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ORIGINAL RESEARCH

Atomic Layer Deposition of Zirconia on Titanium Implants Improves Osseointegration in Rabbit Bones

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Purpose: Atomic layer deposition (ALD) is a method that can deposit zirconia uniformly on an atomic basis. The effect of deposited zirconia on titanium implants using ALD was evaluated in vivo.

Methods: Machined titanium implants (MTIs) were used as the Control. MTIs treated by sandblasting with large grit and acid etching (SA) and MTIs deposited with zirconia using ALD are referred to as Groups S and Z, respectively. Twelve implants were prepared for each group. Six rabbits were used as experimental animals. To evaluate the osteogenesis and osteocyte aspects around the implants, radiological and histological analyses were performed. The bone-to-implant contact (BIC) ratio was measured and statistically analyzed to evaluate the osseointegration capabilities.

Results: In the micro-CT analysis, more radiopaque bone tissues were observed around the implants in Groups S and Z. Histological observation found that Groups S and Z had more and denser mature bone tissues around the implants in the cortical bone area. Many new and mature bone tissues were also observed in the medullary cavity area. For the BIC ratio, Groups S and Z were significantly higher than the Control in the cortical bone area ($P < 0.017$), but there was no significant difference between Groups S and Z.

Conclusion: MTIs deposited with zirconia using ALD (Group Z) radiologically and histologically showed more mature bone formation and activated osteocytes compared with MTIs (Control). Group Z also had a significantly higher BIC ratio than the Control. Within the limitations of this study, depositing zirconia on the surface of MTIs using ALD can improve osseointegration in vivo.

Keywords: atomic layer deposition, ALD, zirconia, machined titanium implant, bone-to-implant contact ratio, BIC, osseointegration

Introduction

Titanium is a biocompatible material mainly used in dental implants. It has mechanical strength and osseointegration capabilities. When titanium is exposed to air, it forms an oxide film, preventing corrosion and promoting osseointegration.[1,2] Titanium dental implants, which were initially machined and used, were reported to have a low success rate depending on bone quality and implantation site.[3,4] Accordingly, various physical and chemical surface treatment methods of titanium implants have been studied to improve osseointegration and increase the implant success rate.[5] One of the implant surface treatment methods is sandblasting with large grit and acid etching (SA), which is a method of spraying metal oxides on dental implant surfaces and then corroding them with acid. This method improves contact area and promotes osseointegration, resulting in a higher clinical success rate.[6,7] However, it has been found to have side effects, including foreign body reaction to the remaining non-absorbable materials and fine cracks on the implant surface due to acid corrosion.[8] Therefore, absorbent or biocompatible materials are sprayed onto the surface,[9] or electrochemical surface treatment methods, including anodized oxidation, are applied.[10]

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Zirconia (zirconium oxide; ZrO_2) is a biocompatible ceramic material with excellent mechanical strength and wear resistance. Since it has little bone resorption caused by bacterial adhesion and does not interfere with the activity of osteoblasts, it has been attracting attention as a potential substitute material for titanium.[11] Since there is a risk of fracture and difficulty in surface treatment when zirconia is used directly as an implant fixture,[12] various physical and chemical methods of coating zirconia particles onto titanium have been studied. One of the physical coating methods, blasting of zirconia particles, showed higher removal torque than treating the surface of zirconia implants directly.[13] The colloidal suspension method, a chemical method, has the advantage of maintaining the properties related to macro, mini, and micro designs of the implant.[14] It has been found that zirconia-coated implants have more reliable osseointegration than titanium implants, thereby being composed of histologically mature bone.[14] However, Hayashi et al[15] reported that while many studies have suggested various surface modification procedures of zirconia, appropriate standards have not yet been established.

Osseointegration is defined as direct contact between implant and bone without the involvement of peri-implant soft tissues.[16] Osseointegration is essential in successful implants, which are evaluated based on the bone-to-implant contact (BIC) ratio.[16,17] The osseointegration process occurs sequentially, starting from the absorption of blood clots and proteins on implant surfaces, through the recognition process by osteoblasts and mesenchymal stem cells, and then to bone remodeling.[18] Since this process occurs in the nanoscale microscopic area, various surface treatment methods have been studied with the goal of developing implant surfaces with a nanoscale biomimetic structure.[18,19]

