Comparison of the Stability of Sandblasted, Large-Grit, and Acid-Etched Treated Mini-Screws With Two Different Surface Roughness Values: A Histomorphometric Study

Elif Dilara Seker, DDS, DS,[*] Ibrahim Yavuz, DDS, PhD,[†] Turker Yucesoy, DDS, PhD,[‡] Ebru Cenkci, DDS, MA,[§] and Arzu Yay, DDS, PhD[||]

Purpose: To evaluate the effects of 2 different surface roughness values produced by sandblasted, large-grit, and acid-etched treatments at different loading conditions on the stability of mini-screws.
Material and Methods: A total of 56 mini-screws (Group 1; 28 with Ra value of 1 μm, Group 2; 28 with Ra value of 1.5 μm) were inserted into the tibia of fourteen New Zealand rabbits. Surface analysis was performed before the placement of the miniscrews using multi-technique characterization. The mini-screws were loaded with 500 grf after different healing times: unloaded, immediate, 4 and 8 weeks. Resonance frequency analyses were performed immediately after mini-screw placement and at the end of loading. Biomechanical and histomorphometric analyses were also performed at the end of the loading period.
Results: All mini-screws preserved their stability at the end of the loading period. However, the resonance frequency analyses showed higher implant stability quotient scores for 8-week group, unlike the immediate loading and unloaded groups ($P < 0.05$). According to the infinite focus microscopy results, prolongation of healing time resulted in a greater bone area on the loaded mini-screws in Group 2 ($P < 0.05$). Similarly, the histomorphometric analysis revealed higher bone-to-implant contact values in the 8-week group. There was no significant difference in the stability between the miniscrews with the Ra values of 1 and 1.5 μm.
Conclusions: Sandblasted, large-grit, and acid-etched treated mini-screws showed significantly higher stability with healing time under heavy forces. Sandblasted, large-grit, and acid-etched treated mini-screws can be removed without fracture of the screw or the bone surfaces.

Key Words: Bone implant interactions, orthodontics, surface chemistry

(*J Craniofac Surg* 2022;33: 41–47)

The use of a stable orthodontic anchorage increases the possibility of the orthodontic treatments without tooth extraction or orthognathic surgery.[1] Recently, distalization of the whole mandibular arch and flattening of the occlusal plane with mini-screws anchorage placed into the retromolar region or the buccal shelf have been introduced as an alternative approach for treatment of Class III malocclusions relative to orthodontic treatment with extraction or orthognathic surgery.[2] Identically, Class II malocclusions are also treated with distalization of the entire maxillary arch by using mini-screws inserted into the infrazygomatic crest.[2,3] The mini-screws, which ensure absolute orthodontic anchors prevent undesirable reciprocal side effects in transverse, vertical, and anteroposterior dimensions.[4] In recent years, the treatment planning including mini-screws was preferred more frequently in the clinical practice to optimize the orthodontic treatment duration or to avoid invasive procedures such as orthognathic surgery. On the other hand, the mini-screws would endure relatively higher amounts of orthopedic heavy forces. Therefore, the stability of the mini-screws under heavy forces remains a clinical challenge.[5]

Mechanical retention and partial osseointegration at the interface between the mini-screw and the cortical bone are the key factors for the stability of a mini-screw.[6] This necessity to ensure the stability in mini-screws might lead to relatively higher failure rates (11%–30%) in comparison to osseointegrated implants or miniplates.[7,8] Therefore, the numerous surface modifications have been evaluated to improve the osteoconductivity of screws.[9–11] Surface treatments produce a biologically active surface for screws. Surface roughness promotes osteoblast proliferation and osteoblasts migration to the screw-bone interface and lead an increase in the alkaline phosphatase, growth factor beta, prostaglandin 2 levels.[11]

Surface treatment with SLA procedure was reported as a safe method that generates reliable and predictable surfaces.[12,13] It was reported that the rough surface produced by the sandblasted, large-grit, and acid-etched (SLA) procedure ensured higher bone-to-implant contact (BIC) and better stability compared to the surface features modified by other surface treatment methods.[14] Although the favorable effects of roughened surfaces on bone growth have been well documented, the optimal surface modification and roughness for selective adsorption of osteoblastic cells and rapid osseointegration are still unknown.[9,11,15]

