Histologic and Biomechanical Evaluation of Osseointegrated Miniscrew Implants Treated with Ozone Therapy and Photobiomodulation at Different Loading Times

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Purpose: The aim of this study was to evaluate how continuous heavy orthopedic forces affect the stability of sandblasted, large-grit, acid-etched (SLA)-surfaced miniscrew implants and surrounding bone tissue healing at three different loading periods with treatment of photobiomodulation and ozone therapy. Materials and Methods: Miniscrew implants were applied on the tibias of 9-month-old rabbits (n = 18). The animals were randomly divided into three groups: control, photobiomodulation, and ozone therapy. In all groups, miniscrew implants were loaded with 500 gf at 0, 4, and 8 weeks, respectively (G1, G2, and G3). Several biomechanical and histologic analyses were performed in different centers to measure the implant stability quotient level, bone volume, and bone-to-implant contact. Results: According to the results of the Infinite Focus Microscopy, the ozone therapy group revealed significantly higher scores than the control group and photobiomodulation group at the 4-week loading time, whereas the photobiomodulation and ozone therapy groups revealed significantly higher scores than the control group at the 8-week loading time in terms of bone volume measurements in mm³ (P < .05). According to the histologic analysis, the ozone therapy and photobiomodulation groups revealed significantly higher scores than the control group at the 4-week loading time, whereas the photobiomodulation group showed the highest scores among the 8-week loading groups (P < .05). Conclusion: This is the first study in the literature that reveals a better osseointegration process in miniscrew implants when treated with photobiomodulation and ozone therapy compared with control groups. Although the photobiomodulation and ozone therapy groups did not reveal significantly higher scores in immediately loaded miniscrew implants (G1), these treatments were significantly more effective when loaded after 4 or 8 weeks of osseointegration (G2 and G3). SLA-surfaced miniscrew implants are successful in the orthopedic forces (500 gf) and can be removed without complications. Int J Oral Maxillofac Implants 2019;34:1337–1345. doi: 10.11607/jomi.7601

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Miniscrew implants are used in orthodontics because of the great advantages in clinical practice, such as low cost and simple surgical placement.¹ The success of miniscrew implants in providing definitive anchorage depends on their stability, and pure titanium implant treatment might fail due to its mobility, before or during orthodontic force application.² Various surface treatments have been investigated to improve the stability of miniscrew implant treatment at the bone-to-implant contact (BIC) surface. Within these techniques, sandblasted, large-grit, acid-etched (SLA) surfaces have been shown to have higher BIC values and a positive effect on the osseointegration rate.

To measure the miniscrew implant stability in bone, a resonance frequency analysis (RFA) device is used with the wireless SmartPeg attached to the miniscrew implant. In this technique, ISQ values (from 1 to 100)
were obtained by using a variable frequency magnetic pulse produced by the RFA device. The stability of the miniscrew implant is evaluated by using the resonance frequency of vibration.3

Furthermore, there are some biomechanical techniques on the evaluation of surface characteristics in terms of miniscrew implant stability, such as scanning electron microscopy (SEM) and Infinite Focus Microscopy. Because SEM has high imaging capabilities for examination of material surface geometries on sensitive surfaces, it is likely to be used safely for miniscrew implant surfaces. On the other hand, Infinite Focus Microscopy is a new and promising technique that allows detailed examination with high-resolution images of each point on the surface where it is represented by three-dimensional coordinates. The technique is based on an optic microscope and focus variation technology.4,5

