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Original Article

Analysis to evaluate novel separable dental implant stability: An experimental study in rabbits

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KEYWORDS

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Abstract *Background/purpose:* Peri-implantitis is a representative etiology that affects the long-term survival of dental implants. It is known that decontamination of the implant surface is essential for the successful outcome of regenerative therapy for peri-implantitis. In the present study, the stability of a novel separable dental implant (SDI) was evaluated and compared with a conventional non-separable dental implant (NDI) using biomechanical and histomorphometric analyses.

Materials and methods: In this animal study, 40 rabbits were implanted with two SDI fixtures in the left tibia and two NDI fixtures in the right tibia. The rabbits were sacrificed 3 and 6 weeks after implantation, and the implant samples were evaluated using resonance frequency analysis (RFA), micro-computed tomography (CT), removal torque testing, and histomorphometric analysis.

Results: SDI exhibited comparable or better osseointegration and implant stability to NDI. In particular, SDI showed significantly higher implant stability quotient (ISQ) values immediately and 6 weeks after implantation, while removal torque values were significantly higher at both

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3 and 6 weeks. In addition, microgaps on the histomorphometric images were not observed and abnormal signs or inflammation did not occur at the connection between the top and bottom parts of the SDI.

Conclusion: The novel SDI fixture demonstrated sufficient osseointegration and biomechanical stability compared with NDI in this animal study. In addition, the changeable top part of SDI indicates that it may be effective in easily treating peri-implantitis in clinical practice. Additional future studies on the stability and clinical application after loading to the fixture are necessary.

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Introduction

The use of dental implants has significantly increased in recent decades due to its high success rates with advanced techniques. With the increased use of implants, peri-implant inflammatory conditions are frequently observed in the population with dental implants, and the prevalence of peri-implantitis has been increasing.^{1,2} Peri-implantitis is a site-specific infectious disease that causes an inflammatory reaction with loss of supporting bone surrounding an osseointegrated implant. The prevalence of peri-implantitis was 5–8% in one previous study,¹ and approximately 22% in the meta-analysis by Derks and Tomasi.² Atieh et al.³ reported an 18.8% prevalence of peri-implantitis, and patients with a history of periodontitis were estimated to have a 21.1% incidence of peri-implantitis in subgroup analysis. Therefore, peri-implantitis poses a challenge to the long-term success of implant osseointegration for stable masticatory function.

Peri-implantitis is managed with various methods including chemical and mechanical treatments such as local or systemic antibiotics, ultrasonic cleansing, and debridement.⁴ The surgical management of peri-implantitis is indicated in cases where non-surgical treatment failed, with recurrence of peri-implantitis.⁵ Surgical treatments of peri-implantitis can be divided into regenerative and non-regenerative classifications. The presence of bacteria around the implant is an important factor in determining the success of peri-implantitis treatment.⁶ Reportedly, implantoplasty, which smooths the implant surface to prevent adherence of bacteria and aids in soft tissue adaptation, is helpful in reducing peri-implantitis.⁷ Implantoplasty, which is classified as a non-regenerative therapy, results in a significant decrease in bleeding on probing and probing depth and may result in superior implant condition compared to non-surgical treatment.⁵ However, this non-regenerative therapy is not a method to reverse bone loss that has already occurred. To solve this issue, a xenogenic bone graft with implantoplasty was investigated to regenerate the alveolar bone.⁸ For successful regenerative therapy, decontamination of the implant surface infected with bacteria is important.⁹ However, this is not always successful, and issues with titanium particles from this process can be encountered.^{10,11} The optimal treatment of peri-implantitis is regeneration of the supporting alveolar bone loss around an implant (re-

osseointegration). In another study, the long-term results of regenerative therapy using a bone graft in patients with peri-implant bone loss resulted in implant loss without adequate osseointegration in some patients.¹² Attempts have been made over the last several years to resolve peri-implantitis, but a standard treatment protocol has not been established.¹³

To fundamentally solve the decontamination and regenerative issues of dental implants, the implant surface must be remodeled. A new dental implant fixture that is separable to allow easy retrieval of the top part in patients with peri-implantitis was designed in a previous study.¹⁴ When progressive peri-implantitis-induced alveolar bone loss occurs and a fixture is exposed, only the exposed upper part of the separable fixture is removed and replaced, allowing the unaffected lower part of the fixture to be maintained. Therefore, bacterial infection as a major cause of peri-implantitis can be mechanically removed and the area decontaminated. Successful osseointegration can be achieved by bone grafting on the newly replaced top part of the implant. Progressed peri-implantitis is effectively treated by replacing the infected top part of the fixture and grafting the bone materials for new osseointegration.

