Original

The Effect of Super-Hydrophilic Treatment on Zirconia Implant Osseointegration in Rats

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Abstract: Surface modifications of implants can improve the rate of osseointegration. The aim of this study was to determine the effect of super-hydrophilic modification on tetragonal zirconia polycrystals (TZP) implant surface and its subsequent effect on the rate of osseointegration. The TZP implants were rendered super-hydrophilic by the use of ultraviolet light (UV) or via atmospheric-pressure plasma treatments (PL), on their surface and were compared to control specimen that any surface modification wasn’t performed (NC). According to the surface wettability and x-ray photoelectron spectroscopy (XPS) analysis, the contact angle of water droplets on the surface of UV and PL was 0 degree, and their C1s peak was less than that of NC. The push-in test and histological analysis revealed that the super-hydrophilic modification enhanced the bone-implant integration and the formation of new bone around the TZP implants. Additionally, carbon removal and surface wettability enhancement likely improved the osseointegration rate. The study, therefore, demonstrates the design of future TZP implants, particularly for dental applications.

Key words: Animal study, Osseointegration, Rough surface, Super-hydrophilic treatment, Tetragonal zirconia polycrystals implant

Introduction

Titanium and its alloys are the most frequently used material for bone implants due to their high success rate1–10. However, its dark color starkly contrasts with the light pink color of the gingiva, especially in patients with thin gingiva. Furthermore, the titanium may become visible if the soft tissue recedes. Additionally, some studies have reported hypersensitive reactions to titanium implants11–14. Metal-free implants, such as zirconia, have gained attention for dental applications due to their ability to alleviate some of these challenges. However, titanium implants have a survival rate of 98.8% after ten years, according to previous clinical studies15, whereas zirconia implants have a survival rate of only 77.3% after seven years16. Correspondingly, modifications to the surface of zirconia implants may improve their osseointegration and, consequently, their survival rate.

The implant surface properties are categorized into “surface topography” and “surface physicochemistry”17, both of which are critical for osseointegration. The rough surfaces of current titanium implants have been successfully demonstrated clinically18,19. We previously demonstrated that the micro- and nano-topography of TZP’s rough surface benefit the cellular environment20. Nonetheless, implant treatment times are longer than those for bridges and dentures, owing primarily to slow osseointegration. The present study aims to accelerate the osseointegration rate by modifying the surface physicochemistry of implants via super-hydrophilic modification. The most super-hydrophilic modification is done using the ultraviolet light (UV) and atmospheric-pressure plasma treatment (PL), both of which have shown to be effective for titanium implants21–30. There are, however, only a few reports on the efficacy of super-hydrophilic modification in zirconia implants. Zirconia, particularly tetragonal zirconia polycrystals (TZP), exhibit superior aesthetic, biocompatibility, and mechanical properties31,32, which has resulted in TZP being used as a standard material for dental restorations33,34. These characteristics may compensate for the disadvantages of titanium implants. TZP is more aesthetic and biocompatible than titanium. As a result, TZP implants are an excellent option for patients seeking a metal-free treatment.

Thus, the purpose of this study was to elucidate the effect of super-hydrophilic modification using ultraviolet light and atmospheric-pressure plasma treatment on the creation of micro- and nano-topographies of TZP implant as well as the overall effect on osseointegration rate.

Materials and Methods

Sample preparation

To evaluate the osseointegration rate, Yttria stabilized TZP implants (ZrO2: balanced, Y2O3: 5.16 mass%, Al2O3: 0.250 mass%, Na2O: 0.021 mass%, SiO2: 0.007 mass%, Fe2O3: 0.003 mass%) (TZP, TZ-3YB-E, Tosoh Corp., Tokyo, Japan) of 1 mm diameter and 1.5 mm length were used (Fig. 1). TZP disks measuring 13 mm in diameter and 1 mm in thickness were used to evaluate the surface properties. The TZP implants and disks were sandblasted from a distance of 10 mm with 150 μm alumina particles (White morundum, Showa Denko K.K., Tokyo, Japan) at 0.4 MPa air pressure (330750 Whirlwind, JELENKO, Morrisville, USA). Additionally, they were immersed for 15 min at room temperature in 47% hydrofluoric acid (HF) (NC). All specimens were ultra-
sonically cleaned for 10 min with acetone and distilled water and then sterilized for 15 min at 121°C in an autoclave. All specimens were stored aseptically in desiccators for two weeks before use. The implants were subjected to the physicochemical treatments listed in Table 1. The ultraviolet light treatment (UV) was performed for 1 h using a UV irradiator (PC440, BioForce Nanosciences, Ames, IA, USA). This equipment generates UV radiation with a total power density of 19 mW/cm² and excitation wavelengths of 185 and 254 nm, which correspond to ultraviolet C, and that of 365 nm, which corresponds to ultraviolet A.