From the *Department of Orthodontics, Faculty of Dentistry, Bezmialem Vakif University, Istanbul; †Department of Orthodontics, Faculty of Dentistry, Erciyes University, Kayseri; ‡Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Bezmialem Vakif University, Istanbul; §Atilim University, Metal Forming and Excellence Center, Ankara; and ||Histology and Embryology Department, Erciyes University, Medicine Faculty, Kayseri, Turkey.
Received March 1, 2021.
Accepted for publication April 18, 2021.
Address correspondence and reprint requests to Elif Dilara Seker, DDS, DS, Department of Orthodontics, Faculty of Dentistry, Bezmialem Vakif University, Fatih/Istanbul 34093, Turkey;
E-mail: dilaraarsln@hotmail.com, elseker@bezmialem.edu.tr
The experimental protocols of this study were approved by the Erciyes University Local Ethics Committee for Animal Experiments (17/032).
This study was supported by the Erciyes University Scientific Research Projects Coordination Unit (TSD-15-6098).
The authors report no conflicts of interest.
Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jcraniofacialsurgery.com).

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000007837

Even if it is known that roughened surface leads to the strongest bone response for dental implants, the optimal parameters on the nano-sized level for rapid bone apposition remain unknown.[16,17] Furthermore, mini-screws are temporary anchorage devices that are removed at the end of orthodontic treatment, and they are smaller in size in comparison to dental implants. Therefore, surface treatment and roughness studies specifically for mini-screws should be carried out to develop a mini-screw surface that promotes faster and more effective osseointegration but allows removal of the mini-screw safely at the same time. To our knowledge, no previous study has been published on the effects of surfaces with differing roughness values produced by the SLA procedure on the stability of mini-screws. Besides surface roughness, the effects of loading time on mini-screw stability under heavy forces have frequently been discussed in the literature. However, there is no consensus on the waiting time before loading with heavy forces following the placement of mini-screws.[18]

The aim of this study was to evaluate the effects of 2 different surface roughness values produced by SLA treatments on the clinical stability of orthodontic mini-screws used for anchorage. The second purpose of this study was to investigate the effects of different waiting times before loading with heavy orthodontic forces on BIC. This study set out to test the hypothesis that different surface roughness values (1) and healing times (2) before loading would not significantly affect the clinical stability of mini-screws.

MATERIALS AND METHODS

Mini-Screw Designs and Experimental Animals

Fifty-six conical pure titanium (grade 4) orthodontic mini-screws were specially designed and fabricated for this study. The heads of the mini-screws had custom-made external screw heads. The mini-screws measuring 8 mm in length, 1.8 mm in outer diameter, 1.45 mm in inner diameter, and 0.5 mm in thread pitch were used in this study. Figure 1A presents the geometric features of the mini-screws. Following the manufacturing step, among the 56, 28 mini-screws were prepared by the SLA procedure to have an Ra value of 1 micrometer (μm) (Group 1), and 28 mini-screws were prepared to have an Ra value of 1.5 μm (Group 2). The SLA surface treatment included the following steps. The mini-screws were blasted with Al_2O_3 (large grits of 0.25–0.5 mm). Then, they were etched in a boiling mixture of HCl/H2SO4 acid (ratio of 1:6). Etching conditions of 90°C and 15 minutes for mini-screws with an Ra value of 1 μm and 18 minutes for mini-screws with an Ra value of 1.5 μm. After that, all the mini-screws were ultrasonically cleaned with acetone, 70% ethanol, and distilled water for 20 minutes each, and then dried in air. The mini-screws were maintained under sterile conditions after gamma sterilization. In this study, fourteen 12-month-old New Zealand adult female rabbits weighing at 3000 to 3200 grams were used. All rabbits were given a 1-week adaptation period. This study was approved by the Experimental Animal Committee of Erciyes University (17/032).