Nanoscale surface treatments can be largely classified into two methods: physical or chemical vapor deposition. Of these, physical vapor deposition (PVD) uses a physical method of depositing thin coatings by spraying laser pulses or ionized gas.[20] Since coatings deposited by this method suffer from low adhesion to surfaces, it is not as suitable for complex surfaces.[21] Chemical vapor deposition (CVD) is a method of chemically changing a solid material into a gaseous phase and reacting and growing it on the surface. Compared to PVD, CVD produces coatings with higher surface adhesion and can be applied even to complex structures.[21] However, it has the disadvantages of requiring a high temperature of over 600°C and containing many impurities.[20,21]

Atomic layer deposition (ALD), proposed to compensate for these shortcomings, is a chemical method derived from CVD. ALD allows for a finely and uniformly thin film deposited on an atomic layer basis at a low temperature of 500°C or less.[20,21] Since the shape of the implant surface is a complex screw structure, if the coating on the surface has not been deposited uniformly, it can easily peel off.[22] ALD is a suitable method for processing screw-shaped implant surfaces, as it enables us to control the thickness of the film uniformly on an atomic layer basis. Studies on depositing zirconia using ALD have shown excellent results in inhibiting bacterial adhesion and activating osteoblasts.[15,22] However, to date there has been no research that has reported the osseointegration effect of implants deposited with zirconia using ALD in vivo.

The purpose of this study was to radiologically and histomorphometrically evaluate the effect of osseointegration by implanting machined titanium implants (MTIs), SA-treated MTIs, and MTIs deposited with zirconia using ALD into the femur of rabbits. The null hypothesis was tested under the assumption that zirconia deposited on the implant surface by ALD would not affect the BIC ratio.

Materials and Methods

Experimental Materials

Internal system dental implants (JUST®, KJ Meditech, Gwangju, Korea) of the same dimensions (a diameter of 4 mm and a length of 7 mm) were used in this study. Machined implants were used as the control group. SA treated (sandblasted with alumina particles of 120μm and acid etched with HCl-HNO_3) implants used as the Group S. And zirconia-deposited with ALD implants were used as Group Z. Twelve implants were prepared for each group.

Atomic Layer Deposition of Zirconia

Zirconia deposition on the surface of MTIs was performed using atomic layer deposition equipment (Compact ALD 150, Ultech, Daegu, Korea). Tetrakis[ethylmethylamido]zirconium (TEMAZr, UPchem, Gyeonggi-do, Korea) was used as a precursor, H_2O was the reaction gas, and argon was the carrier and purge gas. According to the method reported by Jo et al[22] one cycle of the ALD process consists of four steps: the precursor is first injected into the reactor chamber for 0.05 seconds and purged with argon

Table 1 Processing of Atomic Layer Deposition of Zirconia

Processing Temperature (°C)	Ar_2 Flow Rate (SCCM)	ALD Sequence				Growth Cycle
		TEMAZ Pulse(s)	Ar_2 Purge(s)	H_2O Pulse(s)	Ar_2 Purge(s)	
200	350	0.05	50	0.5	50	150

gas for 50 seconds; next, when H_2O is injected for 0.5 seconds, amorphous zirconia is formed; then argon gas is injected for 50 seconds to purge impurities and unreacted substances (Table 1). This process was repeated for 150 cycles. Based on the pilot study, since a zirconia film with a thickness of 0.3741 to 0.4137 nm is formed in each cycle, zirconia was deposited to a thickness of approximately 56.11 to 62.05 nm in this study.

