The objective of the present study was to assess hard and soft tissue around dental implants made of three different materials with microgrooves on the collar surface. Microgrooved implants were inserted in the mandibles of five male beagles. Implants were made of three kinds of material: titanium (Ti), yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) and ceria partially stabilized zirconia/alumina nanocomposite (Ce-TZP/Al2O3). The animals were euthanatized at three months after implantation, and harvested tissue was analyzed by means of histology. All kinds of implant were osseointegrated, and there were no significant differences in any histomorphometric parameters among the three groups of microgrooved implants made of different materials. Within the limitations of this study, implants with microgrooves integrated into the surrounding bone tissue, without statistically significant differences among the three tested materials, Ti, Y-TZP, and Ce-TZP/Al2O3.

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

**INTRODUCTION**

The success of dental implants depends on various factors. The surface type is listed as a factor to affect healing after implantation. It was presumed that the coronal bone and connective tissue attached to a greater degree on concave-machined implants than on straight-machined implants in the early stages after implantation. Microgrooves on the implant collar part and abutment have recently been the focus for superior effects on the surrounding tissue. Microgrooved abutments of titanium (Ti) induce a biological response, such as inhibition of epithelial downgrowth. In addition, Ti implants with a microgrooved abutment enhance subepithelial connective tissue attachment and maintain crestal bone levels. It was also reported that the mean plaque index values of a Ti implant with a laser-microtextured collar and a Ti implant with machined collar did not show any significant differences at 6 or 12 months after implantation; therefore, the presence of a microgroove does not appear to increase plaque adhesion.

The materials are also considered to influence the condition of peri-implant soft tissue and peri-implant bone resorption; for example, abutments made of Ti, ZrO2, and AuPt-alloy were reported to have differences concerning these points. Osseointegration of zirconia that is used as a substitute for Ti, appeared to be similar to that of Ti in various animal studies. Zirconia Oxide surfaces significantly reduce bacterial adhesion when compared with Ti, which is important for the health of peri-implant soft tissues. Another study using implants described the similarities between yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) and Ti with regard to soft tissue integration in addition to osseointegration. There were also no marked differences between Y-TZP and Ti in the health of mucosa around implants and abutments. Furthermore, ceria partially stabilized zirconia/alumina nanocomposite (Ce-TZP/Al2O3) was developed recently, and it has greater fracture toughness and greater cyclic fatigue strength than Y-TZP. It is therefore anticipated that Ce-TZP/Al2O3 will lead to the clinical application of zirconia; for instance, dental implants and implant abutment. We previously discussed the peri-implant tissue around the one-piece implants, of which the collar part was a machined surface, made of Ti, Y-TZP and Ce-TZP/Al2O3. Implants without grooves using these materials demonstrated no significant differences histomorphometrically. We revealed that Ce-TZP/Al2O3 implants are potentially useful for clinical application.

Despite improvements in the surface style and material, we found no studies to evaluate the tissue response to zirconia implants with microgrooves on part of the implant collar. In particular, microgrooves on Ce-TZP/Al2O3 implants have never been investigated. Our hypothesis was that zirconia microgrooved implants would be comparable or superior to Ti microgrooved implants due to combined advantages of microgrooves.
and the good biocompatibility and esthetics of zirconia. The aim of this study was to assess hard and soft tissue responses around one-piece implants with microgrooves on their collar surface using three different materials in a dog model.

MATERIALS AND METHODS

Animals
Five male beagles aged approximately 24 months and weighting 11–14 kg, received standard feed and water ad libitum. Their behavior, posture, reactivity and appearance were monitored throughout the entire treatment period. The present study was approved by the Animal Experiment Ethics Committee of The Nippon Dental University School of Life Dentistry at Niigata (No. 2013-156).