The atmospheric-pressure plasma treatment (PL) was carried out for 5 sec at room temperature using a plasma-surface modification apparatus (NJZ-2820, Nagano Japan Radio Co., Ltd., Nagano, Japan). Fig. 2 illustrates a UV Ozone cleaner and a plasma-surface modification apparatus.

**Surface observation**

The surfaces of the TZP discs were observed and reconstructed as three-dimensional (3D) images using an electron beam surface roughness analyzer (ERA-8900FE, Elionix Inc., Tokyo, Japan) at an accelerating voltage of 15 kV.

**Surface wettability**

The surface wettability of NC and super-hydrophilic modified TZP implants were determined by measuring contact angles with a contact angle meter (Phoenix α, Kromtek, Selangor, Malaysia), 3 s after applying a droplet of 4 μl distilled water to 5 samples (Fig. 3). The contact angle $\theta$ was defined as the angle based on the water droplet on the surface of the TZP implant. When the value of $\theta$ reached close to 0 degree, the specimens were defined as super-hydrophilic.

**X-ray photoelectron spectroscopy (XPS) analysis**

XPS was used to determine the composition of the outermost surface of the TZP discs and chemical shift was determined using an X-ray photoelectron spectrometer (AxisUltra, Kratos-Shimadzu, Kanagawa, Japan) equipped with an Al Kα (monochromator) X-ray source operating at 15 kV and 15 mA where the intensity of C for the TZP implants was also evaluated at a depth of 1 nm from the sample surface. The binding energy of each spectrum was calibrated using a C1s of 285.0 eV.

**TZP implant placement**

In this experiment, 12 ten-week-old male Sprague Dawley rats (Japan SLC Inc., Tokyo, Japan) were used. The anesthetic was prepared as a mixture of 3 drugs: MED (Domitor® Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan), MID (Dormicum®, Astellas Pharma Inc., Tokyo, Japan), and BUT (Vetorphale®, Meiji Seika Kaisha, Ltd., Tokyo, Japan) at a clean bench in a sterile manner. The mixed solution is composed of the following; 0.3 mg of MED, 4 mg of MID, and 5 mg of BUT/kg body weight/mouse and added sterilized distilled water to adjust it to an administrative volume of 0.1 ml/10 g body weight/mouse. A TZP im-
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Plant was placed in each of the right and left central femurs by drilling with a 0.8 mm round bur and enlarging it using #ISO 090 and 100 reamers (Fig. 4). Implant placement was limited to one location per femur so that it did not affect other implants. In addition, the implant was placed in the central part of the femur so that it did not penetrate the bone. Two weeks after implantation, the rats were euthanized under deep anesthesia to evaluate the rate of osseointegration. The rats were housed and fed solid food and water until sacrificed. This animal experimental study was approved by Tokyo Dental College Animal Experiment Committee (Permission number: 213304) and carried out according to the Institutional Animal Experiments Guidelines.

Push-in test

Six rats were implanted with each of the modified TZP implants (UV and PL) and NC. One implant was placed in each of the right and left central parts of the femur. The push-in test was used to determine the strength between the bone and the TZP implants\(^7\). The removed femurs were immediately embedded in an autopolymerizing resin after the rats were euthanized. Subsequently, the TZP implants were vertically loaded at a cross-head speed of 1 mm/min using a precision universal testing machine (Autograph AG-1, Shimadzu Corp., Tokyo, Japan) that was equipped with a 100 N load cell and a 0.8 mm diameter stainless steel rod. The push-in test value was determined by measuring the peak of the load-displacement curve.

Histological observation

TZP implants treated with two different approaches (UV and PL) and NC were placed in 6 rats. One implant was placed in each of the right and left central parts of the femur. Removed femurs were fixed in 10% neutral-buffered formalin after the rats were euthanized. Fixed samples were dehydrated using a graded series of ethanol solutions, embedded in photocurable resin, and sliced longitudinally to a final thickness of 100 μm using a cutting unit. They were stained with toluidine blue/pyronine G and observed under a standard microscope (Axiophot 2; Carl Zeiss, Oberkochen, Germany).

Results

Surface observation

Each TZP disc exhibited a nanoscale morphology with a relatively large-waved configuration (Fig. 5). The implants treated with UV and PL exhibited no change in surface topography and had the same topog-
Surface wettability
NC had a contact angle of 126.2° ± 3.9°, whereas UV and PL had a contact angle of 0°, indicating super-hydrophilicity (Figs. 6 and 7).

