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# Comparison of osseointegration in commercial SLA-treated dental implants with different surface roughness: a pilot study in beagle dogs

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**PURPOSE.** This pilot study investigated the effect of surface roughness on osseointegration by comparing two types of commercial SLA-treated dental implants with different surface roughness levels: moderately rough ($S_a$ = 1 – 2 μm) and rough surfaces ($S_a$ > 2 μm). **MATERIALS AND METHODS.** Two implant groups were studied: TS (rough surface) and ADD (moderately rough surface) groups. Surface characteristics were analyzed using optical profilometry and SEM. *In vitro* studies using BRITER cells assessed cell adhesion, proliferation, and osteogenic differentiation through CCK-8 assay and qRT-PCR for osteopontin (OPN), osteocalcin (OCN), and alkaline phosphatase (ALP) expression. The *in vivo* study involved 12 implants (six per group) placed in mandibular defects of two beagle dogs. After 8 weeks, histomorphometric analysis evaluated bone to implant contact (BIC) and inter-thread bone density (ITBD). Statistical analysis used Student's t-test and two-way ANOVA for *in vitro* data, and Mann-Whitney U test for *in vivo* data. **RESULTS.** Surface analysis revealed $S_a$ values of 2.50 ± 0.27 μm for the TS group and 1.80 ± 0.06 μm for the ADD group. *In vitro* studies showed no significant differences in cell adhesion and proliferation between the groups ($P$ > .05). However, gene expression patterns differed, with ADD group showing higher OPN expression ($P$ < .001) and TS group showing higher ALP expression ($P$ < .01). The *in vivo* study revealed no statistically significant differences in BIC and ITBD between the two groups ($P$ > .05). **CONCLUSION.** Surface roughness influenced osteoblast differentiation *in vitro*, but did not significantly affect osseointegration outcomes *in vivo*. Both moderately rough and rough surfaces appeared to support comparable levels of osseointegration. Larger studies are needed to confirm these findings and determine optimal implant surface characteristics. [J Adv Prosthodont 2024;16:348-57]

**KEYWORDS**
Dental implant; Osseointegration; Surface roughness





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## INTRODUCTION

Dental implants have revolutionized the restoration of masticatory function and aesthetic improvement for patients with tooth loss.[1] While the success of implant procedures is determined by various factors, osseointegration between the implant and surrounding bone tissue plays a crucial role.[2] Osseointegration, the direct structural connection between the implant surface and bone, is essential for functional load transfer and maintaining implant stability.[3] Recent studies have explored various approaches to optimize the osseointegration process, with a particular focus on implant surface characteristics.[4,5] This focus has become increasingly important as surface modifications can significantly influence the biological response and healing process at the bone-implant interface.[4,5]

The implant surface microstructure, especially its roughness, is vital in promoting osseointegration.[6] Rough surfaces enhance implant-bone interlocking and promote cell attachment and proliferation.[7] Among various surface modification methods, sandblasted, large-grit, acid-etched (SLA) treatment has gained widespread adoption.[8] SLA treatment creates a uniform, complex microstructure on the implant surface by combining sandblasting with large-grit particles to create macro-roughness and acid-etching to superimpose micro-roughness.[9] This dual-scale topography enhances the initial healing response in the osseointegration process by promoting protein adsorption and thrombus formation, ultimately leading to improved bone formation and mechanical interlocking between the implant and surrounding bone tissue.[10]

As surface treatment technologies have advanced, research has extensively explored the impact of implant surface roughness levels on osseointegration.[11-13] Notably, Wennerberg and Albrektsson classified implant surface roughness into four categories based on the $S_a$ parameter (arithmetic mean height deviation): smooth ($S_a$ < 0.5 μm), minimally rough ($S_a$ = 0.5-1 μm), moderately rough ($S_a$ = 1-2 μm), and rough ($S_a$ > 2 μm).[13] While they reported that moderately rough ($S_a$ = 1-2 μm) surfaces exhibited optimal bone response,[13] subsequent studies have shown varying results regarding the relative effectiveness of moderately rough versus rough surfaces.[14,15] Shalabi *et al*.,[14] in their systematic review, found a positive correlation between surface roughness and bone-implant contact, suggesting potential benefits of rougher surfaces. In contrast, Almas *et al*. found that while excessive surface roughness might accelerate initial bone formation, it could potentially compromise long-term osseointegration stability.[15] Although these varied research outcomes have led to the widespread adoption of both moderately rough and rough surfaces in current commercial implants, their comparative effectiveness in achieving optimal osseointegration remains unclear.[16,17] Consequently, there is still no clear consensus on the optimal surface roughness for enhancing osseointegration in SLA-treated implants.[16,17]

In this context, this pilot study investigated the comparative osseointegration performance of two commercial SLA-treated implants representing moderately rough and rough surfaces. By examining these two most commonly used surface roughness ranges in current clinical practice, we sought to provide insights into their relative effectiveness in achieving osseointegration. The null hypothesis was that there would be no significant difference in osseointegration outcomes between these two types of SLA-treated implants.

