Bone-Miniscrew Contact and Surface Element Deposition on Orthodontic Miniscrews After Ultraviolet Photofunctionalization

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Purpose: The aim of this study was to evaluate the effect of ultraviolet (UV) photofunctionalization on peri-implant osteogenesis of miniscrews. Materials and Methods: Titanium orthodontic miniscrews were placed in the maxillary premolar-molar region of 17 patients undergoing fixed orthodontic treatment. This was a split-mouth study wherein the miniscrews on one side were treated with UV photofunctionalization and those on the other side were left untreated. Photofunctionalization was performed by placing the miniscrews in a chamber consisting of UV-A and UV-C lights for 15 minutes immediately prior to implantation. Efficacy of the UV chamber was assessed by examining stereomicroscopic images of a 10-µL droplet of double-distilled water placed on a UV-treated titanium pellet. Retrieved miniscrews were evaluated for bone-miniscrew contact (BMSC) using scanning electron microscopy (SEM) based on a custom-devised 4-point objective scoring system. Surface element deposition of miniscrews was estimated using energy-dispersive x-ray spectrometry (EDX). Ratios of Ca/Ti and Ca/P were calculated for upper, middle, and lower regions of all miniscrews.

Results: Increased spread of the water droplet over the UV-treated pellet showed that photofunctionalization converted the titanium surface from hydrophobic to superhydrophilic. SEM imaging revealed that BMSC was greater in the photofunctionalized group, but only in the lower third of miniscrews, and this was not statistically significant. EDX analysis revealed that Ca/Ti and Ca/P ratios in both groups were similar. Thus, there was no significant difference between peri-implant osteogenesis of UV-treated and untreated miniscrews. Conclusion: These results suggest that UV photofunctionalization did not enhance the biologic potential of titanium orthodontic miniscrews in clinical application.


Keywords: miniscrews, osteogenesis, photofunctionalization, stability, superhydrophilic, ultraviolet

Orthodontic miniscrews are a group of temporary anchorage devices (TADs) that provide absolute and compliance-free intraoral anchorage. Miniscrews have had a considerable impact on modern orthodontic treatment, not only by providing a new source of anchors for anchorage-demanding cases but also for force management and control.¹ Miniscrews are minimally invasive and cost-effective, permit immediate loading, can be inserted at various alveolar locations, and can also be easily removed.²,³ However, they may become loose during treatment, and therefore, stability of orthodontic miniscrews is paramount to their ability to reinforce anchorage. The clinical stability of miniscrews in patients has been reported to be exceptionally high. While a few studies show success rates higher than 90%,⁴⁻⁶ others have slightly lower success rates.⁷⁻⁹ A recently published systematic review and meta-analysis conducted by Alharbi et al reported a success rate of 86.5%.¹⁰

Miniscrews are generally made of commercially pure titanium or titanium alloy (Ti-6Al-4V) and are manufactured with an untreated, smooth surface.¹¹ Various techniques to enhance osseointegration of orthodontic miniscrews based on titanium surface modifications and improvements in implant surface topography have been proposed. Sandblasted, large-grit, and acid-etching (SLA)¹²; microgrooving¹³; anodization¹⁴; plasma ion implantation¹⁵; resorbable blasting media (RBM)¹⁶; and nanoscale modifications¹⁷ are a few such proven techniques. These techniques are aimed at altering the surface texture leading to increased intercuspation between the roughened screw surface and bone, thereby favoring peri-implant osteogenesis.¹⁸ However, this may also cause fracture of miniscrews or bone chipping due to enhanced removal torque.¹⁹⁻²¹ Therefore, there is a need for miniscrews produced without modification of the surface topography.
Ultraviolet (UV)-mediated photofunctionalization is a recently reported method of surface modification that uses a combination of UV-A and UV-C for pre-treatment of titanium implants. This process alters the physicochemical properties of titanium via: (1) the generation of superhydrophilicity, (2) surface carbon reduction, and (3) electrostatic surface charge conversion from negative to positive. It also enhances the biologic capacity of titanium surfaces via increased: (1) protein adsorption, (2) osteogenic cell attachment and cell spread, (3) cell retention and proliferation, and (4) osteoblastic differentiation. All such physicochemical and biologic surface enhancements result in improved peri-implant osteogenesis.