Differences may exist between separable dental implants (SDIs) and conventional non-separable dental implants (NDIs). The SDI exhibited equivalent biomechanical stability to the conventional NDI in a previous finite element analysis.¹⁴ Proper evaluation of a new implant, including biomechanical stability and *in vivo* osseointegration evaluation, is mandatory prior to clinical application.

In the present experimental study, the stability of a newly designed SDI was compared with that of a conventional NDI and the histomorphometric features were analyzed.

Materials and methods

Implant fixture preparation

Two groups of sandblasted, large-grit, and acid-etched (SLA) surfaced implant fixtures were prepared: SDI fixtures and NDI fixtures. The exterior designs and dimensions of the SDI and the NDI were the same at 4 mm wide, 8 mm long, and 0.4 mm screw thread pitch, with the top part being 3 mm long and bottom part being 5 mm long (Fig. 1A

and B). The SDI had a standard hex on top of the bottom part of the fixture and was characterized by a passive fit with the top part of the fixture (Fig. 1B). The external hex type of the bottom part and internal hex type of the top part were fit and passively separable (Fig. 1C and D). The implants were manufactured with titanium grade 5 ELI (Ti-6Al-4V extra low interstitial elements). A total of 232 implants, 116 SDI and 116 NDI, were prepared; of these, 72 implants were used for implant stability testing on synthetic bone models and 160 implants were used for the rabbit tibia experiment.

Implant stability test on the synthetic bone model

In a previous study, finite element analysis demonstrated that SDI was biomechanically stable.¹⁴ Based on that study, implant stability was tested in a synthetic bone model. Holes of three sizes ($\varnothing$ 2.2, 2.8, and 3.3 mm) were created in the synthetic bone, and the fixtures were inserted ($n = 72$, 24 fixtures in each size of hole). Resonance frequency analysis (RFA) was performed six times from four

directions using an Osstell™ Mentor (Integration Diagnostics Ltd., Göteborg, Sweden).

Experimental animals and surgical procedure

This study was conducted at the Laboratory Animal Resource Center of Dental Research Institute at Seoul National University and was approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC). Ethical clearance for *in vivo* experiments was obtained from the IACUC prior to the experimental procedure (SNU-1712222-2).

A total of 40 male New Zealand white rabbits (DOOYEOLBIOTECH, Seoul, Republic of Korea) with an average weight of 3.3 kg were used for the experiments. For implant placement, a 3.3 mm hole was prepared using an implant drill, and a fixture was placed with a torque of 30–40 N. Two SDI fixtures were installed in the left tibia and two NDI in the right tibia. Prophylactic antibiotic (cefazolin) and general anesthesia (xylazine and tiletamine-zolazepam) were applied intravenously through

