

# Microbiological cleaning and disinfection efficacy of a three-stage ultrasonic processing protocol for CAD-CAM implant abutments

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**PURPOSE.** Computer-aided design and manufacturing (CAD-CAM) of implant abutments has been shown to result in surface contamination from site-specific milling and fabrication processes. If not removed, these contaminants can have a potentially adverse effect and may trigger inflammatory responses of the peri-implant tissues. The aim of the present study was to evaluate the bacterial disinfection and cleaning efficacy of ultrasonic reprocessing in approved disinfectants to reduce the microbial load of CAD-CAM abutments. **MATERIALS AND METHODS.** Four different types of custom implant abutments (total N = 32) with eight specimens in each test group (type I to IV) were CAD-CAM manufactured. In two separate contamination experiments, specimens were contaminated with heparinized sheep blood alone and with heparinized sheep blood and the test bacterium *Enterococcus faecium*. Abutments in the test group were processed according to a three-stage ultrasonic protocol and assessed qualitatively and quantitatively by determination of residual protein. Ultrasonicated specimens contaminated with sheep blood and *E. faecium* were additionally eluted and the dilutions were incubated on agar plates for seven days. The determined bacterial counts were expressed as colony-forming units (CFU). **RESULTS.** Ultrasonic reprocessing resulted in a substantial decrease in residual bacterial protein to less than 80 µg and a reduction in microbiota of more than 7 log levels of CFU for all abutment types, exceeding the effect required for disinfection. **CONCLUSION.** A three-stage ultrasonic cleaning and disinfection protocol results in effective bacterial decontamination. The procedure is reproducible and complies with the standardized reprocessing and disinfection specifications for one- or two-piece CAD-CAM implant abutments. [J Adv Prosthodont 2022;14:273-84]

## KEYWORDS

CAD-CAM abutments; Contamination; Disinfection; Bacterial decontamination; Ultrasonic cleaning

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## INTRODUCTION

Dental implant abutments are part of the prosthetic superstructure with direct contact with the surrounding oral tissues. In addition to prefabricated stock abutments, computer-aided design and manufacturing (CAD-CAM) enables the fabrication of customized abutments.<sup>1</sup> They facilitate the compensation of axial divergences between the implant and the corresponding crown as well as the individualization of the abutment shoulder with an anatomical emergence profile for implant-supported single-tooth restorations.<sup>2,3</sup> The peri-implant mucosa is commonly known as hypovascular and hypocellular scar tissue. Immunologically, it is inferior to the periodontal tissue around teeth, as it exhibits lower resistance to bacterial induced infections.<sup>4</sup> Surface properties of the abutment such as topography, roughness, hydrophilicity, surface energy, contaminants, and macromolecular conditioning affect the biological response at the hard- and soft tissue interface in multiple ways.<sup>5</sup> Hence, recurrent hazards with potential negative impact on the attachment of the peri-implant tissues should be prevented.<sup>6,7</sup> CAD-CAM production for custom abutments have been proven to result in surface contamination from microwear particulates, cooling lubricants, and general laboratory debris.<sup>8,9</sup> Analysis revealed contamination on their outer and inner surfaces following laboratory procedures.<sup>10,11</sup> The presence of these micro-residues at the critical abutment-tissue junction may provoke inflammatory responses of the peri-implant tissues and mechanically compromise the stability of the implant-abutment junction.<sup>12</sup> Cleaning and subsequent disinfection of the abutment surface is therefore mandatory. European health regulations and the guidelines of the American Dental Association (ADA) both approved cleaning and disinfection regimens for semi-critical medical devices, including CAD-CAM implant abutments.<sup>13,14</sup> This refers to either dry heat sterilization, steam treatment of the components under pressure at 134°C (autoclaving) or ultrasonic cleaning by means of approved disinfectants. While heat-stable metallic implant abutments (e.g. titanium or titanium nitride) can be safely autoclaved without compromising their material properties,<sup>15</sup> sterilization of mono-