## MATERIALS AND METHODS

SLA-treated titanium implants with different surface roughness from two manufacturers were used in this study. To minimize the influence of variables other than surface roughness on the results, implants with identical dimensions were selected. All experimental implants were of the submerged type, with a diameter of 3.5 mm and a length of 8.5 mm. The implants were divided into the following groups based on their product: TS group (TSIII SA implants; Osstem, Seoul, Korea) and ADD group (ADDplant ON implants; PNUADD, Busan, Korea).

To characterize the implant surfaces, surface roughness was measured using a HD-3D surface and film thickness optical profiler (Talysurf CCI HD; Taylor Hobson, Leicester, UK) based on ISO 25178 protocol.

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It was then analyzed using a software (Talymap Platinum software; Taylor Hobson, Leicester, UK) under conditions excluding the 4th and 2nd polynomial expressions. The surface roughness parameter for arithmetic mean height ($S_a$) was calculated for each group. This test was conducted at the Korea Testing Laboratory (KTL, Jinju, Korea), a registered international testing and certification organization. Surface images were obtained using a scanning electron microscope (SEM) (SUPRA 40VP system; Zeiss, Oberkochen, Germany) at magnifications of ×11, ×1000, and ×3000, with an accelerating voltage of 10 kV.

For cell studies, the BMP-responsive immortalized reporter (BRITER) cell line was purchased from Kerafast (Boston, MA) and cultured in DMEM 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco), at 37°C in an atmosphere containing 5% $CO_2$. The medium was refreshed every other day. To seed cells on the surface of implant fixture, we cut an implant fixture vertically and placed it in a polydimethylsiloxane (PDMS) mold with the same cross-sectional area as the cut implant fixture.

For cell adhesion analysis, BRITER cells were seeded onto implant fixture at a density of $5 \times 10^5$. The measurement of adhesion was performed at 8 h after cell seeding. Cells were fixed with 4 % paraformaldehyde and permeabilized with 0.2 % Triton X-100. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA) and imaged using EVOS FL Auto imaging system (ThermoFisher Scientific, MA, USA). The number of DAPI-positive nuclei per image was determined using ImageJ program (version 1.53q, National Institutes of Health, Bethesda, MD, USA). Cell proliferation was also assessed using the same seeding density over a period of 3 days after cell seeding by CCK-8 solution (Dojindo, Rockville, MD, USA). Absorbance was measured at 450 nm using an Opsys MR micro-plate reader (DYNEX Technologies Inc., Denkendorf, Germany).

To evaluate osteogenic differentiation, BRITER cells were cultured on implant fixtures at a density of $5 \times 10^5$ for 24 hours, followed by exposure to an osteogenic medium. This medium consisted of DMEM supplemented with 10% FBS, 10 mM β-glycerophosphate, 50 μg/mL ascorbic acid (Sigma-Aldrich, Milan, Italy), and 100 ng/mL BMP2 (PeproTech, Rocky Hill, NJ, USA). The differentiation process was maintained for 4 days, with medium refreshed every other day. To analyze gene expression, total RNA was purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, and 1 μg of RNA was reverse-transcribed under standard conditions using Superscript II (Invitrogen, Waltham, MA, USA). For qPCR analysis, 40 ng of cDNA was mixed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and amplified for 40 cycles using an AB7500 system (Applied Biosystems, Foster City, CA, USA). Experiments were performed in triplicate and the data were normalized to those of β-actin. The data were analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were as follows: OPN (Osteopontin), 5′-TGCCTGTTTGGCATTGCCTC-3′,5′-ACAGCATTCTGTGGCGCAAG-3′; OCN (Osteocalcin), 5′-GCAATAAGGTAGTGAACAGACTCC-3′, 5′-GTTTGTAGGCGGTCTTCAAGC-3′; ALP (Alkaline phosphatase), 5′-TGACCTTCTCTCCTCCATCC-3′, 5′-CTTCCTGGGAGTCTCATCCT-3′; β-actin, 5′-TCTGGCACCACACCTTCTAC-3′, 5′-TACGACCAGAGGCAT ACAGG-3′.

