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Original

Zirconia Implants with Laser Surface Treatment: Peri-Implant Bone Response and Enhancement of Osseointegration

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Abstract: The aim of this study was to prepare zirconia implants with laser-modified surfaces and to evaluate peri-implant tissue response and osseointegration in an animal study. The experimental zirconia implants received one of the following surface treatments and were placed in the tibiae of SD rats: vertical irradiation with a fiber laser (vertical fiber laser), horizontal irradiation with a fiber laser (horizontal fiber laser), vertical irradiation with a Neodymium-doped yttrium orthovanadate (YVO4) laser (vertical YVO4 laser), and horizontal irradiation with a YVO4 laser (horizontal YVO4 laser). The control implants were smooth surfaced. Tibiae with implant bodies were collected 28 days after implant placement, and removal torque values were measured. Tissue sections were prepared for light microscopy, and the bone-implant contact (BIC) ratio and the peri-implant bone area (BA) were measured. The vertical fiber-laser implants had a mean BIC that was significantly higher than other implants. The mean BIC of the vertical fiber-laser implants was approximately 4.2 folds of the value of the control implants on the cortical bone side and approximately 2.7 folds of the value of the control implants on the bone marrow side. The mean BA was significantly higher in the vertical YVO4-laser implants. The vertical fiber-laser implants had a mean torque removal value that was approximately 2.4 folds of the value of the control implants and approximately double of the value of the vertical or horizontal YVO4-laser implants. The horizontal fiber-laser implants had a mean removal torque value that was approximately double of the value of the control implants and approximately 1.7 folds of the value of the vertical or horizontal YVO4-laser implants ($p<0.05$). Both types of lasers were useful in implant surface treatment to enhance osseointegration of zirconia implants.

Key words: Animal study, Laser treatment, Osseointegration, Rough surface, Zirconia implant.

Introduction

The development of CAD/CAM technology has rapidly widened the application of zirconia ceramics, which have already been used in crown restoration for natural teeth, bridge prostheses for missing teeth, abutments on dental implants, and superstructures. Zirconia ceramics have excellent mechanical strength to enable esthetic restoration in areas where the use of a ceramic material was previously difficult due to its insufficient strength[1,2]. Zirconia ceramics have such excellent characteristics and consequently are very attractive as a next-generation material for implant bodies. Zirconia implant bodies are already commercially available from some manufacturers. The surface modification of zirconia implant body surface is thought to enhance osseointegration between the implant surface and bone[3-9]. There are some reports on the surface modification of zirconia ceramics and enhancement of osseointegration. However, surface modification methods for zirconia ceramics have not yet been established. Thus, much is still unknown regarding what surface topography optimizes osseointegration.

The aim of this study was to prepare zirconia implants with new surface topography by laser irradiation, to place these implants in rat tibiae, and to evaluate peri-implant tissue response and osseointegration.

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Materials and Methods

Experimental implant bodies

Threaded zirconia ceramic implants were made from yttria-tetragonal zirconia polycrystal (Y-TZP) ceramics (NANTO PRECISION, Shizuoka, Japan). Each implant was φ1.6 mm × length: 8 mm (Fig. 1).

The experimental implant bodies underwent one of four surface treatments: vertical irradiation (axial direction) with a fiber laser (vertical fiber laser), horizontal irradiation (direction of the threads) with a fiber laser (horizontal fiber laser), vertical irradiation (axial direction) with a YVO4 laser (vertical YVO4

Figure 1. Experimental Zirconia implant. 1.6mm in diameter, 8mm in length.