In the past decade, numerous reports have been published on the effectiveness of this photofunctionalization technique. However, its clinical application with orthodontic miniscrows has not yet been thoroughly investigated. So far, only three studies have been conducted on orthodontic miniscrows, all of which include an animal model. Therefore, these results cannot be perfectly extrapolated to human patients. The objectives of this study were to evaluate in vivo bone-miniscrew contact (BMSC) and surface element deposition on photofunctionalized miniscrows and to compare the BMSC and surface elements between experimental and control groups.

**MATERIALS AND METHODS**

This study was approved by the Institutional Ethics Committee of Bharati Vidyapeeth Deemed To Be University Dental College and Hospital, Pune, Maharashtra, India (ECR/328/Inst/MH/2016), and was conducted on patients undergoing treatment in the Department of Orthodontics and Dentofacial Orthopedics at the hospital. The sample size was selected based on a type I error frequency of 5% and power of the statistical test set at 85% (P = .85, β = 0.2). The inclusion and exclusion criteria are summarized in Table 1. Fixed orthodontic mechanotherapy was started for all patients following extraction of maxillary first premolars. After leveling and aligning of the dental arches, space closure was commenced using sliding mechanics. Each patient received self-drilling titanium orthodontic miniscrows (AbsoAnchor, Dentos; diameter: 1.4 mm; length: 7 mm) under topical anesthesia on both sides of the maxillary arch. Thus, a total of 34 miniscrows were used in 17 patients. This was a split-mouth study design wherein after sterilization, the miniscrew to be inserted on one side was photofunctionalized (experimental group) and the miniscrew to be inserted on the opposite side was used as received (control group). This was a randomized, double-blinded study, in which sides for the experimental and control groups were selected via lottery method. The site of miniscrew placement selected was the intradicular space between the maxillary first permanent molar and second premolar, and the height of miniscrew placement was at the mucogingival junction. A retraction force of 150 g was measured with a Dontrix gauge (Dentsply Sirona) and applied on each side with the help of calibrated nickel-titanium closed coil springs (Dentsos).

Photofunctionalization of miniscrows was performed by placing the miniscrows in a custom-fabricated UV chamber consisting of UV-A (power: 15 W, wavelength: 350 ± 20 nm, intensity: 0.1 mW/cm²) and UV-C (power: 15 W, wavelength: 250 ± 20 nm, intensity: 2.0 mW/cm²) lights (Philips) for 15 minutes immediately prior to placement in the patient’s mouth. This chamber was made as per recommendations by previous authors. In order to demonstrate the change in titanium surface hydrophilicity, a Grade 2 titanium pellet (Orotig) with a 10-μL droplet of double-distilled water (ddH₂O) was observed under a stereomicroscope (Apex Industrial Electronics) before and after UV pretreatment. Photographs were taken with a DSLR camera (Canon EOS 1300D), and the nature of spread of water droplets was assessed. This was done in order to demonstrate the effectiveness of the UV chamber.

