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CLINICAL RESEARCH

Evaluating the efficiency of three methods to clean and disinfect screw- and cement-retained prostheses

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ABSTRACT

Statement of problem. Screw- and cement-retained prostheses (SCRPs) may be contaminated during fabrication in a dental laboratory, leading to mechanical and biological complications related to the implant treatment. Studies that explored methods to efficiently and conveniently clean and disinfect SCRPs are sparse.

Purpose. The purpose of this clinical study was to compare the efficiency of 3 methods to remove contaminants and microorganisms present on the surface of an SCRP.

Material and methods. Forty-eight 1-unit SCRPs fabricated in a dental laboratory were randomly divided into 3 groups: wiping, soaking, or ultrasonic cleaning. The presence of contaminants was determined by scanning electron microscopy, and microbial cells were cultured before and after treatment. Bacterial colony-forming units (CFUs) on the surface of the SCRPs and contamination density at the implant-abutment interface and emergence profile area were assessed. Statistical tests including ANCOVA were used to compare the efficiency of different methods before and after treatment (α=.05).

Results. Significant differences in contamination density were noted during the treatment at the implant-abutment interface and at the emergence profile area in the 3 groups (P<.05), but no significant differences were observed in the number of CFUs (P>.05). There were significant differences among the 3 methods for cleaning efficiency both at the implant-abutment interface (P=.023) and the emergence profile area (P=.038). At the implant-abutment interface, the contamination density after treatment was lower in the ultrasonic cleaning group than that in the soaking group (P=.007), whereas at the emergence profile area, the contamination density after treatment was lower in the ultrasonic cleaning group than that in the wiping group (P=.019) and the soaking group (P=.048).

Conclusions. All 3 treatment methods reduced contaminants on the SCRP surface, but ultrasonic cleaning yielded the most favorable results. However, none of the methods provided additional disinfection for SCRPs previously disinfected by ozone and UV in a dental laboratory. (J Prosthet Dent 2022;127:775-82)

Screw-retained prostheses are widely applied in implant dentistry. The prosthesis can be either a 1-piece cast crown or a 2-piece prosthesis that comprises a titanium base on which a custom zirconia crown is cemented. This 2-piece prosthesis is termed a screw- and cement-retained prosthesis (SCRP). However, the abutment surface may be contaminated during fabrication in a dental laboratory, leading to mechanical and biological complications related to the implant treatment.[1-6] Steam is routinely used in a dental laboratory to clean prostheses and abutments, but the presence of contaminants and bacterial residues has still been reported on their surface.[1,2,7,8] Good cleaning is the premise of effective disinfection, and disinfection cannot replace cleaning to eradicate surface contaminants. Therefore, prostheses and abutments should be cleaned, disinfected, and under special circumstances, even sterilized before they are clinically connected to the implants.[9-13] For example, dental implants are typically sterilized by the manufacturer to ensure successful osseointegration. However, the importance of cleaning and disinfecting abutments, which are directly attached to the implants through the gingival margin, has

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Clinical Implications

Considering the risk of biological and mechanical complications, more attention should be paid to the surface contamination of SCRPs and appropriate cleaning before delivery. Ultrasonic cleaning with 75% ethanol represents an effective treatment option in clinical practice.

not drawn enough attention, and practices are inconsistent. Canullo et al[14] conducted a study involving 100 universities to evaluate cleaning, disinfection, and sterilization protocols for custom abutments. Of the respondents, 26.7% stated that no pretreating protocols were adopted, and only 2.4% stated that they performed all 3 procedures. Based on the current scientific evidence, a uniform cleaning standard for abutments does not exist. In comparison to abutments, SCRPs, which undergo more complex manufacturing steps, have a higher risk of being contaminated. Conventional methods of sterilizing abutments involve the use of an autoclave sterilizer, ethylene oxide, and disinfectants; however, such methods will complicate the working procedure and prolong treatment times. High temperature may also adversely affect a zirconia framework and bond strength.[15-18] Additionally, disinfectant concentration and soaking duration must be controlled, so the procedure is complicated, leading to poor compliance.[9] Industrial ultrasonic cleaning (with pure acetone solution, ethanol solution, and antibacterial cleaner for 10 minutes at 60 °C) and argon plasma treatment (with long-distance accelerated electrons and ions at a low temperature and under vacuum) are more efficient than steam treatment for removing contaminants and microorganisms from custom titanium abutments.[1] In addition, argon plasma treatment may activate the surface to facilitate cell attachment.[1,2,19-21] Despite the efficiency, industrial ultrasonic cleaning is not recommended for clinical practice because of the effect on the bond and the toxicity of the reagents, and the use of argon plasma is not widespread because the sophisticated equipment and technology is expensive.