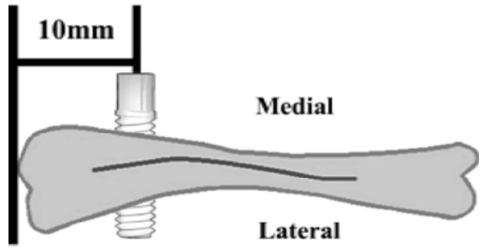

Figure 2. Implant placement in rat tibia. An experimental implant was placed in the rat tibia approximately 10mm from the knee joint and in the direction from the medial to the lateral side. The arrow shows the direction of implant placement.

laser), and horizontal irradiation (direction of the threads) with a YVO4 laser (horizontal YVO4 laser). The control implant bodies were smooth surfaced and not treated with any laser.

Observation of implant surface

The surfaces of implant bodies were observed using a scanning electron microscope (SEM) (JSM-6330F, S3500N, JEOL Ltd., Tokyo, Japan). Surface topography and changes in thread morphology were compared by laser surface treatment.

Measurement of surface roughness

Surface roughness was measured using a laser microscope for profile measurement (VK-X100; KEYENCE Co., Osaka, Japan), and the three-dimensional arithmetic mean roughness (Sa) was compared among implant surfaces. Measurements were made at the top of the thread, flank, and valley. Three points (arbitrary area of 50× 50 μm) were measured in each of these sites. The mean value of the total of these 9 points was calculated for each type of implant surface treatment.

Animal study

Forty 8-week-old male SD rats were used as experimental animals. One implant body was placed on each of the right and left tibiae. The rats were placed under isoflurane general anesthesia (Forane, Abbott Japan, Tokyo, Japan) and treated. The implant site was located 10 mm distal from the most superior part of the knee joint. An implant socket was prepared that penetrated from the medial to lateral side of the tibia, and a zirconia implant was placed (Fig. 2). The implant bodies placed on the right and left tibiae had different surface treatment but otherwise randomly chosen. The experiment was conducted with the approval of the Animal Experimentation Committee of Fukuoka Dental College (approval number: 10027).

Histological evaluation

The rats were sacrificed with an anesthetic overdose (diethyl ether, Wako Pure Chemical Industries, Ltd., Osaka, Japan) 28 days after implant placement. Tibiae, including the implant bodies, were collected as specimens. Immersion fixation was performed on the specimens in 10% formalin (pH 7.4). Dehydration was performed, and the specimens were embedded in MMA resin. Longitudinal slices were made so that the cross-section of the center of the implant body could be observed. Hematoxylin and eosin staining was performed, and undecalcified sections were made.

A light microscope (BX51-DP 12 OLYMPUS, Tokyo, Japan) was used to make observations of the peri-implant tissues. Image analysis and measurement software (WinROOF, MITANI CO., Tokyo, Japan) was used to measure the bone-implant contact (BIC) ratio and the bone area (BA) in the threads (between the threads). Measurements were made in a total of 6 sites within the areas of 3 threads: 4 sites on the cortical bone side in the peri-implant area and 2 sites on the marrow side. The mean values were calculated for the cortical bone region and bone marrow region (Fig. 3).

Evaluation of osseointegration enhancement

The rats were sacrificed 28 days after implant placement, and tibiae were collected, including implant bodies. Soon after the specimens were collected, the implant bodies were turned counterclockwise, and torque gauges (BTG60CN-S and ATG12NCN-S, Tohnichi Mfg., Ltd., Co. Tokyo, Japan) were used to measure the removal torque values.

Statistical analysis

Statistical analysis was performed using one-way ANOVA (surface type × experimental period) with Bonferroni's post-hoc test. The level of significance was set at $p<0.05$.