Photobiomodulation, which is also known as photobiostimulation, biostimulation, or low-level laser therapy, has positive effects on miniscrew implant stability via fibroblastic and chondral proliferation.2,6 While photobiomodulation is being accepted and getting more and more popular in medical treatments, ozone therapy can also be used to improve healing. Due to the biocompatibility of ozone with oral epithelial cells, gingival fibroblasts, and periodontal cells, and improvement of bone healing, it is therefore safely and effectively used in dentistry and maxillofacial surgery.7 Activation of the protein synthesis mechanism and an increase of the amount of ribosomes and mitochondria are achieved; therefore, the changes on the cellular level explain elevation of functional activity and regeneration potential of tissues and organs.8 Although it is reported that both laser therapy and ozone therapy with prolonged application time increase implant stability in the immediate slightly functional loading of single implants,9 both alternative treatment methods have not been compared in the literature earlier in terms of different loading times of miniscrew implants in orthodontics. Therefore, because of their biostimulation capacities and positive effects on bone healing, it was hypothesized that photobiomodulation and ozone therapy could be effective and safe treatment methods for increasing the osseointegration level of miniscrew implants at different loading times even under orthopedic forces.

**Aim of the Study**

The aim of the present study was to evaluate how continuous heavy orthopedic forces affect the stability of SLA-surfaced miniscrew implants and surrounding bone tissue healing at three different loading times with the treatment of photobiomodulation and ozone therapy.

**MATERIALS AND METHODS**

**Ethical Statement**

The experimental protocols of this study were approved by the Erciyes University Local Ethics Committee for Animal Experiments (09.12.2015, 15/159-15.03.2017,17/032).

**Miniscrew Implant Designs and Experimental Animals**

A total of 72 cylindrical pure titanium orthodontic miniscrew implants (S.C. Medikal Urunler San. ve Tic), 8 mm in length and 1.8 mm in diameter, were specially designed and manufactured for the present study. The head of the mini-implants was also produced with an external implant head (Fig 1).

In this study, 9-month-old New Zealand Oryctolagus Cuniculus L. male rabbits weighing 3,000 to 3,400 g were preferred randomly (n = 18) (Table 1). The animals had access to food and water ad libitum under an artificial 12-hour light-dark cycle.

**Study Design and Experimental Procedures**

The animal experiments were performed in the Hakan Çetinsaya Experimental and Clinical Research Centre of Erciyes University, Kayseri, Turkey. Also, histologic analysis was performed in the Erciyes University.
Faculty of Dentistry Research Laboratory, Kayseri, Turkey, and Erciyes University Medicine Faculty, Histology and Embryology Department, Kayseri, Turkey. The biomechanical analysis was performed in the Attilim University Metal Forming and Excellence Center, Ankara, Turkey. This study followed the ARRIVE guidelines, which are part of the EQUATOR guidelines.

All surgical procedures were performed under sterile conditions in an operating room. The animals were sedated using an intramuscular injection of ketamine (35 mg/kg, Ketalar, Pfizer) and xylazine hydrochloride (5 mg/kg, Rompun, Bayer). Thereafter, the hairs on both tibias were shaved, and the skin was decontaminated with povidone-iodine 10% (Biokadin). After administration of local anesthesia (2% lidocaine with 1:100,000 epinephrine) via local infiltration, the operation site was isolated with surgical drapes. All surgical procedures for placement of miniscrew implants were performed by the same operator (T.Y.). The tibial metaphysis was exposed by a 40-mm incision that was done on the proximal-anterior part of the tibia. After that, the periosteum was ripped off with an anteromedial incision through the periosteum. Four corresponding miniscrew implants were inserted in the right tibia at the standard distance of 15 mm (Fig 2). The animals were divided into three groups (photobiomodulation, ozone therapy, and control groups), and each group was then divided into three subgroups in terms of loading times: G1, immediate loading; G2, 4-wk waiting period; and G3, 8-wk waiting period (Table 1; Fig 3). Administration of photobiomodulation and ozone therapy was performed for 21 days after the loading periods of each group, respectively. The tibias of the rabbits were collected for the histologic and biomechanical measurements.

**Administration of Photobiomodulation and Ozone Therapy**

Rabbits in the control group received no treatments, whereas the rabbits in the study groups were administered photobiomodulation and ozone therapy.