Studies exploring methods of efficiently and conveniently cleaning and disinfecting SCRPs are sparse. Thus, the purpose of this clinical research was to compare 3 methods of removing contaminants and microorganisms present on the surface of SCRPs to determine the most effective method for clinical practice. The null hypotheses were that no differences would be found in the efficiency of the 3 methods to remove contaminants and microorganisms present on the surface of SCRPs.

MATERIAL AND METHODS

Ethics committee approval of this study was obtained from the Peking University School of Stomatology Biomedical Institutional Review Board (PKUSSIRB-201523068). Participants were provided with written and verbal information about the study, including the level of involvement required, the voluntary nature of participation, and the right to withdraw at any time. Written informed consent to participate was obtained, and assurances were given regarding confidentiality and privacy. The 1-unit SCRPs were obtained from the Fourth Clinical Division of Peking University School and Hospital of Stomatology between January 2018 and July 2018.

The 1-unit SCRPs were fabricated in the dental laboratory of Peking University School and Hospital of Stomatology. Specifically, 48 standard titanium abutments (SPI EASY; Thommen Medical AG) were used and/or customized as necessary. Zirconia crowns were fabricated with computer-aided design and computer-aided manufacturing (CAD-CAM) technology, glazed, and attached to the abutments by using a self-adhesive resin cement (RelyX U200; 3M ESPE). After 24 hours, SCRPs and their casts were steam cleaned at 4 MPa for 5 seconds, and they were then disinfected by placing them in an ozone and UV disinfection cabinet (ZYW-170 Z; Zhongyi) for 30 minutes.[22] Subsequently, the SCRPs were placed on the definitive casts, packed with bubble-wrap, and transferred to a clinic for evaluation and delivery.

The SCRPs were randomly divided into 3 groups by using the random number table method. For the wiping group, all internal and external surfaces of the specimens, including the emergence profile area, implant-abutment interface, and screw channel, were wiped with 75% ethanol. Each surface was wiped clockwise and counterclockwise 3 times for at least 10 seconds each until no visible stains were detected. The screw channel within the crown was cleaned by using cotton wrapped on the tip of an explorer. For the soaking group, the specimens were soaked in 75% ethanol for 5 minutes, and, for the ultrasonic cleaning group, the specimens were immersed in a 20-mL stainless-steel medicine cup containing 75% ethanol and then ultrasonically cleaned (BioSonic UC50; Coltène) in distilled water for 5 minutes at 60 °C.[23,24]

After these treatments, the specimens were rinsed with sterile normal saline and dried with an air stream. The presence of contaminants and microorganisms was determined both before and after the treatment. All procedures were performed by using sterile forceps and gloves to avoid contamination. All observers were trained for the analysis process and blinded to the treatment methods.