Surface Characterization

Three SLA-treated mini-screws from each group were randomly selected and scanned for analysis of their surface properties. The surface analysis prior to the placement of the SLA-treated mini-screws was performed at the Atilim University Metal Forming and Excellence Center, Ankara. Microstructural and elemental analyses of the SLA-treated mini-screws were carried out with a scanning electron microscope (SEM, Zeiss Evo LS 15, Carl Zeiss, Jena, Germany) and an energy dispersive X-ray spectrometer (EDS, Bruker Quantax 400 The Flash 5000 series, Berlin, Germany), respectively.

Surface roughness evaluation was performed using an infinite focus microscope to verify whether the desired amount of surface roughness was achieved. The Ra value, which provides the average roughness of a surface profile, is the roughness parameter that is the most commonly used to measure the roughness of implants.[19] The Ra value was measured three-dimensionally with an Alicona device (Infinite Focus Microscopy [IFM], Imaging GmbH, Graz, Austria). Three mini-screws were selected randomly from each mini-screw group. Three-thread valley and 3-thread flanks of these mini-screws were measured and the average roughness value was recorded as the roughness value of the mini-screw. However, these mini-screws were not placed to the tibias of the rabbits.

Design and Experimental Procedures

The mini-screws for each group that were created according to the specified surface roughness values were randomly divided into 4 subgroups in terms of loading times. Identification of the groups which included mini-screws with an Ra value of 1 μm (Group 1) and those with an Ra value of 1.5 μm (Group 2) was carried out as follows: immediate loading (n = 8); loading after 4 weeks (n = 8); loading after 8 weeks (n = 8); unloaded control group (n = 4) (Fig. 2). The experiments were performed at the Experimental and Clinical Research Center of Erciyes University, Kayseri. All surgical procedures were performed under sterile conditions and anesthesia in an operating room by the same operator T.Y. The pilot hole was drilled with a 1 mm-diameter-drill because of the high percentage of cortical bone in the rabbit tibia. After pilot hole drilling, the mini-screws were placed using a specially designed screwdriver under saline irrigation because of the high percentage of cortical bone in the rabbit tibia. Two corresponding mini-screws were inserted into each tibia of the rabbits at a standard distance of 15 mm. The mini-screws were loaded with horizontal continuous forces of 500 g by nickel-titanium closed-coil springs (DB Orthodontics, West Yorkshire, United Kingdom) (Fig. 1B). In immediate loading group, the mini-screws were loaded after the placement into the bone at the same session. On the other

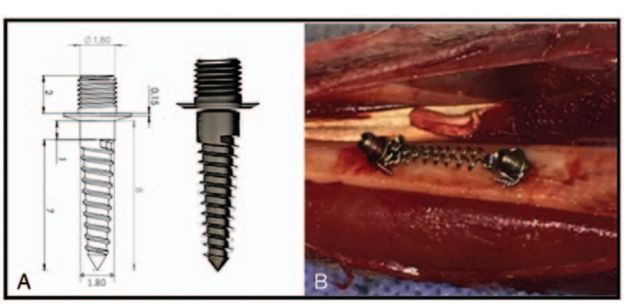

FIGURE 1. (A) The SLA-treated titanium mini-screw design used in this study. (B) The localization of mini-screws with an Ra value of 1 μm on rabbit tibia. SLA, sandblasted, large-grit and acid-etched.

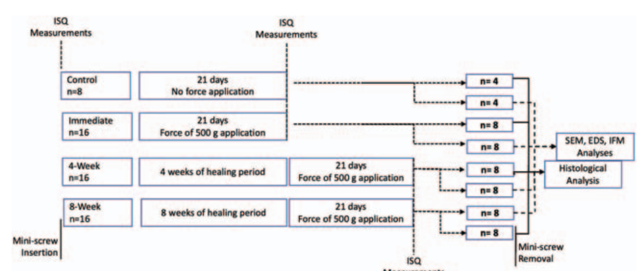

FIGURE 2. Time diagram for the groups. EDS, energy dispersive X-ray spectrometer; IFM, infinite focus microscopy; ISQ, implant stability quotient; SEM, scanning electron microscope.

hand, a second surgical procedure was performed to place coil springs and apply the force in the groups which 4-week waiting and 8-week waiting before loading.

