Comparison of different cleaning procedures to decontaminate zirconium oxide surface after polishing

Evelina Gineviciute

Jonas Alkimavicius
Department of Dental and Oral Pathology, Lithuanian University of Health Sciences, Kaunas, Lithuania; Vilnius Research Group, Vilnius, Lithuania. Address: Eiveniu 2, Kaunas, Lithuania

Rolandas Andrijauskas
Dental technician, private practise, Vilnius, Lithuania.

Danas Sakalauskas
Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania. Address: Naugarduko 24, Vilnius, Lithuania

Laura Linkeviciene
Associate Professor, Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania; Vilnius Research Group, Vilnius, Lithuania; Vilnius Implantology Center, Vilnius, Lithuania. Address: Zalgirio 117, Vilnius, Lithuania

Corresponding author:
Linkevicius Tomas DDS, Dip Pros, PhD
Professor, Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania; Vilnius Research Group, Vilnius, Lithuania; Vilnius Implantology Center, Vilnius, Lithuania. Address: Zalgirio 117, Vilnius, Lithuania
Email: linktomo@gmail.com
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Abstract

Purpose: To evaluate the efficacy of cleaning protocol in decontamination of organic compounds from polished zirconium oxide samples. Materials and Methods: A total of 24 rectangular plate specimens were sintered from zirconium oxide. All samples were polished with commercially available polishers (course, fine, and superfine) and polishing paste. During the first step of the protocol, all specimens were cleaned with steam. After that, samples were randomly assigned to one of three groups (n = 8 each): A, B, and C. In group A, no additional cleaning was performed, while specimens of group B underwent an ultrasonic cleaning in distilled water. Group C specimens were cleaned in an ultrasonic bath with a special detergent solution. After washing, samples were subjected to energy-dispersive x-ray spectroscopy (EDX) and scanning electron microscope examination. In order to detect organic materials, the level of carbon atoms was measured. Results: EDX analysis revealed that samples in group A had the highest percentage levels of carbon atoms (9.57 ± 3.67) on the surface compared to the other cleaning protocols. The group B cleaning protocol resulted in lower carbon levels (4.73 ± 3.56), but the difference was not significant from only steam-blasted ones (P = .439). All specimens in group C had no detectable carbon atoms (0), which implies that all wax molecules had been
removed ($P < .05$). **Conclusion:** Only following the group C cleaning protocol can total decontamination of the polished zirconium surface from organic compounds be expected. Therefore, it is advised to employ an ultrasonic bath with detergent solution for cleaning procedures of zirconium abutments before delivery. *Int J Prosthodont* 2021. doi: 10.11607/ijp.6896

**Introduction**

It is well known that the integrity of peri-implant soft tissue seal is mandatory for a long-term success of dental implants 1. The condition of soft tissues around implants depends on many factors, including properties of prosthetic abutment. With the advancement of milling technologies, customized zirconia abutments became more popular due to superior mucogingival esthetics 2-4, durability, simplified CAD-CAM production and biological advantages 5,6. However, manufacturing processes, like milling and polishing, inevitably result in contamination of produced abutments 7,8. This contamination might take its biological toll, as it was shown, that even micron-sized debris particles can stimulate immune response in hard tissues 9. Simultaneously, microbiological pollution of the abutment surfaces has been observed 7. Organic and inorganic debris might compromise not only biological soft tissue seal 10, but implant-abutment connection as well, resulting in screw loosening and increased microbial leakage at the microgap 11. Besides polishing rubbers, processing of custom zirconium abutment may involve the use of special polishing paste to achieve ultra-smooth surface. Polishing pastes contain various waxes, which, if not removed, might harbor bacteria, potentially causing the inflammatory response of the tissues. Logic dictates that some kind of cleaning must be done prior seating the abutment on the implant. It has been shown that there is a considerable
heterogeneity in abutments’ treatment before clinical use\textsuperscript{12}. Steam vapor cleaning is widely used; however, several papers have shown that steaming is not effective enough to remove all contaminants form surfaces \textsuperscript{7,8,13}. Furthermore, Canullo and co-workers showed that implants, restored with steam-cleaned titanium abutments had more bone loss compared to those treated with argon plasma \textsuperscript{14-17}, putting generally in question the use of steam as a method for cleaning. An ultrasonic bath with distilled water is suggested as another procedure to clean abutments after laboratory work \textsuperscript{18,19}. Several in vitro studies have shown that ultrasonic cleaning is an effective method \textsuperscript{7,13,20,21}. High frequency sound waves generated in an aqueous medium mechanically clean the surface, but up to date there is no standardized protocol for the procedure, as there are various suggestions regarding the medium, temperature and duration of the process. Moreover, studies addressing the removal of polishing paste remnants are lacking. It is obvious that an easy and clear abutment cleaning protocol is required, which could be used by ordinary dental lab, as sophisticated protocol with argon laser is less likely to be implemented.