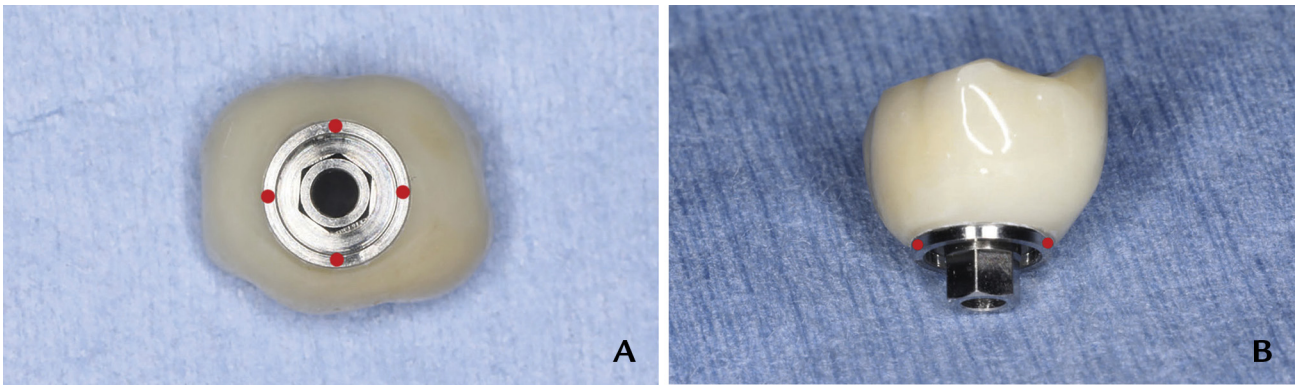

Figure 1. A, Four locations at implant-abutment interface (mid-buccal, mid-lingual, mid-mesial, and mid-distal). B, Two locations at emergence profile area (mid-buccal and mid-lingual).

Representative specimens were observed under a scanning electron microscope (SEM) (EVO-18; Zeiss) at ×50, ×150, and ×500 magnification. To evaluate contamination density, 4 locations at the implant-abutment interface (mid-buccal, mid-lingual, mid-mesial, and mid-distal) were observed (Fig. 1A). Two locations were selected on the mid-buccal and mid-lingual sides at the emergence profile area (Fig. 1B). Each location was observed and photographed by SEM at ×150 magnification to determine the presence of surface contaminants before and after treatment. The images were then exported into a graphics editing software program (Adobe Photoshop CS6; Adobe), and the brightness and contrast were adjusted until the clean and polluted areas were clearly distinguishable. A rectangular area (150×400 pixel) was used as the measuring space according to the reference marks. The images were then analyzed with an image-processing software program (Image-Pro Plus 6.0; Media Cybernetics, Inc). The polluted surface was determined by gray differences, and the contamination density (the ratio of the polluted surface area to the total surface area) was calculated for each image. The mean values of the contamination density at the implant-abutment interface (4 locations) and the emergence profile area (2 locations) were calculated. Typical contaminants at 2 regions were analyzed by using energy-dispersive X-ray spectroscopy (EDS) (Inca X-Act; Oxford Instruments) before treatment.

A sterile cotton swab was used to wipe the surface of the SCRP, including the implant-abutment interface, emergence profile area, and screw channel (clockwise and counterclockwise, 3 times). Subsequently, the sterile swab was washed in 1 mL of normal saline, which was present in a microcentrifuge tube; 100 μL of normal saline was added to BHI-sheep blood agar. Each specimen was plated on 5 culture dishes, and after air-drying, the dishes were incubated at 37 °C and 5% CO_2 for 72 hours. A solution obtained by soaking fresh carious extracted teeth was used as the positive control, while sterilized normal saline was used as the negative control. Colonies on each dish were counted, and the results were expressed as colony-forming units (CFUs)/abutment=the sum of the colony number on the 5 culture dishes×2.

Numerical variables were calculated as mean ±standard deviation. The Wilcoxon signed ranks test was used to compare the contamination density before treatment at the implant-abutment interface with that at the emergence profile area. The contamination density was compared before and after the treatment of each region in the 3 groups. After square root-arcsine transformation, analysis of covariance (ANCOVA) was used to compare the cleaning effect of the 3 treatment methods at the 2 regions. The contamination density before treatment was used as a covariate to compare the contamination density after treatment. The least significance difference (LSD) method was used for multiple comparison. In addition, the Wilcoxon signed ranks test was used to compare the number of bacterial colonies before and after treatment. Statistical analysis was performed by using a statistical software program (IBM SPSS Statistics, v22.0; IBM Corp). Hypothesis testing was based on bilateral tests (α=.05).