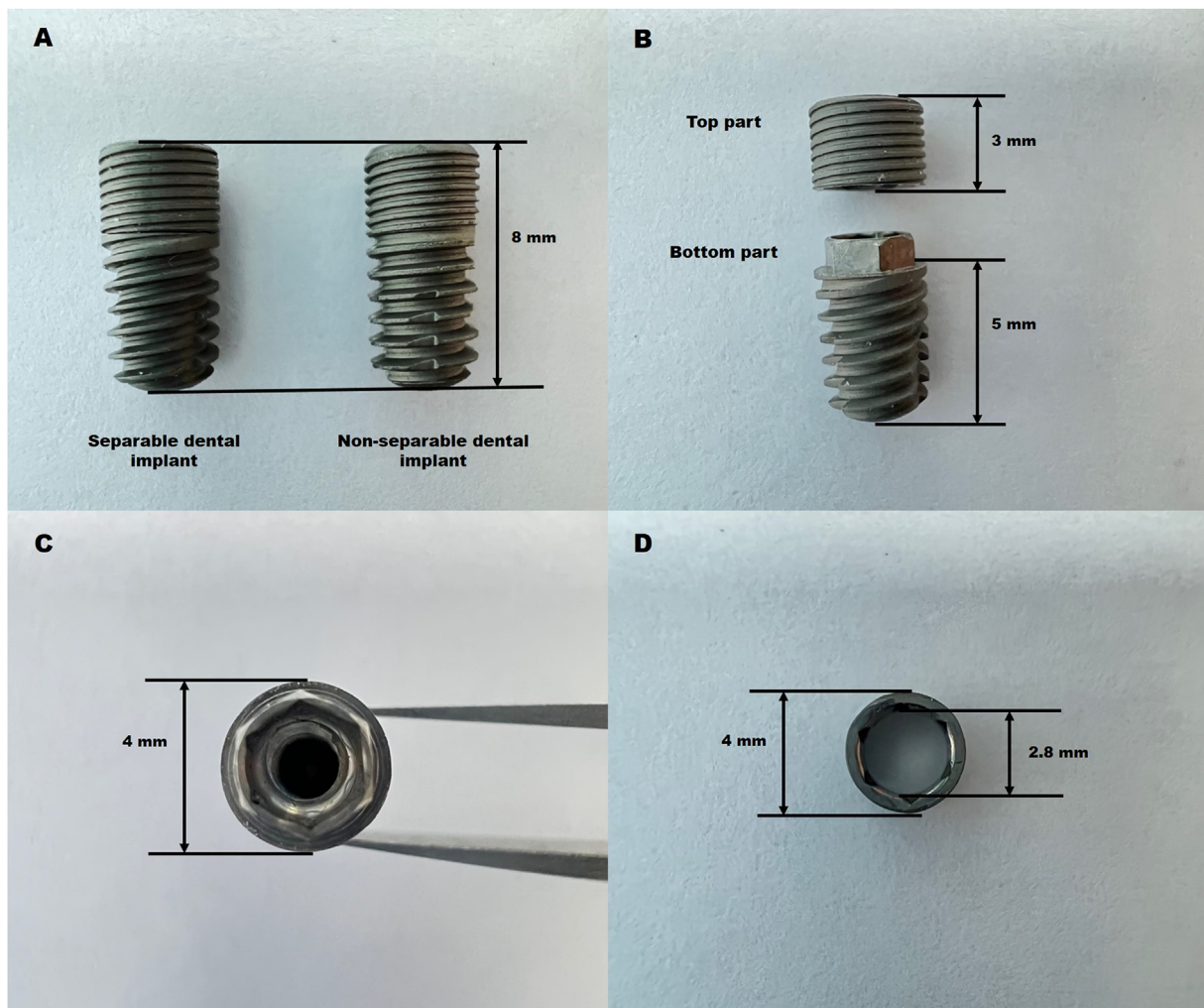


Figure 1 (A) Overall structures of the separable dental implant (SDI) and non-separable dental implant (NDI) (B) Top and bottom parts of SDI (C) Cross-sectional image of the bottom part (D) Cross-sectional image of the top part.

marginal ear veins. Radiographic photographs were collected immediately and 3 and 6 weeks after surgery.

Since implant location in the tibia may influence osseointegration due to different bone features from the epiphysis to diaphysis, two fixtures were positioned on the medial surface of the tibial medial condyle. Therefore, the fixtures were installed mono-cortically in spongy bone and not into a marrow cavity. One fixture was positioned anteriorly and the other posteriorly of the tibial medial condyle, with an 8-mm gap between the fixtures. In all, 40 rabbits received a total of 160 implants ($n = 80$ per group) in the tibia.

Twenty randomly selected rabbits were sacrificed at 3 or 6 weeks after implantation. Immediately after sacrifice, the tibia specimens containing the fixtures were harvested in blocks and fixed in 10% neutral buffered formalin.

Resonance frequency analysis

Immediately after implant placement, initial implant stability was measured with an Osstell™ Mentor for RFA. Duplicate implant stability quotient (ISQ) measurements were repeated for each implant in two directions: proximal to distal and lateral to medial. After a healing period of 3 or 6 weeks, ISQ was measured again with the same method as when the rabbits were initially sacrificed.

Bone volume fraction in micro-computed tomography

All specimens were scanned with high-resolution computed tomography (CT) at a spatial resolution of $9.945\ \mu\text{m}$ (voxel dimension). Section reconstruction was performed using a program in Skyscan NRecon software version 1.7.0.4 (Bruker, Kontich, Belgium). For each specimen, a total of 2240 consecutive micro-CT slices was acquired. Bone volume fraction (bone volume to total volume ratio, %BV/TV) was directly calculated in the VOI from the 2D and 3D datasets using CTAn software version 1.17 (Bruker).

