Effect of titanium or zirconia implant abutments on epithelial attachments after ultrasonic cleaning

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Abstract: Zirconia is widely employed as a material during dental implant work because of its superior esthetics. This study sought to evaluate the impact of titanium or zirconia implant abutments on epithelial attachments after ultrasonic cleaning. These implants were inserted into the extraction socket of rat maxillary first molars. Then, the length of the horseradish peroxidase (HRP) reaction was measured. In addition, titanium and zirconia disks were cleaned using an ultrasonic scaler, surface morphology changes were observed, and the number of epithelial cell attachments to the surface was measured. Ultimately, the surfaces of the titanium disks were easier to damage than those of the zirconia ones. There was no difference in the number of epithelial cell attachments between the two materials with the ultrasonic cleaning. The length of the HRP reaction was shorter on the zirconia implant abutment surface than on the titanium one after mechanical cleaning. In conclusion, zirconia is harder than titanium and a better choice for use in the epithelial tissue attachment. Zirconia is more suitable as a material for implant abutments than titanium.

Keywords: epithelial cell, epithelial tissue, implant surface, laminin-332, titanium, zirconia

Introduction

Dental implants can greatly improve patients’ quality of life and continue to be more widely adopted in dental treatment. However, the success of oral implants requires not only strong osseointegration but also a tight seal between the implant itself and the soft tissue surrounding it [1, 2]. Like natural teeth, the presence of a strong soft-tissue attachment is the first barrier against invasion by foreign bacteria [3, 4]. The degree of soft-tissue attachment around the implant depends on the attachment of the epithelial tissue to the dental material. Therefore, implant abutment materials must have good biocompatibility to ensure optimal outcomes. Plaque may cause implant failure [5], and plaque build-up begins with the implant abutment. Therefore, daily maintenance of the implant abutment is very important.

Two materials of the implant abutment exist at this time: titanium and zirconia. Specially, zirconia has the high strength and white color like teeth. Given the patients’ increasing desire for better aesthetic results, zirconia abutment has been more widely used in clinical treatment. Many studies have shown that during the removal of plaque from the surface of the implant abutment, ultrasonic scaling may increase the degree of surface roughness and provoke harmful changes in the neck or abutment of implants [6-8]. However, few studies to date have assessed the effects of this treatment on the surface of zirconia. In addition, although many studies have compared titanium and zirconia implant abutments [9-11], there has been little research published concerning the attachments of the two materials to epithelial tissue after plaque removal.

During implant placement, the epithelium around the implant first forms a basal lamina on the surface of the implant, and the epithelial cells then attach to the substrate through hemidesmosomes. During this process, epithelial cells produce laminin, which enhances the degree of adhesion between epithelial cells and the various layers of molecules within the basal lamina [12]. The detection of laminin-332 (Ln332) can be used as a marker for evaluating the strength of the attachment of the implant to the surrounding epithelial tissue.

In the present study, changes in the surface of titanium and zirconia materials after cleaning using an ultrasonic scaler were evaluated by scanning electron microscopy (SEM) and roughness (Ra) values. The rat model is an important tool for exploring the pathogenesis of bacterial infections [13]. Here, the rat model was adopted to study foreign-body penetration from the oral cavity to the gingival tissue to verify the sealing ability of the tissue surrounding the implant. The impacts of titanium and zirconia on oral epithelial cell attachment following cleaning by ultrasonic scaling were also evaluated.

Thus, the primary objective of this study is to evaluate the effects of titanium and zirconia materials on the sealing of epithelial tissue around dental implants after mechanical cleaning using an ultrasonic scaler.

Materials and Methods

In vitro experimental materials and tools

The experimental group consisted of six titanium (US-Ti, Skyblue, Fukuoka, Japan) (15 mm in diameter and 1 mm in thickness, 99.9% Ti; TiCP ASTM F67 Grade2) and six zirconia (US-Zr, Skyblue) (15 mm in diameter and 1 mm in thickness) disks cleaned for 1 min using an ultrasonic scaler (Various 750; NSK, Kanama, Japan). The control group consisted of six titanium (C-Ti) and six zirconia (C-Zr) disks that had not been cleaned (Fig. 1A).