**Ozone Therapy.** Rabbits in this group received ozone therapy with CA probes (CA probe, Mymed) of ozone generator (Ozonytron XL, Mymed) by contacting the tibia. This ozone generator uses the power of high frequency and voltage. The glass probe is formed by a double glass camera, and only the noble gas mixture provides conducting and emitting electromagnetic energy. Contact usage of the tip of the probe with the body emits energy around the treated area and splits environmental diatomic oxygen into singular atomic oxygen and ozone. The achieved concentration of ozone therapy in the operation field is between 10 and 100 μg/mL. Therefore, ozone can be applied to the places that are difficult to reach, eg, gingival pockets or root canals.\(^\text{10}\)

In this study, ozone therapy was administered seven times during the study (once every 3 days). Administrations were performed with 90% intensity and 30 seconds as recommended by the instructions of the ozone generator manufacturer.

**Photobiomodulation Therapy.** Rabbits in this group received photobiomodulation therapy with an Osseo Pulse LED Device (Biolux Research) in contact

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**Fig 2** (Above) Miniscrew implant localizations on rabbit tibia.

**Fig 3** (Right) Time diagram of study. MSI = miniscrew implants; PBM = photobiomodulation; OT = ozone therapy.
Photobiomodulation therapy was administered with 618 nm wavelength, and the output power was 20 mW/cm²; the therapy was performed for 5 minutes and continuously for 21 days. The amount of total energy was 6 J/cm².

**SEM and Infinite Focus Microscopy Analysis**
Assessment of surface characteristics and biomechanical analyses of miniscrew implants were performed in the Atilim University Metal Forming and Excellence Center in Turkey. The designation and morphology of miniscrew implants were evaluated first with SEM (Fig 4a). However, roughness levels of miniscrew implants were evaluated and corrected with Infinite Focus Microscopy analysis (Alicona Imaging, Infinite Focus Microscope). The Ra value, which gives the average roughness of the surface profile, was used to measure the roughness of the miniscrew implants. This measurement was performed in three different grooves for each miniscrew implant, and the main value was determined as the roughness value. After the study period, the miniscrew implants were collected from the right rabbit tibias, and SEM analysis was performed (Fig 4b). However, Infinite Focus Microscopy analysis was performed the second time for measurements of bone volumes (Vp [µm³]).

**Histologic Analysis**
Thirty-six miniscrew implants with the surrounding bone tissue were fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol (70% to 99%) over a period of 10 days, and embedded in methyl methacrylate (Technovit 7200 VLC, Heraeus Kulzer). Specimens with 300- to 350-µm thickness were provided by Exakt 300 CL (Exakt Apparatebau). After that, the specimens were thinned to 40 µm with Exakt 400 CS (Exakt Apparatebau). Histomorphologic staining was performed with hematoxylin-eosin as described. These samples were evaluated histomorphometrically with a digital camera (Olympus DP 71), which was connected to a light microscope (Olympus BX51). After the screening process, the ImageJ program (ImageJ 1.33u, National Institutes of Health) was used for BIC measurements. This percentage of BIC was calculated as Yano et al recommended in their previous study with the following formula:

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\text{BIC} = \frac{\text{Length of cortical bone in contact with the miniscrew implants}}{\text{Total length of the miniscrew implants in contact with the cortical bone}} \times 100
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**Statistical Analysis**
A power analysis indicated that a sample size of 72 miniscrew implants in total gave 80% power to identify significant differences with an effect size of 0.50 at a significance level of \( \alpha = .05 \). Descriptive statistics were shown as mean and standard deviations of final ISQ, bone volume (mm³), and BIC values. Normality of data was tested using the Shapiro-Wilk test. The final
ISQ scores passed the normality test. The homogeneity variance was determined by the Levene test, and one-way analysis of variance (ANOVA) with Tukey’s post hoc test was performed to compare the control and study groups. Parameters of bone volume (mm³) and BIC showed non-normal distribution. Therefore, Kruskal-Wallis analysis and Dunn’s post hoc test were used to compare bone volume and BIC scores between groups.