Removal torque value

Removal torque value (RTV) was measured to evaluate the shear strength of the bone-implant interface for both implant types by applying a counterclockwise rotation to the implant axis at 3 and 6 weeks after implantation. Peak resistance values of reverse torque rotation when displacing the implants from the tibia specimens were measured automatically using a digital torque gauge (MTT03-100, Mark-10, Copiague, NY, USA). An axis of the torque gauge

device was aligned to an axis of the implant with a mount to apply axial movements during the test.

Histomorphometric analysis

Peri-implant bone regeneration was assessed histomorphometrically with percent bone implant contact (%BIC) and percent bone density (%BD) at 3 and 6 weeks after implantation. The specimens were stained with hematoxylin and eosin (H&E) and viewed under a light microscope to evaluate new bone formation around the fixtures. All measurements were performed under $\times 100$ magnification.

Statistical analysis

All data were analyzed using SPSS22 statistical software (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was evaluated using Kolmogorov-Smirnov test. Two-way analysis of variance (ANOVA) was performed to verify the main effects of fixture type and healing period on ISQ, %BV/TV, RTV, %BIC, and %BD and their interaction effects. Values were considered statistically significant when the P value was less than 0.05.

Results

Resonance frequency analysis

In the synthetic bone model, SDI exhibited reasonable stability in all tests.¹⁵ Based on two-way ANOVA, ISQ did not significantly differ between SDI and NDI in $\varnothing 2.2$ - and $\varnothing 2.8$ -sized holes. However, SDI showed significantly higher ISQ (76.8 ± 0.7) compared to NDI (74.3 ± 3.3) in the $\varnothing 3.3$ -sized hole (Table 1, Fig. 2A).

In the rabbit tibia model, SDI was approximately 4.2% higher immediately after implantation and approximately 2.0% higher at 6 weeks compared to NDI, with statistically significant difference ($P < 0.0001$ and $P < 0.05$, respectively). However, at 3 weeks after implant placement, SDI was approximately 1.7% higher than NDI but without significant difference (Table 1, Fig. 2B).

Bone volume fraction in micro-computed tomography

Statistically significant differences were not observed between SDI and NDI within each healing period. However, at both 3 and 6 weeks, SDI exhibited approximately 0.9% higher bone volume fraction than NDI. SDI and NDI demonstrated significant differences in bone volume

Table 1 Resonance frequency analysis (RFA) of separable dental implant (SDI) and non-separable dental implant (NDI) in a synthetic bone model and rabbit tibia.

	Synthetic bone ($n = 72$, 36 SDI and 36 NDI)			Rabbit tibia ($n = 160$, 80 SDI and 80 NDI)		
	$\varnothing 2.2$ ($n = 24$)	$\varnothing 2.8$ ($n = 24$)	$\varnothing 3.3$ ($n = 24$)	Immediately ($n = 160$)	3 weeks ($n = 80$)	6 weeks ($n = 80$)
SDI	67.7 ± 1.2	74.2 ± 1.1	76.8 ± 0.7	65.2 ± 0.7	60.9 ± 1.5	65.2 ± 1.1
NDI	67.0 ± 3.6	72.3 ± 1.7	74.3 ± 3.3	62.6 ± 0.7	59.9 ± 0.7	63.9 ± 0.8

