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ORIGINAL ARTICLE

Surface changes and bacterial adhesion on implant abutment materials after various clinical cleaning procedures

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Abstract

Background: Supportive treatments are essential to long-term dental implant success; however, professional cleaning procedures may alter the surfaces of implant abutments and lead to adverse biological responses. This study aimed to evaluate four clinically used cleaning procedures by examining surface changes and subsequent bacterial adhesion on abutment materials.

Methods: Discs of titanium and zirconia were polished and divided into five groups: titanium curette treatment, carbon fiber reinforced plastic curette treatment, ultrasonic scaling with carbon fiber tip treatment, air polishing with glycine powder, and control group without any treatment. After instrumentation, the arithmetical mean roughness (Ra), hydrophilicity, and surface free energy were recorded. The bacterial adhesion was evaluated after 1 h of *Streptococcus mitis* incubation by optical microscope and quantified by turbidity test.

Results: Among the titanium samples, titanium curette treatment group showed significant surface morphology changes, increased Ra, hydrophilicity, surface free energy, and higher optical density of adhered bacteria. As for the zirconia samples, the differences in surface morphology, Ra, and bacterial adhesion between groups were nonsignificant.

Conclusion: Comparing to titanium, zirconia was less susceptible to surface changes after tested cleaning procedures. Titanium curette should be used with care on titanium abutments.

Keywords: Bacterial adhesion; Cleaning; Dental implant abutment; Titanium; Zirconia

1. INTRODUCTION

Dental implants are commonly used to restore missing teeth. With proper treatment planning and execution, they can be expected to function for a long time.[1] However, the ubiquity of implants has led to increased reports of complications, among which infection-related peri-implant disease is one of the most common complications reported.[2–5] Uncontrolled diabetes, prior periodontal disease, cigarette smoking, and poor oral hygiene have been identified as risk factors for infection around implants.[6,7] Regular supportive care by professionals is essential to long-term implant success.[8] Failures in detecting and treating peri-implant mucositis limited to soft tissue may allow its progression into hard tissue destructive peri-implantitis. Among patients with early peri-implant mucositis, the odds ratio of disease progression was 5.92 for those patients who failed to receive regular dental supportive care.[9] Progressed infections may lead to implant loss and ridge deformity, which can hinder attempts at reimplantation. Thus, it is very important to detect and manage the infection at the beginning.

Nonetheless, the exact procedures that must be taken during supportive treatments have yet to be determined. Besides thorough examinations, removing plaque and calculus around implant system is considered as key to supportive treatment. Both chemical and mechanical methods have been developed. As for chemical methods, chlorhexidine, essential oils, and delmopinol have proven to be safe and effective agents against dental plaque in natural dentition. Unfortunately, when applied to implants, the results have been less than satisfactory.[10,11] As for mechanical methods, ultrasonic scaling devices, metal curettes, plastic curettes, titanium instruments, and air polishing devices have all been studied[12] with different results. Conventional stainless steel Gracey curettes are not recommended for regular implant supportive therapy because they leave more biofilm on the implant than powered instruments do[13] and commonly damage the surfaces of the substrates.[14] Plastic curettes are relatively safe for implant surfaces; however, their effectiveness in cleaning is poor.[12] Ultrasonic scaling is a more efficient approach; however,

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they may create scratches on smooth surface. Numerous scaling inserts have been developed to minimize the alteration of abutment surfaces, including carbon tips, plastic tips, and metal tips with plastic sheath.[15] Unfortunately, succeeding instruments residue deposits on implants raised clinical concerns.[16] Air polishing is a common approach to remove dental plaque from natural dentition and implants with very little damage to both surfaces.[17,18] However, only limited additive effects was reported in cases of peri-mucositis.[19] Early case report of submucosal emphysema after air polishing applied around implant also left some doubts in its usage.[20] Up to now, the best instrument for implant cleaning during supportive treatment remains an issue of debate.