Results

Comparison of threads using SEM

The thread structure of the experimental zirconia implants was observed using SEM before implant placement. There was no damage that had bad influence for the role of thread in any implant type (Fig. 4). The grooves of the vertical fiber-laser implants were observed in the axial direction. The grooves of the horizontal fiber-laser implants were observed in the direction of the threads. Thread morphology was slightly rounded for both the vertical and

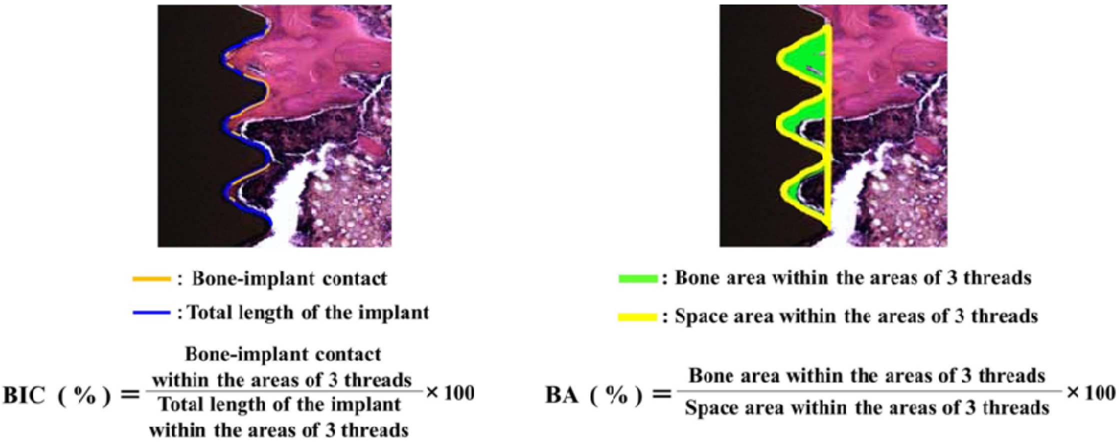

Figure 3. Measurements were made in a total of 6 sites within the areas of 3 threads (4 sites on the cortical bone region in the peri-implant area and 2 sites on the bone marrow region).

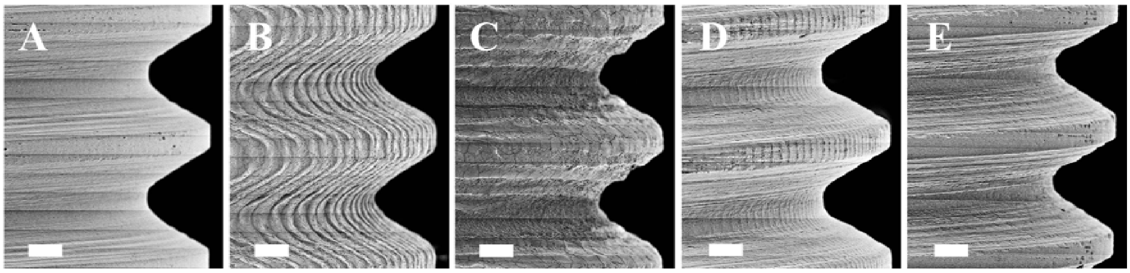

Figure 4. SEM observations: comparison of threads from different implants.
A: control; B: vertical fiber laser; C: horizontal fiber laser; D: vertical YVO4 laser; E: horizontal YVO4 laser. Scale Bar: 100μm.