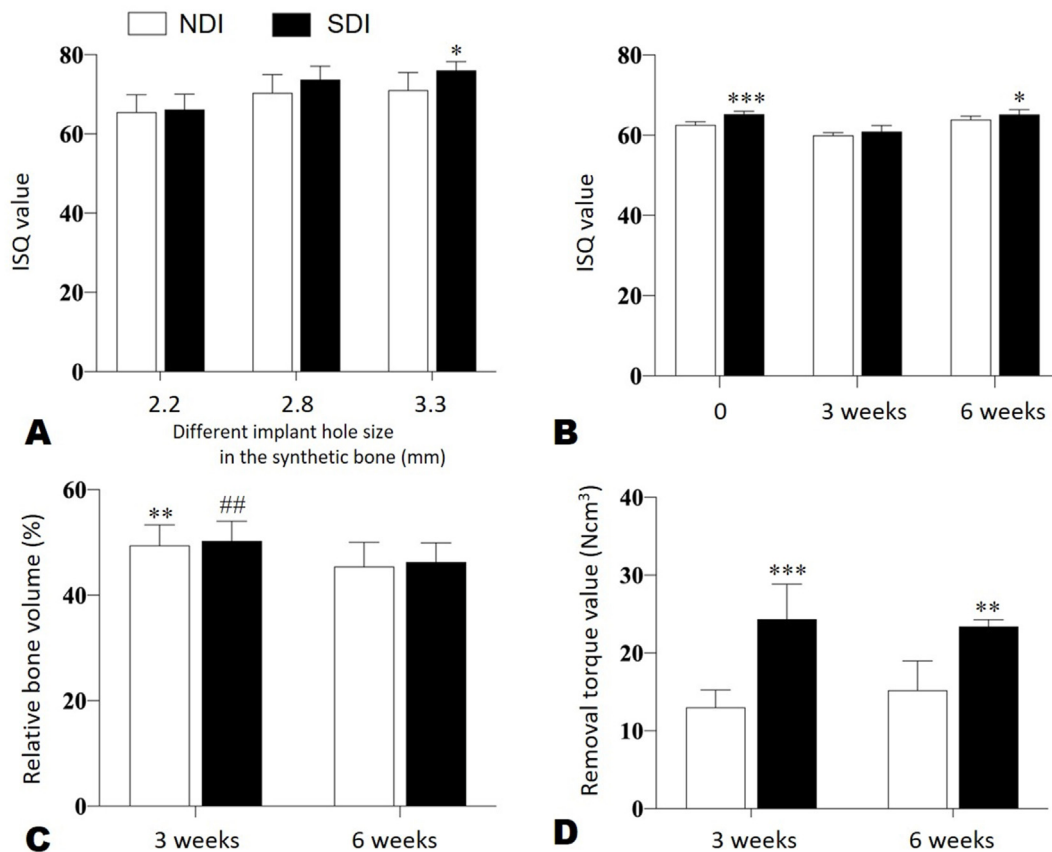


Figure 2 (A) In the synthetic bone model, the implant stability quotient (ISQ) is shown based on the size of implant holes ($\varnothing$ 2.2, 2.8, and 3.3 mm) between the separable dental implant (SDI) and non-separable dental implant (NDI). The SDI exhibited a higher ISQ compared with the NDI (* $\varnothing$ 3.3, $P < 0.05$). (B) In the rabbit tibia model, SDI and NDI showed a significant difference immediately and at 6 weeks (*** $P < 0.0001$, * $P < 0.05$). (C) In the comparison of bone volume (%BV/TV), a statistically significant difference was not observed between SDI and NDI during each healing period. However, statistically significant differences were observed between 3 and 6 weeks in both NDI and SDI (**NDI $P < 0.001$, ##SDI $P < 0.001$). (D) In the comparison of removal torque value (RTV), significant differences were observed between SDI and NDI at 3 weeks and 6 weeks (***3 weeks $P < 0.0001$, **6 weeks $P < 0.001$).

fraction between 3 and 6 weeks (SDI group, 4.0%, $P < 0.001$; NDI group, 4.0%, $P < 0.001$; Table 2, Fig. 2C).

Removal torque value

For both SDI and NDI groups, significant differences were not observed in RTV between 3 and 6 weeks. However, at both 3 and 6 weeks, SDI showed significantly higher RTV than NDI. At 3 weeks, SDI showed approximately 86.9% higher RTV than NDI, and at 6 weeks, SDI showed approximately 53.9% higher RTV than NDI ($P < 0.0001$ and $P < 0.001$, respectively; Table 2, Fig. 2D).

Histomorphometric analysis

Histomorphometric analysis verified that the bone and implant fixture in both SDI and NDI had stable osseointegration, which was not noticeably different between the two implant types. Both sections revealed osteoids in the woven bone around the fixture, indicating new bone

formation (Fig. 3). The fixtures were predominantly in contact with marginal cortical bone along the upper edge of the fixture and in tight contact with newly formed trabecular bone around the middle body of the fixture. Prominent inflammation or soft tissue involvement between the bone and the fixture was not observed. Furthermore, when analyzing the area where the top and bottom parts of the SDI were connected, osseointegration was achieved and did not differ from that of NDI. A gap between the two parts was also not found.