The statistical analysis of the cell study data involved comparing differences between the two groups using Student's t-tests. For multiple comparisons, two-way analysis of variance (ANOVA) with Bonferroni post hoc test was used. Differences were considered statistically significant at $P < .05$. All data are presented as the mean ± standard deviation (SD), and the results are representative of at least three independent experiments.

The *in vivo* studies were conducted following approval from the Chonnam National University Biomaterial R&BD Center Animal Experimental Ethics Committee (BMC-IACUC-2022-21). Two male beagle dogs, aged up to 12 months and weighing between 10 and 15 kg, were used in this study. For anesthesia of the beagles, medetomidine (Tomidin®; Provet Veterinary Products Ltd., Istanbul, Turkey) 0.005 – 0.02 mg/kg was administered intramuscularly. For anesthesia induction, alfaxalone (Alfaxan Multidose; JUROX Pty Ltd., Rutherford, Australia) 1.5 – 2 mg/kg was administered intravenously. Inhalation anesthesia was performed using Isoflurane (Hana Pharm Co., Ltd., Seoul, Korea), and for local anesthesia, bupivacaine (Myung-

moon Pharm Co., Ltd., Seoul, Korea) 1 mg/kg was injected. For pain control and infection prevention, carprofen (Rimadyl injectable 50; Zoetis Korea, Seoul, Korea) 2.2 mg/kg, tramadol hydrochloride (Tramadol HCl Huons Inj.; Huonc Co., Seongnam, Korea) 5 mg/kg, and cefazolin (CEFOZOL Inj.; Hankook Korus Pharm. Co., Ltd., Seoul, Korea) 20 mg/kg were administered intravenously. After full-mouth scaling, bilateral mandibular premolars (P1-P4) and first molars (M1) were extracted and sutured with 4-0 Vicryl (Mersilk; Ethicon Co., Livingston, UK). Following the first surgery, the beagles were allowed to rest for an 8-week healing period.

Eight weeks after the first surgery, a second surgical procedure was performed using the same anesthesia and postoperative care protocols. Cylindrical defects with a diameter of 5.5 mm and depth of 3 mm were created in the alveolar bone using trephine and carbide burs under saline irrigation. Twelve implants from the two experimental groups (six per group) were randomly placed in each defect using a random number generator. To evaluate peri-implant bone regeneration, three implant threads were exposed from the bottom of the defect. The surgical sites were sutured with 4-0 Vicryl (Mersilk; Ethicon Co., Livingston, UK). Fig. 1 illustrates the surgical procedure described above.

Following the implant placement, a comprehensive postoperative care regimen was implemented, which included the daily oral administration of famotidine (Gaster tab; Dong-A, Seoul, Korea) 1 mg/kg, carprofen (Rimadyl® Chewable Tablets 25 mg; Zoetis Korea, Seoul, Korea) 4.4 mg/kg, tramadol hydrochloride (Tramadol Retard Tab.; Huonc Co., Seongnam, Korea) 5 mg/kg, gabapentin (Neurontin Cap 100 mg; Pfizer Biopharmaceuticals Korea Ltd., Seoul, Korea) 10 mg/kg, and enrofloxacin (BaytrilFlavour Tablet; Bayer Korea Ltd., Seoul, Korea) 10 mg/kg for 7 days to manage pain and prevent infection. After 8 weeks, the beagles were anesthetized using the same method as the first surgery and euthanized by intravenous injection of potassium chloride (10 – 20 mL/body). The mandibles of the beagles were harvested and fixed in 10% neutral formalin for 14 days.