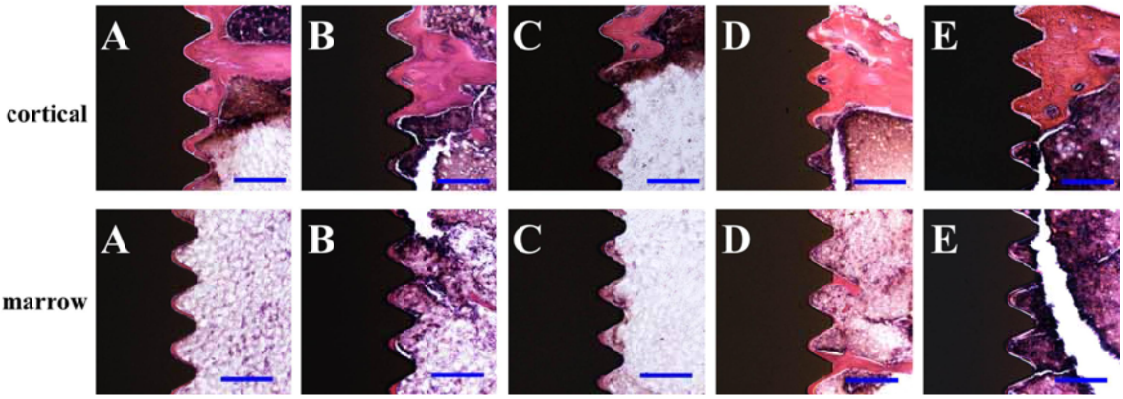

Figure 5. Tissue specimen light microscopic observation 28 days after implant placement.
Upper: Cortical bone side (cortical). Lower: Bone marrow side (marrow).
A: control; B: vertical fiber laser; C: horizontal fiber laser; D: vertical YVO4 laser; E: horizontal YVO4 laser. Scale Bar: 500μm.

horizontal fiber-laser implants. The grooves of the vertical and horizontal YVO4-laser implants were narrower in width and shallower compared with the grooves of fiber-laser implants.

Surface roughness measurement

The Sa values of the control implants, implants treated with a fiber laser, and implants treated with a YVO4 laser were 1.084±0.551μm, 1.84±0.690μm, and 1.44±0.634μm, respectively. The fiber laser-treated implants showed the largest value than the YVO implants and the control implants.

Histological observations

The results of light microscopy revealed that new bone formation occurred around control implants, the vertical fiber-laser implants, the horizontal fiber-laser implants, the vertical YVO4-laser implants and the horizontal YVO4-laser implants at 28 days after placement. On the side of the cortical bone, new bone formation was observed along the implant surface. Thus,

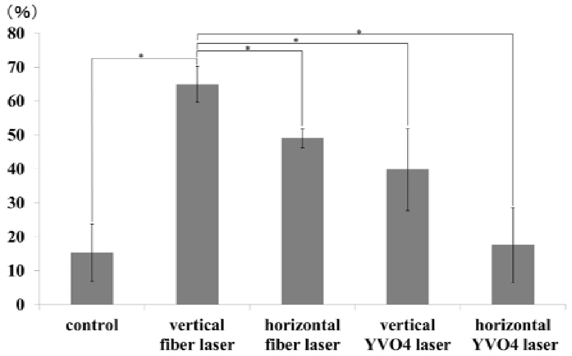

Figure 6. BIC on the cortical bone side.
The asterisk denotes significant difference (p<0.05).

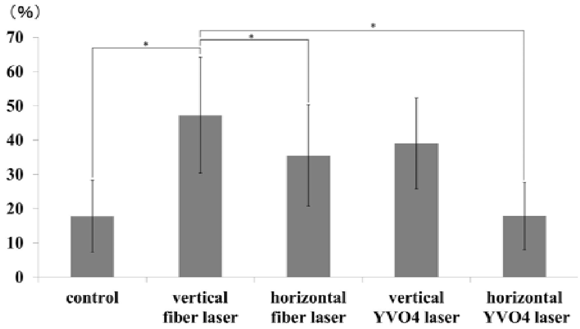

Figure 7. BIC on the bone marrow side.
The asterisk denotes significant difference (p<0.05).

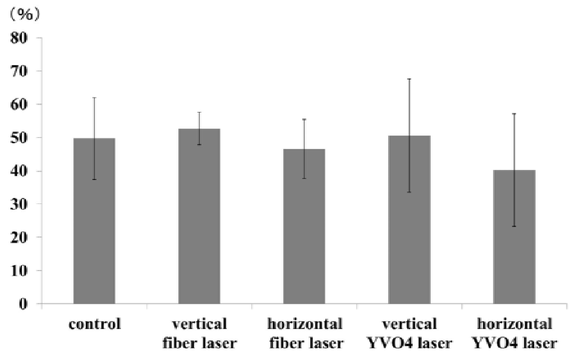

Figure 8. BA on the cortical bone region.

