The aim of this study was to assess hard and soft tissue responses using three dental implants made of different materials. Implants made of titanium (Ti), yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) and ceria partially stabilized zirconia/alumina nanocomposite (Ce-TZP/Al2O3) were used in a dog model. Five male beagles were sacrificed at three months after implantation, and harvested mandible were observed and analyzed. Histological observations were similar in all groups. There were no significant differences in any histomorphometric parameters. Our results suggested the possibility of Ce-TZP/Al2O3 as a dental implant material, similar to Ti and Y-TZP.

**Keywords**: Dental implant, Ce-TZP/Al2O3, Y-TZP, Tissue responses, Dog model

**INTRODUCTION**

Zirconia dental implants have emerged as an alternative to titanium (Ti) implants in recent years. Yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) are now commonly used, achieving good long-term prognosis as an artificial joint material in medical disciplines. Y-TZP has superior biocompatibility because it shows little elution of metal ions. Furthermore, it has high bending strength, high toughness and excellent operability when compared with alumina. Y-TZP also shows similar osseointegration as Ti implants. Various modifications have been applied to experimental implants in order to improve the properties of zirconia implants for bone response, inflammatory response, biocompatibility and bacterial adherence.

Y-TZP has been compared with Ti in *in vivo* study and peri-implant bone response was not apparently different. Moreover, implant abutments, which have machined surfaces, showed no differences between Y-TZP and Ti with regard to qualitative histological features in peri-implant soft tissue. Y-TZP surfaces showed significant reductions of bacteria, and this is important for the health of peri-implant soft tissues. For these reasons, Y-TZP is currently used clinically in dental implants.

On the other hand, Y-TZP is associated with several disadvantages, including its difficult manufacturing process. One disadvantage is its low fracture toughness, a clinical case report that was followed for 3 years showed fracture and loss of the zirconia implant body. To resolve these disadvantages of Y-TZP, ceria partially stabilized zirconia/alumina nanocomposite (Ce-TZP/Al2O3) was recently developed. Ce-TZP/Al2O3 has a higher flexural strength than Y-TZP and about 3 times the toughness of Y-TZP. Ce-TZP/Al2O3 is also more stable at low temperatures, giving it superior long-term stability *in vivo*. Ce-TZP/Al2O3 is therefore expected to expand the clinical application of zirconia; for instance, in abutments of dental implants and implant bodies.

There have been few long term follow-up reports on zirconia implants and a few detailed studies on hard and soft tissue responses to zirconia implants. Thus, some of the properties of zirconia remain unclear. In addition, no studies have compared Ti, Y-TZP and Ce-TZP/Al2O3 *in vivo*. The aim of this study was to assess the hard and soft tissue responses to three dental implants made of different materials, including Ce-TZP/Al2O3, in a dog model.

**MATERIALS AND METHODS**

**Animals**

We used five male beagles, aged approximately 24 months and weighting 11–14 kg. Dogs received standard feed and water *ad libitum*. Throughout the entire treatment period, we monitored the behavior, posture, reactivity and appearance of the dogs. This study was approved by the Animal Experiment Ethics Committee of The Nippon Dental University School of Life Dentistry.
Implant design
A total of 18 implants were used in this study (Fig. 1, Table 1). All implants were processed by Nanto (Shizuoka, Japan), and comprised one-piece (3 mm in diameter and 7 mm in length). Samples were grouped for dental implants made of different materials; Ti implants \( (n=6) \), Y-TZP implants \( (n=6) \) and Ce-TZP/Al\(_2\)O\(_3\) implants \( (n=6) \). For each implant material, the collar surface was machined and was observed by scanning electron microscopy (SEM, JSM-6101LA, JEOL, Tokyo, Japan). In addition, the surface roughness \( (Ra) \) of implant collars was assessed using a laser microscope (VK-X100, KEYENCE, Osaka, Japan). Measuring devices (VK Viewer and VK Analyzer, KEYENCE) were used to show the roughness of the implant collar. Ra values were calculated by averaging the Ra values of several line profiles over the analyzed surface.