After the operations, the subcutaneous tissues and skin were sutured with 3-0 vicryl suture and 3-0 silk suture, respectively. The animals received the antibiotherapy and an analgesic agent. The animals were sacrificed with an intravenous overdose of sodium pentothal after the loading periods. The tibias were dissected for histological measurements and mechanical tests.

Mechanical Tests

Resonance Frequency Analysis

Resonance frequency analysis (RFA) with an Osstell device (Ostell AB, Göteborg, Sweden; implant stability quotient [ISQ]) was used for measuring mini-screw stability in this study. All of the mini-screws that were used in this study were assessed using RFA by an experienced orthodontist. A custom-made headpiece had been placed on the external screw to provide the connection with a SmartPeg abutment (Integration Diagnostics AB) for measurement of the ISQ values by the Osstell device. First, the stability measurements were performed at the mini-screw placement session before the loading. After 21 days of force application, the stability of mini-screws was remeasured by removing the closed-coil springs. Similarly, the stability of unloaded mini-screws (control group) was measured at placement and 21 days after placement. Three ISQ values were obtained for each mini-screw at each time, and the average of the 3 ISQ values was calculated. The baseline ISQ values and change of ISQ values that occurred from placement till the end of the loading phase were used for the statistical analyses.

Scanning Electron Microscope, Energy-Dispersive X-Ray Spectrometry, and Infinite Focus Microscopy Analyses

Scanning Electron Microscope and Energy-Dispersive X-Ray Spectrometry Analyses

After the experimental period, half of the mini-screws for each group were removed from the tibias by reverse torque rotation with the same screwdriver that was used at the time of placement for mechanical tests (Fig. 2). The mechanical tests of the mini-screws were performed at the Atilim University Metal Forming and Excellence Center in Ankara. The mini-screws were evaluated with a SEM (Zeiss Evo LS 15 Microscope) at high vacuum (1×10^{-6} mbar), under 55 to 500× magnification, and with 20 kV of energy. The scanning electron microscopy images were recorded for assessment of surface topography and composition (Fig. 3A). Additionally, the mini-screws were investigated with an EDS detector (Bruker Quantax 400 The Flash 5000 series) to determine their surface composition (Fig. 3B).

Infinite Focus Microscopy Analysis

Infinite focus microscopy is a technique that provides an accurate optical 3D measurement of the mini-screws. It also allows the data overlap of 2 different measurements. In this study, the surfaces of the unused mini-screws with different Ra values were initially scanned to determine the surface properties using IFM (Alicona, IFM, Imaging GmbH). At the end of the experimental period, all of the retrieved mini-screws were measured using the same technique. The 3D data which was obtained from both unused and retrieved mini-screws were overlapped, and the differences between 2 measurements were calculated using IFM analysis software (IFM software 3.5.0.1; Alicona Imaging)[20] and these differences was considered as bone contact area on the mini-screw's surface.

Figure 3C presents an example of the outcome of these measurements. The colored image shows the data overlapping of the

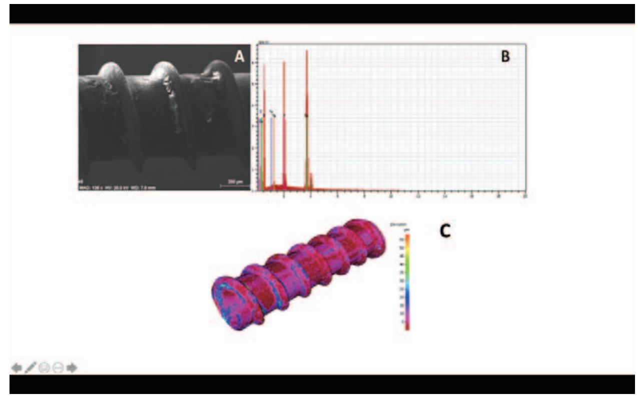

FIGURE 3. (A) Representative secondary electron microscopy image of mini-screw with an Ra value of 1.5 μm (8-week waiting group). (B) Energy-dispersive X-ray spectrometry (EDS) of retrieved mini-screw with an Ra value of 1.5 μm (8-week waiting group). (C) Three-dimensional analysis of the surface of retrieved mini-screw's with an Ra value of 1.5 μm (8-week waiting group) using infinite focus microscopy (Alicona, Imaging GmbH, Graz/Austria). EDS, energy dispersive X-ray spectrometer.

unused and retrieved mini-screws, and it also contains a micron bar to visualize the deviation of the compared specimens. Moreover, the numeric results of this comparison were provided by IFM analysis software.