Surface morphology analysis

All disks were examined by SEM (S-4800; Hitachi, Tokyo, Japan), and screenshots were saved at a magnification of ×500. The surface Ra (µm) values were measured using a roughness analyzer (Surftest 401 Analyzer Series 200; Mitutoyo, Kawasaki, Japan) (Fig. 2).

Cell culture

Oral epithelial cells (OECs) were prepared according to the methods described in previous report [14]. Briefly, OECs were derived from 4-day-old Wistar rats (weighing 100-120 g). After measuring the number of cells, 1 mL of culture medium containing OECs was added to each dish containing the titanium and zirconia disks. Cell attachment was analyzed 3 days after inoculation (Fig. 3A).

Immunofluorescence staining of adhesion protein

Immunofluorescence staining of adhesion protein was performed as described in a previous research [15]. Briefly, actin was stained for 1 h with tetramethylrhodamine isothiocyanate-conjugated phalloidin (dilution ratio of 1:100; Sigma-Aldrich, St. Louis, MO, USA) at 37°C. Nuclei were stained with Vectashield (Vector Laboratories, Burlingame, CA, USA). Then, stained cells were observed using a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan).

Attachment analysis

Fluorescence microscopy was adopted to scan the panorama of titanium and zirconia disks, and the ImageJ software program (National Institutes of Health) was used to determine the number of actin-positive cells on the abutment surfaces.
of Health, Bethesda, MD, USA) was chosen to count the number of cells.

In vivo implants

The in vivo experiments involved two screw-type implants (Skyblue): a titanium implant and a titanium implant with a zirconia abutment (Fig. 1B). Animals were treated in accordance with the animal care guidelines established by Kyushu University (A29-227-0). The details of the surgical implantation are described in a previous research [16]. Briefly, this experiment involved five-week-old male Wistar rats weighing 120-150 g (n = 6 for each group). After anesthesia (0.15 mg/kg of medetomidine, 2.0 mg/kg of midazolam, and 2.5 mg/kg of butorphanol) administration, the right maxillary first molar was extracted from each rat. The implant was placed in the cavity one week after extraction (Fig. 4A).

Topical application of horseradish peroxidase (HRP)

Rats were sacrificed according to the methods described in a previous research [17]. HRP solution was added around the experimental implants every 5 min for 1 h before the rats were killed (Fig. 4B). The implants were removed, and 10-µm-thick sections were cut using a cryostat (Leica CM1860; Leica Camera, Wetzlar, Germany) at −20°C. The sections were stained with hematoxylin and observed using an optical microscope.

Statistical analysis

Data are expressed as means ± standard deviations. Normality was assessed using Shapiro-Wilk test, and homoscedasticity was assessed using Levene test.
The outcomes of both tests showed that the results of all groups were consistent with the normality ($P > 0.05$) and homogeneity of variance ($P > 0.05$). Then, Scheffé test was used to compare inter-group variables, where $P$-values less than 0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics version 22 software program (IBM, Armonk, NY, USA).

**Results**

**Surface morphology analysis**

As shown in Fig. 2A, under SEM × 500 magnification, the titanium disk was characterized by a straight, regular vertical texture before the surface was cleaned using an ultrasonic scaler. After ultrasonic scaler cleaning; however, the vertical texture on the surface was interrupted by deep grooves. In some areas, the vertical texture had disappeared entirely and was replaced by irregularly shaped scars. Meanwhile, the zirconia disk was initially characterized by crisscrossing lines with clear boundaries before the surface was cleaned using an ultrasonic scaler, and after cleaning, a few irregularly shaped spots were observable in some areas. Although the surface of the two materials were both altered after cleaning using the ultrasonic scaler, the changes in the surface of the zirconia were minimal relative to those in the surface of the titanium. This result also was apparent via the Ra test (Fig. 2B): the Ra value in the C-Ti group was 0.86 ± 0.033 μm, whereas that in the C-Zr group was 0.13 ± 0.025 μm. After treatment, the Ra value in the US-Ti group was 1.84 ± 0.021 μm, whereas that in the US-Zr group was 0.23 ± 0.033 μm. Both of the study materials experienced an increase in the surface roughness after ultrasonic scaler treatment. The Ra value among the four groups was significantly different ($P < 0.05$).