Experimental Animals and Procedures

This study was conducted using six New Zealand white rabbits aged 13–16 months, weighing 2.6–3.0 kg. This study was approved by the Institutional Animal Care and Use Committee of OBEN (OBEN-IACUC-1004-01, Suwon, Korea). All experimental procedures, including animal surgery, preoperative preparation, surgical considerations, and postoperative management, were performed according to ethical guidelines, such as the 3R principles (Replacement, Reduction, and Refinement).[23] Six implants were installed per rabbit, three each in the left and right femurs. Implants in the Control, S, and Z groups were alternately assigned to the three positions to prevent any influence of location. Anesthesia and surgical procedures were performed by referring to the methods by Sollazzo et al.[14] Anesthesia was administered through the intramuscular injection of 5 mg/kg of zolazepam hydrochloride (Zoletil 50, Virbac Korea, Seoul, Korea) and 5 mg/kg of xylazine (Rompun, Bayer Korea, Seoul, Korea). Implants were placed under local dental anesthesia using 1.8 mL of 2% lidocaine with 1:100,000 epinephrine (Lidocaine epinephrine, Yuhan, Seoul, Korea). During the placement process implants were drilled into the bone according to the sequential process (Starter, ø2.0, ø2.7, ø3.3, ø3.6) of the implant drilling system (JUST®, KJ Meditech, Gwangju, Korea). The implant placement depth varied, considering the rabbit's femur thickness and medullary cavity location. For the thin parts, implants were placed into the bone 4–5 mm deep with 2–3 mm of the fixture exposed on top of the bone. After the implant placement, 1.5 mL of tolfenamic acid (Tolfed, Komipharm, Gyeongi-do, Korea) was injected intramuscularly as an analgesic, and 5 mg of enrofloxacin (Ashienro 50, Ashish Life Science, Mumbai, India) per day was administered for three days, as an antibiotic. Following the procedure reported by Robert et al[24] all experimental animals were sacrificed six weeks after implant placement, which is the minimum osseointegration period for implants to function, and their femurs were extracted and fixed in 10% neutral buffered formalin.

Micro-CT Analysis

The specimens were fixed to the jig with parafilm and scanned using a micro-CT scanner (SkyScan1173, Bruker, Kontich, Belgium) to acquire 800 images. The images were reconstructed into cross-section images using NRecon software (SkyScan NRecon, Bruker, Kontich, Belgium). Each cross-section was analyzed using DataViewer software (DataViewer v.1.5.4.0, Bruker, Kontich, Belgium).

Histological and Histometric Analyses

The specimens were fixed in 10% neutral buffered formalin and dehydrated using alcohol. To fabricate resin blocks in which the tissue/implant specimens were embedded, light-curing acrylic resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany) was infiltrated into them and light-cured using a UV curing machine. The tissues embedded in resin blocks were cut using a diamond band saw (EXAKT 300, EXAKT, Norderstedt, Germany) and ground with a micro grinder (EXAKT 400 CS, EXAKT, Norderstedt, Germany) to produce the final specimens. For histological observations, specimens were stained with Goldner's Masson trichrome staining solution to color-code them according to bone maturity. In this staining method, mature bones are colored green, immature new bones are colored red, and cell nuclei is colored dark brown to black.[25] The images of the stained specimens were captured using a digital slide scanner (PANNORAMIC 250 Flash III, 3DHISTECH, Budapest, Hungary). The captured images were analyzed using dedicated viewing software (Caseviewer, 3DHISTECH, Budapest, Hungary). For the length of direct contact between the bone and implants, the area where three or more consecutive screw threads contacted the bone was measured by the method Wennerberg et al[26] published. Based on this, the bone-to-implant contact (BIC) ratio was calculated. The measured areas were classified into the screw threads in the cortical bone area and those in the medullary canal area. The BIC rate in both areas was estimated.

Statistical Analysis

The mean and standard deviation of the BIC values measured in the three groups (Control, S, Z) were calculated and compared. All statistical analyses were performed using SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). First, the normality was assessed using the Shapiro–Wilk test to select a parametric or non-parametric test. If the data followed a normal distribution, a significance test was performed using a one-way ANOVA and post-hoc test with Tukey's test. If the *P*- value was less than 0.05, statistical significance was confirmed. If the data did not follow a normal distribution, statistical significance was calculated using the Kruskal–Wallis and a post-test was performed using the Mann–Whitney *U*-test. If the *P*- value is less than 0.017, the statistical significance can be confirmed.

Results

Radiographic Evaluation

The cortical bone was radiopaque, and its thickness and pattern varied depending on the area. For most implants, more than half of the total length of the screw thread was observed to be in contact with the cortical bone without any radiolucency. Most of the medullary cavity was radiolucent, and no cortical bone in clear contact with the screw threads located within the medullary cavity was observed (Figure 1a–c). However, compared to the Control, radiopaque mineralized bones were observed around the screw threads within the medullary cavity in Groups S and Z (Figure 1d and e).