type ceramic or hybrid abutments under moist heat and pressure may lead to permanent damage to the crystal-ceramic framework (degradation) or to the adhesive bond of hybrid abutments and is therefore controversially discussed.<sup>16-19</sup> In addition, it should be noted that the physical process of sterilization by autoclaving using a combination of appropriate heat and pressure is able to kill all viable forms of microbiota, but it cannot effectively remove particulate debris from CAD-CAM abutments. The reprocessing of implant abutments by steam cleaning in the laboratory (vaporization), although frequently employed, is an ineffective method and fails to achieve the normatively required efficacy of disinfection (DIN EN 14885:2018).<sup>20,21</sup> In addition to cleanliness, the surface topography within the submucosal region of the abutment structure should be considered. Roughness, wettability and surface energy are important properties in this context. These parameters exhibit some correlation, as roughness can strongly influence wetting behavior.<sup>22</sup> It can be assumed that there is a critical threshold for surface roughness, where the accumulation of bacteria and plaques is low, at the same time best supporting the attachment of fibroblasts and the adaptation of the peri-implant mucosa.<sup>23</sup> One of the novel cleaning techniques is plasma pretreatment of implant abutments, which has been the subject of promising pilot studies.<sup>24,25</sup> However, it should be considered that this is not a validated cleaning method for laboratory-fabricated implant prosthetic components.<sup>14,26</sup> Due to the lack of legal validation and the fact that plasma devices are not widely used in dental laboratories or practices,<sup>21</sup> the primary focus should be on cleaning methods with a realistic relationship between the technical efforts and costs of the devices and a reliable cleaning efficacy.

Alternatively, recent investigations have demonstrated that a validated three-step ultrasonic cleaning procedure significantly reduces surface contamination of monotype and hybrid CAD-CAM abutments without adversely affecting their tensile bond strength.<sup>9,19</sup> The abutments are cleaned in three successive ultrasonic baths, one with an antibacterial cleaning solution, the other with 80% ethyl alcohol and finally with medically pure water (Cleaning System; Bredent GmbH & Co. KG, Senden, Germany) at 30°C for 5 min each,

yielding a reduction in deposit particles and organic and inorganic contaminants.<sup>10</sup> While qualitative and semi-quantitative effectiveness in removing these contaminants from CAD-CAM-fabricated surfaces has been demonstrated by microscopic and chemical analyses,<sup>9,10</sup> data on the disinfection efficacy of the three-step ultrasonic procedure are lacking. Therefore, the aim of the present study was to evaluate its potential for ultrasonic disinfection to reduce the microbial load of CAD-CAM implant abutments. The null hypothesis tested was that the three-step ultrasonic cleaning protocol results in a normatively required reduction of residual protein  $\leq 80 \mu\text{g}$  per sample<sup>27</sup> and a decrease of microbiota by at least 5 decadal logarithms (lg) of colony-forming units (CFU) relative to the positive control and is therefore suitable for disinfection of implant abutments.

## MATERIALS AND METHODS

The laboratory procedures for sample production have been described in detail in a previous publi-

cation.<sup>10</sup> Briefly summarized, a total of 32 CAD-CAM implant abutments were virtually constructed (Implant Studio; 3Shape, Copenhagen, Denmark) and fabricated (CADAbut F and CADAbut D; BEGO Implant Systems GmbH & Co. KG, Bremen, Germany). The master cast of a clinical case involving the replacement of the right maxillary central incisor with an implant (Semados SCX D 4.1/L 11.5; BEGO Implant Systems GmbH & Co. KG, Bremen, Germany) served as the basis for the digital abutment design. The geometry of the virtual design had uniform dimensions of 10.5 mm height and 6.5 mm shoulder width for all abutment specimens. According to AAMI TIR30:2011/R2016, 3 test samples per test setup are required. Due to the validation of the reprocessing efficacy of the process according to EN ISO 17664, it was decided to test representative abutments of 4 different types with a sample size of 3 per setup. The 32 CAD-CAM abutments were divided into four groups (n = 8 each) depending on the material used and CAD-CAM manufacturing process (Fig. 1): monotype abutments (one-piece) and hybrid abutments (two-piece). Lab-pro-