For histomorphometric analysis, the specimens were dehydrated sequentially in 70%, 95%, and 100% ethanol. After infiltration for 7 days using the Technovit 7200 VLC system (Heraeus Kulzer, Hanau, Germany), the specimens were embedded using a UV curing system (KULZER EXAKT 520; Heraeus Kulzer, Norderstedt, Germany). The embedded specimens were cut to a thickness of 400 μm at the center of the implant using a diamond cutter (KULZER EXAKT 300 CP Band System; Exakt Apparatebau, Norderstedt, Germany), and then ground to a thickness of 30 μm using an EXAKT grinder (KULZER EXAKT 400 CS; Exakt Apparatebau, Norderstedt, Germany). The ground specimens were mounted on slides and stained with Goldner's trichrome (GT). Images of the GT-stained tissues were acquired using a digital slide scanner and observed using Caseviewer (ver. 2.1, 3D HISTECH Ltd., Budapest, Hungary). Bone to implant contact (BIC) and inter-thread bone density (ITBD) were calculated by a trained investigator using the ImageJ program. The region of interest (ROI) was set as the area within 1 mm around the implant and up to the third thread

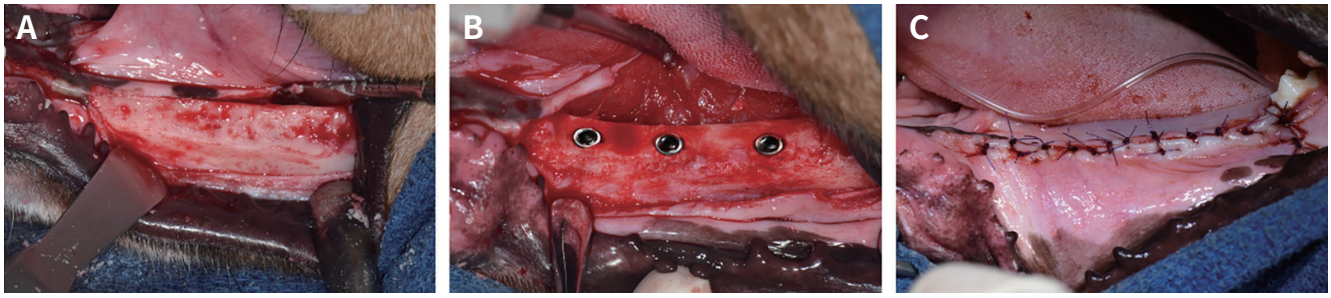


**Fig. 1.** Surgical procedure for implant placement in the mandible of beagle dogs. (A) Post-extraction alveolar ridge, (B) Implant placement in cylindrical defect, (C) Gingival suture.

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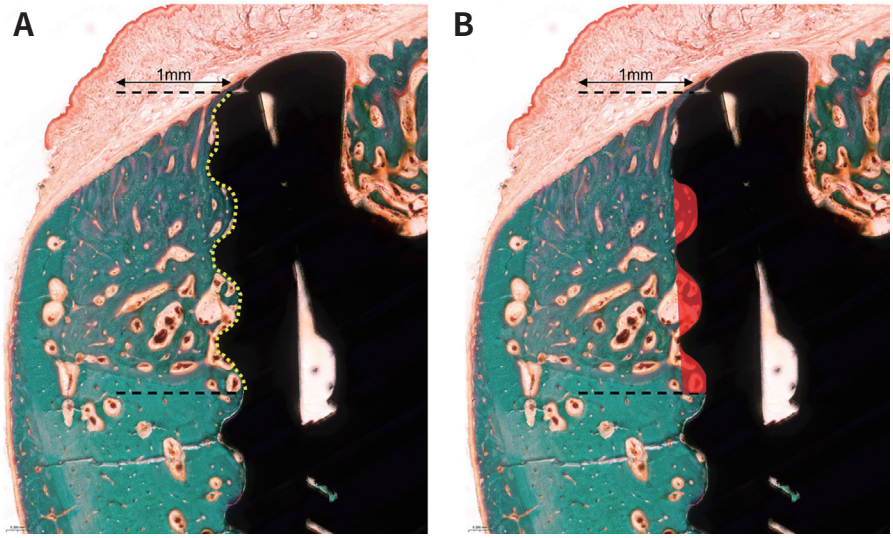


**Fig. 2.** Histomorphometric parameters within the region of interest (ROI). The ROI was established from the implant platform to the third thread, extending 1 mm around the implant in the occlusal view. (A) BIC: Bone to implant contact (yellow dotted line), (B) ITBD: Inter-thread bone density (red area).

from the platform (Fig. 2). BIC and ITBD were calculated using the following formulas: BIC (%) = Length of new bone in contact with implant ($mm^2$) / Total ROI implant length ($mm^2$) × 100, ITBD (%) = New bone area between threads ($mm^2$) / Total area between threads ($mm^2$) × 100.