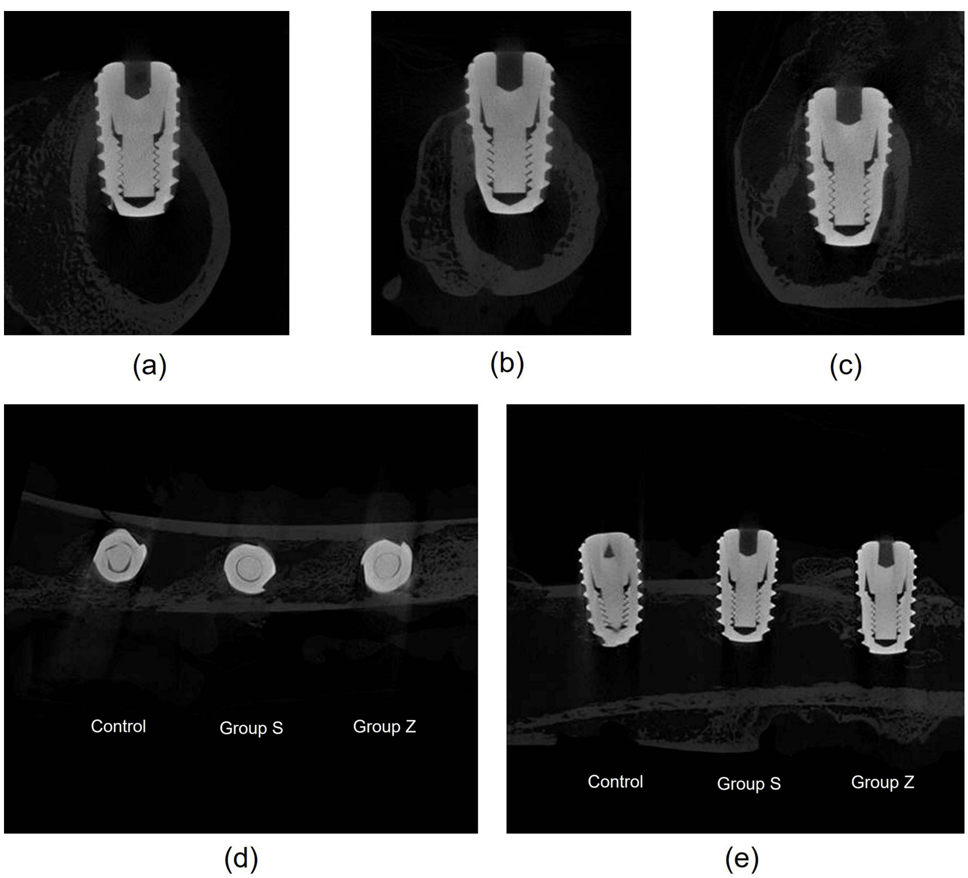

Figure 1 Radiographic images of specimens (**a**) Control group, (**b**) Group S, (**c**) Group Z, (**d**) Cross sectional images of the three implants (Control, Group S, and Group Z), (**e**) Longitudinal sectional images of the three implants (Control, Group S, and Group Z).

Histological Evaluation

Control Group (Machined Titanium Implants)

Mature bone tissues were observed at the implant interface in the cortical bone area, with some new bone tissues between the mature bone tissues. In contrast, almost no mature bone tissues were observed around the implant interface in the medullary cavity area, but new bone tissues and osteocytes were observed (Figure 2).

Group S (SA on Machined Titanium Implants)

In the cortical bone area, mature bones in contact with implant screw threads were greater in number and denser than those in the Control. New bone tissues were being formed in many areas. In the medullary cavity area, more new bone tissues and osteocytes were observed compared with the Control, and mature bone tissues were also clearly observed (Figure 3).

Group Z (Zirconia ALD on Machined Titanium Implants)

Mature bone tissues were observed in the cortical bone area, and new bone tissues were also being formed. In the medullary cavity area, more new bone tissues and osteocytes were observed compared with the Control, occupying most of the area (Figure 4).

Histometric Evaluation

Within the cortical bone area, the BIC ratios of the three groups were 45.2% for the Control, 64.2% for Group S, and 62.4% for Group Z. After the normality was tested using the Shapiro–Wilk test, a non-parametric method was selected, and the statistical significance was confirmed using the Kruskal–Wallis test ($P < 0.05$). A post hoc test for the significance between groups was performed using the Mann–Whitney *U*-test. It was found that Groups S and Z had higher significance than the Control ($P < 0.017$). However, there was no statistical significance between Groups S and Z (Figure 5). The BIC ratio of the three groups in the medullary cavity was 27.8% for the Control, 26.9% for Group S, and 35.2% for Group Z, with no statistical significance (Figure 6).