Surgical procedures
Surgical procedures were performed on dogs under both systemic (ketamine, 10 mg/kg; xylazine, 3 mg/kg) and local anesthesia (2% lidocaine containing 1:80,000 epinephrine), followed by intramuscular injection of atropine sulfate (0.5 mg/mL). All four premolars and first molar were extracted from each side of the mandible. Root furcation areas of mandibular premolars and first molar were cut using a fissure bur, and mesiodistal roots were carefully extracted. After confirming no residual roots, the extracted socket was covered with oral mucosa to aid healing. After a healing period of 3 months, for implant placement, a full-thickness flap was raised to separate it from the gingiva. Bone was removed using a guide drill (2.2 mm first drill, 2.6 mm pilot drill, and 3.0 mm final drill) at 800 rpm under saline irrigation. Following this, 6–8 implants were placed in each dog at a torque of 30 Ncm. Implants were placed 15 mm apart, and in such a way that the implant shoulder matched the crest of alveolar bone. We inserted 3 types of implant in each dog mandible without inclination of implantation site throughout this study. To prevent infection, all animals received antibiotics (Convenia®, Zoetis Japan, Tokyo, Japan: s.c. 8 mg/kg) a single injection of which is effective for 14 days, immediately after surgery, and flaps were sutured using 5-0 monocryl. Placement of implants was performed to avoid contact with opposing teeth and soft dog food was provided for one week after implant placement. At 3 months after implantation, dogs were sacrificed using ketamine hydrochloride, xylazine hydrochloride and thiopental sodium. The mandible block was then cut and harvested. Harvested blocks were fixed in 10% neutral buffered formalin for 2 weeks.

Micro-CT
Before histological processing, harvested blocks were subjected to radiography. Images obtained with a micro-CT system (SMX-100CT-ST, Shimadzu, Kyoto, Japan) were reconstructed using 3D structural analysis software (MPR, Shimadzu).

Histological analysis
After fixation, part of the soft tissue around each implant was removed for paraffin sections. The remaining part of the sample was dehydrated using an alcohol series, and was embedded in methylmethacrylate. Embedded specimens were cut along the long axis of the implant.
in the mesiodistal direction at 800 μm thickness using a slow-speed diamond saw (Varicut® VC-50, Leco, St. Joseph, MI, USA). After mounting on acrylic glass slabs, sections were ground and polished to a final thickness of about 300 μm by using a polisher (Knuth-Rotor-3, Struers, Rodovre/Copenhagen, Denmark). The most central sections were processed for staining with toluidine blue combined with fuchsin. Stained sections were observed under a microscope (AxioObserver.Z1, Carl Zeiss, Oberkochen, Germany), and the following landmarks were identified for histomorphometry (Fig. 2): marginal portion of mucosa (PM), apical portion of the junctional epithelium (aJE), implant shoulder (IS), and first bone-implant contact (fBIC). Based on these landmarks, we measured PM-aJE (epithelial tissue length, ETL), aJE-fBIC (connective tissue contact, CTC), PM-fBIC (biological width, BW) and IS-fBIC (bone resorption, BR). We also estimated the bone-implant contact (BIC) on the whole surface where implants touched the bone. We measured the mesiodistal side of all samples. One side of soft tissue was removed from each sample for observation of inflammatory cells and the other side was measured for parameters related to soft tissue. Bone resorption and BIC were measured on both sides of the implant.

Soft tissue removed from fixed samples was dehydrated through an ascending ethanol series and was embedded in paraffin. Paraffin sections were cut at 4.5 μm thickness and were stained with Hematoxylin and eosin (H-E) for histological observation. Photographs of soft tissue were taken under high magnification using a digital camera connected to a microscope (Axioplan, Carl Zeiss).

Statistical analysis
Statistical analysis was performed using SPSS ver. 17.0 (SPSS, Chicago, IL, USA). All values were presented as means and standard deviation. One-way analysis of variance (ANOVA) was used for analysis. Values of p<0.05 were considered to be significant.