Another issue pertaining to long-term implant success is the implant system itself. Selecting an implant abutment with appropriate chemical and physical characteristics that resists bacterial adhesion is crucial. Besides titanium, zirconia is widely used as implant abutment recently because of its good mechanical properties, aesthetic appearance, biocompatibility, and resistance to bacterial adhesion.[21,22] Nonetheless, most studies dealing with professional cleaning around implant abutments have used titanium as substrates. The impact of professional cleaning on zirconia abutment has rarely been examined. In the present study, we evaluated four clinical cleaning devices commonly used for implant cleaning: titanium curette, carbon fiber reinforced plastic curettes, ultrasonic scaling with carbon fiber inserts, and air polishing using glycine powder. Both titanium and zirconia samples were included to study the cleaning-related changes in their surface characteristics as well as subsequent bacterial adhesion. Our null hypothesis was that all of the tested procedures would not alter the surfaces of titanium and zirconia abutments or affect their subsequent bacterial adhesion.

2. METHODS

2.1. Sample preparation

Two dental implant abutment materials were used in the present study: commercially pure titanium (Ti) (grade IV; Ultimate Materials Technology, Hsinchu, Taiwan) and zirconia (ZrO_2) (yttria-stabilized tetragonal zirconia; Coalition Technology, Tainan, Taiwan). Samples were fabricated in the shape of round disc and polished to simulate clinical implant abutments. Sixty samples of each material were prepared, cleaned with 75% ethanol using a sonicator and then stored in a dry cabinet for 24 h before testing.

2.2. Cleaning procedures

Both titanium and zirconia samples were randomly divided into five groups. Four types of clinical implant cleaning instruments employed in present study were as follows: titanium curettes (Langer 5; American Eagle, Missoula, MT, USA), carbon fiber reinforced plastic curettes (Implant Deplaquer; Kerr, Orange, CA, USA), an ultrasonic scaler with carbon fiber tip (PH1 tip with P5 Newtron; Acteon, Merignac Cedex, France), and air polishing using glycine powder (Air-flow powder Perio with Air-flow S1; EMS, Nyon, Switzerland). Another group of samples without any surface treatment were served as a control group. These four types of instruments were chosen because they are commercially well-known for implant cleaning, particularly with emphasis on minimal alteration to the implant surface. They are also easy to operate without demand for special training, which is favorable to massive use by general dentists and beneficial to reduce the study errors from operator in present study.

The individual sample was positioned on a silicon mold to avoid movement during the cleaning procedure. One well-trained periodontist performed all of the cleaning procedures. For the titanium curette treatment and the plastic curette treatment groups, the application of the curette blade was perpendicular to the sample surface. Cleaning was performed using overlapping strokes without any fluid or lubricant. For the ultrasonic scaling groups, the power level was set at level 2 with water irrigation in accordance with the manufacturer's recommendations. Cleaning was performed using smooth, overlapping movements all over the sample surfaces. For the air polishing groups, the water and power outputs were both set at medium level. The nozzle was kept at a distance of 0.5 to 1 cm away from the sample and perpendicular to the surface throughout the cleaning process. The glycine powder was loaded repeatedly to the recommended level before working on each sample. The control groups were stored in dry cabinet and did not undergo any instrumentation.

After these cleaning procedures, the samples were washed with large amount of distilled water then underwent sonication using 75% ethanol to remove all debris. The surface morphology of samples was observed using an optical microscope (Olympus BX51M; Olympus corporation, Tokyo, Japan). As for further morphology observation, atomic force microscope (AFM; Dimension Edge, Bruker corporation, Santa Barbara, CA, USA) was used. The three-dimensional AFM reconstruction images provide a more straightforward and precise morphology evaluation for the surface that bacteria adhere to. Considering the size of bacteria, a large area of 50 μm × 50 μm was randomly selected and scanned.

2.3. Surface roughness

Three samples from each tested groups were used for surface roughness measurements. The arithmetical mean roughness (Ra) was calculated from measurements obtained using a profilometer (Surtronic 3+; Taylor Hobson, Leicester, UK). Three random areas of each sample were selected. The cut-off length for measurement was 0.8 mm.

2.4. Hydrophilicity and surface free energy

A contact angle goniometer (Model 100SB, Sindatek, New Taipei City, Taiwan) was used with its corresponding software (MagicDroplet). Three samples from each group were included. The hydrophilicity of the sample was analyzed by capturing the side view of deionized water droplet on sample surface. The contact angles were calculated. The sessile drop method was used to evaluate the surface free energy. Deionized water and diiodomethane were used as representative polar and nonpolar liquids. Owens-Wendt method was used to calculate the surface free energy.