Implant design
The dental implants comprised one-piece (3.0 mm in diameter, and 13.3 mm in length; 7.5 mm in length and 2.7 mm in width for implant body) and the collar surfaces (1.8 mm in length) were microgrooved by laser (width: 30 μm, depth: 5 μm), which were processed by Nanto (Shizuoka, Japan) (Table 1). All parts, except for the collar, were made in the same way as the previous paper14. We established three groups of 6 implants each according to implant materials: Ti implants with microgrooved collar (Ti-g); Y-TZP implants with microgrooved collar (Y-TZP-g); and Ce-TZP/Al2O3 implants with microgrooved collar (Ce-TZP/Al2O3-g). The width and depth of microgrooves were confirmed using a measurement device (VK Viewer and VK Analyzer, KEYENCE, Osaka, Japan). Subsequently, we performed observations of the microgrooved surface by scanning electron microscopy (SEM; JSM-6101LA, JEOL, Tokyo, Japan) and measurement of surface roughness (Ra) of implant collars using a laser microscope (VK-X100, KEYENCE).

Surgical procedures
Throughout the present study, all surgical procedures were performed as described previously14. Animals were anesthetized with ketamine (i.m. 10 mg/kg, Ketalar®; Daiichi Sankyo, Tokyo, Japan) and xylazine (i.m. 3 mg/kg, Selactar®; Bayer Yakuhin, Tokyo, Japan). Local anesthetics (2% lidocaine containing 1: 80,000 epinephrine, Ora®; Showa Yakuhin Kako, Tokyo, Japan) were injected for intra-operative analgesia. We administered appropriate therapy if the behavior of animals indicated any adverse reactions or pain. All four premolars and first molars were extracted bilaterally from the mandible. After three months, a surgeon with sufficient experience inserted three types of implant with microgrooves in the present study and the implants without grooves in the previous study14, and these implants were inserted at different positions of the mandible in rotation to prevent occurrence of unevenness in implant location by the implant type. Surgical stents made from models before the surgery were used to determine the detailed position of implants. The three or four implants on each side were placed 15 mm apart at a torque of 30 Ncm bilaterally, so that the bottom of the implant collar was located at the level of the crest of alveolar bone (Fig. 1). Implants were placed in order to avoid contact with opposing teeth, and then flaps were sutured using 5-0 monory. Immediately after the surgery, antibiotic (Convenia®, Zoetis Japan, Tokyo, Japan; s.c. 8 mg/kg) was injected into all dogs, which is effective for 14 days, in order to prevent infection. Soft dog food was provided for one week after implant placement.

Sample preparation
After three months, dogs were euthanatized by intravenous overdose infusion of thiopental sodium (Ravonal®, Mitsubishi Tanabe Pharma, Osaka, Japan).

![Fig. 1 Intraoperative view of treated area.](image)

Implants made of three different materials were placed in the mandible (a). Microgrooved collars (asterisks) on a Ti-g (b-1), Y-TZP-g (b-2) and Ce-TZP/Al2O3-g (b-3) are located above the alveolar ridge.

### Table 1 Implants with microgroove used in this study

<table>
<thead>
<tr>
<th>Implant</th>
<th>Product name (Manufacturer)</th>
<th>Composition</th>
</tr>
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<tbody>
<tr>
<td>Ti-g</td>
<td>ASTM F67 Grade4 (Allegheny Technologies Japan)</td>
<td>CP-Ti</td>
</tr>
<tr>
<td>Y-TZP-g</td>
<td>TZ-3YS-E (Tosoh)</td>
<td>3 mol Y2O3-ZrO2</td>
</tr>
<tr>
<td>Ce-TZP/Al2O3-g</td>
<td>P-NanoZR (Panasonic Health Care)</td>
<td>10 mol CeO2-ZrO2, 30 vol% Al2O3</td>
</tr>
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</table>
under systemic ketamine and xylazine. Mandibles were harvested and divided into blocks for each implant. Blocks were fixed in 10% neutral formalin for two weeks. Soft tissue on either the mesial or distal side of the implant was randomly removed for paraffin sections.

**Micro-CT**
Images of the bone around the implant were obtained using a micro-CT system (SMX-100CT-ST, Shimadzu, Kyoto, Japan) followed by histological processes, and were reconstructed using 3D structural analysis software (MPR, Shimadzu).