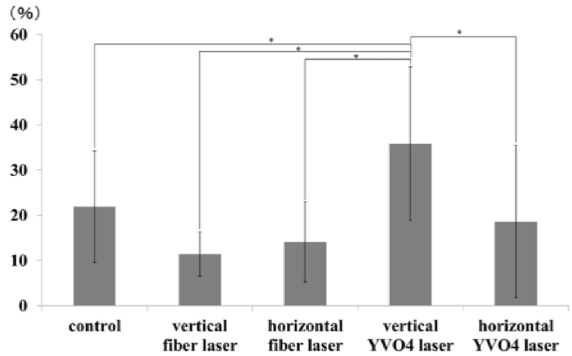

Figure 9. BA on the bone marrow region.
The asterisk denotes significant difference (p<0.05).

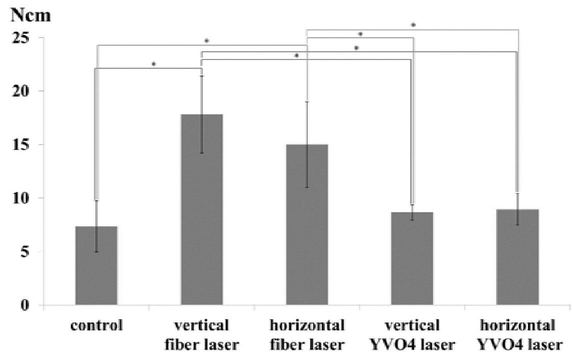

Figure 10. Removal torque value.
The asterisk denotes significant difference (p<0.05)

achievement of osseointegration was confirmed (Fig. 5).

BIC evaluation

The mean BIC on the cortical bone side was 15.3±8.4 % for the control implants. The vertical fiber-laser implants and the horizontal fiber-laser implants was 64.9±5.3% and 49.0±2.8 % respectively. The vertical YVO4-laser implants and the horizontal YVO4-laser implants was 39.8±12.1 % and 17.5±11 %, respectively (Fig. 6). Markedly the vertical fiber-laser implants had a mean BIC that was significantly higher than other implants and that was approximately 4.2 folds of the value of the control implants (p<0.05). The horizontal fiber-laser implants had a BIC that was approximately 3.2 folds of the value of the control implants and approximately 2.8 folds of the value of the horizontal YVO4-laser implants, indicating a significantly higher value (p<0.05). The vertical YVO4-laser implants had a mean BIC that was approximately 2.6 folds of the value of the control implants, indicating a significantly higher value (p<0.05). The vertical and horizontal fiber-laser implants had significantly higher BIC than the control implants or the YVO4 implants (p<0.05) on the cortical bone.

The mean BIC on the bone marrow side was 17.8±10.5 % for the control implants. The vertical fiber-laser implants and the horizontal fiber-laser implants was 47.3±16.9 % and 35.5±14.8 %, respectively. The vertical YVO4-laser implants and the horizontal YVO4-laser implants was 39.0±13.3 % and 17.9±9.8 %, respectively (Fig. 7). The vertical fiber-laser implants had a mean BIC that was approximately 2.7 folds of the value of the control implants and approximately 2.6 folds of the value of the horizontal YVO4-laser implants, indicating a significantly higher value (p<0.05).

BA evaluation

The mean BA on the cortical bone side was 49.7±5.8 % for the control implants. The vertical fiber-laser implants and the horizontal fiber-laser implants was 52.7±4.7 % and 46.6±5.4 %, respectively. The vertical YVO4-laser implants and the horizontal

YVO4-laser implants was 50.6±9.3 % and 40.2±7.9 %, respectively (Fig. 8). In the cortical bone region, the mean BA did not differ significantly by surface treatment.