The *in vivo* results of BIC and ITBD were presented as mean ± SD. As the data did not satisfy the normality assumption, a non-parametric method, the Mann-Whitney U test, was used to analyze the differences between the two groups. All statistical analyses were performed using SPSS Ver. 25 (SPSS Inc., Chicago, IL, USA) software, and the level of significance was set at $P < .05$.

## RESULTS

The surface roughness values of the implants are presented in Table 1. The TS group exhibited a $S_a$ value of 2.50 ± 0.27 μm, while the ADD group showed a $S_a$ value of 1.80 ± 0.06 μm. SEM analysis revealed that both groups displayed similar surface morphologies due to SLA treatment. However, at higher magnifications (× 1000 and ×3000), the TS group surface exhibited relatively larger and more irregular protrusions and depressions compared to the ADD group (Fig. 3).

**Table 1.** Surface roughness values of the implants used in the experiment

| Roughness parameter | Group | |
|---|---|---|
| | TS | ADD |
| $S_a$ (μm) | 2.50 ± 0.27 | 1.80 ± 0.06 μm |

Cell adhesion and proliferation assays revealed no significant differences between the two implant groups ($P > .05$) (Fig. 4). However, osteogenic differentiation analysis showed distinct gene expression patterns. In the presence of osteogenic medium, both groups demonstrated increased mRNA expression of OPN and ALP. OCN mRNA expression was elevated only in the ADD group. Notably, the ADD group exhibited significantly higher OPN mRNA expression ($P < .001$), while the TS group showed higher ALP mRNA expression levels ($P < .01$) (Fig. 5).

Histomorphometric examination at 8 weeks post-implantation revealed favorable healing with new bone formation in both groups, without evidence of abnormal inflammation (Fig. 6). Quantitative analysis showed mean BIC values of 62.83 ± 10.23% for the TS group and 66.48 ± 10.06% for the ADD group. Mean ITBD values were 66.54 ± 10.42% for the TS group and 72.26 ± 8.99% for the ADD group. Although the ADD group demonstrated slightly higher mean values for both BIC and ITBD, these differences were not statistically significant ($P > .05$) (Table 2 and Fig. 7).

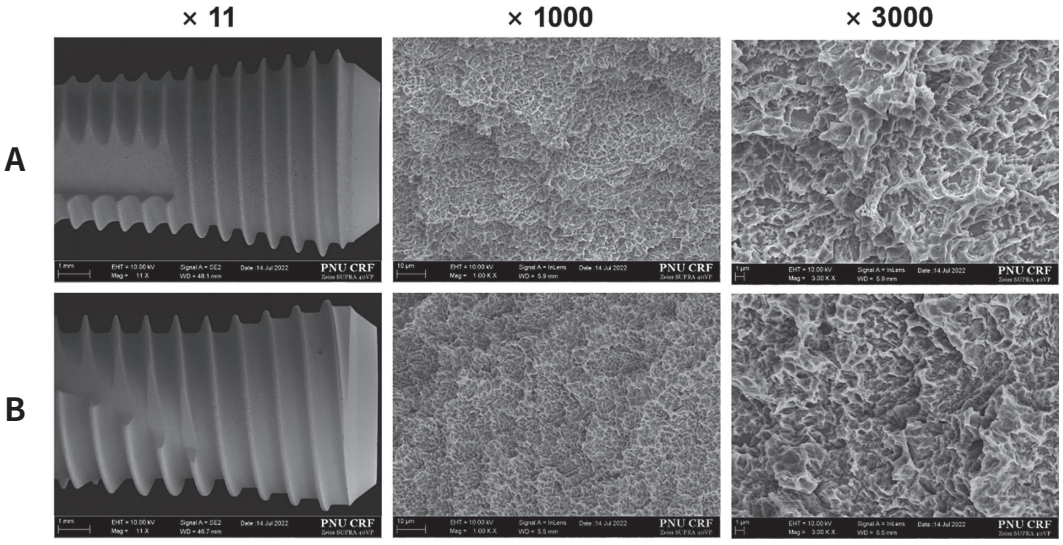


**Fig. 3.** Scanning electron microscopy (SEM) images of implant surfaces. (A) TS group, (B) ADD group. [Original magnifications: × 11, × 1000, and × 3000].