**Table 1 Inclusion and Exclusion Criteria for Selection of Patients**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tr>
<td>Male and female</td>
<td>Gingival inflammation (gingivitis)</td>
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<td>Age range, 18–45 years</td>
<td>Poor oral hygiene for more than 2 visits</td>
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<tr>
<td>Class I bimaxillary dentoalveolar protrusion or Class II Division 1 malocclusion, with extraction of both maxillary first premolars</td>
<td>Current or past evidence of periodontal disease</td>
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<tr>
<td>Critical anchorage and en-masse space closure</td>
<td>Dental caries</td>
</tr>
<tr>
<td>Adequate intradicular distance between the maxillary first permanent molar and second premolar for insertion of miniscrows</td>
<td>Long-term use of antibiotics, phenytoin, cyclosporine, anti-inflammatory drugs, systemic corticosteroids, and calcium channel blockers</td>
</tr>
<tr>
<td>Good gingival and periodontal health</td>
<td>Smoking habit</td>
</tr>
<tr>
<td>No systemic disease</td>
<td>Current or past evidence of systemic illness</td>
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Out of 17 patients, 2 were lost to follow-up and 3 were excluded from the study due to miniscrew failure resulting from poor oral hygiene maintenance. Thus, 24 miniscrews from 12 patients were eventually included in the analyses. All miniscrews were retrieved after 6 to 8 months during which period the extraction spaces were closed. The retrieved miniscrews were dipped once in normal saline to remove any possible tissue debris and then stored in autoclaved, labeled centrifuge tubes. These tubes were sun-dried to make sure that no/minimum damage/alteration of tissue attached to the specimens occurred. The miniscrew surface was examined using a scanning electron microscope (SEM; Quanta200, ThermoFischer Scientific) to assess BMSC, and energy-dispersive x-ray spectrometry (EDX; EDAX, Ametek) was performed to assess elemental deposition of calcium and phosphorus on the miniscrew surface. Each miniscrew in its dry state was placed on the SEM platform. After adjusting the working distance at low vacuum mode, each specimen was imaged at 100× magnification in the upper, middle, and lower regions starting from below the screw head. Additionally, in each of these three regions, scans were also taken at 500× magnification at the crest of selected threads. For EDX analysis, the area with maximum tissue adherence was selected from the 100× scan of each of the three regions of each specimen. The elements included in the EDX analysis were titanium (Ti), calcium (Ca), and phosphorus (P). The Ca/Ti (at%) and Ca/P (at%) ratios for both groups in all three regions were calculated and analyzed. The SEM images obtained were scored according to a specially devised 4-point grading scale based on the amount of remnant bone tissue observed (Table 2).

Statistical Analysis
The hypothesis to be tested in this study was that UV photofunctionalization of titanium orthodontic miniscrews enhances its peri-implant osteogenesis in humans. Twenty-four samples were included in the study due to miniscrew failure resulting from poor oral hygiene maintenance. Thus, 24 miniscrews from 12 patients were eventually included in the analyses. All miniscrews were retrieved after 6 to 8 months during which period the extraction spaces were closed. The retrieved miniscrews were dipped once in normal saline to remove any possible tissue debris and then stored in autoclaved, labeled centrifuge tubes. These tubes were sun-dried to make sure that no/minimum damage/alteration of tissue attached to the specimens occurred. The miniscrew surface was examined using a scanning electron microscope (SEM; Quanta200, ThermoFischer Scientific) to assess BMSC, and energy-dispersive x-ray spectrometry (EDX; EDAX, Ametek) was performed to assess elemental deposition of calcium and phosphorus on the miniscrew surface. Each miniscrew in its dry state was placed on the SEM platform. After adjusting the working distance at low vacuum mode, each specimen was imaged at 100× magnification in the upper, middle, and lower regions starting from below the screw head. Additionally, in each of these three regions, scans were also taken at 500× magnification at the crest of selected threads. For EDX analysis, the area with maximum tissue adherence was selected from the 100× scan of each of the three regions of each specimen. The elements included in the EDX analysis were titanium (Ti), calcium (Ca), and phosphorus (P). The Ca/Ti (at%) and Ca/P (at%) ratios for both groups in all three regions were calculated and analyzed. The SEM images obtained were scored according to a specially devised 4-point grading scale based on the amount of remnant bone tissue observed (Table 2).²⁹

**Table 2**  
**Specially Devised Four-Point Grading Scale for Rating SEM images**

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
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<tbody>
<tr>
<td>No bone present</td>
<td>0</td>
</tr>
<tr>
<td>Very little bone present—scattered or concentrated</td>
<td>1</td>
</tr>
<tr>
<td>Moderate amount of bone present</td>
<td>2</td>
</tr>
<tr>
<td>Extensive amount of bone present</td>
<td>3</td>
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A chi-square test was used to compare the BMSC scores for the upper, middle, and lower regions of untreated and UV-treated groups. Cohen’s kappa statistic was used to check for intraobserver and interobserver reliability/agreement. Normality of data was checked using the Shapiro-Wilk (SW) test, and it was found to be normally distributed. An unpaired Student t test was used to compare the mean outcomes of Ca/Ti and Ca/P ratios between the untreated and UV-treated miniscrew groups. All data were analyzed using SPSS 24.0 (IBM Analytics). A P value < .05 was considered significant.