The mean BA on the bone marrow side was 21.9±12.3 % for the control implants. The vertical fiber-laser implants and the horizontal fiber-laser implants was 11.4±4.87 % and 14.1±8.93 %, respectively. And the vertical YVO4-laser implants and the horizontal YVO4-laser implants was 35.9±17% and 18.6±16.9%, respectively (Fig. 9). In the bone marrow region, the vertical YVO4-laser implants had a mean BA that was approximately 3.15 folds of the value of the vertical fiber-laser implants, approximately 2.5 folds of the value of the horizontal fiber-laser implants, and 1.9 folds of the value of the horizontal YVO4-laser implants. Thus, the vertical YVO4-laser implants had a most value of BA ($p<0.05$).

Evaluation of osseointegration enhancement

The mean removal torque value was 7.35±2.38 Ncm for the control implants. The vertical fiber-laser implants and the horizontal fiber-laser implants was 17.8±3.62 Ncm and 15.0±4.0 Ncm, respectively. And the vertical YVO4-laser implants and the horizontal YVO4-laser implants was 8.65±0.72 Ncm and 8.95±1.5 Ncm, respectively (Fig. 10). The vertical fiber-laser implants had a mean removal torque value that was approximately 2.4 folds of the value of the control implants and approximately double of the value of the vertical or horizontal YVO4-laser implants. Thus, the vertical fiber-laser implants had a significantly higher removal torque value ($p<0.05$). Furthermore the horizontal fiber-laser implants had a mean removal torque value that was approximately double of the value of the control implants and approximately 1.7 folds of the value of the vertical or horizontal YVO4-laser implants. Therefore, the horizontal fiber-laser implants had a significantly higher removal torque value ($p<0.05$).

Discussion

Pure titanium and titanium alloys are currently the main materials used for implant bodies, and these materials have been reported to have high success rates in clinical practice[10-14]. In recent years, there have been some reports on titanium allergy and hypersensitivity[15-24]. These complications can become a big problem as the use of titanium implants increases. Sicilia et al. analyzed 1500 patients with dental implants for titanium allergy[17]. Nine of these patients displayed positive reactions to titanium allergy tests. There are also clinical reports on facial dermatitis and gingival hyperplasia after titanium implant placement[15,16,21]. Metal implants can cause esthetic problems when peri-implant tissue recedes and metallic color becomes visible. Zirconia ceramics have been used in dentistry for crown restoration, bridge prostheses for missing teeth, abutments and superstructures on implants. In reports, zirconia ceramics have been favorably rated for their high mechanical strength, biocompatibility, and esthetics. Zirconia ceramics are an attractive material for implant bodies, and some zirconia implant systems are commercially available[1,2]. Oliva et al. reported that the success rate was 95% for zirconia implants after 5 years of follow-up[25]. Borgonovo et al. reported that the survival rate was 100% for sandblasted zirconia implants[26]. Considerably fewer basic research studies have been conducted on zirconia implants compared with titanium implants. In particular, there are only a small number of reports on surface topology[3-9]. Therefore, we have conducted previous studies to examine the zirconia-bone interface. In our studies, zirconia implants with modified surface topography were prepared and placed in rat tibiae[27]. Light microscopic observations were made on the healing process of implant-bone interface until osseointegration was achieved. Our results showed that the YAG laser-modified implants had a higher removal torque value than the implants without surface modification. However, YAG laser treatment damages the shape of the threads of implant bodies. Thus, this treatment is not suitable for clinical application. In our present study, experimental implants were prepared using surface treatment with fiber laser or YVO4 laser irradiation. Peri-implant tissue response and osseointegration enhancement were evaluated.

Fiber lasers use optical fibers doped with rare-earth ions, such as ytterbium, as the lasing medium to amplify light. A laser beam is generated by directing excitation light into the optical fiber. The fiber laser beam is stable and has no spatial fluctuation. Another advantage of fiber laser is that it generates high energy because of the short wavelengths (1000-1100 nm). Therefore, it is suitable for preparing uniform surface topography of implants.