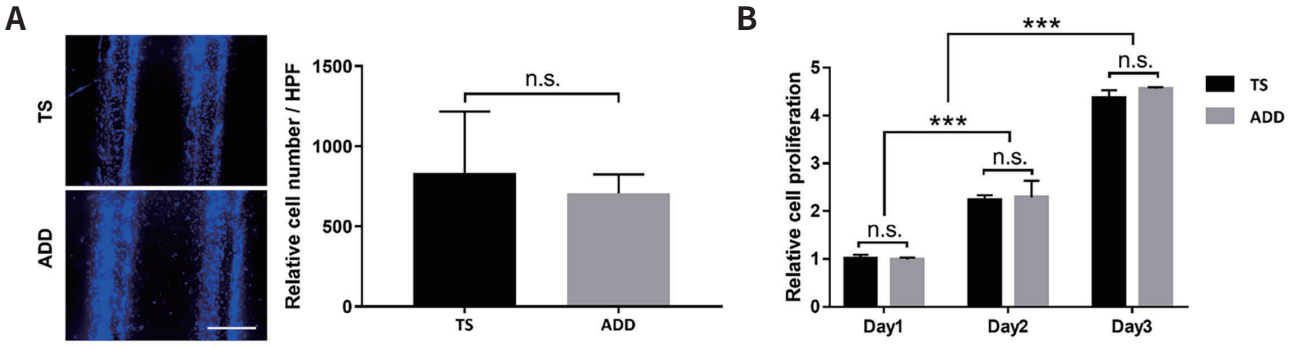


**Fig. 4.** Effects of surface roughness on adhesion and proliferation of BRITER cells. (A) BRITER cells were seeded onto both groups. After 8 hours, nuclei staining was performed with 4′,6-diamidino-2-phenylindole (DAPI). Representative images and quantitative analysis of DAPI-positive cell numbers are shown. Scale bars = 1 mm. (B) Proliferation of BRITER cells in the two groups was measured by CCK-8 assay. Data represent the mean ± SD. *** $P < .001$ by Student t-tests or two-way ANOVA. n.s., not significant ($P > .05$).

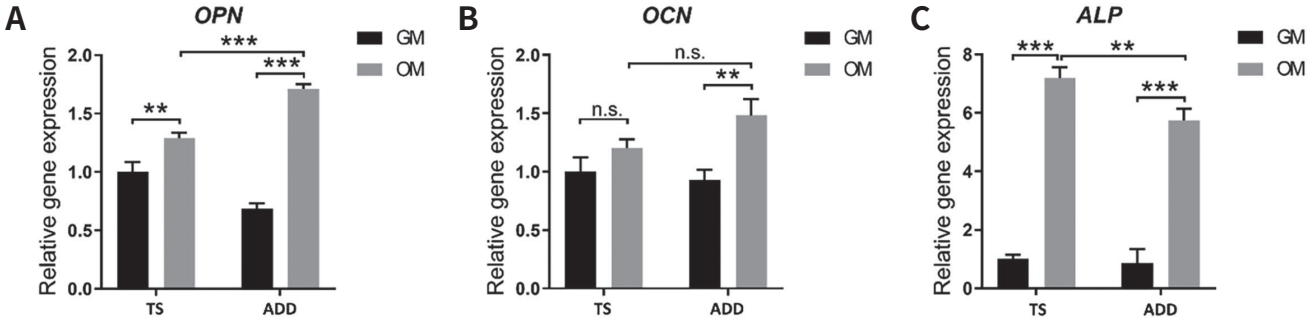


**Fig. 5.** Effects of surface roughness on osteogenic differentiation of BRITER cells. Cells were seeded onto both implant groups and osteogenic differentiation was induced by osteogenic medium for 4 days. mRNA expression of (A) OPN, (B) OCN, and (C) ALP was determined by qRT-PCR and normalized to β-actin expression. Data represent the mean ± SD. ** $P < .01$ and *** $P < .001$ by two-way ANOVA. n.s., not significant ($P > .05$); GM, growth medium; OM, osteogenic medium.