RESULTS

In total, 44 specimens (4 specimens were excluded because of missing data) and 254 locations (10 locations were excluded because the images had poor lighting) were included in the surface contamination evaluation. Before treatment, the presence of obvious contaminants was observed on most of the outer surface of the SCRPs, including the implant-abutment interface, emergence profile area, and antirotation feature (Fig. 2). The differences in the contamination density before treatment between at the implant-abutment interface and at the emergence profile area were statistically significant (P<.001) (Table 1). The components of contaminants

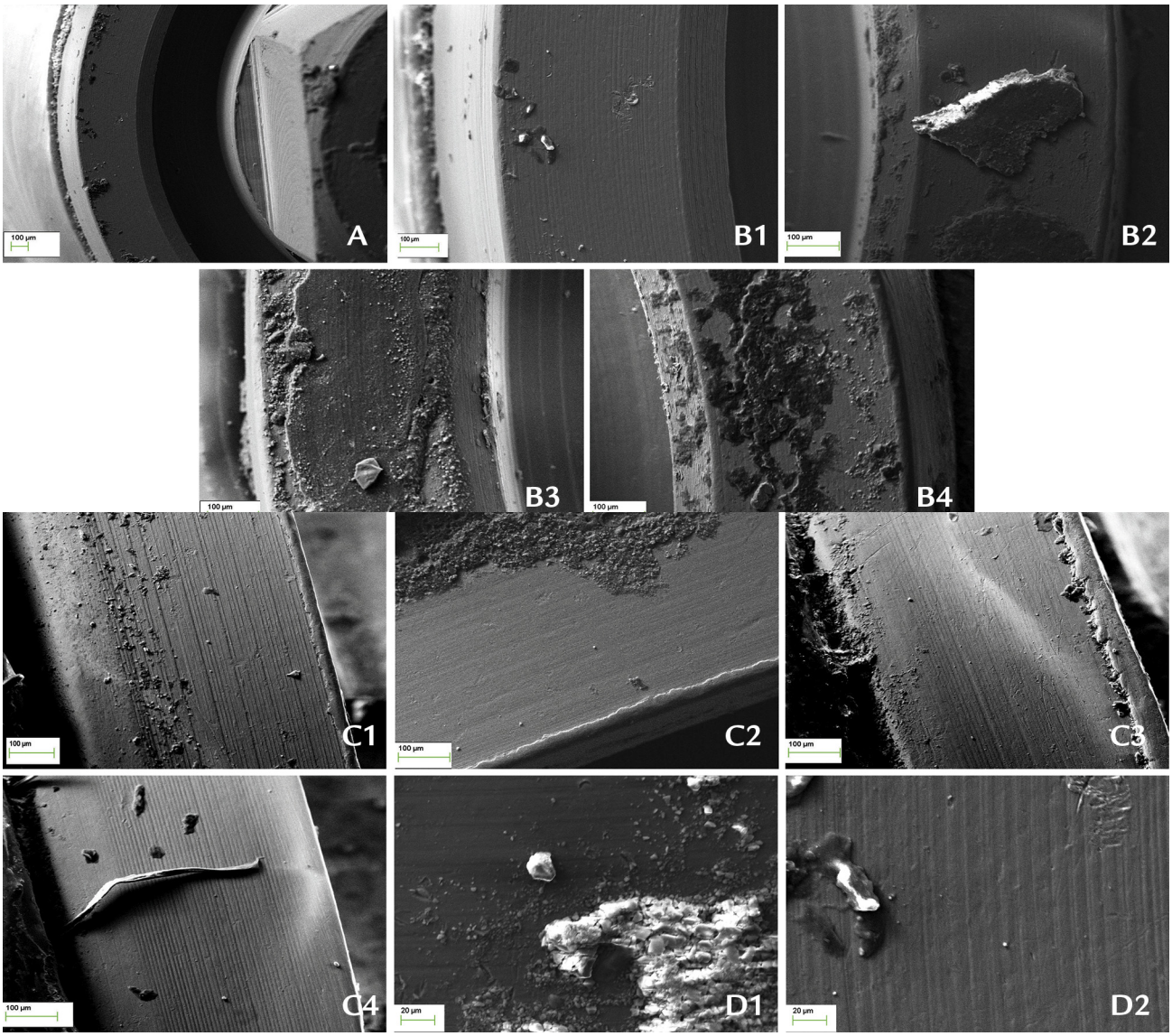

Figure 2. Obvious contaminants observed on surface of SCRPs before treatment. A, Outer surface of SCRPs, original magnification×50. B, Implant-abutment interface, original magnification×150. C, Emergence profile area, original magnification ×150. D, Outer surface of SCRPs, original magnification ×150. SCRP, Screw- and cement-retained prosthesis.