In this study, percentage of the bone-screw contact area (BSC) was used for the comparison of the data. The percentage of the BSC was calculated by using the formula below:

$$BSC\% = \frac{\text{Bone} - \text{screw} - \text{contact area}\,(\mu m2)}{\text{The area of unused miniscrew}\,(\mu m2)} \times 100$$

Histological Analysis

Histological analysis was performed at the Erciyes University Faculty of Dentistry Research Laboratory, Kayseri, Turkey and Erciyes University Faculty of Medicine, Histology and Embryology Department, Kayseri, Turkey. Fourteen mini-screws from each group (Group 1 and 2) with at least 5 mm of the surrounding bone tissue were fixed in 10% buffered formalin and embedded in methyl methacrylate (Technovit 7200 VLC; Heraeus Kulzer, SouthBend, IN). Bone blocks with 300 to 350 μm of thickness were obtained by using EXAKT 300 CL (EXAKT Apparatebau, Norderstedt, Germany). Afterward, the specimens were thinned to 40 μm with EXAKT 400 CS (EXAKT Apparatebau). Histomorphological staining was performed with hematoxylin-eosin as described. The specimens were analyzed histomorphometrically by a digital camera (Olympus DP 71, Tokyo, Japan) which was connected to a light microscope (Olympus BX51, Tokyo, Japan), and histological images were recorded. After this step, BIC values were measured and calculated with the ImageJ software (Image J 1.33u; National Institutes of Health, Bethesda, MD). The BIC ratio was calculated using the following formula[21] (Fig. 4):

$$\text{BIC ratio} = \frac{\text{Length of cortical bone in contact with the minscrew}\,(\mu m)}{\text{Total length of the miniscrew in contact with the cortical bone}\,(\mu m)}$$

Statistical analysis

The statistical analyses were performed using SPSS (version 11.5, SPSS Inc., Chicago, IL). The power analysis revealed that a sample size of 56 mini-screws would provide more than 95% power

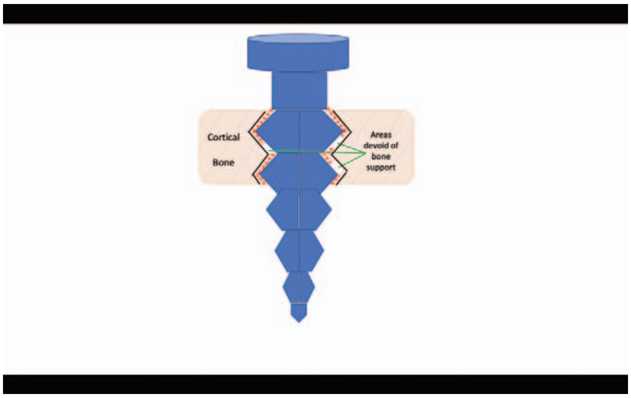

FIGURE 4. Method for measuring the bone-implant contact (BIC) ratio. BIC ratio = length of cortical bone in contact with the mini-screw (dotted-line)/total length of the mini-screw in contact with the cortical bone length of screw surface at cortical bone (solid line) × 100.

to detect significant differences with an effect size of 0.80 and a significance level of $\alpha = 0.05$.

Descriptive statistics are presented as the mean values and standard deviations. Shapiro–Wilk test was performed on all variables to test normal distribution. Independent-samples *t*-test was used for the intergroup comparisons of the roughness values, RFA and IFM results. The differences in the RFA and IFM outcomes among 4 different loading protocols were analyzed using one-way analysis of variance. Mann–Whitney *U* test was used for comparison of the BIC values between groups, and Kruskal-Wallis test was used for the intragroup comparison of the BIC values for 4 different loading protocols. Additionally, Bonferroni test for multiple comparisons was applied. A significance level of 0.05 was chosen.