Therefore, this research was carried out to evaluate the efficacy of different cleaning protocols of zirconia samples to remove remnants of the commercially available polishing paste composed of silica and aluminum particles embedded in wax filler. Focus was given to organic residues i.e. waxes. Null hypothesis was formulated that there is no difference between different cleaning protocols of polished zirconia samples.

**Materials and Methods**

For this in vitro experiment, 24 rectangular specimens of predetermined size (10x10x3mm) were milled from zirconium oxide (Lava Classic 3M ESPE). All samples were polished using diamond impregnated polishing system (NTI CeraGlaze, Kerr, Switzerland) and polishing paste
(Zirkopol, Feguramed, Germany) using protocol, suggested recently by Linkevicius et al. Polishing procedures were carried out under a microscope (ZEISS EyeMag Pro, ZEISS) at x 3.2 magnification. Coarse green polisher (P301) for pre-polishing wheel was used for 2 minutes at 16000 rpm (rotations per minute), fine blue polisher (P3001) to refine - for 1 minute at 12000 rpm and superfine yellow polisher wheel (P30001) to reglaze-high shine - for 1 minute at 6000 rpm. Finally, goat-hair brush and diamond polishing paste (Zirkopol, Feguramed, Germany) was used on all specimens for 1 minute at 15000 rpm. Polishing materials and sequence is summarized in the Figure 1. All instrumentation was carried out following manufacturers’ instructions by a professional dental technician imitating regular zirconia processing. All samples were randomly assigned to one of the 3 groups (n=8): A, B or C. Then each sample was blasted with steam vapor for 30 seconds at 4 MPa at around 5 cm distance. Group A specimens after steam cleaning were immediately put in sterile tubes and taken for subsequent analysis to serve as a control. Group B samples after steam cleaning underwent treatment in an ultrasonic bath with distilled water for 10 min at 50°C temperature. In Group C specimens were cleaned for 10 min in an ultrasonic bath with detergent for ultrasonic polishing paste cleaner (Siladent, Dr. Bohme & Schops GmbH, Germany) at 50°C temperature. Detergent contained 2-(2-Butoxyethoxy) ethanol, Benzenesulfonic acid, mono-C10-13-alkyl derivatives, compounds with ethanolamine. The solution was prepared by adding 1 part of detergent cleaner and 20 parts of distilled water. After removing from ultrasonic bath Group C samples were washed with distilled water for 1 minute. All specimens during cleaning procedures were handled with clean sterile tweezers. Immediately after cleaning all samples were dried using nitrogen and subjected to scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDX) analysis using scanning electron microscope (Hitachi TM3000, Hitachi, Japan). At the moment of
analysis accelerating voltage were 15 keV, magnification of 1000, area of measurement 3777 micrometres. Each sample was scanned 3 times at three different locations: approximate middle, approximately 1 mm right from middle area and approximately 1 mm left from middle area. Out of these three measurements mean atomic % of carbon was calculated for each sample for quantitative assessment. SEM images were captured as well for qualitative visual inspection. Statistical analysis was done using IBM SPSS Statistics 20 (IBM Corp). Mann-Whitney U tests with Bonferroni correction were utilized to detect differences between groups. Significance level was set to $\alpha = 0.05$.

**Results**

According to EDX analysis, samples cleaned only with steam (Group A) had highest levels of carbon atoms on the surface (Table 1), meaning that samples were contaminated with wax of polishing paste. The cleaning of the samples ultrasonically with water for 10 mins (Group B) removed part of the molecules containing carbon (Table 1), yet the difference was not significant from steam processed plates ($p = 1$) (Table 1). Only samples treated ultrasonically with detergent (Group C) had no detectable carbon atoms on the surface and differed significantly from A group ($p = 0.002$) and B group ($p = 0.036$), (Figure 2, Table 1). Visual inspection of SEM images showed stains and/or foreign bodies on A group samples (Figure 3A). No foreign bodies were observed in group B, but stains were still present (Figure 3B). Group C specimens were visually free from stains or other impurities (Figure 3C). EDX spectrums are presented in Figure 4. Spectrums of all samples presented peaks of aluminum (Al) and silicon (Si) as these elements might be found in yttria-stabilized zirconia ceramics.