**Histological analysis**
Whole samples except for soft tissue removed for paraffin sections were embedded in methylmethacrylate and were then cut in the mesiodistal direction at 800-μm thickness using a slow-speed diamond saw (VC-50, Leco, St. Joseph, MI, USA). Sections were polished to about 400 μm using a polisher (LaboPol-21, Struers, Ballerup, Denmark), and were superficially stained with toluidine blue combined with basic fuchsin. The most central sections were observed and photographed under a light microscope equipped with a digital imaging system (Axio Observer.Z1/Axiocam 506 color, Carl Zeiss Microscopy, Jena, Germany). Histomorphometry was performed based on the following landmarks (Fig. 2): marginal portion of mucosa (PM), apical portion of the junctional epithelium (aJE), implant shoulder (IS) as the bottom part of the collar that was the level of the alveolar bone crest when the implant was placed, and first bone-implant contact (fBIC). We performed linear measurement around the implant as follows: PM-aJE (i.e., epithelial tissue length, ETL), aJE-fBIC (i.e., connective tissue length, CTL), PM-fBIC (i.e., soft tissue length as the sum of ETL and CTL, STL) and IS-fBIC (i.e., bone resorption, BR). For BR, positive values were set when the fBIC was located more apically than the IS. The percentage of bone-implant contact (BIC) was measured on the implant surface from fBIC to last bone-implant contact, as determined for bone deposition between first and last bone-implant contact, excluding the area related to bone resorption and apical area without bone tissue. The opposite side of soft tissue removed for paraffin sections was applied for parameters relating to soft tissue; on the other hand, BR and BIC were measured on both sides of the implant, and the mean of the values for both sides was calculated as each sample value.

Soft tissue removed from fixed samples was embedded in paraffin. Paraffin blocks were cut at 4.5–5.0-μm thickness and sections were stained with hematoxylin and eosin (H-E). Stained sections were observed and photographed using a digital camera connected to a microscope (Axioplan, Carl Zeiss Microscopy).

**Statistical analysis**
Mean and standard deviation were calculated. Differences among the three groups were assessed by one-way ANOVA following the test for homogeneity of variance. Values of p<0.05 were considered to be significant differences.

**RESULTS**
The postoperative process was universally uneventful in all animals. During the experimental procedures, neither allergic reactions nor infections were noted. No implants were lost throughout the healing period after implantation. Although there were no fractures of Ti-g and Ce-TZP/Al2O3-g, two Y-TZP-g fractures above the crestal bone were excluded from observation. Ra values of microgrooved collar of the Ti-g, Y-TZP-g and Ce-TZP/Al2O3-g were 0.40, 0.74 and 0.42 μm, respectively.

**SEM imaging**
SEM images of the implant collar part differed among the three groups (Fig. 3). The microgrooved surface on Ti looked regular. On the other hand, the high magnification image of the microgroove applied on Y-TZP appeared irregular, and Ce-TZP/Al2O3 indicated an uneven surface with cracks.

**Micro-CT imaging**
In most samples of the Ti-g group, there was slight bone resorption around the implant (Figs. 4a-1, 2). The Y-TZP-g group indicated bone resorption to varying degrees from a little resorption around the implant on both the buccolingual and mesiodistal sides (Figs. 4b-1, 2) to notable bone resorption of the buccolingual side. Bone resorption around Ce-TZP/Al2O3-g was seen on both the mesiodistal and buccolingual sides in most samples, and was occasionally comparatively large (Figs. 4c-1, 2).
Bone resorption on the micro-CT images varied among samples within the group.

**Histological observation**

All samples, including those with bone resorption, achieved osseointegration. Bone far from the implant showed the appearance of mature bone. Although each group had a variety of samples with regard to bone resorption, the implant surface above IS was covered with new bone in one and more samples each group. Inflammatory cells existed widely or locally in soft tissue around the implant in most samples in all groups. In soft tissue surrounding several implants, the border between epithelium and connective tissue was unclear. A narrow gap was observed between the implant and surrounding tissue in all groups. Histological structures such as dental calculus were scarcely observed in this space. Within this space there was a little plaque near the top of the epithelium in only a few samples and slight pieces of tissue appeared to be peeled off the
attached tissue, and the inside surface of tissues facing the spaces appeared to correspond the implant surface. In one or two samples from each group, the epithelium covered the majority of the microgrooved surface via the space, often turning the top of the epithelium down. These samples showed advanced bone resorption. The gap below the groove was frequently observed in the Y-TZP-g and Ce-TZP/Al₂O₃-g groups in addition to the above samples with the groove covered with epithelium, which indicated bone resorption.

