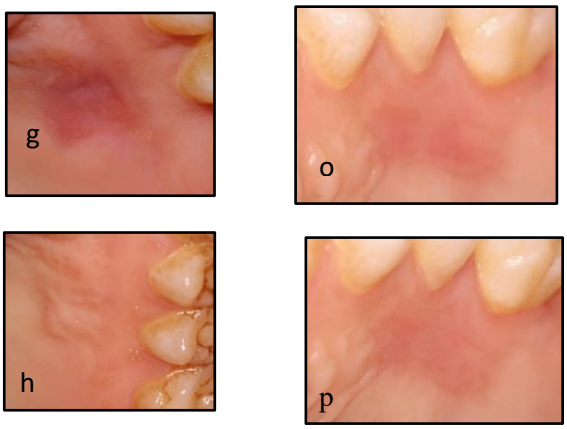


Fig. 1 Clinical pictures of the palatal wound at baseline (a), ozone application at day 0 (b), day 3 (c), H_2O_2 test (d), day 7 (e), after 2 weeks (f), day 21(g) and after 28 days (h) in the (OT) group. Palatal wound dressing at baseline (i), periodontal dressing (j), day 3 (k), at 1 week (l), day 14 (m), presence of bubble formation (n), day 21 (o) and after 4 weeks (p) in the control group.

Statistical Analysis

Data presented as mean, standard deviation (SD), median, minimum, and maximum when appropriate. Data explored for normality using Shapiro Wilk test. VAS score, Palatal Healing Index (Landry's index), and re-epithelialization test showed non-normal distribution, so Mann Whitney test used to compare between control and test groups. The Friedman test was used to compare time intervals followed by multiple comparisons with Dunn Bonferroni adjustment. The number of tablets and age showed normal distribution, so independent t-test was used for comparison between control and test groups. The significance level was set at $p < 0.05$. Statistical analysis was performed with IBM® SPSS® (ver. 26. SPSS Inc., IBM Corporation, Armonk, NY, USA).

RESULTS

Generally, the patients did not report any complications except one patient in each group complained from bleeding. In both groups bleeding was controlled by applying pressure to the palatal wound with wet gauze. Patients in the control group received another periodontal dressing. In both groups it was possible to harvest FGGs of comparable lengths (11.4 ± 3.2mm in the test group and 11.6± 3.3mm in the control group) with no statistical significance between the two groups ($p = 0.579$).

Postoperative pain

The OT group showed significantly lower VAS scores on day 3 and day 7 compared to the control group. There were no significant difference in VAS scores between (OT) and control groups on Days 0, 14, 21, and day 28. (**Table.1**)

Table.1 Mean, Standard deviation (SD) and median, results of VAS score for (OT) and control groups at different intervals.

		Control					(OT)					p-value
		Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
VAS	Day 0	6.6a	4.0	8	1	10	3.8a	4.0	4	0	10	0.052 NS
	Day 3	3.4a	2.8	4	0	10	1.1ab	1.2	1	0	3	0.029*
	Day 7	3.2a	2.6	2	1	10	0.8ab	0.9	1	0	2	0.002*
	Day 14	1.2ab	1.8	1	0	6	0.3b	0.7	0	0	2	0.089 NS
	Day 21	0.1b	0.3	0	0	1	0.0b	0.0	0	0	0	0.739 NS
	Day 28	0.0b	0.0	0	0	0	0.0b	0.0	0	0	0	1.00 NS
p-value				<0.001*					<0.001*			

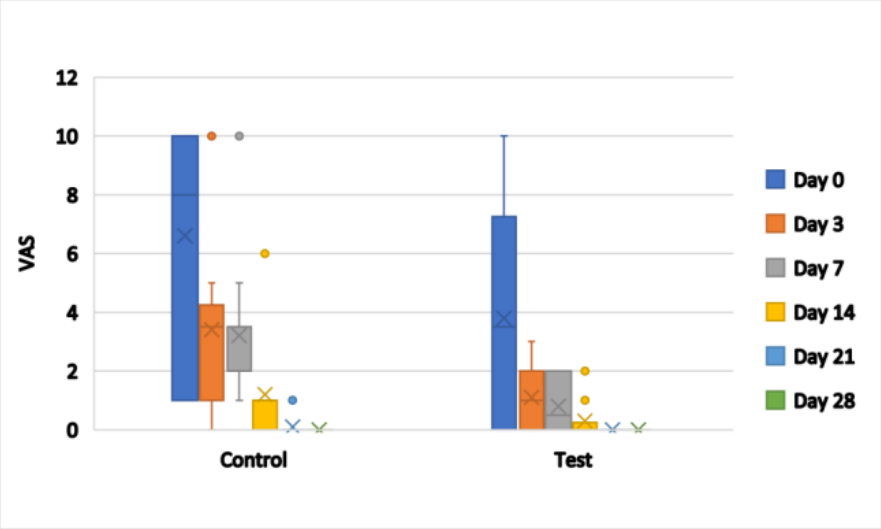


Fig.2 Box plot showing VAS Scores for test (OT) and control groups.