2.5. Bacterial adhesion

A frozen pure strain of *Streptococcus mitis* (ATCC49456) was revived using brain heart infusion medium (Bacto Brain Heart Infusion, Becton, Dickinson and Company, Sparks, MD, USA) in an anaerobic environment at 37°C. The bacteria were cultured to its logarithmic phase before use. The optical density (OD, wavelength = 600 nm) of the bacteria suspension was measured with a spectrophotometer (U-1900; Hitachi High Technologies America, Waltham, MA, USA) and controlled at approximately 0.9.

Six samples from each group were used in bacterial adhesion test. Following disinfection with ultraviolet irradiation, 200 μL of the bacteria suspension was loaded on each sample. In the present study, our focus was on initial bacterial adhesion, which included both reversible and irreversible attachment phases. Although the exact timeline for these two attachment phases may vary from person to person, it is reasonable to set our initial

bacterial adhesion observation time in hour instead of day. The bacterial incubation time was set for 1 h in this study. After 1 h of incubation for initial bacterial adhesion, the discs were gently washed with phosphate buffered saline (PBS) to remove non-adhered bacteria. One disc from each group was sent for optical microscopic observation after Gram staining (BaSO Rapid Gram Stain; BaSO, Taipei, Taiwan). Images of the samples were captured under ×500 magnification. Quantitative data was obtained by placing the other five samples from each group into the wells of a new polystyrene culture test plate loaded with PBS. The adhered bacteria were transferred to the PBS solution via sonication. The adhered bacteria were quantified using a turbidity test based on OD.

2.6. Statistical analysis

Data of surface roughness, hydrophilicity, surface free energy, and OD of bacteria suspension were analyzed using two-way analysis of variance. The post-hoc Tukey HSD test was adopted for comparison between different cleaning procedures. All of the calculation was done by SPSS statistics (IBM, Armonk, NY, USA). The significance level (α) was set at 0.05.

3. RESULTS

3.1. Surface roughness

Table 1 showed the Ra of the tested samples. In the titanium samples, the highest Ra was in the titanium curette treatment group. Significant differences were only observed between the titanium curette treatment group and the other groups ($p < 0.05$). In the zirconia samples, the highest Ra was also noted in the titanium curette treatment group. However, none of the intergroup difference reached the level of significance.

3.2. Hydrophilicity and surface free energy

The hydrophilicity of the test samples is shown in Table 2. Zirconia samples showed higher hydrophilicity than corresponding titanium samples with the same treatment ($p < 0.05$). As for the effects of different cleaning treatments, comparing to the control groups, air polishing treatment groups of both materials showed significantly reduced hydrophilicity ($p < 0.05$); while titanium curette treatment and ultrasonic scaling treatment groups showed significantly increased hydrophilicity ($p < 0.05$).

The surface free energy of the test samples is listed in Table 3. Similar to the hydrophilicity, zirconia samples showed greater surface free energy than corresponding titanium samples ($p < 0.05$). For both test abutment materials, titanium curette treatment group showed the significantly highest surface free energy ($p < 0.05$).

3.3. Bacterial adhesion

Quantified results, in terms of OD value, of bacterial adhesion are listed in Table 4. Among the titanium samples, the OD of the bacteria suspension was the highest in titanium curette treatment group ($p < 0.05$). The differences between the other four treatment groups were not statistically significant. Among the zirconia samples, no significant differences in OD were observed between any groups.

3.4. Surface observation

Fig. 1 illustrated the surface morphologies of the test samples from each group as viewed under an optical microscope. Among the titanium samples, the control group presented an overall smooth surface with several shallow carving marks that were randomly distributed. The morphology of the air polishing group was similar to the control group. The surface of the

Table 1

Arithmetic mean roughness (Ra; μm) of the test samples

	Treatment groups				
Materials	Control	Ultrasonic scaling	Air polishing	Plastic curette	Titanium curette
Ti	0.10 (0.02)^{a,A}	0.18 (0.03)^{a,A}	0.09 (0.01)^{a,A}	0.14 (0.02)^{a,A}	0.45 (0.11)^{b,A}
ZrO_2	0.07 (0.00)^{a,A}	0.08 (0.01)^{a,B}	0.07 (0.01)^{a,A}	0.11 (0.03)^{a,A}	0.13 (0.06)^{a,B}

Values are given as mean (SD).
The same capital letter in the same column indicates no significant difference ($p > 0.05$). The same lowercase letter in the individual rows indicates no significant difference ($p > 0.05$).