RESULTS

At the end of the observation period, all mini-screws for the loading and nonloading groups were in situ, and no mobility was observed. The mini-screw survival rates were 100% for all groups. Moreover, at the end of the observation period, all SLA-treated mini-screws were removed safely without fracture of the screw or the bone surfaces.

Topographic Findings

The SEM analysis demonstrated that the mini-screws had a homogenous, regular surface, and an acceptable body structure. Moreover, no defects such as pores or craters on the surfaces of the mini-screws were observed. Furthermore, the elemental composition of the surfaces was quite similar in both mini-screw groups.

The mean values and standard deviations of surface roughness (Ra value) for Groups 1 and 2 were recorded as 1.11 ± 0.18 μm and 1.53 ± 0.02 μm, respectively. A significant difference between groups was found in the Ra values ($P = 0.01$). From these results, it could be stated that the surface characteristics which were planned before production of the mini-screws for this study were achieved.

Results of Resonance Frequency Analysis

The initial ISQ values of all groups were compared, and no statistically significant differences were observed ($P > 0.05$) as shown in Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C826. No significant differences were found in the changes of the ISQ values from mini-screw placement to the end of follow-up between Groups 1 and 2 ($P > 0.05$). However, significant differences for both groups were observed in the changes of ISQ values in the intragroup comparisons ($P < 0.05$). The statistical comparisons of the ISQ changes among different healing times before loading are shown in Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C826.

Scanning Electron Microscope, Energy-Dispersive X-Ray Spectrometry, and Infinite Focus Microscopy Findings

Scanning electron microscope analysis was performed on the retrieved mini-screws, and no damage or deformation was observed in the structure of the mini-screws. Moreover, amorphous layers were detected on the secondary SEM images of the retrieved mini-screws. The quantitative elemental analysis with EDS demonstrated that these layers contained titanium, oxygen, calcium, carbon, phosphorus, magnesium, sodium, sulfur, nitrogen, and fluoride peaks. The elemental composition of these layers on the retrieved mini-screws was consistent with the mineralized matrix in bone (Fig. 3A-B). Therefore, it was thought that these could be bone particles adhering to the surface of the mini-screw.

IFM analysis was performed to measure the percentage of BSC (%) (Fig. 3C). The significant intergroup differences were observed (Group 1 versus Group 2), ($P < 0.05$). In the intragroup comparisons for the percentage of BSC, no significant difference was observed among the 4 different loading protocols in Group 1 ($P > 0.05$), whereas a significant difference was observed in Group 2 as shown in Supplementary Digital Content, Table 2, http://links.lww.com/SCS/C827 ($P < 0.05$).

Histomorphometric Findings

The mean BIC ratios and statistical evaluation results are shown in Supplementary Digital Content, Table 3, http://links.lww.com/SCS/C828. No significant differences were observed in the BIC ratio between Groups 1 and 2 ($P > 0.05$). The intragroup comparisons for Group 1 showed no significant difference among the 4 different loading protocols ($P > 0.05$), whereas a significant difference was observed in Group 2 ($P < 0.05$). Figure 5 illustrates the histological images of the specimens stained with hematoxylin-eosin under light microscopy.

DISCUSSION

Though all clinical advantages, the failure rate of mini-screws was reported that vary from 11% to 30% in the literature.[8] However, the higher forces applied in the orthopedic treatment of skeletal class II and class III malocclusions could affect the success of the mini-screw negatively. There is no defined rate of failure in the literature because of the limited study on the relationship between orthopedic forces and mini-screw stability.

In order to improve the success rates of mini-screws, various surface treatment methods have been studied.[22] Among these techniques, it was reported that SLA surface treatments lead to a higher success rate and stability.[23] This is because the sandblasting and acid-etching procedures produce relatively homogeneous surface roughness values and allow more cell adhesion than machined surfaces.[19] However, comparative studies with different mini-screw surfaces are rarely performed. To the best of our knowledge, no previous study has investigated the effects of surfaces with different roughness values derived from SLA treatment on bone response surrounding mini-screws and the stability of mini-screws. Therefore, 2 different surface topographies (approximate Ra values of 1 and 1.5 μm) were created by SLA treatment for this study.