included carbon, oxygen, titanium, calcium, silicon, aluminum, magnesium, and sulfur. They were mainly derived from calcium carbonate, silicon dioxide, wollastonite, titanium, ferrous sulfide, aluminum oxide, and magnesium oxide (Fig. 3). Figure 4 shows the SEM images of each group before and after treatment.

Contamination density results before and after treatment at each region are shown in Table 2. The contamination density was significantly decreased after cleaning by using the 3 methods ($P \leq .001$). Significant differences were found among the 3 methods for the cleaning efficiency both at the implant-abutment interface ($P=.023$) and the emergence profile area ($P=.038$). In the case of the implant-abutment interface, the contamination density after treatment was lower in the ultrasonic cleaning group than that in the soaking group ($P=.007$), whereas in the case of the emergence profile area, the contamination density after treatment was lower in the ultrasonic cleaning group than that in the wiping group ($P=.019$) and the soaking group ($P=.048$). The differences in the contamination density after treatment among the other groups were not significant. Table 3 shows the number of

Table 1. Contamination density at 2 regions before treatment

Region	*n*	$\bar{\chi} \pm s$	*Z*	*P*
Implant-abutment interface	44	6.263 ±7.990	-4.009	<.001
Emergence profile area	44	1.581 ±1.592	—	—