Table 2

Hydrophilicity, in terms of water contact angle (°), of the test samples

	Treatment groups				
Materials	Control	Ultrasonic scaling	Air polishing	Plastic curette	Titanium curette
Ti	89.83 (0.42)^{a,A}	77.77 (1.53)^{b,A}	98.43 (0.35)^{c,A}	92.97 (0.58)^{d,A}	63.87 (1.37)^{e,A}
ZrO_2	57.60 (1.08)^{a,B}	49.47 (1.95)^{b,B}	67.87 (1.10)^{c,B}	53.77 (2.63)^{ab,B}	44.20 (0.62)^{d,B}

Values are given as mean (SD).
The same capital letter in the same column indicates no significant difference ($p > 0.05$). The same lowercase letter in the individual rows indicates no significant difference ($p > 0.05$).

Table 3

Surface free energy (mN/m) of the test samples

	Treatment groups				
Materials	Control	Ultrasonic scaling	Air polishing	Plastic curette	Titanium curette
Ti	33.13 (0.62)^{ab,A}	42.07 (1.46)^{c,A}	34.36 (0.85)^{a,A}	31.14 (0.68)^{b,A}	43.94 (0.65)^{c,A}
ZrO_2	50.70 (0.68)^{a,B}	46.53 (0.42)^{b,B}	44.64 (1.22)^{b,B}	54.64 (1.12)^{c,B}	59.80 (0.30)^{d,B}

Values are given as mean (SD).
The same capital letter in the same column indicates no significant difference ($p > 0.05$). The same lowercase letter in the individual rows indicates no significant difference ($p > 0.05$).

Table 4

Optical density of the adhered bacteria on the test samples

Materials	Treatment groups				
	Control	Ultrasonic scaling	Air polishing	Plastic curette	Titanium curette
Ti	0.017 (0.005)^{ab,A}	0.010 (0.004)^{a,A}	0.010 (0.003)^{a,A}	0.021 (0.005)^{b,A}	0.041 (0.006)^{c,A}
ZrO_2	0.013 (0.002)^{a,A}	0.012 (0.001)^{a,A}	0.012 (0.001)^{a,A}	0.014 (0.001)^{a,B}	0.013 (0.001)^{a,B}

Values are given as mean (SD).

The same capital letter in the same column indicates no significant difference ($p > 0.05$). The same lower-case letter in the individual rows indicates no significant difference ($p > 0.05$).