XPS analysis
The XPS spectrum of the TZP disk used in this study displayed peaks of C1s. The C1s peak at 285 eV for NC, UV and PL were 11,066 cps, 6,150 cps and 5,158 cps, respectively. XPS analysis of NC, UV and PL revealed that ultraviolet light and atmospheric-pressure plasma treatment reduced the C1s peak. The results indicated that carbon contamination was present in atmospherically preserved TZP implants, indicating that UV and PL treatment effectively remove carbon contamination on the surface of TZP (Fig. 8).

Push-in test
The push-in test value of NC, UV and PL were 7.5 ± 1.8 N, 19.3 ± 1.7 N, and 22.5 ± 2.5 N respectively. At two weeks post-implantation, the push-in test value of UV and PL were significantly greater than that of NC (Fig. 9). There was no significant difference between push-in test values of UV and PL.

Histological observation
Histological analysis of the bone surrounding NC, UV and PL were performed. Immunostaining results two weeks after implantation revealed that all TZP implants had osseointegrated and formed new bone. In comparison to NC, more new bone formation was observed around the UV and PL (Fig. 10; An area surrounded by a dotted line indicates new bone formation area).

Statistical analysis
Statistical analysis was performed using a one-way analysis of variance followed by the Bonferroni test. For all statistical analyses, the significance level was set to $p < 0.01$ **.
Discussion

This study explored the effect of super-hydrophilic surface modification on TZP implants and its subsequent effect on the rate of osseointegration in rats. In this study, UV and PL were used. These treatments had no detrimental effect on the TZP implant surface and demonstrated sufficient capacity for carbon removal and super-hydrophilicity for TZP implants. UV and PL TZP implants demonstrated improved osseointegration rate compared to NC.

Super-hydrophilic surface treatments have been used to improve the osseointegration rate of titanium implants. Ultraviolet light exposure enhances protein adsorption, bone marrow cell adhesion, osteoblast differentiation, and osseointegration rate of titanium implants. A previous study demonstrated that 4-week-aged, Ultraviolet light treated titanium surfaces increased bioactivity to a level comparable to or greater than that of freshly prepared surfaces, owing to the restoration of super-hydrophilicity. Plasma-treated titanium surfaces promoted osteoblast proliferation and increased bone-to-implant contact. Ultraviolet light treatment enhanced osteoblast adhesion, proliferation, and mineralization in zirconia implants and improved bone-to-implant contact and osseointegration. Additionally, Plasma treatment enhanced the biocompatibility of zirconia implants. The findings of this study are consistent with those reported in the previous studies.

In this study, the values of push-in tests of UV and PL TZP implants were 19.3 ± 1.7 N and 22.5 ± 2.5 N respectively, indicating that the same level of osseointegration could be achieved. In a similar experiment with titanium implants (1 mm in diameter and 2 mm in length), the push-in test value for titanium implants without super-hydrophilic treatment was approximately 12 N. The value of untreated TZP implant was 7.5 ± 1.8 N, which is inferior to titanium. Performing super-hydrophilically treated implants solved a problem due to changing material. It was suggested that zirconia implants treated with super-hydrophilic agents could achieve a higher rate of osseointegration than titanium implants not treated with super-hydrophilic agents (also known as conventional titanium implants). It is possible that zirconia implants treated super-hydrophilically may achieve the same clinical outcomes as conventional titanium implants. Although there are few reports comparing UV and PL TZP implants, this study found no difference in the strength of osseointegration or bone formation between the two implants. However, due to its high output, PL requires less processing time than UV. As a result, PL may be more suitable for clinical applications.

Super-hydrophilic surfaces improve osseointegration of TZP implants, in several ways: improved wettability allows proteins and cells to diffuse more freely immediately post-implantation, removing carbon from the implant surface reduces factors that impair the adhesion of proteins and cells, and positive surface polarity adsorbs more negatively charged proteins and cells.

On the other hand, the blasted and acid-etched micro- and nano-topographies on TZP implants suggested a promising method for enhancing osteogenic differentiation of osteoblast-like cells and hMSCs. Nanoscale modification of the implant surface may aid in simulating bone cellular environments, thereby promoting bone formation. According to a previous study, nanoscale groove-ridge patterns on the surface of polystyrene were similar in profile and size to collagen fibrillar architecture and served as a scaffold for osteoblast-like cell orientation, spreading, and directional mineralization. The micro- and nano-topographies, as well as super-hydrophilic modifications (UV and PL), were...
applied to the TZP implant surface in this study. These synergistic effects of the TZP implant surface treatment may promote rapid osseointegration in vivo. These surface treatments may enhance the clinical outcome of TZP implants.

Previous study has reported that blasted and acid-etched TZP implants promoted osteoconductive differentiation of osteoblast-like and mesenchymal stem cells\(^ {11,29}\). Within the constraints of this study, further superhydrophilic modification after this micro- and nano-topographies promoted bone-implant integration and new bone formation around the TZP implant.

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Conflict of Interest

The authors have declared that no COI exists.

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