RESULTS
In all animals, postoperative healing was generally uneventful. Throughout the study period, no complications such as allergic reactions, abscesses or infections were noted. Experimental procedures had no influence on the health status, behavior or feeding habits of each animal. No implants were lost in the three months after implantation. Although no fractures of Ti implants occurred, one Y-TZP implant and two Ce-TZP/Al2O3 implants fractured above the crestal bone.

The average Ra was 0.11 μm in the Ti group, while the Ra values in the Y-TZP and Ce-TZP/Al2O3 groups were 0.11 μm and 0.09 μm, respectively.

SEM characterization of materials
SEM images of the surface of the implant collar showed almost the same roughness in all three groups, but the surface of Ti and Y-TZP included scratches, and some spots were observed on the Ce-TZP/Al2O3 implant (Fig. 3).

Micro-CT image
Bone resorption of the buccolingual side was notable. On the mesiodistal side, bone resorption was less than that on the buccolingual side and the differences in bone resorption were slight among the groups. In the Ti group, marked bone resorption was rarely observed on the mesiodistal side (Figs. 4a-1, 2). However, bone resorption was observed on the buccolingual side in a few samples. There was a little bone resorption on the mesiodistal and buccolingual sides in many samples in the Y-TZP group, although the surrounding bone sustained its height on the mesiodistal side in a few samples (Figs. 4b-1, 2). In some CT images from the Ce-TZP/Al2O3 group, there was marked bone resorption on the buccolingual side and a little bone resorption on the mesiodistal side (Figs. 4c-1, 2).

Histological description
There were narrow spaces between the implant and surrounding tissue in many samples in all groups. The space looked like an artifact because the interface of tissue corresponded to the implant surface and nothing was observed in these spaces. The bone around the upper area of the implant appeared to be compact bone; on
Fig. 3  SEM image of implant collar: Ti (a), Y-TZP (b) and Ce-TZP/Al₂O₃ (c). These are low-magnification (a-1, b-1, c-1) and high-magnification images (a-2, b-2, c-2).

Fig. 4  Micro-CT image: mesiodistal side of Ti (a-1), Y-TZP (b-1) and Ce-TZP/Al₂O₃ (c-1), buccolingual side of Ti (a-2), Y-TZP (b-2) and Ce-TZP/Al₂O₃ (c-2). The surrounding bone around the Ti implant is not resorbed markedly (a-1, 2). The bone resorption around the Y-TZP implant is observed a little on the mesiodistal (b-1) and buccolingual sides (b-2). The image of Ce-TZP/Al₂O₃ implant shows bone resorption (c-1, 2).
the other hand, cancellous bone mostly surrounded the apical area of the implant. Both of these bones directly contacted implants. Although three samples showed marked bone resorption (one sample in each group), osseointegration was established on at least one wall of the implant, even in these samples (Figs. 5a-1, 5b-1, 5c-1). The new bone was located around the resorbed pre-existing bone (Figs. 5a-3, 5b-3, 5c-3). In one sample of the Ti group, there was marked resorption of the superficial bone (Fig. 5a-1). In only this sample, soft tissue facing the implant was connective tissue, not epithelium (Fig. 5a-2). A sample from the Y-TZP group demonstrated thick epithelium and severe bone resorption (Figs. 5b-1, 2). A thin layer of new bone was located at the interface where osseointegration was not complete (Fig. 5b-3). One sample in the Ce-TZP/Al2O3 group showed marked resorption and numerous inflammatory cells in wide areas (Figs. 5c-1, 2).

**Ti group**

Osseointegration was achieved on the surface of at least one part of all implants. Bone resorption of the area around the implant neck was rare (Fig. 6a-1). Epithelium around the implant continued from the external marginal epithelium, and extended along the longitudinal axis of an implant (Fig. 6a-2). The

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**Fig. 5** Peri-implant tissue of samples with bone resorption: Ti (a), Y-TZP (b) and Ce-TZP/Al2O3 (c).