RESULTS

In the present study, no clinical mobility or loss of miniscrew implants was observed. No rabbits died, and only one rabbit in ozone therapy–G3 had an infection 4 weeks after surgery. The rabbit received antibiotherapy with an intramuscular injection of 20 mg/kg cefazolin sodium (Cefamezin 500 mg, Eczacıbaşı) for 5 days, and healing was uneventful after 1 week. Another rabbit in photobiomodulation-G2 showed a slight swelling on the tibial area; however, the animal did not show infectious symptoms, so antibiotherapy was not administered. The swelling was healed uneventfully after 1 week. All miniscrew implants undergoing biomechanical examination were removed from bone blocks without any complications by applying reverse torque. Thus, no rabbits or miniscrew implants were excluded from the study.

Presurgical Outcomes

SEM and Infinite Focus Microscopy Evaluation. The miniscrew implants had a homogenous surface in the images obtained by the SEM examination before surgery. Acceptable general shape, symmetrical grooves, and body structure were observed (Fig 4a). The roughness value (Ra score) obtained by presurgical Infinite Focus Microscopy analysis is indicated in the histogram. The SLA surface was found to have a medium roughness value.

Postsurgical Outcomes

Resonance Frequency Analysis (Osstell Systems). According to the final scores of RFA, no statistical difference was observed between the groups (Table 2).

SEM Outcomes. As a result of SEM analysis performed on the removed miniscrew implants, no deformation was observed in the structure of the miniscrew implants, and layers thought to have calcification texture from tibia bone of rabbits on the grooves were detected (Fig 4b).

Infinite Focus Microscopy Outcomes. Volume of Bone. Half of the total numbers of miniscrew implants (n = 36) were collected from tibial bones of the rabbits after sacrifice, and the total amount of remnant bone tissue between grooves was measured with the Infinite Focus Microscopy technique. The measurements were used for determination of osseointegration levels between groups. According to the results of the scores, ozone therapy–G2 revealed significantly higher scores than the control-G2 and photobiomodulation-G2 groups, whereas photobiomodulation-G3 and ozone therapy–G3 revealed significantly higher scores than control-G3 for bone volume measurements (mm³) (Table 3).
The other half of the collected miniscrew implants (n = 36) were also evaluated histologically, and the percentage of osseointegrated areas were measured with the ImageJ program (ImageJ 1.33u, National Institutes of Health). According to the statistical analysis, ozone therapy–G2 and photobiomodulation-G2 revealed significantly higher percentage scores than control-G2, whereas the photobiomodulation-G3 group showed the highest scores among G3 groups (Figs 5a to 5d; Table 4).

**DISCUSSION**

Anchorage control is one of the key factors in the success of orthodontic treatment. Although there are many devices used for anchoring, the skeletal anchoring devices, such as miniscrew implants, that provide an absolute anchorage regardless of patient cooperation, have become popular. These anchor devices can be used in combination with many dental movements, as well as in functional treatments of class II patients and in the orthopedic treatments of class III patients.12,13

Although all the advantages are well-known in the literature, a systematic review study reported that the failure rate of miniscrew implants may vary from 11% to 30%.14 The most common reason for miniscrew implant failure is the loss of primary stability in the initial stage. Moreover, an increase of orthodontic force magnitude could affect the success of the miniscrew implant in the orthopedic treatment in which much heavier forces should be applied.15 Extra mini plaques are preferred to miniscrew implants in order to increase anchorage in the treatment of class III malocclusions.16 There is no acceptable rate of success and failure in the literature due to the limited study of the relationship between orthopedic forces and stability.

On the other hand, although forces between 25 and 500 gf have successfully been applied in studies of the stability of mini-implants against forces, it is not possible to determine the upper and lower thresholds that negate a positive balance from the available evidence.
in the literature. Therefore, the stability of miniscrew implants under heavy orthopedic forces is still a clinical challenge. For these reasons, the present study aimed to investigate the stability and clinical success of SLA-surface mini-implants under orthopedic heavy forces, and it was preferred to apply 500 gf.