Histological observation in Ti-g group
Little or no bone resorption was seen in half of the samples (Fig. 5a). The other samples showed resorption; in particular, one of these showed advanced bone resorption extending the epithelium with inflammatory cells along the microgrooves. In most samples, epithelium around the implant was slender (Fig. 5b). The microgrooved part was relatively close or almost adhered to the connective tissue with or without epithelium in all samples except for the notably resorbed sample (Fig. 5c). Newly formed bone was observed in the superficial bone and around the implant (Fig. 5d). Woven bone surrounding blood vessels was present near the implant surface (Fig. 5e), and also sprinkled over the mature bone.

Histological observation in Y-TZP-g group
Samples in the Y-TZP-g group varied markedly. Half of the samples showed remarkable bone resorption around the implant neck (Fig. 6a). In these samples, the epithelium extended to the vicinity of the bottom part of the microgrooves and soft tissue was detached from the surface of microgroove and below (Figs. 6b, c). Even these resorbed samples showed osseointegration, forming new bone on the surface of resorbed bone (Fig. 6d) and around the implant body (Fig. 6e). Conversely, sections of the other samples showed comparatively modest bone resorption and no resorption, despite the presence of inflammatory cells in the soft tissue (Figs. 7a, b). A gap between soft tissue and the implant surface was also observed (Fig. 7c); however, the gap below microgroove was absent and very slight in the sample without bone resorption and that with modest resorption, respectively. New immature bone was recognized near the implant (Fig. 7d) and around vascular canals surrounding the implant (Fig. 7e).

Histological observation in Ce-TZP/Al₂O₃-g group
All samples except for one showed bone resorption in varying degrees, although osseointegration was achieved (Fig. 8a). There were both samples with soft tissues detached from the microgroove (Fig. 8b) and...
Fig. 8 Peri-implant tissue around Ce-TZP/Al₂O₃-g with bone resorption.
There is marked bone resorption (a). The epithelium extends to the microgrooved collar and below, which is distant from the surface under the grooves (b, c). New bone (black arrowheads) is formed near the implant surface and around the vascular canal (d, e). Toluidine blue with basic fuchsin stain.

Fig. 9 Peri-implant tissue around Ce-TZP/Al₂O₃-g without bone resorption.
There is no bone resorption around the implant (a). Inflammatory cells gather within soft tissue near the implant (b). Very thin space exists between the microgrooved part of the implant and soft tissue (c). New bone (black arrowheads) is located on the upper surface of the surrounding bone (d) and near blood vessels around the implant (e). Toluidine blue with basic fuchsin stain.

 Histological observation of inflammatory cells in soft tissue on paraffin sections
In all groups, histological differences were not specifically observed (Figs. 10a-1, b-1, c-1). Inflammatory cells, including plasma cells, were noted in connective tissue (Figs. 10a-2, b-2, c-2).

 Histomorphometric analysis
Sixteen samples were used for measurements; six samples each from the Ti-g and Ce-TZP/Al₂O₃-g group, and four samples from the Y-TZP-g group excluding the fractured implants. Mean values for histomorphometric measurements are shown in Table 2. The mean value of all measurements related to the soft tissue (ETL, CTL and STL) and BIC was the largest in Ce-TZP/Al₂O₃-g, followed by Ti-g and Y-TZP-g. The mean BIC in all groups was more than 60%. For mean values of BR, the Ti-g and the Ce-TZP/Al₂O₃-g groups showed the lowest and highest values, respectively. On one-way ANOVA, there were no significant differences among the three groups for all parameters (p>0.05).

DISCUSSION
We investigated the detailed responses of hard and soft tissues around implants with microgrooves on the implant collar. To our knowledge, this is the first study to simultaneously compare microgrooved implants made of Ti, Y-TZP and Ce-TZP/Al₂O₃.