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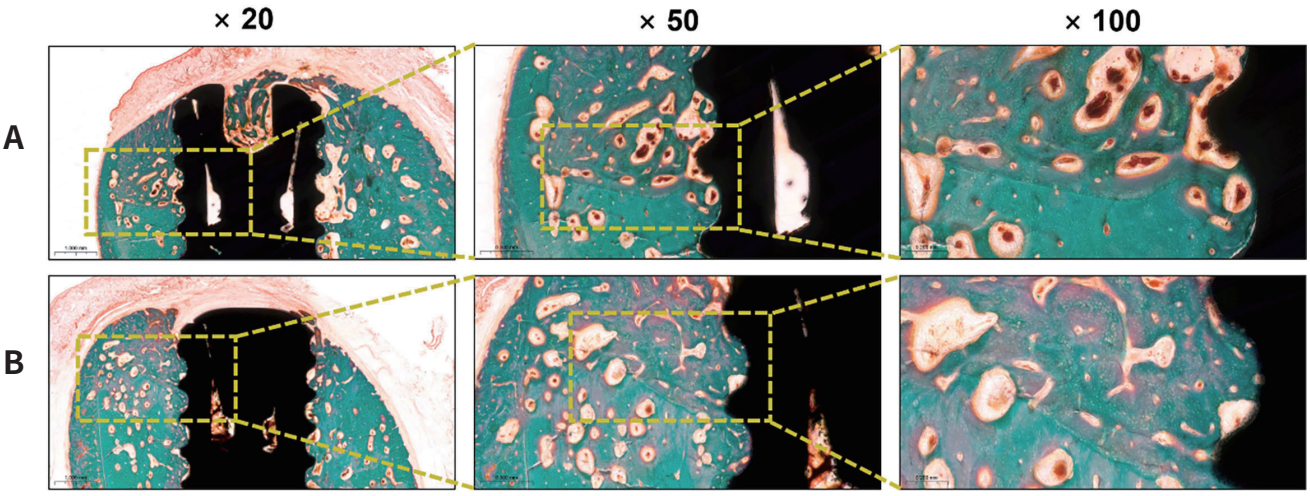


**Fig. 6.** Goldner's trichrome stained sections at 8 weeks post-implantation. (A) TS group, (B) ADD group. [Original magnifications: × 20, × 50, and × 100].

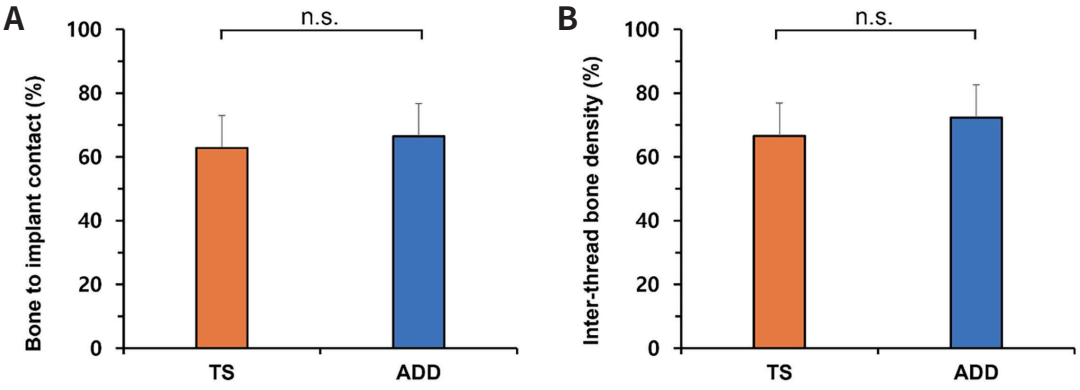


**Fig. 7.** Histomorphometric analysis within the region of interest (ROI). (A) Bone to implant contact (%), (B) Inter-thread bone density (%). n.s., not significant ($P > .05$).

**Table 2.** Mean values of bone to implant contact (BIC) and inter-thread bone density (ITBD) obtained from the histomorphometric analysis

| Measurement | Group | Mean ± SD | *P* value |
|---|---|---|---|
| BIC (%) | TS | 62.83 ± 10.23 | .686 |
| | ADD | 66.48 ± 10.06 | |
| ITBD (%) | TS | 66.54 ± 10.42 | .486 |
| | ADD | 72.26 ± 8.99 | |

## DISCUSSION

This pilot study explored the influence of surface roughness on osseointegration by examining two types of SLA-treated dental implants with different surface roughness levels. Previous research by Wennerberg has shown that implants with moderately rough surfaces ($S_a$ around 1.5 μm) demonstrate stronger bone responses compared to smoother or rougher surfaces.[14] These bone responses include improved osseointegration, reduced risk of peri-implantitis, and maintained bone height levels.[14] Building on this

foundation, our study aimed to investigate whether this difference in bone response persists in currently marketed implants with moderately rough and rough surfaces, specifically in terms of their clinical osseointegration levels.