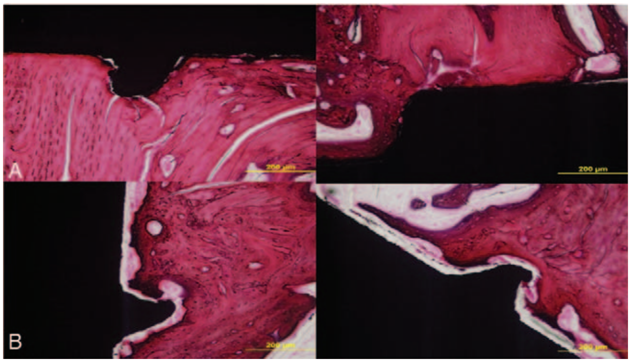

FIGURE 5. Histology images of (A) SLA-treated mini-screw with an Ra value of 1.5 μm after 21 days of force application (8-week waiting group), (B) unloaded SLA-treated mini-screw with an Ra value of 1.5 μm after 21 days. The osseointegration rate in the 8-week waiting time group was higher compared to the unloaded SLA-treated mini-screws. SLA, sandblasted, large-grit and acid-etched.

The mini-screws were analyzed for characterization of their topography, elemental composition, and surface roughness (Ra value) using SEM, EDS, and IFM, (Alicona, Imaging GmbH) respectively. This was similar to the method used by Eliades et al[24] and Yucesoy et al.[25] The results of the surface analyses showed that the surface characteristics which were planned before production of the mini-screws for this study were achieved, and the topographic features and elemental compositions of the mini-screws were quite similar in both groups. In order to understand the effects of surface topography and roughness on mini-screw stability, mini-screws with controlled and standardized surfaces should be included in studies on the stability of mini-screws. This is because a heterogeneous topography may affect protein adsorption and adhesion of osteoblastic cells, and thus, it may decrease the osseointegration rate.[26] Therefore, the mini-screws with standardized surfaces were included in the study.

Loading time, as well as the surface feature of a mini-screw, is also an important factor for the success of mini-screws during orthodontic treatment.[27] It was reported that 4 weeks are a critical time point for osseointegration of mini-screws, the stability continued to increase after 8 weeks of healing.[28] Therefore, the mini-screws were loaded with continuous forces of 500 grf after 0, 4, or 8 weeks of healing.

The stability of the mini-screws was measured using RFA (Osstell AB; ISQ) at the mini-screw placement session and 21 days after force loading. The connection between SmartPeg and mini-screw to measure the ISQ values was provided using a previously reported method.[29] RFA is an accurate technique for early assessment of osseointegration.[30] Furthermore, this technique has a high degree of repeatability for measurement of stability.[31] This study showed that the ISQ values of the mini-screws were similar in both groups and the mean ISQ values of the mini-screws in this study were between 67.75 and 76.63 as shown in Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C826. It was reported that the mean ISQ levels should be in the range of 57 to 82 for normal implant systems.[32] In this study, the SLA-treated mini-screws provided sufficient primer stability after insertion. Therefore, the first null hypothesis, which stated that different surface roughness values produced by SLA treatments would not significantly affect the stability of mini-screws, was not rejected. At the end of the loading period, there was no sign of implant failure, the SLA-treated mini-screw survival rates were 100% for all groups, and this was similar to the results of a previous study.[23] However, the secondary stability of the SLA-treated mini-screws which were loaded immediately after implantation remained almost unchanged, while delaying the load on the mini-screw by 8 weeks resulted in a higher ISQ value in comparison to the immediate loading and control groups as shown in Supplementary Digital Content, Table 1, http://links.lww.com/SCS/C826. Application of the orthodontic forces after the healing process positively affected the bone remodeling surrounding the SLA-treated mini-screws.[28,33] Furthermore, it may be speculated that relatively low secondary stability for the immediate loading might be a result of microfractures in the bone surrounding the mini-screw.[29]