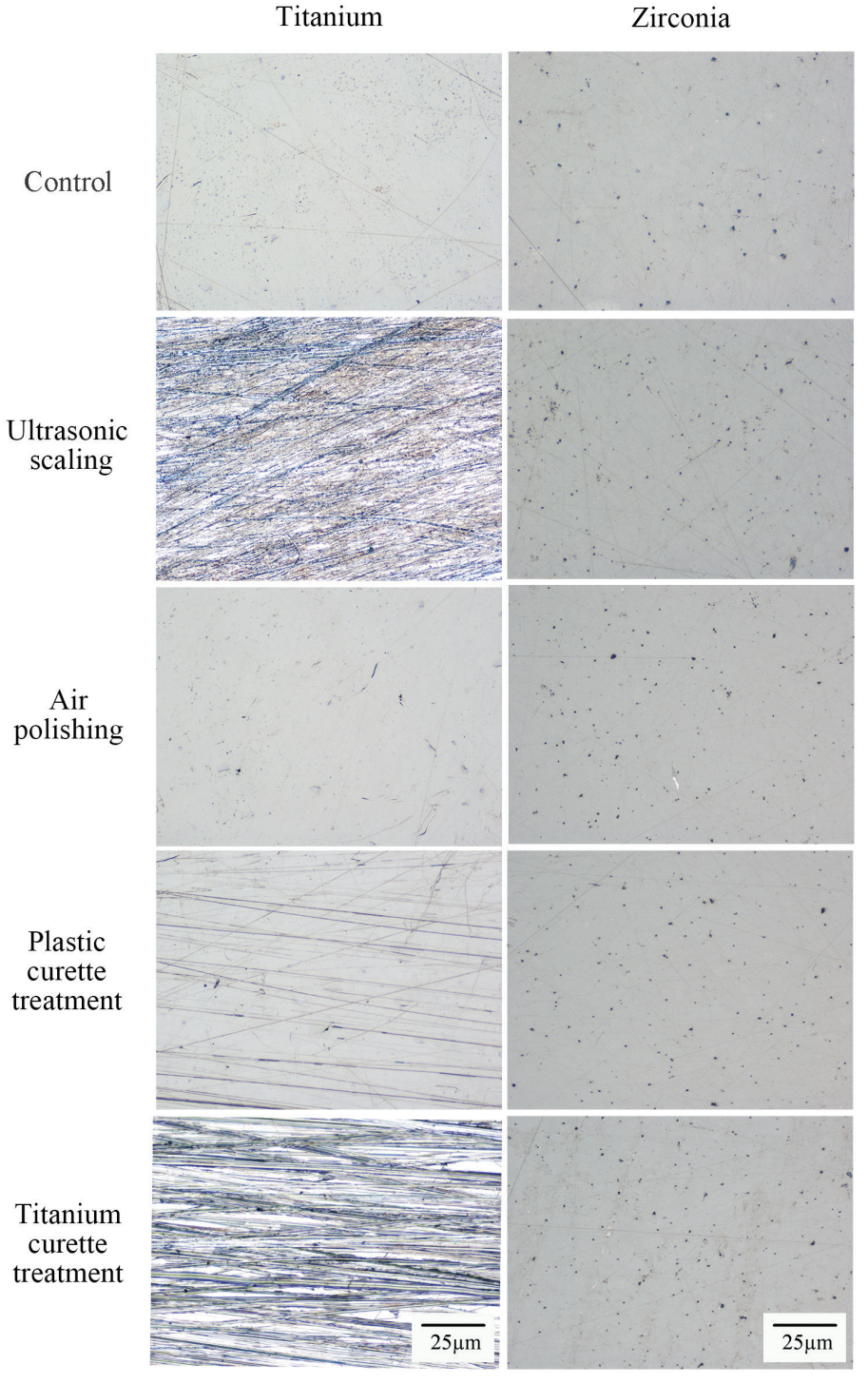

Fig. 1 Optical microscopic images of surface morphology of the test samples after cleaning procedures.

ultrasonic scaling group showed multiple linear carving marks almost covered the whole surface. Linear carving marks were also observed in the plastic curette treatment group; however, they were sparse and relatively shallow. The alignment of carving marks in the titanium curette treatment group was similar to those in the plastic curette treatment group; however, they were far deeper. In the zirconia samples, there were some shallow and randomly distributed carving marks with a number of evenly distributed pits, which might be attributed to the manufacturing process. There were no obvious intergroup differences observed between all zirconia samples.

The three-dimensional images observed by AFM are shown in Fig. 2. For titanium samples, the air polishing treatment group and control group showed similar surface morphology