In our study, we examined two implant groups: ADD and TS. Both implant groups had similar fixture shapes, allowing for a focused comparison of surface roughness effects. The surface roughness of each group's implants was optically measured and evaluated using a non-contact optical surface profiler according to ISO 25178 protocol. The results were then analyzed with appropriate filtering conditions, providing a quantitative representation of the implants' surface microstructure.[18] Our results showed that the ADD group had a moderately rough surface ($S_a$ = 1.80 ± 0.06 μm), while the TS group had a rough surface ($S_a$ = 2.50 ± 0.27 μm). These findings not only enabled a comparison between two surface types widely used in current clinical practice but also offered an opportunity to better understand the impact of surface roughness on osseointegration.

The observed difference in surface roughness between the two groups influenced gene expression patterns related to bone formation. In our study, the ADD group exhibited relatively higher mRNA expression of osteopontin (OPN), a non-collagenous protein crucial in the late stages of bone formation,[19] whereas the TS group demonstrated elevated alkaline phosphatase (ALP) levels, an enzyme essential in the early phases of mineralization.[20] These results suggest that variations in implant surface characteristics may modulate osteoblast differentiation through alterations in bone-related gene expression patterns.[21] Our findings correlate with previous studies demonstrating that microstructured titanium surfaces can influence mesenchymal stem cell differentiation and subsequent osteoblast behavior.[22,23] The cellular response is mediated through focal attachments and cytoskeletal arrangements, which modifies cell shape and differentiation via signal transduction pathways.[22]

Interestingly, the histomorphometric analysis revealed no statistically significant differences in bone to implant contact (BIC) and inter-thread bone density (ITBD) ratios between the ADD and TS groups, despite the ADD group showing relatively higher mean values for both BIC (66.48 ± 10.06% vs. 62.83 ± 10.23%) and ITBD (72.26 ± 8.99% vs. 66.54 ± 10.42%) compared to the TS group ($P$ > .05). These results highlight the complexity of the osseointegration process, which is influenced by various surface characteristics beyond just roughness.[24] A systematic review by Junker *et al*. demonstrated that factors such as chemical composition and surface energy can significantly affect the biological response to implant surfaces.[25] Indeed, the chemical composition and wettability of the implant surface are known to affect protein adsorption and cell attachment.[26,27] Therefore, our findings underscore the need for a comprehensive understanding of the interactions between implant surface characteristics and biological responses in terms of osseointegration.[28]

This study provides several important implications for implant design and clinical applications. While surface roughness influences the osteoblast differentiation process *in vitro*, our findings suggest that both moderately rough and rough surfaces can achieve successful osseointegration *in vivo*. This observation aligns with previous long-term clinical research demonstrating that both surface types can provide predictable clinical outcomes.[29] The choice between moderately rough and rough surfaces might therefore depend more on specific clinical scenarios than on absolute performance differences. For instance, implants similar to the TS group, which showed elevated ALP expression indicating enhanced early bone formation, could be particularly beneficial for immediate loading protocols or in patients with compromised healing capacity. Conversely, implants like the ADD group, which demonstrated higher OPN expression associated with late-stage bone formation, might be more suitable for conventional loading protocols or when simultaneous bone augmentation is planned.

Several limitations of this study suggest the need for future research. Cochran demonstrated that differences due to implant surface characteristics are more likely to be found under less ideal conditions, such as in areas with diminished cortical bone or higher biomechanical forces.[30] Furthermore, this experiment focused only on the early stages of osseointegration. Current implant research trends emphasize the com-

plex interactions between various surface characteristics, including nano-level topography and chemical properties.[31,32] Future studies should therefore include larger sample sizes with diverse patient groups and longer observation periods. The inclusion of wider bone defects would also provide a more comprehensive assessment of osseointegration and bone regeneration capabilities. Moreover, investigation of synergistic effects between surface roughness and other modifications, such as hydrophilicity or bioactive coatings, could further contribute to optimizing implant design for various clinical situations.

## CONCLUSION

This pilot study investigated the effects of dental implant surface roughness on osseointegration. While surface roughness influenced osteoblast differentiation *in vitro*, it did not significantly affect final osseointegration levels *in vivo*. These findings suggest that both moderately rough and rough surfaces may support osseointegration, but larger scale studies are needed to validate these results and determine optimal surface characteristics for dental implants.

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