Based on mean %BIC, after 3 weeks of healing, SDI was approximately 0.9% higher than NDI, and after 6 weeks of healing, SDI was approximately 4.1% higher than NDI. At 3 and 6 weeks, %BIC increased by approximately 11.2% in SDI and by approximately 7.0% in NDI. No %BIC values were statistically significant (Table 2, Fig. 4A).

Based on the mean %BD, after 3 weeks of healing, SDI was approximately 1.1% lower than NDI, and after 6 weeks of healing, SDI was approximately 0.3% lower than NDI. There were no statistically significant differences in %BD values between two groups. Compared to the %BD at 3

Table 2 Bone volume fraction (%BV/TV), removal torque value (RTV), percent bone implant contact (%BIC), and percent bone density (%BD) quantitative values based on micro-computed tomography (CT) measurement and histomorphometric analysis.

	Interval	Fixture	Mean $\pm$ SD	P-value*
%BV/TV	3 weeks	SDI (n = 40)	50.3 $\pm$ 3.8	<0.001
		NDI (n = 40)	49.4 $\pm$ 3.9	
	6 weeks	SDI (n = 40)	46.3 $\pm$ 3.7	<0.001
		NDI (n = 40)	45.4 $\pm$ 4.6	
RTV (Ncm ³)	3 weeks	SDI (n = 40)	24.3 $\pm$ 3.7	<0.0001
		NDI (n = 40)	13.0 $\pm$ 2.0	
	6 weeks	SDI (n = 40)	23.4 $\pm$ 0.8	<0.001
		NDI (n = 40)	15.2 $\pm$ 3.4	
%BIC	3 weeks	SDI (n = 40)	76.2 $\pm$ 10.3	NS
		NDI (n = 40)	75.3 $\pm$ 9.5	
	6 weeks	SDI (n = 40)	84.7 $\pm$ 10.4	<0.001
		NDI (n = 40)	80.6 $\pm$ 12.2	
%BD	3 weeks	SDI (n = 40)	69.7 $\pm$ 10.4	<0.001
		NDI (n = 40)	70.8 $\pm$ 5.7	
	6 weeks	SDI (n = 40)	85.2 $\pm$ 9.5	<0.001
		NDI (n = 40)	85.5 $\pm$ 4.3	

Abbreviations: SD, standard deviation; SDI, separable dental implant; NDI, non-separable dental implant; NS, no statistical significance.

*P-value evaluated by two-way analysis of variance.

weeks, at 6 weeks, SDI showed a 15.5% increase in %BD, and NDI showed a 14.7% increase in %BD (SDI, $P < 0.001$; NDI, $P < 0.001$; Table 2, Fig. 4B).

Discussion

An SDI is designed to have a regenerative effect after replacing the upper part with a new implant surface when bone loss occurs due to peri-implantitis. However, since SDI is divided into two parts, the stability of the implant is uncertain. The microgap between the implant fixture and the abutment can cause peri-implantitis and bone loss by housing bacterial colonies.¹⁶ Therefore, since the SDI fixture is divided into two parts, a microgap and any issues with osseointegration and implant stability must be prevented. In the present study, SDI exhibited no difference or better values compared with NDI in both osseointegration and implant stability as measured based on various parameters. In particular, SDI showed higher ISQ values immediately and 6 weeks after implantation, and RTV also demonstrated significantly higher values at both 3 and 6 weeks. In addition, no microgap or abnormal signs were observed at the connection of the top and bottom parts on the histomorphometric image. Therefore, SDI has equal or higher implant stability than conventional NDI.

Rabbits are the smallest and most suitable animals for testing dental implants. Albrektsson et al.¹⁷ suggested biocompatibility, implant design and surface condition, surgical method, and load control during the healing period as factors for achieving successful osseointegration. Reportedly, using rabbits for experiments is advantageous due to the low cost, standardization of experimental

conditions, fast bone turnover rate,^{18,19} and similarities to humans in bone density and fracture toughness in the mid-diaphysis.²⁰ In the present study, to evaluate the newly designed SDI, ISQ in the synthetic bone was compared between SDI and NDI, and biomechanical stability was evaluated based on ISQ, %BV/TV, RTV, %BIC, and %BD in the rabbit tibia.