**RESULTS**

**Superhydrophilic Conversion of Titanium**

In Fig 1a, the stereomicroscopic image of an untreated titanium pellet shows that a 10-µL droplet of ddH2O remained hemispherical and did not spread over the surface, owing to a very high contact angle characteristic of hydrophobic titanium surfaces. In Fig 1b, the photofunctionalized titanium pellet shows a greater spread of the water droplet over the surface, due to a reduced contact angle, thereby indicating a superhydrophilic conversion of the titanium surface.

**Evaluation of Bone-Miniscrew Contact**

SEM images of miniscrew specimens at 100× magnification from untreated and UV-treated groups in the upper, middle, and lower regions were rated by three independent, blinded observers as per the 4-point grading scale given in Table 2. Representative SEM images of untreated and UV-treated groups are shown in Fig 2. The scores given by the principal observer are summed up in Table 3. In a comparison of the two study groups, the chi-square test revealed a score of 56 for the UV-treated group and 40 for the untreated group, with a P value of .7742 and DF of 2 (Table 4). It was found that there was no statistically significant difference in the scores between the untreated and UV-treated group in any of the three regions. The most frequently given score (ie, arithmetic mode) was calculated for each of the three regions of miniscrews based on the SEM images (Table 5). Intraobserver reliability tests showed an agreement of 75% for the untreated group and 85.71% for the UV-treated group. Interobserver reliability tests showed a highest agreement of 85.71% and lowest agreement of 66.66% between Observers 1 and 2 and a highest agreement of 100% and lowest agreement of 85.71% between Observers 1 and 3.

**Evaluation of Calcium and Phosphorus on Titanium Miniscrew Surface**

It was observed that there was no statistically significant difference in either Ca/Ti ratio or Ca/P ratio...
between the untreated and UV-treated groups. Large standard deviations were found in most regions indicating the variability of the results, especially in the Ca/Ti ratio comparison between the lower regions of the two groups (Table 6).

**DISCUSSION**

Orthodontic miniscrews are intended to be removed at the end of their clinical application. There is a dilemma...
regarding miniscrews: They should have long-term stability for clinical applications, yet be easy to remove after treatment. Consequently, osseointegration of miniscrews is not highly desirable because it would complicate later removal. Surface modification of miniscrews by roughening may achieve stability, but may also result in breakage.\textsuperscript{19–21} UV photofunctionalization is a technique that achieves surface modification by alteration of physiochemical surface characteristics, while maintaining the original surface topography and roughness.\textsuperscript{23} Yamazaki et al reported increased peri-implant bone volume (1.5- to 2-fold) after UV pretreatment at both early and late stages of healing.\textsuperscript{30} Hirota et al reported elemental peaks of calcium and phosphorus on UV-treated implants as well as 20 times higher Ca/Ti ratio compared with untreated implants.\textsuperscript{31} Some animal studies using photofunctionalized orthodontic miniscrews have shown promising results as well.\textsuperscript{26–28} Tabuchi et al and Takahashi et al have reported greater biomechanical strength, enhanced osseointegration, and improved stability in immediate loading cases, following photofunctionalization of orthodontic miniscrews.\textsuperscript{26–28} However, the results of this study are in agreement with Mehl et al, who reported that UV photofunctionalization did not cause any significant improvement in the osseointegration of titanium dental implants in pigs.\textsuperscript{32} Notwithstanding that these experiments were done on animals, it shows that photofunctionalization can improve surface characteristics of titanium without altering the existing topography, roughness, or other morphologic features.\textsuperscript{33} It can therefore be considered as neither an additive nor a subtractive method of implant surface modification. This technique does not affect implant biocompatibility, and no side effects have been reported to date. In this study, it was decided to evaluate the effects of UV photofunctionalization on titanium orthodontic miniscrews in human patients.