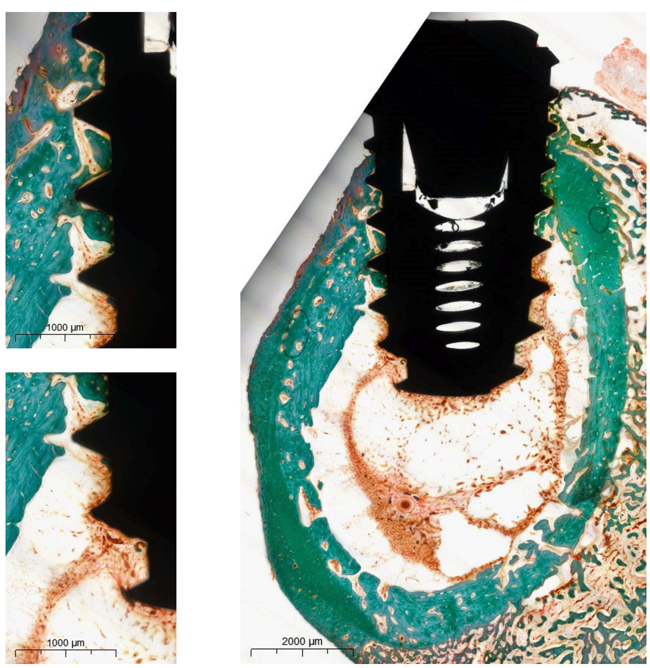

Figure 2 Histological images of the machined surface of the implant (Control).

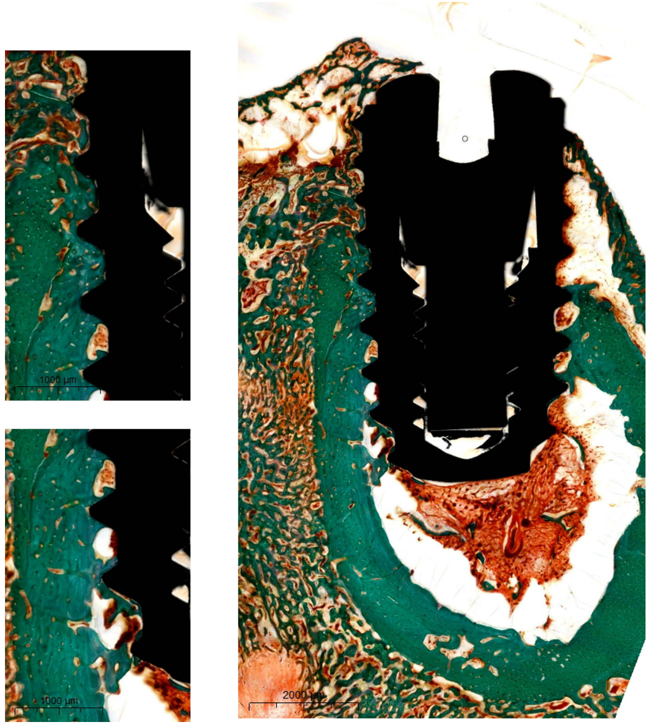

Figure 3 Histological images of the SA surface of the implant (Group S).

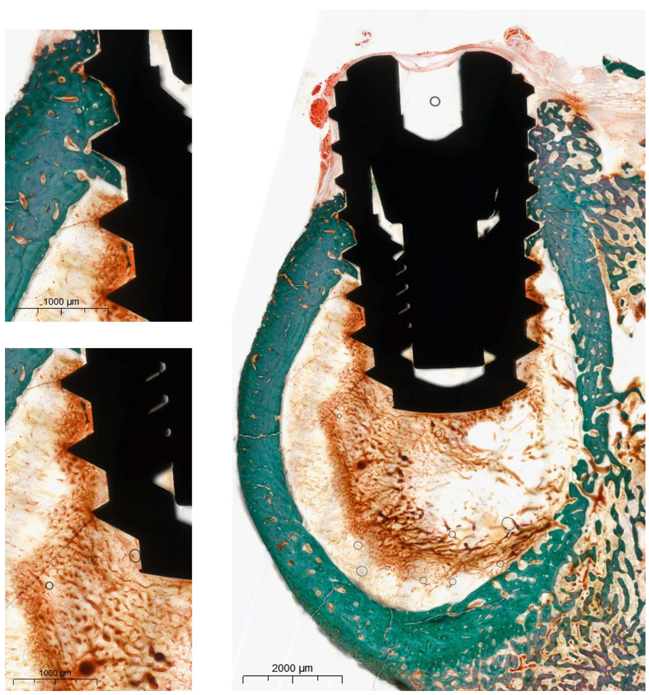

Figure 4 Histological images of zirconia ALD Coating on the machined surface of the implant (Group Z).