**Discussion**
The aim of the study was to find out how effective is the use of ultrasonic bath with distilled water and special detergent in removing organic debris from polished zirconia plates. The results have shown that it is possible to completely remove organic remnants with the use of C protocol technique, and based on this result, the null hypothesis was rejected. In addition, there was no statistical difference between steam cleaning and ultrasonic bath with distilled water, indicating that distilled water cleaning is as ineffective as single steam-cleaning.

Smooth zirconia surface is the ultimate goal of polishing procedure, meant to prepare the material for contact with peri-implant soft tissues. As a rule, zirconia CAD-CAM abutments arrive from milling centers in a rough state, not suitable for exposure to peri-implant tissues. Thus, certain amount of polishing is necessary before abutment is delivered to the implant. Of course, the level of polishing might be different, ranging from 0.2 to 0.06 \( \mu \text{m} \) of Ra, as different opinions exist regarding the smoothness of abutments. Quirynen et al. has shown more soft tissue recession on ultrapolished titanium abutments, compared to medium smoothness; however Bollen et al. showed no statistical difference in probing depth between ultrapolished zirconia and moderately polished titanium abutments. In either case, smoothening of zirconia results in polishing paste being rubbed into the surface. Polishing paste remnants, mostly composed of wax filler, might harbor bacteria on the subgingival prosthetic parts which could potentially cause the inflammatory response of the tissues leading to peri-implant bone loss, when the abutment is seated on the implant. It could be argued that use of polishing paste, as the last step of this protocol, may not be necessary, as there is no reliable evidence, that ultrapolished zirconia has any clinical advantages over less smooth state of this material. This would reduce the contamination, as most of contaminants come from waxes in polishing paste. From the other
hand, this does not reduce the importance of sufficient cleaning after polishing, as pollution can originate from rubber wheels as well.

Within awareness of the authors, up-to-date there are no studies specifically addressing the contamination of the custom abutments after processing with organic compounds, such as waxes. EDX was elaborated in this study to detect any contaminants containing carbon atoms. Carbon is one of the main components in organic substances that may be of different origin: bacterial, polishing paste or other, such as fingerprints. However, we are inclined that most of the carbon pollution on the abutment comes from polishing paste, where waxes are used as filler for abrasive particles. During polishing procedure, the waxed might get melted into small irregularities of the surface which might lead to detection and removal of waxes unpredictable.

Results of this study have confirmed the fact, that steam vapor cleaning is not sufficient to remove organic contaminants, which is in accordance with other in vitro studies\textsuperscript{7,8,13}. It is interesting to note, that ultrasonic treatment only with distilled water is not adequate in complete removal of polishing paste remnants as well. This might be due to the fact that waxes are hydrophobic and do not dissolve in the water, thus the detergent solution, which is specifically produced to resolve organic waxes, have increased the efficacy of the cleaning significantly. Canullo et al. showed samples free of contaminants in SEM visual analysis after cleaning in ultrasonic bath with three different solutions - antibacterial detergent (Cl 4%), pure acetone and pure ethyl alcohol\textsuperscript{7}. However, Gehrke et al. used the same ultrasonic cleaning protocol, but found small amounts of residual particles of zirconium, carbon, oxygen, aluminum and hafnium in EDX analysis\textsuperscript{13}. According to the authors, aluminum and hafnium elements were components of the polishing paste used in that study, however the origin of the carbon atoms was not discussed. Another experiment by Farronato and coworkers revealed that ultrasonic cleaning at
60°C in 4% sodium bicarbonate for 5 min, 2 bathes of 15% nitric acid for 15 min each and pure acetone for 10 min with subsequent autoclave could decontaminate 99.99% of the analyzed surface with SEM, yet EDX analysis was not performed. It could be speculated that organic contaminants such as waxes are not necessary visually detected by SEM and EDX analysis should be elaborated for analyzing cleaning efficacy. Furthermore, specific polishing paste cleaner might be needed to remove organic debris entirely as show in this study.