The effectiveness of the UV chamber and subsequent hydrophilic change caused by the photofunctionalization technique can be evaluated accurately with a contact-angle meter.\textsuperscript{23} However, visual observation through a stereomicroscope has also proven to be an equally effective and reliable method.\textsuperscript{27} On retrieval, miniscrews were dipped once in normal saline to remove any possible tissue debris and then were stored in autoclaved centrifuge tubes and sun-dried. This method seems to be better than previous ones,\textsuperscript{26,28} as it minimizes any damage/distortion or alteration of biologic tissues adhered to the miniscrews.

<table>
<thead>
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<th>Region</th>
<th>Group</th>
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<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
<td>Total</td>
</tr>
<tr>
<td>Upper</td>
<td>7 (7.92%)</td>
<td>12 (11.08%)</td>
<td>19</td>
</tr>
<tr>
<td>Middle</td>
<td>13 (13.75%)</td>
<td>20 (19.25%)</td>
<td>33</td>
</tr>
<tr>
<td>Lower</td>
<td>20 (18.33%)</td>
<td>24 (25.67%)</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>56</td>
<td>96</td>
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<table>
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<tr>
<th>Region</th>
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<th>UV-treated</th>
</tr>
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<tbody>
<tr>
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<td>1</td>
</tr>
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<td>Middle</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lower</td>
<td>2</td>
<td>3</td>
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Table 4: Comparison of Bone-Miniscrew Contact Between Untreated and UV-Treated Groups in Upper, Middle, and Lower Regions

Table 5: Arithmetic Mode of Scores Graded by Principal Observer

Table 6: Comparison of Ca/Ti and Ca/P Ratios Between Surfaces of Untreated and UV-Treated Miniscrews in the Upper, Middle, and Lower Regions

$P = .7742$
In SEM analysis, not all regions of photofunctionalized miniscrews showed a distinct pattern of bone morphogenesis, and this was not in agreement with previous claims of enhanced osseous tissue formation by this technique.²⁶–²⁸ Since no previous literature has reported any method of grading SEM images of miniscrews to assess BMSC, a customized 4-point objective grading scale was devised based on morphologic characteristics in a similar manner to that of Lee et al (Table 2).²⁹ The overall reliability of scoring SEM images was good, but in one particular area, the interobserver agreement was low (66.66%). This type of error is often seen in SEM grading and must not be considered as misclassified images; rather, it could be the human uncertainty contribution to the validation error.³⁴ The grading scores showed that there was very little to moderate BMSC in the upper and middle regions of both untreated and photofunctionalized groups, and this finding was of no significant value. In the lower region, an extensive amount of bone was seen adhering to the miniscrews in the UV-treated group compared with the untreated group, but this too was of no statistical significance. However, it appeared as a thick layer of dried tissue with an uneven contour and cracks along the surface. This might be due to the cohesive nature of bone and mucosal soft tissues, thereby preventing complete adherence of biologic tissues to the miniscrew surface during manual retrieval of implants. Another probability could be distortion or detachment of bone tissue during removal of miniscrews. Increased BMSC in the lower region of the experimental group may hint toward the positive osteogenic potential of UV photofunctionalization, but it is difficult to say with utmost certainty, as SEM imaging is subject to operational errors. These results suggest that the merits of photofunctionalization could be improved stability and resistance to exogenous force by a gain of bone area, especially in the lower third of miniscrews, which is the region that is completely embedded in cortical bone.

Blunting and fracture of miniscrew threads from mechanical wear during insertion and removal has been reported.³⁵ Some of the specimens in the present study showed blunting of threads, but there were no signs of visible fracture on either the miniscrew body or thread, thus possibly indicating that removal torque values were maintained within acceptable levels since photofunctionalization did not increase the tissue-surface intercuspation. Therefore, the use of photofunctionalization to enhance peri-implant osteogenesis can be assumed to be safe for orthodontic miniscrews.