Microgrooves were recognized as being effective for Ti abutment2,3). The microgrooves added in the intraosseous portion of sandblasted zirconia implants were reported to increase BIC and peripheral bone density when compared with sandblasted and acid-etched Ti implants and sandblasted zirconia implants19). Resulting in the present analysis, there were no significant differences among the three groups with regard to histomorphometric parameters. The present
Table 2 The measurement results of epithelial tissue length (ETL), connective tissue length (CTL), soft tissue length (STL), bone resorption (BR) and bone-implant contact (BIC) in three groups

<table>
<thead>
<tr>
<th>Histomorphometric parameters</th>
<th>ETL (mm)</th>
<th>CTL (mm)</th>
<th>STL (mm)</th>
<th>BR (mm)</th>
<th>BIC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Ti-g</td>
<td>2.04±0.42</td>
<td>1.34±0.75</td>
<td>3.37±0.56</td>
<td>0.27±0.34</td>
</tr>
<tr>
<td></td>
<td>Y-TZP-g</td>
<td>1.95±0.59</td>
<td>0.77±0.33</td>
<td>2.73±0.68</td>
<td>0.34±0.58</td>
</tr>
<tr>
<td></td>
<td>Ce-TZP/Al₂O₃-g</td>
<td>2.14±0.45</td>
<td>1.38±0.98</td>
<td>3.52±1.09</td>
<td>0.56±0.49</td>
</tr>
</tbody>
</table>

All values are expressed as means±SD.

Fig. 10 Inflammatory cells in soft tissue around Ti-g (a-1, 2), Y-TZP-g (b-1, 2), and Ce-TZP/Al₂O₃-g (c-1, 2). There are inflammatory cells in samples from all groups (a-1, b-1, c-1). Black boxed areas in the upper figures are enlarged in the lower figures (a-2, b-2, c-2). Inflammatory cells such as plasma cells with cartwheel nuclei are observed in higher magnification images. H-E staining.

The study also gave fairly favorable results, such as the establishment of osseointegration in all samples and new bone deposition on the implant surface above IS in at least one or more implants in all groups, whereas there were individual differences among the samples within each group. Considering that the mean values of BIC in the present study were comparatively high (more than 60%) in all groups, microgrooves on the collar part may be equally efficient for dental implants of all three materials, including Ce-TZP/Al₂O₃.

The lack of significant differences might have been due to the diversity of samples, as well as the small sample size. This diversity may be influenced by conditions in the experiment; for example, bone conditions such as the combination of bone quality and jawbone shape, or bone quality and bone quantity, as well as surgical parameters related to surgeons such as technique, skill and judgement, are likely to have an impact on implant failure. Despite every effort made to standardize surgery and analysis (i.e., surgery by an experienced surgeon, locations alternated between different types of implant, stents for accuracy, and measurement using standard points as shown in Fig. 2), samples may not have been in equivalent situations in the present study. In particular, 3-dimentional unification was difficult. With regard to analysis, to supplement the limitation of our two-dimensional methods, which were histological observation and measurement using stained sections, the addition of micro-CT analysis that has the advantage of providing 3-dimensional information may have yielded new findings. Previous papers have reported some differences with similar trends between data from histomorphometry and micro-CT; these methods are complementary and when performed in tandem, can overcome their respective drawbacks. The lack of significant differences in the present study could therefore have been changed by using more samples, more precise experimental techniques and/or additional analyses.

The discrepancy between the results of the present study and those of a previous study using three types of implant without microgrooves made of the same materials seems to support the notion that the microgrooves have some effect on the surrounding tissue. Y-TZP-g in the present study showed greater BR and lower BIC values than Y-TZP implants without microgrooves in our previous study; on the other hand, Ce-TZP/Al₂O₃-g was better for BR and BIC in the
present study. Considering the present results, the ideal microgroove pattern still needs to be identified for each material. We adopted a wider microgroove, referring to papers on zirconia implant bodies\(^{39}\) and on Ti plate\(^{27}\), when compared to grooves in histological studies using Ti implants\(^{2,4,29}\) because of the difficulty in processing zirconia implants. Ce-TZP/Al\(_2\)O\(_3\)-g not Y-TZP, appeared to be suitable for this type of groove. In addition, according to papers using Ti that showed adhesion of bacteria to be related to surface roughness\(^{29}\) and surface modifications\(^{30}\), we speculate that surface texture, including roughness, changes the effect of microgrooves on the surrounding tissue.