At the end of the observation period, the SLA-treated mini-screws were removed safely without bone or screw fracture from the tibias. The surface analyses were re-performed following removal of the mini-screws. The SEM and EDS results indicated calcified teguments on the surface of the retrieved mini-screws which were attributed to bone particles (Fig. 3A-B). Furthermore, Alicona (IFM, Imaging GmbH), which enables noncontact 3D measurements, was used for the retrieval analysis in our study. The IFM analysis demonstrated a greater percentage of BSC for the mini-screws with an Ra value of 1.5 μm (Group 2). This analysis also demonstrated that delaying loading for 4 or 8 weeks resulted in a higher bone-to-metal contact ratio for Group 2 as shown in Supplementary Digital Content, Table 2, http://links.lww.com/SCS/C827. Alicona (IFM, Imaging GmbH) is an optical microscope that builds a three-dimensional replica of a surface from a specimen (Fig. 3C). This tool details the specimen surface by representing each point on the sample surface in three-dimensional coordinates, and it provides high-resolution images.[34] Cook et al[35] measured mechanical erosion using Alicona on retrieved mini-screws which were used for total hip replacements. We measured the bone–screw contact by using a similar technique.[35] Although IFM technology is widely used for surface imaging, it was used for the first time to measure the amount of bone on the mini-screws in this study, and this made the comparison of the results to those of previous studies difficult.

The results of the histological analysis showed that the BIC values of the mini-screws with an Ra value of 1.5 μm were higher for the 8-week group than the control and 4-week groups as shown in Supplementary Digital Content, Table 3, http://links.lww.com/SCS/C828 (Fig. 5). According to these results, the significantly lower BIC value for the 4-week mini-screws in Group 2 could be attributed to the variation in bone quality and quantity. Furthermore, the histomorphometric analysis, which is the most precise method, provided similar results to those obtained with the IFM analysis. However, the null hypothesis, which stated that healing time before loading would not significantly affect the secondary stability of the SLA-treated mini-screw, was rejected. Taken together, these results suggested that bone apposition surrounding the loaded SLA-treated mini-screws was more active than the unloaded ones, and this result was in accordance with the literature.[36]

Limitations

Finally, some important limitations need to be considered. The first limitation was the differences in the bone microstructure and metabolism between humans and animal models. The results of animal studies may not be a valid predictor of human reactions. However, human's reactions may be simulated more accurately in animal studies when significant improvement in the scientific methods for both human and animal studies. Another limitation was the removal torque values could not be measured for mini-screws. The correlation between results of removal torque, IFM could be investigated. The sectioned site for the histomorphometric analysis may not always provide complete information on the amount of surrounding bone for mini-screws. Furthermore, the study was not designed to include a control group which includes machine surfaced mini-screws (no surface treatment) to reduce the number of the sacrificed animals. Therefore, these issues should be considered while transferring these experimental findings to clinical practice.

Sandblasted, large-grit, and acid-etched treated mini-screws used for orthodontic anchorage delivered promising results from both clinical stability and biomechanical aspects. Sandblasted, large-grit, and acid-etched treated mini-screws under heavy orthopedic forces provided clinically adequate osseointegration and acceptable unscrewing, avoiding bone fracture and tissue destruction. Since this surface treatment enhances the clinical success of the mini-screws, it may provide shorter treatment times and high-quality results without undesired side effects. However, the findings obtained on animals are needed confirmed in further human studies.

CONCLUSIONS

Notwithstanding these limitations, the study suggests that,

(1) The survival rates of SLA-treated mini-screws were 100% under loading with 500 gf and the SLA-treated mini-screws were removed without any complications at the end of the observation period.
(2) The ISQ values and contact area between the bone and screw of the SLA-treated mini-screws which were loaded immediately after implantation remained almost unchanged, while it showed an increase in these parameters for the mini-screws which were loaded after a healing period.
(3) No significant differences were seen in the bone volume on the surfaces of the retrieved mini-screws between the groups.
(4) The rate of osseointegration in the mini-screws with the Ra value of 1.5 μm was found to be higher for 8-week group. However, there was no difference in the extent of osseointegration between the mini-screws with the Ra values of 1 and 1.5 μm.

ACKNOWLEDGMENTS

The authors acknowledge Dr Aysegul Yabaci (Department of Biostatistics and Medicine Informatics, Division of Basic Medical Sciences, Bezmialem Vakif University School of Medicine) for her help in statistical analysis.

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