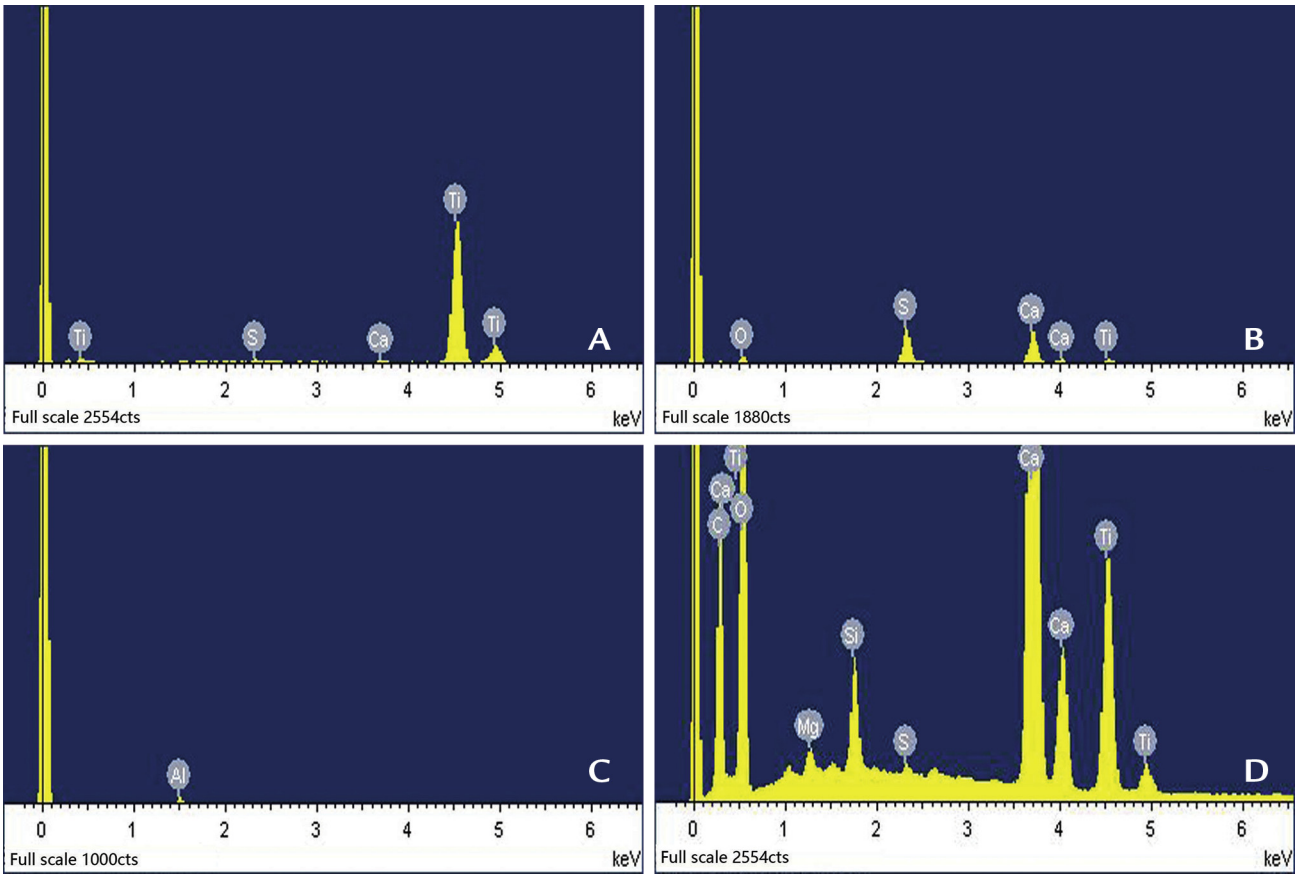

Figure 3. Components of contaminants. A, B, Elements of typical contaminants at implant-abutment interface. C, D, Elements of typical contaminants at emergence profile area.

locations with zero contamination density before and after treatment at the 2 regions.

In total, 43 specimens and 430 parallel dishes were included. The number of CFUs was determined for all specimens before and after treatment. No significant differences ($P>.05$) were found before and after treatment in the 3 groups (Table 4).

DISCUSSION

The results of this clinical study supported rejecting the hypothesis that there were no differences in the efficiency of the 3 methods to remove contaminants present on the surface of SCRPs but supported the hypothesis that there were no differences in the efficiency of the 3 methods to remove microorganisms for SCRPs previously disinfected by ozone and UV in a dental laboratory.

In the study, all the specimens were cleaned with steam before transferring them to a clinic. However, the SEM observations revealed that the surfaces still showed some signs of contamination, and the extent of contamination varied markedly among the specimens. The EDS results indicated that the contaminants may be cement, greasy substances, particles of gypsum, titanium, ceramic materials, or a combination of these. The contaminants on the abutment surface are mainly from 2 sources. One can be attributed to the fabrication process, during which contaminants such as titanium, carbon, and aluminum particles are usually generated. In addition, residues of agents used by technicians can cause contamination. Such debris and stains may exist on the surface, and their presence has been reported even after steam treatments.[1,2,7,8] With regard to SCRPs, processes such as acrylic resin scanning coping preparation, ceramic coating, adjusting, grinding, glazing, and cementing increase the possibility of residual materials, which may not only affect the accuracy of abutment placement and lead to mechanical complications such as screw loosening but also be deposited in the gingival margin,[3,4] thereby adversely affecting the attachment of soft tissues and causing biological complications around the implant.[1,2] Moreover, various processing steps in a dental laboratory and transportation may increase the risk of microbial contamination, potentially influencing the long-term stability of the implant.[5,6]

The extent of contamination at the implant-abutment interface was higher than that at the emergence profile area, which may be because the interface is more easily contaminated during processing. Other elements such as