The bone around the implant is resorbed markedly (a-1, b-1, c-1). Higher magnification of black-boxed and white-boxed area show soft tissue with inflammatory cells (a-2, b-2, c-2) and the new bone formed around the resorbed bone (a-3, b-3, c-3), respectively. White arrowheads indicate newly formed bone. Toluidine blue with fuchsin.

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**Fig. 6** Peri-implant tissue in Ti group.

There is little bone resorption (a-1) with the slender epithelium (a-2; higher magnification of boxed area in a-1). The epithelium around the implant looks similar to junctional epithelium (a-2). The sample shows slight bone resorption (b-1), but has thick epithelium with numerous inflammatory cells (b-2; higher magnification of boxed area in b-1). Fibers in connective tissue run along the implant (c). There is newly formed bone in the superficial bone and around blood vessels (d), with osteoblasts (black arrow) and osteoclasts (white arrow) (e). Toluidine blue with fuchsin.
epithelium in the superficial layer facing the implant resembled the junctional epithelium connected to the enamel (Fig. 6a-2). Fibers of the connective tissue ran along the implant (Fig. 6c). Newly formed bone was seen on part of the superficial bone and the inside of the bone appeared mature (Fig. 6d). There were blood vessels with spindle-shaped osteoblasts and osteoclasts, surrounded by osteoid (Fig. 6e). However, this group included various samples that showed different histological features. Some samples had thick epithelium and the area under the epithelium included numerous inflammatory cells (Figs. 6b-1, 2). Slight resorption of the surrounding bone existed around the implant neck in some samples (Fig. 6b-1).

**Y-TZP Group**

Histological characteristics were not uniform in samples in this group. In many samples, inflammatory cells were present in the upper subepithelium (Figs. 7a-1, 2) and in the tissue under thick epithelium (Figs. 7b-1, 2). In one of these, bone was not resorbed (Fig. 7a-1), but the others showed bone resorption around implant necks (Fig. 7b-1). There was also a sample that showed no inflammation reactions and no bone resorption (data not shown). The epithelium was similar to the tissue around teeth in the other groups (Fig. 7c). Moreover, fibers were seen along the implant (Fig. 7d). Osteoblasts (Fig. 7e-1) and osteoclasts (Fig. 7e-2) were observed on the superficial bone. The bone widely present around the new bone facing the implant appeared mature (Fig. 7f).

**Ce-TZP/Al₂O₃ Group**

Some samples showed bone resorption with inflammation of the epithelium, although it varied from sample to sample. However, a part of the implant was in contact with bone in all samples, including those

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[Fig. 7](#) Peri-implant tissue in Y-TZP group.
There are samples without bone resorption (a-1) and with bone resorption (b-1) in this group. Inflammatory cells are present in connective tissue on enlarged views (a-2, b-2) of the boxed areas in a-1 and b-1. The epithelium around the implant appears similar to the tissue around teeth (c). Fibers run along the implant (d). There are osteoblasts (black arrow) and osteoclasts (white arrow) on the superficial bone (e-1, 2). Blood vessels in new bone (white arrowheads) are seen around the implant (f). Toluidine blue with fuchsin.

[Fig. 8](#) Peri-implant tissue in Ce-TZP/Al₂O₃ group.
There is bone resorption around the implant neck (a-1). This sample has inflammatory cells (a-2) in the boxed area in a-1. The sample with thin epithelium does not show bone resorption (b-1). The appearance of thin epithelium around the implant is similar to that around teeth (b-2) in the boxed area in b-1. Fibers around the implant run along the implant (c). New bone is located on the superficial bone with (d-1) and without bone resorption (d-2), and around blood vessels (white arrowheads) facing the implant (e). Toluidine blue with fuchsin.
with resorption (Figs. 8a-1, 2). On the other hand, there were samples with long slender epithelium and no bone resorption in this group (Fig. 8b-1). The thin epithelium that extended down was similar to that around teeth (Fig. 8d-2). Fibers of connective tissue ran along the implant (Fig. 8c). New bone was located on the superficial bone, both with (Fig. 8d-1) and without bone resorption (Fig. 8d-2). The inside bone further from the bone-implant interface showed the appearance of compact bone in all samples (Fig. 8e). Blood vessels were observed around the implant, similar to the other groups (Fig. 8e).