The present findings did not clearly reveal the histological characteristics in each group because of the large differences within the groups. However, the bone resorption in the samples with epithelium extending to the bottom of the microgrooves in this study is indicative of the importance of preventing and inhibiting epithelial downgrowth\(^2-4\). A human histologic study showed high BIC in the Ti implant with a microgrooved collar covered in connective tissue\(^6\). It was thought that the location of epithelial and connective tissue, as well as balance and length of them, might affect results. In addition, papers on the tissue around implants without microgrooves have indicated that bone resorption may be related to tissue reactions initiated to establish a proper length of BW of the mucosal-implant barrier\(^{31-33}\). The observation that the Ce-TZP/Al\(_2\)O\(_3\)-g group showed the largest values for both STL and BR appears to confirm the relationship between bone resorption and soft tissue.

Moreover, observation of the surrounding soft tissue and the gap between soft tissue and the implant surface may provide some insights. Soft tissue was often distant from even microgrooved surface in the present study, although the groove was reported to induce fibroblasts\(^{27,34}\). Based on the lack of histological structures within the space and the form of soft tissue facing implants, we believe that part of the surrounding tissue was mostly attached to the implant surface. While we regard this gap as an artifact, we speculate that the gap reflects the strength of soft tissue attachment. As the requirement of certain degree of mucosal attachment was suggested\(^{35}\), the strength of soft tissue contact might also have influenced the peri-implant condition as shown by the present results such as bone resorption under the detached soft tissue below the microgrooves. Previous papers demonstrated that the Ti microgrooved abutments gained a connective tissue attachment and retained peri-implant bone levels\(^{2,3}\), and that microgrooved implants may provide more favorable conditions for the attachment of soft and hard tissues\(^{35}\). We assumed that adding a microgroove to the implant collar potentially maintains the condition of soft and hard tissues. Furuhashi et al. showed that greater fibroblast density increased on the microgroove surface when compared with a machined or rough surface\(^{27}\).

In another research, the biological response of human gingival fibroblasts to Y-TZP with microgrooves was comparable to that of Ti in short-term periods\(^{34}\). As soft tissue appeared to be in contact with microgrooves on the Ti-g and Ce-TZP/Al\(_2\)O\(_3\)-g in a few present sections, the shapes of the microgrooves used in this study appeared to suit Ti and Ce-TZP/Al\(_2\)O\(_3\).

On the other hand, the surface other than the grooves on Ce-TZP/Al\(_2\)O\(_3\)-g was mostly separate from the surrounding soft tissue. The roughness characteristics of zirconia and Ti were recognized to change the morphology and proliferation of human gingival fibroblasts, as well as the expression of integrins and collagens\(^{40}\). Osteoblasts attach to Y-TZP and Ce-TZP/Al\(_2\)O\(_3\), and the rough surface on both materials appeared to be able to induce initial cells similarly to Ti\(^{27}\). We should improve the entire surface of the implant in addition to the form, width and location of microgrooves. Since compatibility between the material and implant surface must influence attachment to the peri-implant soft tissue, further investigation of the processing of the implant surface including grooves and their properties is necessary for all materials, particularly zirconia.

**CONCLUSIONS**

To our knowledge, this was the first report on simultaneous comparisons among microgrooved implants using Ti, Y-TZP and Ce-TZP/Al\(_2\)O\(_3\) in a dog model. In the present study, various degrees of bone resorption around the implants and soft tissue with inflammatory cells were observed in all groups. In addition, soft tissue frequently detached from the implant surface, including microgrooves, on sections; however, the bone tissue made contact with all implants. Within the limitations of this study, the microgrooved implants made of the present materials integrated into surrounding bone tissue, without statistically significant differences. Additional studies using various analyses are therefore needed in order to allow microgroove application to further improve the condition of soft and hard tissues around implants made from various materials.

**ACKNOWLEDGMENT**

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