Number of analgesic tablets

The number of analgesic tablets taken by the patients in the control group was significantly higher than the (OT) group during the first post-operative week.

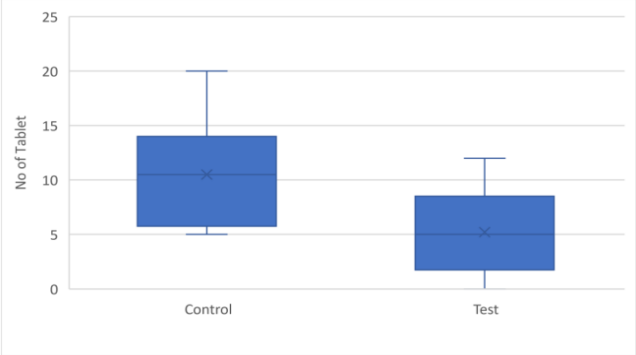


Fig. 3 Box plot showing No of tablets in test and control groups.

Wound re-epithelialization

The wound healing process illustrated partial epithelialization in 60% of the sites in the (OT) group compared to 30% of the sites in the control group after 3 days. After 7 days 80% of the sites showed partial epithelialization in the (OT) group compared to 60% in the control group. After 14 days all sites in the test group showed complete epithelialization while 100% of the sites in the control group showed partial epithelialization. However, complete epithelialization of donor sites was achieved after 21 days. (**Table. 2)**

Palatal Healing Index (Landry et al., Healing Index, 1988)

There was a statistically significant difference between (OT) and the control groups after 7 and 14 days after FGG harvest. The (OT) group showed a statistically significant increase in Landry et al., healing index from the 3rd day up to 14 days after surgical intervention. In the control group there was a statistically significant increase in Landry et al., healing index from the 3rd day up to 21 days after surgical intervention. (**Fig.4)**

The changes in VAS scores were followed for 28 days. This was adopted from the study of (Isler et al.,2018) that evaluated the effects of Laser biomodulation and ozone application on palatal wound healing after free gingival grafts and pain perception up to 30 days. In this study the control group (no treatment), the patients reported burning sensation and changes in dietary habits for more than 3 weeks [16].

The results of our study; although complete epithelialization was achieved after 21 days, nevertheless pain was still perceived in the control group (SD= 0.3) in comparison with the (OT) group. Therefore, a follow up of 4 week period was mandatory for evaluation of pain perception in both groups.

Table. 2 The frequency and the percentage of palatal donor sites that exhibit none, partial or complete wound epithelialization.

		Control				(OT)				p-value
		Yes		No		Yes		No		
		N	%	N	%	N	%	N	%	
Complete wound epithelialization	Day 7	0a	0.0%	10	100.0%	2a	20.0%	8	80.0%	0.146 NS
	Day 14	0a	0.0%	10	100.0%	10b	100.0%	0	0.0%	<0.001*
	Day 21	10b	100.0%	0	0.0%	10b	100.0%	0	0.0%	1.00 NS
	Day 28	10b	100.0%	0	0.0%	10b	100.0%	0	0.0%	1.00 NS
p-value		<0.001*				<0.001*				

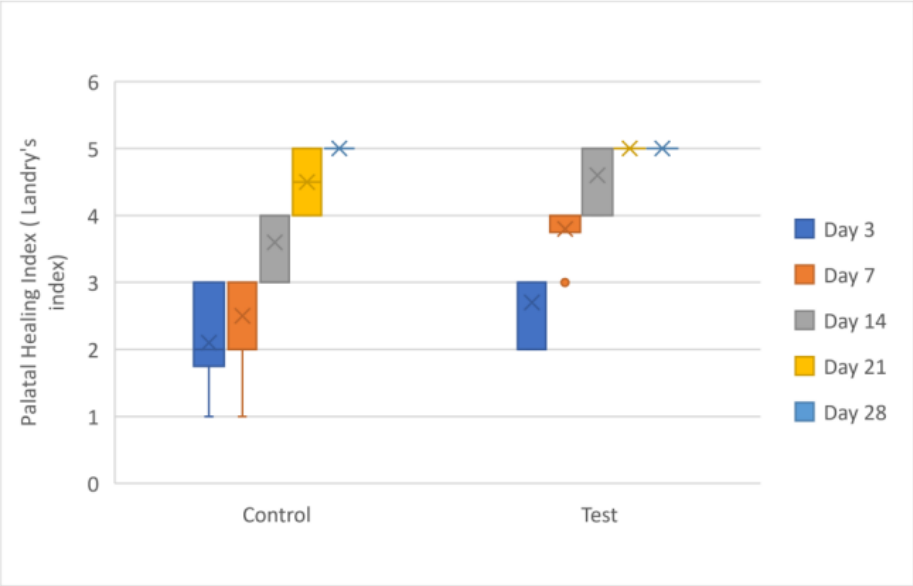


Fig.4 Box plot showing Palatal Healing Index (Landry's index) for test and control groups.

DISCUSSION

FGGs harvested from palatal tissues are often associated with some complications such as pain, bleeding, delayed healing, eating and speech impairment [17]. One of the aims of plastic periodontal surgery is to protect the palatal wound until healing is complete, minimizing patient pain and discomfort.