Park et al reported a significantly high Ca/Ti ratio for the photofunctionalized group in their in vitro study.³⁶ On the contrary, the EDX results of the present study showed that there was no significant difference in Ca/Ti ratio between the two groups in any of the three regions assessed. This suggests that photofunctionalization may not be effective in enhancing bone spread over the miniscrew surface in all clinical cases. Calculating the Ca/P ratio gives an estimate of the quality of bone tissue (ie, degree of mineralization) formed around miniscrews. A few studies have reported that photofunctionalization enhances the mineral quality of new bone that forms around titanium implants by improving their Ca/P ratios, with values nearing that of hydroxyapatite (1.67).³⁶,³⁷ The present study did not show any significant difference in Ca/P ratios between the untreated and UV-treated groups, suggesting that only a thin layer of bone comprising poorly mineralized tissue was formed in both of the groups.

Morphologic and elemental analyses of implants is a useful measure to evaluate the nature and behavior of tissues at the bone-implant interface.³⁸ However, the results require careful interpretation, as this method is subject to technical artifacts such as tissue damage, distortion, and/or detachment during sample preparation. In the case of manually retrieved specimens, it is difficult to be sure if the tissue at the screw surface represents the original tissue or if it represents the damaged surface of remnant tissues. Therefore, since this study involved removal of miniscrews from the patient, the chances of tissue distortion and loss could have been more likely, as opposed to the en-bloc harvested miniscrew-bone complexes used in previous studies in which the miniscrew was not removed from within the bone at all.²⁶–²⁸ This might be a probable reason as to why these studies have reported a much higher BMSC as well as improved Ca/Ti and Ca/P ratios in photofunctionalized miniscrews compared with the present study.

One of the other reasons for the difference in element quantification could possibly be an error in selecting the mapping area in the computer software. Standardizing the site and size of the mapping area could possibly negate this error. However, the present attempts to do so were unsuccessful due to unequal coverage of the miniscrew surface by bone tissue and therefore unequal selection of the mapping area for different specimens. This may be noted for future reference so that better methods for standardization can be selected. In addition, the distribution of crystals is not uniform, but varies depending on the place where the sample is focused to analyze it through EDX. There is also a possibility of organic contaminants being deposited during the manipulation and storage of the samples or during analysis in the SEM. In this sense, the importance of the time elapsed between retrieval of samples and the quantitative x-ray microanalysis must be considered. However, all of the specimens were stored in air-tight centrifuge tubes to prevent atmospheric contamination.
This research was conducted with thorough preparation and utmost precision; nonetheless, certain limitations may be pointed out. First, this study did not include tests for measuring the insertion torque, removal torque, and miniscrew stability, all of which are important clinical parameters that indicate positive biomechanical miniscrew potential. Another limitation was the unavailability of more advanced analytical techniques such as field emission-SEM and transmission electron microscopy. These techniques have the potential to give detailed layer-wise analyses of the biologic tissues present on the miniscrew surface. Incorporating these tests and techniques in future studies may enable researchers to obtain more conclusive and substantive evidence regarding the effects of UV photofunctionalization.

All previously reported studies on this technique have shown positive results of UV pretreatment on the osseointegration potential of dental implants. There have been only a few studies involving orthodontic miniscrews, all of which have been carried out in animal models. This in vivo study provides a proof of concept in first-of-its-kind research evaluating the effects of UV photofunctionalization on peri-implant osteogenesis in titanium orthodontic miniscrews in human patients. This study is intended to serve as a pilot study for future controlled clinical trials. The results of this study showed that photofunctionalization enhanced neither the BMSC nor the surface elements that could be indicative of improved osteogenic potential of the peri-implant tissues. Further research is required to establish the clinical feasibility and effectiveness and to examine the longer-term effects of photofunctionalization. This technique may pave the way for novel orthodontic treatment as a chairside implant conditioning method to enhance miniscrew anchorage, and if proven, may further improve the performance and longevity of orthodontic miniscrews as intraoral load-bearing devices.

CONCLUSIONS

Within the technical limitations and the qualitative nature of this study, the following conclusions can be drawn. Photofunctionalization converted the titanium pellet surface from hydrophobic to superhydrophilic. BMSC in untreated and UV-treated titanium orthodontic miniscrews was the same in the upper and middle regions, but more extensive in the lower region of photofunctionalized miniscrews. Ca/Ti and Ca/P ratios were similar for both untreated and UV-treated titanium orthodontic miniscrews in the upper, middle, and lower regions.

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