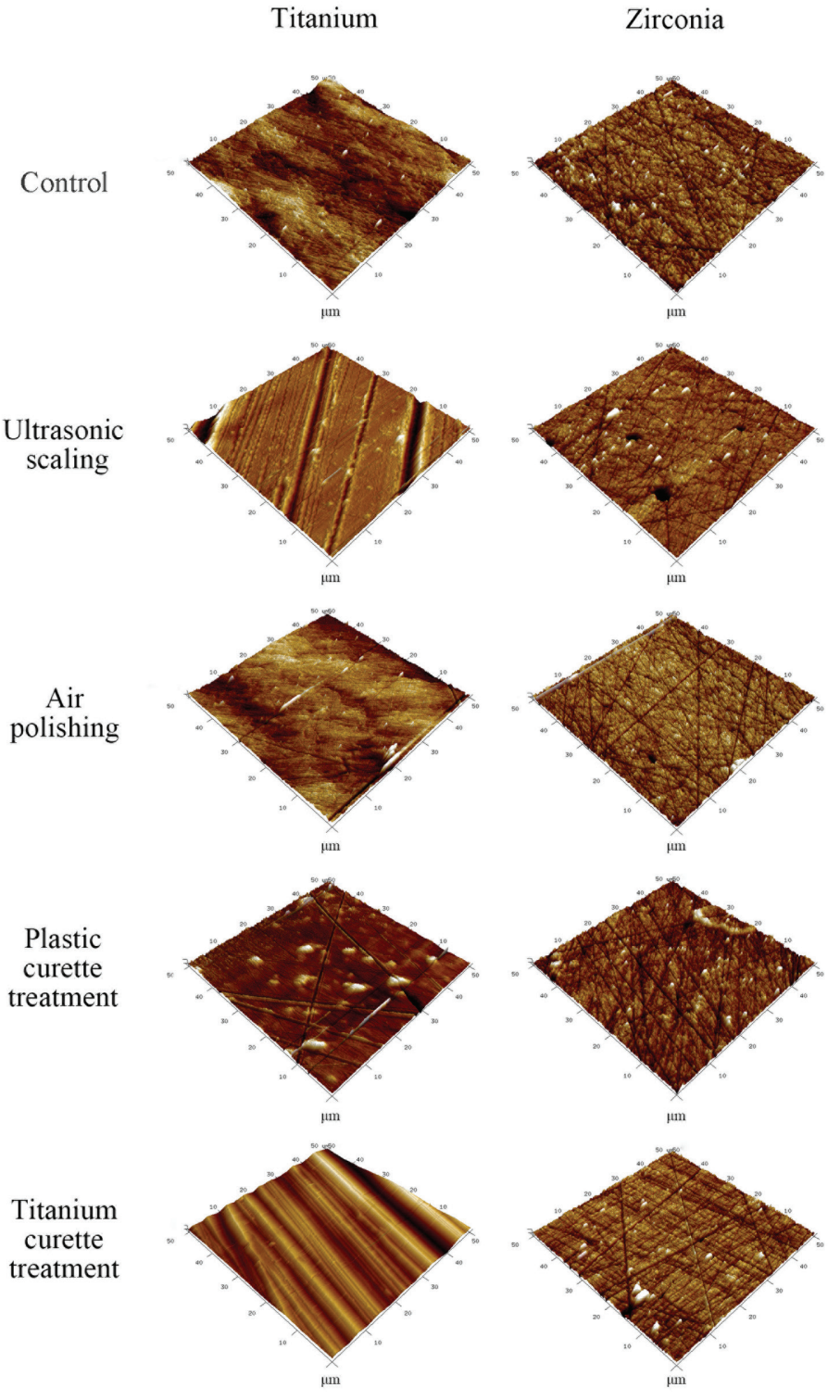

Fig. 2 Three dimensional atomic force microscope (AFM) images of the samples after cleaning procedures.

composed of minor and even waves. Some shallow scraping lines were noted on the sample of plastic curette treatment group. As for ultrasonic scaling treatment and titanium curette treatment groups, the waves over surfaces were more dominant, especially in titanium curette treatment group. For zirconia samples, there were no obvious differences observed among different cleaning treatments. All of the examined samples showed shallow and random orientated carving marks with relatively smooth surfaces.

Following incubation with *Streptococcus mitis*, clusters of bacteria were observed on all sample surfaces (Fig. 3). Among