Discussion

Compared to titanium, zirconia has a risk of fracture when used directly as an implant fixture, and its wear and corrosion resistance are very high, making it difficult to treat the surface physically and chemically.[12] Accordingly, various methods of coating zirconia particles on the surface of titanium implants have been studied, but appropriate standards have not yet been

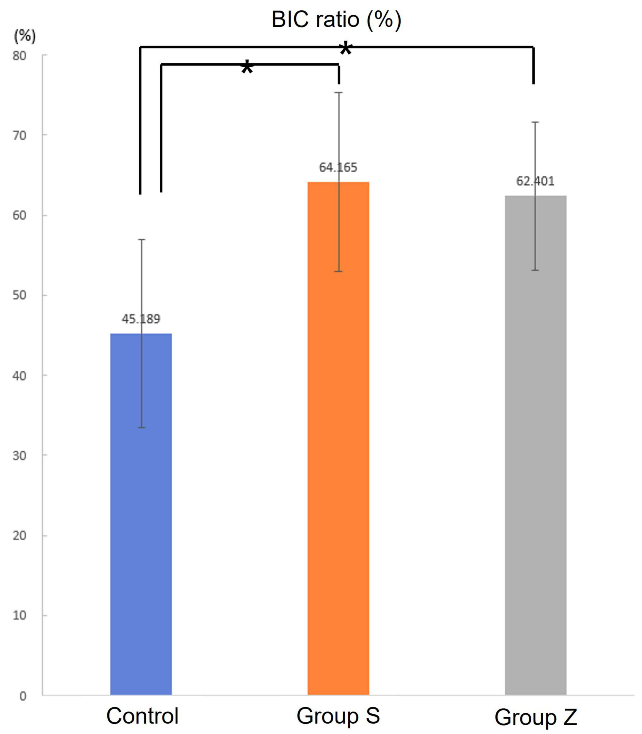

Figure 5 Bone to implant contact ratio (%) on the cortical area (*Significant at $P < 0.017$).

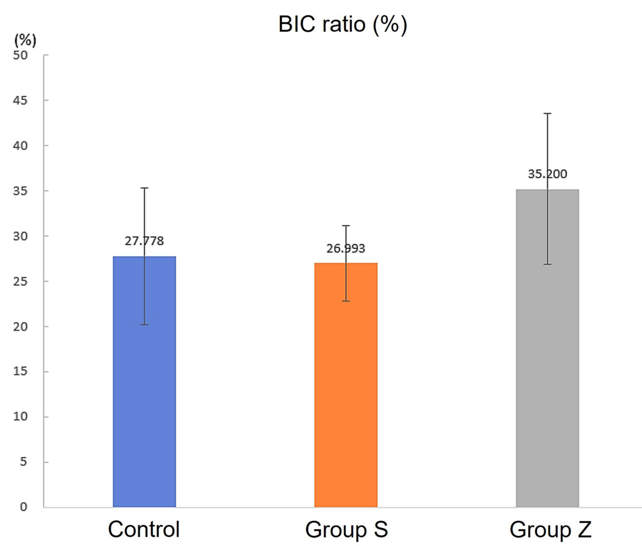

Figure 6 Bone to implant contact ratio (%) on the medullary cavity area.

established.[15] Zirconia undergoes a phase transition into a monoclinic or tetragonal structure depending on temperature or stress, and this changes its strength or volume.[27] If zirconia is deposited by physical or chemical treatments at high temperatures and pressures using the conventional methods, its films may contain impurities or undergo unexpected phase transitions. Furthermore, since the shape of the implant's surface is a complex screw structure, it is expected that if the surface is treated using conventional methods, it will be difficult to obtain a uniform surface, and the coated particles may easily peel off.[22] In contrast, since ALD can process zirconia precursors at a relatively low temperature compared with conventional methods and can uniformly deposit them on an atomic basis without impurities,[20] it is suggested as a suitable method for depositing them on screw-shaped implant surfaces.[15] However, to date no studies have reported the effect of implants deposited with zirconia using ALD on osseointegration in vivo.