Though there is extensive agreement on the use of miniscrew implants for orthodontic anchors in the literature, there is no clear consensus on loading time. Wu et al reported that the appropriate time for osseointegration was 8 weeks, as the secondary remodeling event occurred at 8 weeks. However, Kim et al reported a 4-week waiting period before applying force for stability. Therefore, the present study aimed to compare outcomes of different treatment modalities such as photobiomodulation and ozone therapy for 21 days treatment immediately after surgery simultaneously with the loading (G1), and after 4 (G2) and 8 weeks (G3) of loading. According to the Infinite Focus Microscopy results in the present study, ozone therapy showed better scores after a 4-week loading period, whereas photobiomodulation showed better scores after an 8-week loading period (Table 4).

There are many techniques for surface examination of miniscrew implants prepared with the SLA technique. SEM and Infinite Focus Microscopy (Alicona Imaging, Infinite Focus Microscope) are the techniques that can make a contactless measurement. Although SEM analysis gives a general idea of the structure of the surface, in particular, all parameters affecting the results of the surface treatment must be included in the measurement process to be performed. With Infinite Focus Microscopy (Alicona Imaging, Infinite Focus Microscope), surface depth information can be obtained and high-resolution images are obtained in which each point on the surface is represented by three-dimensional coordinates. Infinite Focus Microscopy can be used, but a light-optic microscope is generally preferred to detect the roughness on the surfaces of miniscrew implants. This makes comparison very difficult with other studies. However, Cook et al have measured the corrosion amount of implanted and then failed miniscrew implants for ilium replacement by using a similar technique. The present study compared the bone volume on the miniscrew implant surface using the Infinite Focus Microscopy technology by Alicona Imaging (Infinite Focus Microscope) and measured the biologic differences at different loading times.

However, histologic outcomes were slightly different than the Infinite Focus Microscopy scores. According to these outcomes, both study groups revealed significantly better scores than the control groups at 4 and 8 weeks after loading (G2, G3). Despite those satisfactory outcomes of the present study, a discrepancy in the results was seen between Infinite Focus Microscopy and BIC scores in the photobiomodulation-G2 group. The difference between Infinite Focus Microscopy and BIC scores is not surprising and may be encountered because they measure different parameters on osseointegration. However, although the total numbers of miniscrew implants were statistically adequate, the difference could only be explained with the authors’ maximum effort to use fewer animals in the present study. Because of ethical reasons, the authors preferred to use four miniscrew implants in one rabbit, but one of the animals in the photobiomodulation-G2 group had swelling in the tibial area, which was perfectly healed even though antibiotic therapy was not administered. Thus, the authors assume that another reason for this discrepancy could be the possible negative effects of the infection around the miniscrew implant(s). Similarly, BIC scores were significantly higher in the ozone therapy–G2 and photobiomodulation-G2 groups compared with the control-G2 group, whereas the photobiomodulation-G3 group was significantly higher than the other groups. However, the ozone therapy–G3 group showed worse scores than the ozone therapy–G2 group. This time, it was expected because of the infected rabbits in this group. Thus, the number of animals used in the present study could be the first limitation.

Another limitation could be the non-measuring of the removal torque of the miniscrew implants. Those results could be used in explaining the difference in outcomes in terms of determination of the relation between BIC values, Infinite Focus Microscopy results, and removal torque of miniscrew implants. On the other hand, although Infinite Focus Microscopy results could be used in evaluation of osseointegration, BIC results should be used more in the final decision. The Infinite Focus Microscopy results show the total amount of bone remnants in grooves where BIC values perfectly reveal the osseointegration of miniscrew implants in bone in histologic sections. Moreover, it is shown that SLA-surfaced miniscrew implants have higher BIC values and positively affect the stability.