**Immunofluorescence staining of the adhesion protein**

As shown in Fig. 3B, after cleaning using an ultrasonic scaler, the expression of Ln332 and the clear boundaries of actin staining on the titanium or zirconia disks were weakened to varying degrees. In the control group, the titanium showed better continuous boundaries for actin and broader expression of Ln332 relative to the zirconia. However, in the treated group, both materials showed discontinuous boundaries for both Ln332 and actin to a similar degree.

**Cell attachment number**

In the epithelial cell attachment experiments (Fig. 3C), the C-Zr group demonstrated a markedly lower number of attachments than that in the C-Ti group. Further, there was a significant difference between the C-Ti, C-Zr, and US-Ti groups ($P < 0.05$) but no differences between the US-Ti and US-Zr groups ($P > 0.05$).

**HRP histochemistry**

As shown in Fig. 4C, the HRP reaction in the titanium implant with a zirconia abutment showed distinct coloration from the upper to the lower area. In each group, the length of the HRP reaction around the implant in the US-Ti group was the longest. Statistical analysis revealed that there were significant differences between each group ($P < 0.05$), except between the C-Ti and C-Zr groups ($P > 0.05$) (Fig. 4D).

**Discussion**

After the implant is placed, plaque will adhere to the implant abutment during daily use. Plaque may cause implant gingivitis and peri-implantitis, ultimately leading to implant failure. Therefore, regular maintenance is important [5,19,20]. In this experiment, the purpose of cleaning the two materials using an ultrasonic scaler was to simulate the cleaning and maintenance of the implant and abutment in clinical practice. It has been shown in previous research that ultrasonic scalers may cause damage to implant abutments during clinical maintenance [6-8,21,22]. Therefore, the implant abutment material must have a certain degree of hardness to resist external damage and enhance the long-term stability of the implant. These findings indicate that zirconia is harder than titanium, and thus, it performs better than titanium in resisting external damage. This is consistent with the findings of a previous study [23]. However, in this experiment, we limited the cleaning time in 1 min. Other research has suggested that if the surface of a zirconia abutment has been treated by grinding or other means for a long time, the risk of abutment fracture increases [24].

The establishment of a tight seal between the implant and the surrounding epithelial tissue depends upon a close attachment of the epithelial cells to the surface of the material. In the *in vitro* experiment, titanium was better than zirconia in facilitating soft-tissue adhesion before treatment [16], but there was no difference in such after treatment. The same conclusion was be obtained during immunofluorescence staining of adhesion proteins *in vitro*. After treatment, the surface roughness of both materials was increased, leading to a decrease in the adhesion of epithelial cells to the surface [25].

The results of the *in vivo* experiments with HRP better explain the results obtained in the above *in vitro* experiments. The shorter the length of the HRP reaction in the experiment, the more closely the implant was attached to the surrounding epithelial tissue, making the implant better able to resist invasion by foreign bacteria. The HRP reaction on the surface of both the titanium and zirconia implants extended from the upper to the lower area, and there was no difference between the two materials before treatment. However, following treatment, the length of the HRP reaction in correlation with the titanium surface was longer than that seen with the zirconia surface. These findings confirm that, because of its stronger resistance to damage by mechanical cleaning, zirconia seems to be more conducive to soft-tissue attachment than titanium after treatment.

In conclusion, zirconia presents better aesthetic outcomes and is harder than titanium, making it more beneficial than titanium for epithelial tissue-sealing after treatment. Zirconia is more suitable as a material for implant abutments than titanium.

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**Conflict of interest**

The authors declare that no conflict of interest exists in this research.

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