This study was conducted under the null hypothesis that zirconia deposited on titanium implants using ALD would not affect the BIC ratio. The comparisons with SA-treated MTIs in addition to the Control, were performed since SA is a method that has shown high clinical success rates in many studies.[6,7] In this study, by measuring the BIC ratio, it was found that Groups S and Z had higher statistical significance than the Control in the cortical bone area ($P < 0.017$), and there was no significant difference between Groups S and Z. The BIC ratio measured in the medullary cavity area was not statistically significant. As such, the results of this experiment rejected the null hypothesis that zirconia deposited on the surface of MTIs using ALD does not affect the BIC ratio.

These results indicate that MTIs with zirconia deposited using ALD are better osseointegrated than MTIs. In addition, MTIs with zirconia deposited using ALD were similarly well-osseointegrated compared with the SA-treated MTIs, suggesting that zirconia-treated MTIs using ALD are as good as SA-treated MITs. The histological results also showed that osteocytes and new bone tissue in Group Z were distinct compared with the Control. This suggests that the effect of zirconia deposition goes beyond biocompatibility as a simple substitute for titanium and promotes osseointegration by inducing the activity of osteoblasts and osteocytes. These results are consistent with the findings of Carinci et al,[28] which demonstrated that zirconia can affect the cell cycle regulation of osteoblasts. Notably, considering that inducing osteogenesis and osseointegration is a cellular process in the nanodomain, ALD, which can coat zirconia finely and uniformly at the atomic level, is more effective. This can also be seen in the findings of Jo et al,[22] which confirmed the enhanced activities of osteoblasts and inhibition of bacterial adhesion by depositing zirconia on titanium using ALD. Based on these studies, zirconia ALD deposited implants can be expected to have several advantages over SA implants. First of all, compared to SA implants, zirconia ALD deposited implants are biocompatible because they are coated with zirconia instead of residual acids. Zirconia surfaces can enhance osseointegration and exhibit lower bacterial adhesion compared to SA surfaces, potentially reducing the risk of peri-implantitis. Zirconia ALD deposition can provide durable protection against corrosion and wear of titanium surfaces compared to SA implants. We can also consider ways to improve the disadvantages while retaining the advantages of SA implants. ALD enables precise control of coating thickness and uniformity, thus allowing zirconia to be coated while preserving the surface properties of SA implants.

The in vivo experiment in this study was conducted using rabbits for six weeks. Since rabbits have a higher metabolic rate and a shorter bone turnover cycle than humans, it can be expected that osseointegration sufficient to withstand the occlusal force will occur even in six weeks.[14,24] However, this study has limitations. One limitation is that the differences across periods within the same experimental group were not compared by extending the experimental period so that immature bone tissue and osteocytes could be more clearly distinguished.

In addition, human bones differ in bone quality between the maxilla and mandible; even within the same jaw, bone quality and occlusal force can vary depending on the area, resulting in differences in implant success rates.[29] As this experiment was performed only using rabbit femurs, differences depending on bone quality and effects on the occlusal force could not be evaluated. Within those limitations, the osteocyte and osteogenic responses occurring in the cortical bone and medullary cavity areas were compared. As many new bone tissues were observed around the implant or more osteocytes in the medullary cavity, it seems that zirconia ALD promotes osseointegration even in unfavorable bone qualities.

This study, demonstrated the improved osseointegration of zirconia-deposited MTIs using ALD in comparison with MTIs. To evaluate the effectiveness of ALD while excluding the effect of zirconia, it is necessary to add and compare experimental implant groups in which zirconia is deposited by spraying, CVD, or PVD methods. Additionally, loss of deposited zirconia may occur due to physical reactions during the implant placement process or metabolic reactions after the placement. Since ALD enables precise control of the zirconia thickness deposited and achieves a thickness of 0.096 ± 0.002 nm per cycle,[20] further research is needed on the change in osseointegration effect depending on the thickness.

Conclusions

The results of this study suggest that MTIs deposited with zirconia using ALD showed radiologically and histologically more mature bone formation and activated osteocytes compared with MTIs. In addition, SA-treated MTIs and zirconia-deposited

MTIs had a significantly higher BIC ratio than MTIs. Within the limitations of this study, depositing zirconia on the surface of MTIs using ALD was found to be a method that can improve osseointegration in vivo.

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Disclosure

The authors report no conflicts of interest in this work.

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