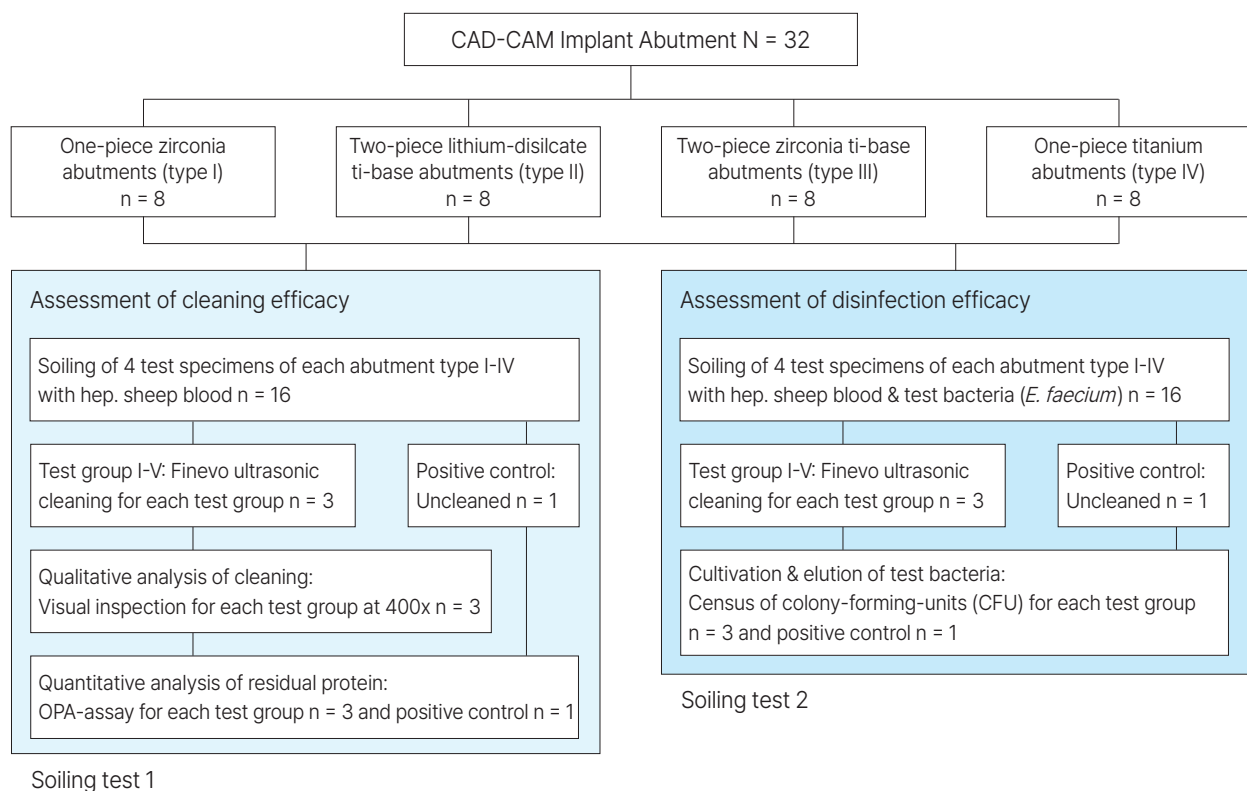


Fig. 1. Study design.

cessed materials for monotype abutments included zirconia and titanium, while hybrid abutments were comprised of zirconia or lithium disilicate CAD-CAM copings that were bonded to prefabricated titanium bases (Ti-Base D 4.1 mm) (Semados SCX; BEGO Implant Systems GmbH & Co. KG, Bremen, Germany). Accordingly, the following four material groups were employed for testing: one-piece zirconia abutments (type I), two-piece lithium disilicate (LDS) meso-abutments bonded to titanium bases (type II), two-piece zirconia meso-abutments bonded to titanium bases (type III), and one-piece titanium abutments (type IV) (Fig. 2). A list of materials and manufacturers can be found in Table 1. The bonding surfaces of the titani-



**Fig. 2.** Samples of the investigated CAD-CAM abutment types I-IV from left to right. Type I: zirconia monotype abutment (CADAbut F, BeCe CAD Zirconia XH), type II: lithium disilicate hybrid abutment (CADAbut D, IPS e.max CAD LT on Ti-Base), type III: zirconia hybrid abutment (CADAbut D, Zirconia LT CAD on Ti-Base), type IV: titanium monotype abutment (CADAbut F, CAD Titanium).

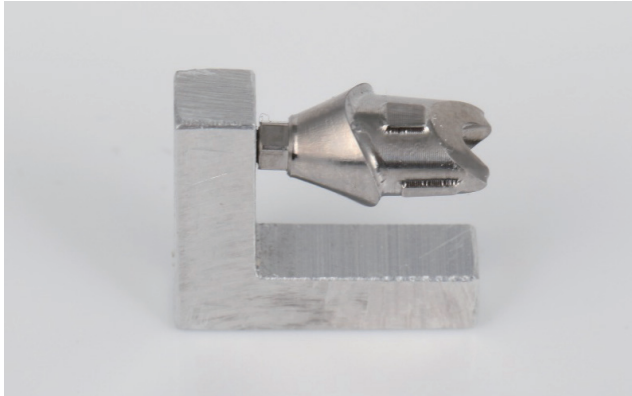
um inserts and the ceramic copings of the two-piece hybrid abutments were grit-blasted (aluminum oxide particles 50  $\mu$ m; 2 bar/0.25 MPa; 10 s; distance 10 mm) and cleaned with ethanol. Afterwards, the titanium inserts were moistened with a metal primer (GC Metal Primer II; GC EUROPE N.V, Leuven, Belgium), while a bonding agent (Monobond Plus; Ivoclar Vivadent, Schaan, Liechtenstein) was applied to the basal portion of the ceramic copings. Each hybrid abutment was luted with a dimethacrylate/hydroxyethyl methacrylate (DMA/HEMA)-based cement (Multilink Implant; Ivoclar Vivadent, Schaan, Liechtenstein) as recommended by the manufacturer. The excess cement was removed and the adhesive joint was polished with silicone polishers and polishing paste according to a previously documented protocol.<sup>28</sup> Upon completion of a defined 3-step ultrasonic cleaning protocol, all test abutment types I-IV were evaluated in terms of both cleaning and disinfection effectiveness.