YVO4 lasers can generate laser light using a lamp to excite a YVO4 crystal doped with neodymium. Excitation occurs at the wavelength of 1.064 μm, and surface treatment of implant bodies can be performed with minimal heat stress. When surfaces are roughened using a laser, there is no contact between the implant and the laser equipment. Therefore, laser treatment poses no risk for surface contamination, while blasting and acid treatment result in such contamination. Thus, the use of a laser is suitable for surface treatment of implant bodies. Albrektsson et al. reported that a high degree of osseointegration was achieved using titanium implants with Sa of 1-2μm[28-31] and that a high activity of osteoblasts was observed for Sa of approximately 2μm[32]. In our study, Sa was 1.84±0.690μm for the zirconia implants treated with a fiber laser. This value suggests that the results from zirconia ceramic implants might be similar to titanium implants with comparable Sa. Hoffmann et al. examined the removal torque of titanium implants and zirconia implants with different surface roughness. The removal torque was higher when the surfaces of implants were roughened[6].

In our animal study, implants treated with a fiber laser had a mean BIC and a mean removal torque value that were significantly higher than other laser treated zirconia implants. The results suggest that surface treatment with a fiber laser is highly useful for zirconia implants. For both fiber laser and YVO4 laser, the removal torque value was higher for implants with vertical irradiation than for implants with horizontal irradiation. The reasons are thought to be increased mechanical fit due to grooves forming in the vertical direction and increased BA with vertical laser irradiation. These results suggest that surface modification using laser irradiation has a direct effect on the enhancement of osseointegration.

Zirconia ceramics have excellent mechanical strength but are brittle. Therefore, fracture can be a problem. Gahlert et al. analyzed fractured zirconia dental implants and found that approximately 10% of the implants were fractured 36.75 months after prosthetic loading[33]. The causes of fracture were the use of implant bodies with a small diameter and occlusal overloading. Sintered Y-TZP bodies are composed almost entirely of tetragonal zirconia and have excellent chemical durability. However, when Y-TZP is placed in a moist environment for a period of time, the tetragonal crystals transform to the monoclinic phase, which is associated with an approximately 4 % increase in volume, resulting in degradation. Even when the ambient temperature is low, the crystals degrade due to phase transformation to the monoclinic phase in the presence of atmospheric moisture. Thus, a marked decrease in mechanical strength is a concern regarding Y-TZP[34,35].

In clinical practice, Y-TZP has been used as a biocompatible material for artificial heads in the hip joints. However, Maccauro et al. reported on damages to the zirconia femoral heads, which were speculated to be from low-temperature degradation due to an increased monoclinic content[36]. Zirconia restorations have been used prevalently in the intraoral region, but serious issues have not been reported that raise the suspicion of low-temperature degradation. It is speculated that even though the intraoral environment is moist, low-temperature degradation does not occur at body temperature. However, a long-term examination is necessary for further investigation.

Our study with rats used implants whose surfaces had been modified by fiber laser irradiation or YVO4 laser irradiation. The results showed that osseointegration was significantly more enhanced in the fiber laser-modified implants compared with the control implants. On the cortical bone side and bone marrow side, histological evaluation showed that BIC was significantly higher in implants with vertical fiber-laser irradiation compared with other implants. In the bone marrow region, the vertical YVO4-laser implants had a significantly higher mean BA than the fiber-laser implants. The results suggest that zirconia surface treated with a fiber laser promoted osteogenesis at the bone-implant interface and that vertical YVO4-laser irradiation promoted osteogenesis in an area at a slight distance from the interface. Both types of lasers were useful in implant surface treatment to enhance osseointegration of zirconia implants.

In Conclusion, implants treated with a fiber laser had a significantly higher mean BIC and a mean removal torque value compared with the control implants or implants treated with a YVO4 laser. The results of this study suggest that laser surface treatment is useful in the clinical application of zirconia implants. Since laser treatment does not involve contact between the implant and the laser equipment, there is no risk for contamination on the implant surface. In addition, osseointegration is enhanced.

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