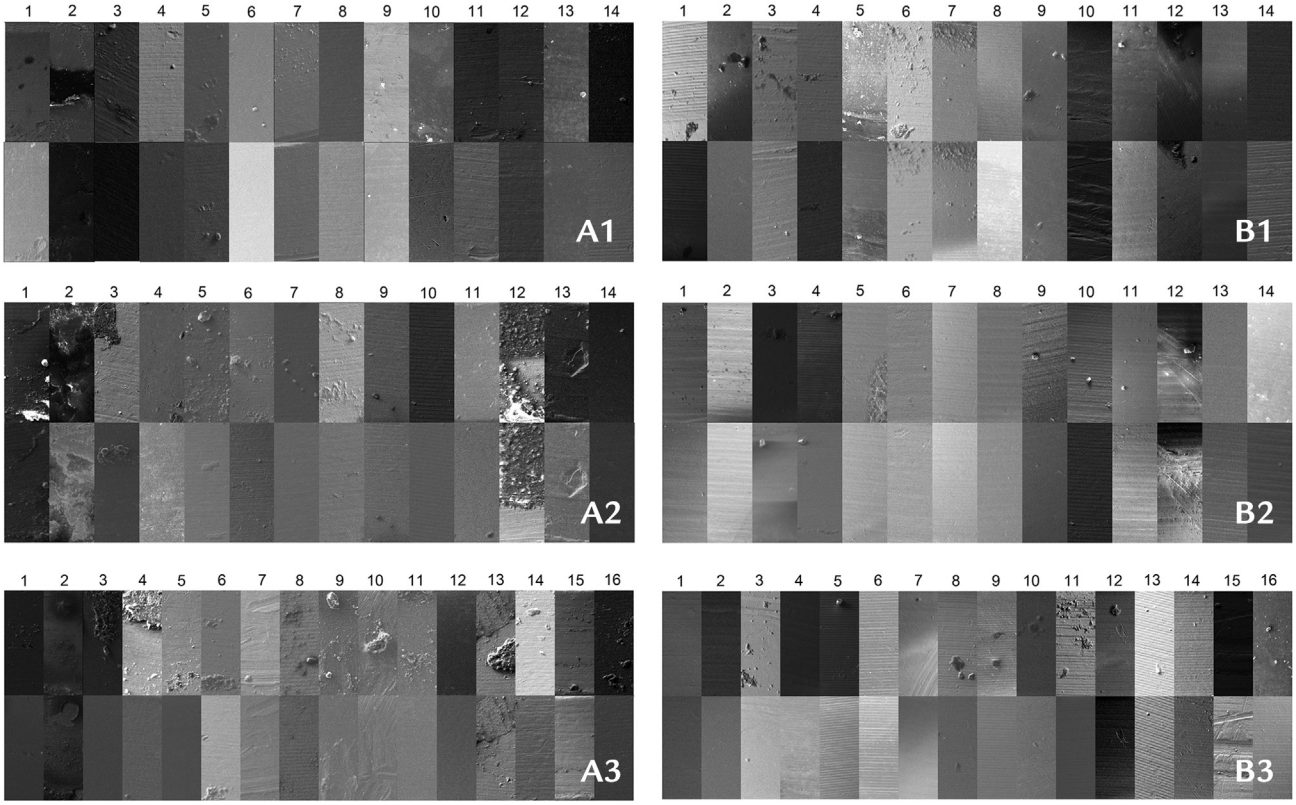

Figure 4. Images of each group before and after treatment. Original magnification ×150. A, Implant-abutment interface. B, Emergence profile area.

Table 2. Contamination density at 2 regions of 3 groups before and after treatment

		Implant-Abutment Interface			Emergence Profile Area		
Group	***n***	**Before Treatment**	**After Treatment**	***P***	**Before Treatment**	**After Treatment**	***P***
Wiping	14	0.051 ±0.066	0.022 ±0.066	.001	0.020 ±0.189	0.008 ±0.013[b]	.001
Soaking	14	0.048 ±0.063	0.027 ±0.042[a]	.001	0.010 ±0.011	0.003 ±0.004[b]	.001
Ultrasonic	16	0.086 ±0.101	0.041 ±0.077[a]	<.001	0.017 ±0.016	0.001 ±0.001[b]	.001
–		*F*=197.379, *P*=.023			*F*=18.488, *P*=.038		

[a]Significant difference between ultrasonic cleaning group and soaking group (*P*=.007). [b]Significant difference between ultrasonic cleaning group and wiping group (*P*=.019) and between ultrasonic cleaning group and soaking group (*P*=.048).