The RFA technique can detect changes in the stability of miniscrew implants, and these changes can reflect biologic events associated with the bone–miniscrew implant interface. The stability values of the implants measured by the RFA method are reported to be related to the BIC ratio. Serra et al reported no statistically significant differences in stability values after 1, 4, and 12 weeks in loaded miniscrew implants. Consistent with the literature, there was no statistically significant difference between groups in terms of final ISQ values of mini-implants in the present study.

The present study aimed to have better miniscrew implant stability in the study groups than the control
group at each loading time with the administration of photobiomodulation and ozone therapy. Many studies in the literature have reported that irradiation by LED and laser may provide increased success rates\(^\text{3,25}\) and that ozone may not only stimulate cell proliferation and soft tissue healing, reducing pain,\(^\text{26}\) but also enhance bone regeneration.\(^\text{27}\) On the other hand, there are not adequate and satisfactory studies, especially, about the effects of ozone therapy on the orthodontic miniscrew implant treatment technique. Photobiomodulation improves angiogenesis and the mechanism of metabolic activities and the revitalization processes, reduces the risk of infection, and accelerates the healing of the damaged tissue. Photobiomodulation therapy could be either administered by LED devices or another low-level laser therapy device. The main advantage of LED devices is to have a less coherent scattering pattern and have fewer side effects because of that. These devices could be used safely in large areas of body skin.\(^\text{28}\) The dose (wavelength, power, frequency, fluency or dose, and energy parameters) applied during laser treatment is an important benefit of photobiomodulation for obtaining good results. However, there is no consensus for determining the dose and wavelength of photobiomodulation. It has been reported that the most suitable wavelength is 550 to 950 nm,\(^\text{29}\) and the dose is 0.001 to 20 J/cm\(^2\) for biostimulation in the literature.\(^\text{25}\)

Although it has been reported that both laser therapy and ozone therapy with prolonged application time increase implant stability in the immediate slightly functional loading of single implants,\(^\text{9}\) no investigations were found concerning miniscrew implant stability using those techniques in orthopedic forces. The evidence that ozone can react with the composition of the clinical application of ozone therapy in dentistry is not extensive. Ozone can positively affect oxygen metabolism, cell energy, the immunomodulator property, antioxidant defense system, and microcirculation.\(^\text{30}\) Alpan et al reported that gaseous ozone application accelerates xenograft resorption and enhances bone regeneration, especially in the early stages of bone healing within the limitations of their animal study.\(^\text{27}\) Similarly, according to the bone volume results, ozone therapy–G2 revealed significantly higher scores among G2 groups, but the photobiomodulation–G3 group showed a similar healing pattern with the ozone therapy–G3 group, which was significantly higher than the control–G3 group. As a final conclusion, the present study reports that miniscrew implants treated with photobiomodulation and ozone therapy are more stable than control groups and that either photobiomodulation or ozone therapy are effective techniques for increasing bone volume, which may lead to better stability for miniscrew implants in orthodontics and oral and maxillofacial surgery.

**CONCLUSIONS**

This is the first study in the literature that reveals a better osseointegration process in miniscrew implants when treated with photobiomodulation and ozone therapy compared with control groups. Therefore, photobiomodulation or ozone therapy are recommended to be preferred as effective and safe treatment methods for increasing bone volume, which may lead to better stability for miniscrew implants. Not only should those results be evaluated in miniscrew implants, which are used in orthodontics, but also in every SLA-surfaced implant in dentistry. Moreover, 500 gf is an acceptable orthopedic force to be applied on SLA-surfaced miniscrew implants in orthodontics, which may lead to increased anchorage, especially in treatment of skeletal Class II and Class III patients.

However, a limitation of the study is that the condition of the experiment cannot be simulated in the mouth. The miniscrew implants are placed in humans in an open oral environment. More accurate results can be obtained with human trials. The authors also recommend reconsidering the power analysis of animal studies in terms of detecting the animal numbers or to place fewer miniscrew implants for each tibia, which may cause more animals to be sacrificed.

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