The aim of this study was to investigate the effectiveness of gaseous ozone on parameters related to the healing of the palatal donor tissue and the patients' pain perception after FGG harvesting. Ozone was chosen in this study to enhance the healing of palatal donor wound. Application of gaseous ozone at 0, 3, 7, and 14 days for 2 minutes at power setting 12 (roughly parallel to 40-60 ug/ml) as recommended by the manufacturer; ozone exerts an antimicrobial effect for few seconds prior to its solubility in wound fluids and oxidation of polyunsaturated fatty acid (PUFA) with formation of H_2O_2 and lipid oxidation products (LOPs); acts as a more stable secondary mediators compared to ozone itself and convey ozone's biologic effects. Ozone can improve the healing process by increasing oxygen delivery to the wound area and its blood supply. Ozone upregulates nitric oxide expression, a powerful vasodilator and promoter of angiogenesis. It enhances cellular metabolism with synthesis of proteins, carbohydrates, and lipids [9].

The palatal donor sites in the control group were protected by non-eugenol periodontal dressing. Conventional periodontal dressings provide an inert mechanical barrier, which neither influences cellular behavior nor play a major role in the biological events that take place during wound healing [18].

In the present study Landry's healing index was used to evaluate palatal wound healing after FGGs harvesting. The donor sites that received ozone exhibited median score of 5 (4-5) after 14 days and all the sites showed a score of 5 after 21 days. This might be attributed to the property of ozone as growth factor inducer by numerous cells involved in wound healing [19]. The application of ozonated oil to acute wounds in guinea pigs upregulated expression of growth factors PDGF, TGF-β and VEGF in both dermis and epidermis by day 7. It stimulates antioxidant response and secretion of TGF-b by fibroblasts [20].

The natural healing at donor sites in the control group of the present study showed a median healing index score of 4 (3-4) after 2 weeks and 5 (4-5) after 3 weeks. This is in parallel with previous studies that exhibited a median score of 4 (3-4) at day 14 and 4 (4-5) after 21 days respectively [21,22].

The process of re-epithelialization can be monitored by clinical inspection of wound margins, in the present study a more objective test was employed using the hydrogen peroxide test. In the absence of epithelial covering H_2O_2 can diffuse into the connective tissue and is acted upon by catalase enzyme producing bubble formation. To reflect the rate of re-epithelialization of palatal wounds, the degree of re-epithelialization was graded as absent, partial, or complete as reported by (Keceli et al.) [23].

This study, the OT group achieved complete re-epithelialization as early as 14 days. These findings could be explained by findings of Patel et al [24]; by using cytological analysis; the rate of migration of epithelial cells and keratinization were enhanced by ozone oil application to the palatal wound during 5,7 and 14 days compared to control group.

Isler et al. [16] evaluated the potential of gaseous OT and laser biomodulation therapy to modulate healing of palatal wound after FGGs; concluded that at 21 days all donor sites showed complete re-epithelialization.

FGG harvest leaves a defect in the palatal mucosa, which heals by secondary intention accompanied by pain and discomfort. This pain affects chewing, daily activities, patient acceptance for treatment and willingness to perform other surgical interventions. In the current study, pain was assessed directly by VAS and indirectly by recording NSAIDs consumption. The postoperative pain after ozone therapy was moderate (3.8±4 with a median score of 4). This was reduced to mild pain starting from day 3 (1.1± 1.2 with a median score of 1) till day 7 (0.8±0.9 with a median score of 1) and at day 14 (0.3 ±0.7 with a median score of 0).

Similar findings have been detailed by Isler et al. [16] that patients in the ozone group reported an intake of a similar number of NSAIDs tablets over a period of 7 days. These findings are similar to a previous study, reporting that ozone application reduced early and late pain after third molar extraction compared to other biological agents such as low level diode laser(LLDL), PRF, cryotherapy and hyaluronic acid when compared with no treatment [25].

It has been shown that peripheral sensitization of nociceptors by inflammatory mediators is an essential mechanism of pain perception during inflammatory conditions [26,27]. Owing to its potent anti-inflammatory and antioxidant actions it can reduce the release of inflammatory mediators. Ozone can induce an increase in some molecules such as serotonin and endogenous

opioids that can stimulate antinociceptive pathways, thus mediating an analgesic effect [28,29].

CONCLUSION

Ozone therapy produces more significant pain reduction at day 3 and day 7, compared to the control sites. Ozone application to the palatal donor site wound, induced complete epithelialization after two weeks.

RECOMMENDATIONS

Randomized clinical trials with larger sample size would be of great importance to shed light on the exact protocol for enhancing postsurgical wound healing & reducing patient morbidity. Clinical trials with application of ozone gas at the recipient bed following mucogingival surgery, to evaluate added benefits on the healing outcomes. The healing process could be monitored by using Doppler-flow meter or Image analysis techniques to confirm the superiority of ozone therapy.

Conflict of interest: No conflict of interest.

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Ethics: This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on: (28/12/2021), approval number: 131221.

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