Table 3. Number of locations with zero contamination density at 2 regions of 3 groups before and after treatment

	Implant-Abutment Interface			Emergence Profile Area		
Group	***n***	**Before Treatment, *n*/%**	**After Treatment, *n*/%**	***n***	**Before Treatment, *n*/%**	**After Treatment, *n*/%**
Wiping	56	2/3.57	23/41.07	28	6/21.43	15/53.57
Soaking	64	3/4.69	17/26.56	32	3/9.38	11/34.38
Ultrasonic	68	1/1.47	36/52.94	34	1/2.94	10/29.41
Total	188	6/3.19	76/40.43	94	10/10.64	36/38.30

Table 4. Number of colony-forming units of 3 groups before and after treatment

Group	***n***	**Before Treatment**	**After Treatment**	***P***
Wiping	14	1.357 ±3.875	0.750 ±1.312	.916
Soaking	13	0.846 ±1.519	0.539 ±1.450	.317
Ultrasonic	16	2.719 ±3.011	0.813 ±1.315	.072

screw channels and grooves around the antirotation feature were not included in the analysis, but stains were observed, and these locations may be difficult to clean because of their complex structures.

Among all types of contamination on the surface of SCRPs, particles are the easiest to clean. If greasy materials are present, specific concentrations of organic solvents should be used for a specific duration, while excess cement is difficult to remove after polymerization. The results of the present study suggested that soaking, wiping, and ultrasonic cleaning with 75% ethanol were efficient cleaning methods, as the specimens in the 3 groups showed fewer surface contaminants after treatment. However, all 3 methods showed limited effectiveness in removing resin cement, as was evident from

the SEM images. When bonding a crown to an abutment in a dental laboratory, excess cement should be examined and removed under a microscope before it has polymerized.

In some specimens, the contamination density increased after soaking, particularly in some restorations with large stained areas. It appears that soaking has little effect on removing large stains mixed with grease or cement. Wiping can remove most visible and accessible stains, but it is ineffective for cleaning complex structures such as screw channels and grooves. Moreover, the efficiency of wiping depends on the meticulousness of the operator. Ultrasonic cleaning, however, takes advantage of the strong cavitation and vibration effects generated by ultrasonic waves to eradicate surface stains. This method can also degrade and emulsify greasy contaminants, and the ultrasonic waves can reportedly reach any part of a work piece, including screw channels and grooves.[23,24] The present study determined that ultrasonic cleaning combined with 75% ethanol treatment can ensure higher cleaning efficiency at the implant-abutment interface and the emergence profile area.

In the present study, the SEM analyses after treatment showed the potential presence of contaminants on the surface of SCRPs even after subjecting the specimens to different treatment methods. In particular, cement and some large stains could not be completely removed by the 3 methods. Therefore, a combination of methods or other detergents should be used. Alternatively, the duration of ultrasonic cleaning may be increased to 10 minutes to improve cleaning efficiency.

All the specimens in this study, both before and after treatment, met the requirements of high-level disinfection after ozone and UV disinfection in the laboratory.[10-13] The number of CFUs decreased after treatment with the 3 methods, but there was no statistical significance. Nevertheless, the ozone and UV disinfection, which is not used in every laboratory, may have contributed to the negative results before treatment. In addition, in other studies, colonies of gram-positive bacteria have been found on custom abutments from dental laboratories.[1] Therefore, the possibility of microbial contamination in a dental laboratory because of the operational environment and various procedures cannot be excluded in spite of quality control measures. Limitations of the present study included the small sample size, and the most efficient method of disinfecting SCRPs remains to be confirmed. Further studies are needed to establish a suitable disinfection protocol for SCRPs.

CONCLUSIONS

Based on the findings of this clinical study, the following conclusions were drawn:

1. Contamination was found on the surface of 1-unit SCRPs from a dental laboratory, and cleaning methods in addition to steam treatment should be used before delivery.
2. Wiping, soaking, and ultrasonic cleaning were efficient at reducing the contaminants, and ultrasonic cleaning combined with 75% ethanol treatment yielded the most favorable results.
3. None of the 3 methods provided additional disinfection for SCRPs previously disinfected by using ozone and UV in a dental laboratory.

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