RTV and histomorphometric analysis can provide reliable data on the strength of the bone-implant interface and the fixation quality between the implant and bone.²¹ However, these destructive assessments are only applicable in an experimental environment. Therefore, RFA was required to predict implant stability in clinical settings. RFA is reportedly a reliable and accurate method for early evaluation of implant stability associated with bone-implant interfaces.²² An increase in implant stability values measured with RFA devices during healing was reported in rabbit tibia and human clinical studies.^{23–26} In the present study, SDI group showed comparable ISQ to NDI group, however, both groups did not show significant increase during the 6-week healing period. It may be due to insufficient bone healing period to achieve secondary implant stability.

The RTV results were similar to the %BIC and %BD values obtained in the histomorphometric analysis. The %BIC and %BD values were higher at 6 weeks than at 3 weeks. RFA is a useful clinical method to predict %BIC, namely the degree of osseointegration, and to evaluate implant stability.²⁷ However, a rough implant surface is easily covered by a thin layer of bone, which is not very supportive for biomechanical stability. In this case, %BIC usually exhibits good results. In addition, Johansson demonstrated that biomechanical tests were more sensitive for predicting implant stability compared to histomorphometric analyses.²⁸ In a previous study, the mean %BIC value of the SLA-treated implant placed in the proximal tibial metaphysis was 29% in the total length of the implant surface after a healing period of 4 weeks.²⁹ Calvo-Guirado et al.³⁰ reported mean %BIC values of 23–40% of four modified SLA surface implants placed in the proximal tibial metaphysis after a healing period of 2–8 weeks. The results from the present study showed higher %BIC values compared with the literature.

In the present study, it was observed that the %BV/TV at 6 weeks after implant placement was significantly lower compared to 3 weeks after implant placement, which may be explained by the use of an immature rabbit model, as suggested in previous studies. Chen et al.³¹ reported that in an immature rabbit model, bone remodeling peaked at 6 weeks, with woven bone and new trabecular formation decreasing after 5 weeks. This resulted in the lowest bone volume fraction at the 6 weeks. In the study by He et al.,³² assessing osseointegration after the placement of titanium and plasma electrolytic oxidation-coated titanium implants in a rat model, both groups exhibited significantly reduced bone volume and bone area fractions at 6 weeks compared to 0, 2, and 4 weeks. In this study, the use of immature rats has been proposed as the cause for the observed decline. Therefore, to assess the osseointegration of SDI, long-term animal studies following implant placement will be necessary.

The stability of implants can be divided into primary and secondary. Bone density, surgical preparation, and implant