**Inflammatory cell in soft tissue**

There were inflammatory cells in subepithelial connective tissue in all groups (Figs. 9a-1, 9b-1, 9c-1). Abundant plasma cells were included in these inflammatory cells (Figs. 9a-2, 9b-2, 9c-2). Differences in histological findings were not clearly confirmed between groups as there were variations in samples in each group.

**Histometric analysis**

A total of 15 samples were used for measurements, excluding fractured implants; 6 in the Ti group, 5 in the Y-TZP group and 4 in the Ce-TZP/Al₂O₃ group. The mean values for histometric measurements are summarized in Table 2 and Fig. 10. ETL in the Ti group was the shortest, and ETL in the Ce-TZP/Al₂O₃ group was the longest. For CTC, the Ti group had the highest value and the Ce-TZP/Al₂O₃ group had the lowest value. BW, which is the sum of CTC and ETL, was approximately 3 mm in all groups. Therefore, ETL showed the opposite tendency of CTC; ETL was longer as CTC was shorter. The mean values for BR were lowest in the Y-TZP group and highest in the Ce-TZP/Al₂O₃ group, respectively. BIC reached almost 60% in all groups. On one-way ANOVA test, no significant differences were seen among the groups (p>0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>ETL (mm)</th>
<th>CTC (mm)</th>
<th>BW (mm)</th>
<th>BR (mm)</th>
<th>BIC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>1.88</td>
<td>1.23</td>
<td>3.11</td>
<td>0.38</td>
<td>68.9</td>
</tr>
<tr>
<td>Y-TZP</td>
<td>2.19</td>
<td>1.09</td>
<td>3.28</td>
<td>0.21</td>
<td>62.7</td>
</tr>
<tr>
<td>Ce-TZP/Al₂O₃</td>
<td>2.29</td>
<td>0.49</td>
<td>2.78</td>
<td>0.67</td>
<td>58.7</td>
</tr>
</tbody>
</table>

Fig. 9  Inflammatory cells in peri-implant soft tissue of Ti (a-1, 2), Y-TZP (b-1, 2), and Ce-TZP/Al₂O₃ (c-1, 2).

There are numerous inflammatory cells in all groups (a-1, b-1, c-1), and plasma cells with cartwheel nuclei are seen in higher magnification images of the boxed areas (a-2, b-2, c-2). H-E staining.
DISCUSSION

The present study demonstrated the detailed response of hard and soft tissues around implants. We showed that Ce-TZP/Al2O3 implants can be used in nearly the same way as Ti and Y-TZP implants, as the tissue surrounding Ce-TZP/Al2O3 implants exhibited similar reactions. Significant differences among the three groups were not observed with regard to any histomorphometric parameters. The results for BIC reached nearly 60% in all groups. A previous study reported no significant differences in BIC between Ti and Y-TZP24. Our results in the present study were consistent with previous studies and provide new information showing that Ce-TZP/Al2O3 implants are comparable to Ti and Y-TZP implants. Histological observations were also similar in all groups, although samples within each group had large inter-individual variations. For example, mature bone was present in the surrounding bone in all groups. Soft tissue attached to Ti implants was reported to be long zones of epithelial and connective tissues25,26. Another report showed that the peri-implant soft tissue of Ti implants and the gingival tissue around a tooth appeared similar27. Furthermore, in all groups, BW was approximately 3 mm, in accordance with the result of Ti showed by Cochran et al.28 and Abrahamsson et al.29. There were the present findings in all groups agreed with these reports. Combined with the clinical record of Ti and the resemblance among the tissues around the three different materials, all three materials appear to be useful for dental implants.