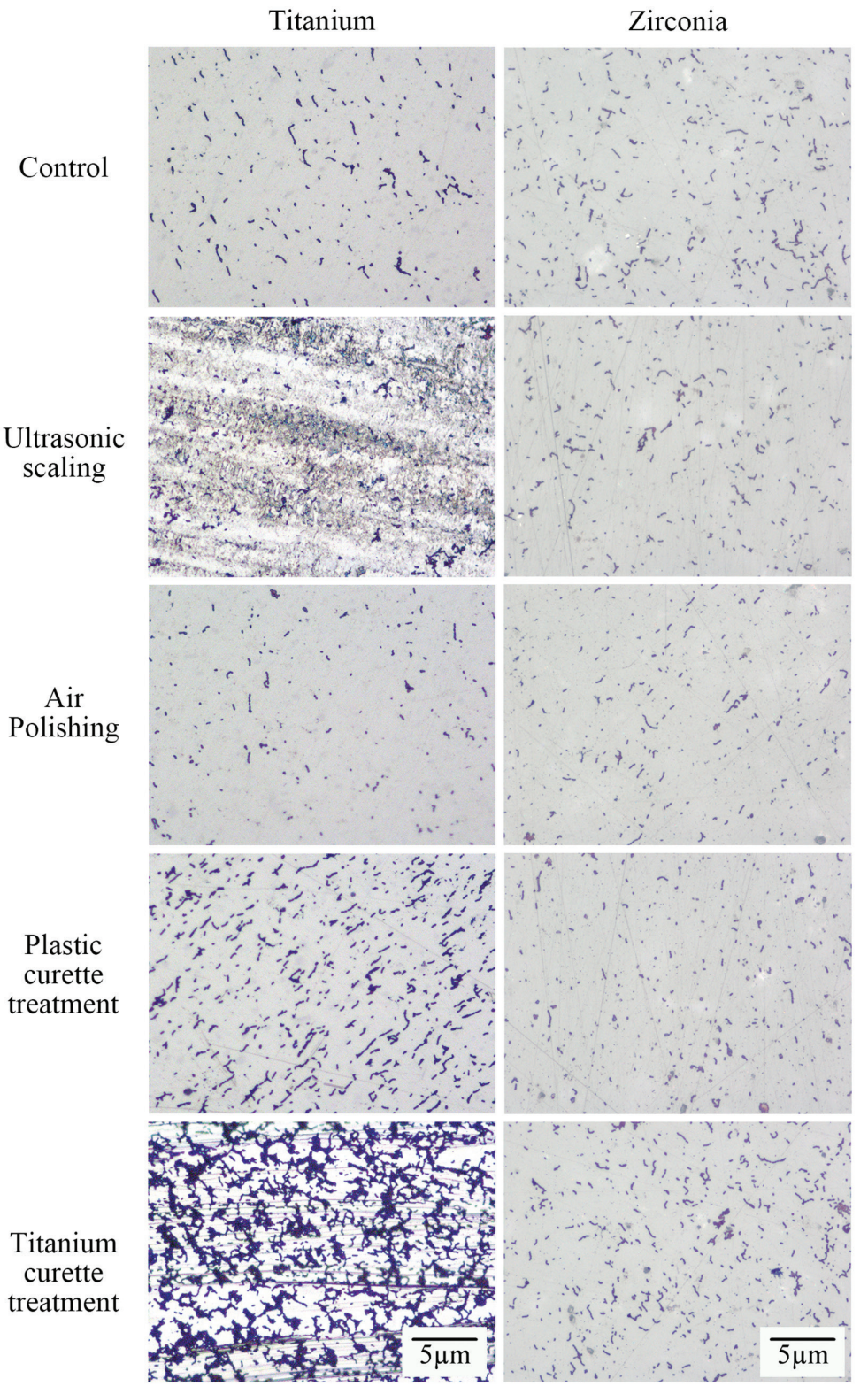

Fig. 3 Optical microscopic images of bacterial adhesion on the test samples after cleaning procedures.

the titanium samples, the density of adhered bacteria was the highest in the titanium curette treatment group, followed by plastic curette treatment group. No obvious differences in the distribution of bacteria were observed between the other groups. Among the zirconia samples, the bacterial distribution patterns and density were similar in all groups.

4. DISCUSSION

Regular removal of biofilm on implant system is crucial to its long-term survival. Nevertheless, the procedure itself can result in negative alterations of abutments' surfaces. In the present study, these alterations included increased roughness, hydrophilicity, and surface free energy. According to literature reviews, the surface roughness, surface free energy, and hydrophilicity of materials are positive-related to bacterial adhesion.[23] For the titanium groups, titanium curette treatment led to increased Ra, hydrophilicity, and surface free energy. These changes favored bacterial adhesion, and as expected, this group showed significantly highest OD of adhered bacteria. Ultrasonic scaling treatment led to increased hydrophilicity and surface free energy, while air polishing and plastic curette treatments led to decreases in these two parameters. Nevertheless, the Ra of these three groups were similar to the control group. Their OD of adhered bacteria also failed to reach significant level. The results indicated that these surface characteristics might have different impacts on bacterial adhesion, while surface roughness seemed to be a particularly significant factor.