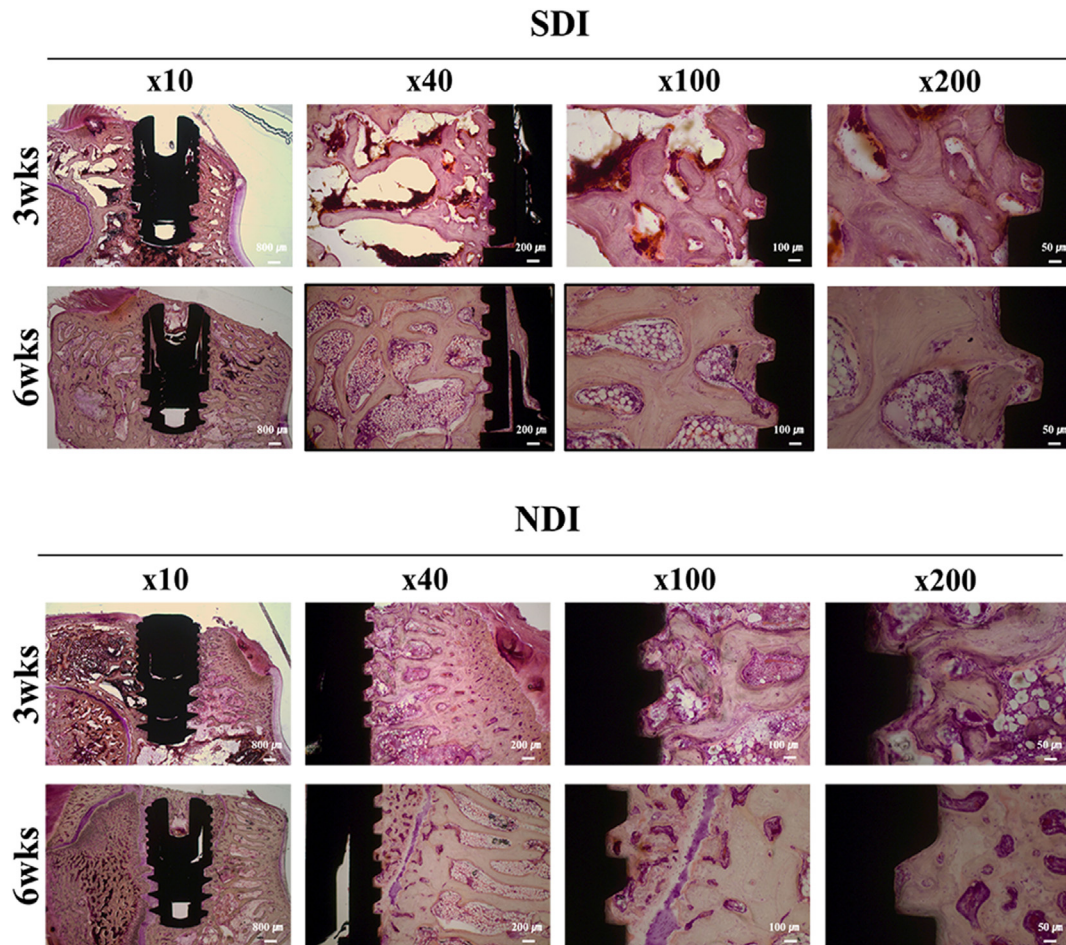


Figure 3 Histological appearance of separable dental implant (SDI) and non-separable dental implant (NDI). Histological analysis of SDI and NDI was compared and analyzed at 3 and 6 weeks, respectively. The magnification of each photomicrograph is 10 × , 40 × , 100 × , and 200 × from left to right, respectively.

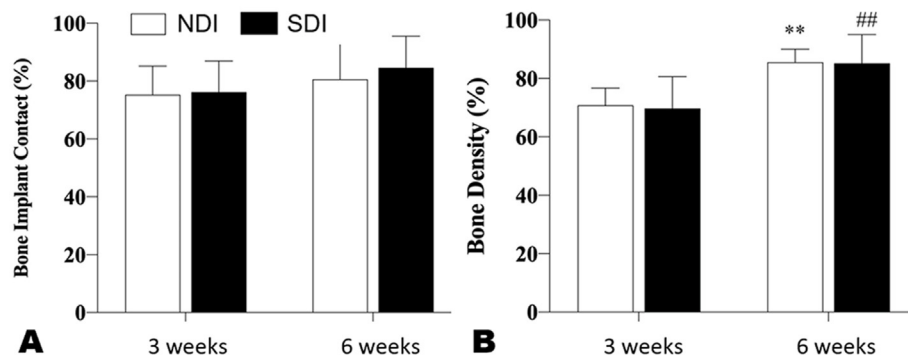


Figure 4 Histomorphometric analysis of separable dental implant (SDI) and non-separable dental implant (NDI). (A) During the healing period, the %BIC values between the SDI and NDI were not statistically significant. (B) Both NDI and SDI were significantly higher at 6 weeks than 3 weeks (**NDI $P < 0.001$, ##SDI $P < 0.001$).

design influence primary stability, and the biodistribution response of implants during osseointegration and functional load is associated with secondary stability.³³ Although the present study showed favorable osseointegration and stability of SDI at 3 and 6 weeks in a rabbit model, functional loading after delivery of implant prostheses may cause

mechanical changes in the implant and changes in the surrounding periodontal tissue, ultimately affecting implant stability. Therefore, in future studies, it is essential to assess potential displacement or loss of the implant during food mastication and to evaluate survival rate and bone mineral density changes after long-term functional

loading. Additionally, friction or corrosion may occur at the junction between the upper and lower parts of SDI when subjected to functional load. Hence, it is also necessary to evaluate the occurrence of microgaps at the junction and changes in these microgaps under the oral bacterial environment. Utilizing miniature pigs as dental implant animal models can be helpful in simulating oral bacterial environments and mimicking the mastication dynamics of real clinical conditions.

In conclusion, the novel SDI fixture exhibited sufficient osseointegration and biomechanical stability compared with NDI in this animal study. Histomorphometric analysis revealed no microgap between the top and bottom parts and no abnormal signs or inflammation at the connection. In addition, the changeable top part of the SDI indicates that it may be effective in easily treating peri-implantitis in clinical practice. Additional future studies on its stability and clinical application after loading to the fixture are necessary.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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