While ultrasonic treatment is effective in mechanically removing debris from the surface, it is not capable to eradicate all microorganisms. Thus, it is suggested to sterilize abutments in an autoclave after ultrasonic cleaning. From the other hand, autoclaving might compromise base-abutment cement strength in custom made zirconia restorations and cause decementation. In addition, there is evidence that sterilization procedure alters the surface of the titanium to the level that it might inhibit soft tissue integration or even change zirconia surface properties. Therefore the sterilization of zirconia abutments should be recommended with caution.

Some researchers add special antibacterial solution to ultrasonic bath to disinfect abutments. Canullo et al reported no bacterial growth after disinfection with Cl 4% solution in an ultrasonic bath; however whether abutments become sterile after such treatment is not clear. Recently argon plasma cleaning was introduced as an efficient method for cleaning and disinfection of prosthetic devices. Moreover, some studies suggested that it may improve cell adhesion to the surface. Yet, implementation of argon plasma cleaning for implant abutments is costly and hardly imaginable in daily clinical practice so far, because the abutment should be seated on the implant immediately after cleaning. On the other hand ultrasonic cleaning with detergents seems approachable and effective method easily applied to daily clinical practice.
Current study used sample size of 8 specimen in each group, which is similar to other in vitro studies on the subject. Gerkhe et al \textsuperscript{13} used 6, while Cannulo et al \textsuperscript{7} studied 10, and Mehl with co-workers\textsuperscript{20} settled with 5 discs in each of the tested groups. This example clearly demonstrates, that still there is lack of consensus, which number is the most appropriate.

In the current study Hitachi TM3000 microscope was used to detect organic pollution on the samples. This device allowed analyzing samples without applying layer of conductor on the surface of specimens, in such manner collecting more precise data. However the drawback was low resolution images which impaired detailed qualitative analysis of the samples. Furthermore, in this study, no other elements were measured in the EDX analysis, as the focus was given to organic remnants. Another limitation of the study was that the rectangular plates of zirconium were used instead of spherical prosthetic abutments.

**Conclusions**

Within limitation of this in vitro study, it can be concluded, that samples cleaned ultrasonically with detergent had no detectable carbon atoms on the surface, implying that all wax organic molecules have been removed. In addition, results indicate that cleaning with air steam or water in ultrasonic bath is not an effective tool to remove organic debris from zirconia plates.

**Acknowledgements.**

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**Abbreviations.**

EDX - Energy-dispersive X-ray spectroscopy  
SEM - scanning electron microscope
Trade names.

Lava™ Classic (3M ESPE AG, Germany).
NTI® CeraGlaze® (Kerr, Switzerland).
ZEISS EyeMag Pro (ZEISS, Germany)
Zirkopol (Feguramed, Germany).
Siladent (Dr. Bohme & Schops GmbH, Germany)
Hitachi TM3000 (Hitachi, Japan)
IBM SPSS Statistics 20 (IBM Corp, USA).

References


**Figure legends**

**Figure 1**

Polishing protocol, sequence, tools and polishing parameters.

**Figure 2**

Box plots of the carbon atomic percentage in the groups. It shows no statistically significant difference between A/B groups ($p = 0.439$), and statistically significant difference between A/C groups ($p=0.018$), and B/C groups ($p=0.037$) (Mann-Whitney U-test, significance $\alpha = 0.05$).

**Figure 3**
SEM images of the groups. (A) visually full of contaminant after steam vapor, (B) cleaner surfaces after distilled water ultrasonic bath; (C) no visual contamination after use of ultrasonic bath with detergent.

Figure 4.

Analysis of EDX spectrum. A and B specimens do contain carbon, while C samples are carbon-free. Aluminum (Al) and silicon (Si) are present in all plates, as these elements might be found in yttria-stabilized zirconia ceramics.
<table>
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<th>Polisher</th>
<th>Green CeraGlaze (P301)</th>
<th>Blue CeraGlaze (P3001)</th>
<th>Yellow CeraGlaze (P30001)</th>
<th>Goat-hair brush with Zirkopol polishing paste</th>
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<td>Parameter</td>
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<td>12000 rpm, polishing time 1 min</td>
<td>6000 rpm, polishing time 1 min</td>
<td>15000 rpm, polishing time 1 min</td>
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Fig. 1
Fig 2.
Fig 3.
Fig. 4.
Table 1. EDX analysis of carbon atoms. Exact significance with Bonferroni correction: * - \( p = 0.1 \), ** - \( p = 0.036 \), *** - \( p = 0.002 \)

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