According to the results of present study, the degrees of cleaning-related surface alterations were greatly influenced by different cleaning procedures. Similar findings were noted in previous studies. FOX et al[24] used relative specular reflectance from helium neon (HeNe) laser to evaluate the titanium implant abutments following treatment using plastic curette, stainless steel curette, and titanium curette. Significant surface alterations were observed in the stainless steel curette and titanium curette groups. The effects of the plastic curette were nonsignificant. In the study of Duarte et al,[14] metal curettes significantly roughened the smooth titanium surfaces, whereas air polishing and plastic curette did not change Ra significantly. In the study of Sahrmann et al,[13] ultrasonic treatment with a metal tip and stainless steel curette greatly altered surface morphology of implant observed under SEM, whereas glycine powder air polishing did not have a notable effect. Schmage et al[12] conducted experiments on four implant surfaces using ten instruments, including plastic curette, ultrasonic device with a carbon composite curette, and air polishing with glycine powder. None of the instruments significantly increased the Ra of smooth surfaces. The overall conclusion to be drawn from these studies is that ultrasonic cleaning with a nonmetal insert, plastic curette, and air polishing with glycine powder do not produce significant alterations on smooth surfaces while metal instruments led to possible damage. These literatures were in consistent to the findings of present study.

Unlike the titanium samples, all cleaning procedures tested in the present study showed neither surface roughness nor surface morphology changes in zirconia samples. Although titanium curette and plastic curette treatment resulted in some increased hydrophilicity and surface free energy, the differences of Ra and surface morphology were nonsignificant. The OD value of adhered bacteria were nonsignificant among all zirconia groups. Zirconia seemed to be less susceptible to these mechanical cleaning procedures than did titanium. This finding may be explained by the superior wear resistance of zirconia. Material wear is influenced mainly by their hardness and surface roughness.[25] Since the Ra values of the nontreated titanium and zirconia samples were similar, the difference in surface changes after cleaning procedures should be attributed to their hardness. The Vickers hardness of titanium and zirconia samples used in present study were 227 kg/mm^2 and 1410 kg/mm^2, respectively. The difference in surface hardness might lead to their different responses to cleaning procedures. When applied to real clinical situations, following repeated cleaning during supportive periods, the superiority in wear resistance of zirconia may be even more dominant and makes zirconia abutments more resisted to bacterial adhesion than titanium ones.

In follow-up visit, since the acute infection and patient's hygiene performing skills are controlled before implant therapy, the majority of implant patients should be with only slight to moderate inflammation and biofilm deposits. Based on this assumption, not to change the surface of the substrates seemed to be a more important criterion than cleaning ability. According to the findings of present study, with the exception of titanium curette applied to titanium samples, all of the tested instruments appeared to be safe for both titanium and zirconia abutments. Nonetheless, it should be kept in mind that in the present study, we adopted a one-time cleaning procedure only. Clinically, patients are expected to receive lifetime supportive implant treatments. Any alterations to the surface may accumulate since cleaning procedures are applied in every follow-up visit. The repeated cleaning possibly leads to significant surface roughness increase eventually. Thus, cleaning procedures should be selected and performed with care.

Unfortunately, clinicians sometimes encounter implant patients with heavy calculus deposition, soft tissue recession, or implant fixture exposure. In these cases, the philosophy and goal of cleaning during supportive treatment might be very different. Due to the unpredictable nature of the re-osseointergration of previously contaminated implant surfaces, elimination of the macro (eg, thread pattern) and micro (eg, cavity after sand-blasting or acid-etching treatment) surface irregularities to facilitate patient's hygiene care seems to be a reasonable option. In such situations, more aggressive instruments, such as a metal scaling tip,[26] titanium curette, titanium brush,[27] or diamond bur would be favored. Clearly, no cleaning procedures would be perfect for all implant systems under all situations. Users must select the most appropriate cleaning procedure to fulfill their own goals of cleaning based on the understanding of the characteristics of the implant systems and cleaning procedures.

According to the results of the present study, the null hypothesis was rejected. The effects of cleaning procedures on implant abutments are influenced by both cleaning instruments and abutment materials. When applied to titanium samples, titanium curettes were shown to create deep scratches and increased surface roughness, thereby facilitating the adhesion of bacteria. During routine implant supportive treatment, titanium curettes should be used with care. Contrarily, zirconia showed superior resistance to damages from all of the cleaning procedures examined in this study. Within the limitations of the present experiments, it appears that zirconia may be a good material for implant abutments, in terms of long-term maintenance.

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