There are some advantages of Ce-TZP/Al2O3. It showed high fracture toughness when compared with Y-TZP30. Ce-TZP/Al2O3 is resistant to low-temperature aging degradation31,32, while Y-TZP shows low-temperature degradation. In addition, there were no significant differences between Y-TZP and Ce-TZP/Al2O3 with regard to attachment of osteoblasts-like cells in a previous study33. Other studies showed superior characteristics for Ce-TZP/Al2O3 when compared with Ti and Y-TZP among common materials for implant body and abutment32,34. Regardless of the above advantages, the Ce-TZP/Al2O3 group had samples with bone resorption in this study. The method and design of implants may have not been appropriate in the present study, which was the first animal experiment to use Ce-TZP/Al2O3 implants. The design of narrow implants is probably one reason why some Ce-TZP/Al2O3 implants were broken. In addition, the surface roughness of materials did not differ markedly, but the surface would also change the response of the surrounding tissue. Considering that the other groups also had samples with bone resorption and all groups showed variations between samples, it is possible that incomplete plaque...
control influenced the inflammation in soft tissue and bone resorption. However, our results and the general advantages of Ce-TiZP/Al₂O₃ indicated that it is useful as an implant material.

Another notable finding is related to the interaction between soft and hard tissues around the implant. A previous study showed that epithelial proliferation occurred in some areas of the ridge that extended deeply into the lamina propria when clinical signs of chronic inflammation appeared in the gingiva⁴⁹, thus, inflammation must affect morphological changes in soft tissue. When observing marked bone resorption, samples showed numerous inflammatory cells in soft tissue and/or unusual forms of epithelium. Samples with few inflammatory cells showed little bone resorption, and most of these had slender epithelium. With regard to the relationship between bone and soft tissue around implants, Lindhe et al. reported inflammatory reactions in the form of recession of gingival tissue and alveolar bone.⁵⁰ On the other hand, bone from several samples did not show resorption, despite the presence of inflammatory cells in the surrounding soft tissue. Thus, inflammation alone might not be responsible. However, inflammation and the type of epithelium are thought to be factors closely related to bone resorption. Moreover, the condition of tissue around the implant would alter the characteristics of connective tissue. A 2 mm connective tissue band was reported to attach tightly to the abutment surface and act as a resistant barrier.⁷⁷,⁷⁸ Although information on the function of this zone where connective tissue contacts adjacent to implants was scarce in the present study, it suggests the importance of connective tissue. The connective tissue displayed numerous fibers along the implant in many samples, and was shorter than the epithelium. Particularly in the Ce-TiZP/Al₂O₃ group, connective tissue was much shorter than the epithelium. The mean value for bone resorption in Ce-TiZP/Al₂O₃ samples was highest among all groups; therefore, we considered that connective tissue helps to prevent bone resorption. This notion appears to be supported by the observation of inflammatory cells and bone resorption in some samples with short connective tissue. It has been reported that adhesion of connective tissue to implants would prevent epithelial apical migration, implying that connective tissue plays numerous roles related to the condition around the implant. Taken together with previous results, our data suggested that connective tissue is as important as epithelium, and that insufficient connective tissue is not preferable. The association between soft and hard tissues in this study was demonstrated to be important for maintenance after implantation. However, the number of samples in our study was not sufficient and detailed information on the interaction between tissues is lacking. Further studies on the soft and hard tissues around these materials are considered necessary in order to provide insights into clinical applications.

CONCLUSION

This was the first study to assess Ce-TiZP/Al₂O₃ implants in a dog model. Within the limitations of this study, the tested materials showed no significant histological differences. The results suggested that Ce-TiZP/Al₂O₃ implants are comparable to titanium and Y-TiZP implants. Further studies on these materials are necessary in order to provide insights into clinical applications.

ACKNOWLEDGMENTS

This study was supported by Nanto. The authors would like to thank Dr. Sumio YOSHIE of the Department of Histology, The Nippon Dental University School of Life Dentistry at Niigata for advice regarding the study. The authors would also like to thank Dr. Hidekazu AOYAGI of the Advanced Research Center, The Nippon Dental University School of Life Dentistry at Niigata for his support and for use of the micro-CT device. The authors are grateful to all the staff of the Department of Crown and Bridge Prosthodontics, The Nippon Dental University School of Life Dentistry at Niigata for their cooperation.

REFERENCES