To test cleanability, in a first trial a test soil was prepared from heparinized sheep blood (Fiebig Xebios Diagnostics GmbH, Düsseldorf, Germany, lot number 31406500/01) diluted 1:5 with 0.85% NaCl and reactivated with protamine and 1% mucin according to DIN EN ISO 15883-5<sup>29</sup> and AAMI TIR 30:2011.<sup>30</sup> For contamination, four test specimens of each abutment type (type I-IV, total n = 16) were immersed in the test soil and subsequently dried for one hour at  $22 \pm 2^\circ\text{C}$  under laminar room air.

After soiling, three test specimens per abutment type were subjected to a three-stage ultrasonic cleaning protocol using ultra-high frequency waves in combination with disinfecting agents (Finevo Cleaning

**Table 1.** Type, material, manufacturer, and sample size of tested CAD-CAM abutments

Abutment type	Abutment design	Material	Product & Manufacturer	No. of samples
Type I	Monotype Abutment	Y-TZP Zirconia	CADAbut F, BeCe CAD Zirconia XH, Semados SCX, BEGO Implant Systems	8
Type II	Hybrid Abutment	Lithium Disilicate Coping/ Ti-Base	CADAbut D, IPS e.max CAD LT/ Ivoclar Vivadent AG on Ti-Base, Semados SCX, BEGO Implant Systems	8
Type II	Hybrid Abutment	Y-TZP Zirconia Coping/ Ti-Base	CADAbut D, Zirconia LT CAD on Ti-Base, Semados SCX, BEGO Implant Systems	8
Type IV	Monotype Abutment	Titanium Grade 5	CADAbut F, CAD Titanium, Semados SCX, BEGO Implant Systems	8



**Fig. 3.** Set-up of type IV titanium monotype abutment for visual inspection after ultrasonic reprocessing.

System; Bredent GmbH & Co. KG, Senden, Germany) (Fig. 3). According to this protocol, the samples were cleansed three times in an ultrasonic device (Finevo-clean; Sirius Ceramics, Frankfurt, Germany) in separate glass beakers at 30°C for 5 min each. The first bath contained an antibacterial cleaning solution (FINEVO 01, serial number 841117230. BEGO Implant Systems GmbH & Co. KG, Bremen, Germany), the second bath contained 80% ethyl alcohol, and the third bath contained medically purified water (Aqua Destillata, Sanismart GmbH, Waltrop, Germany). The total cleaning time amounted to 15 min. Declared by the manufacturer, the cleaning device solution contains 1.5 g of chlorhexidine gluconate and 15 g of cetrimide per 100 g as active ingredients. The fourth test specimen of each abutment type remained uncleaned as a positive control.

The cleanliness of this first group of test specimens was qualitatively assessed after ultrasonic reprocessing by visual inspection with a magnifying glass at 400 × magnification (Fig. 3). Visible cleanliness served as the acceptance criterion in accordance with the requirements for reprocessed instruments (Guideline German Society of Hospital Hygiene).<sup>31</sup> Specimens visually free of contamination were subsequently subjected to quantitative analysis for protein residues using a modified o-phthalaldehyde (OPA) spectrophotometric method.

Prior to protein analysis, the reprocessed test abutments were subjected to elution according to DIN EN

ISO 15883-5:2021-11.<sup>29</sup> For this purpose, they were eluted in 5 ml elution solution (1% sodium dodecyl sulfate, SDS, pH 11) in a 15 ml tube on a rotary shaker for 10 min at 300 rpm. Glass beads were added for better retrieval of the test soil. Screening of protein in the SDS eluate was based on the modified OPA method. The principle of the OPA assay is the chemical conversion of o-phthalaldehyde and free amino groups in the presence of a thiol component to form fluorescent isoindole compounds (absorbance 340 nm; emission 450 nm). Since the thiol reagent mercaptoethanol is not suitable for general use,<sup>32</sup> it is replaced by N,N-dimethyl-2-mercaptoethylammonium chloride in the modified OPA method. Advantageous is a more stable extinction behavior compared to mercaptoethanol. The quantity of residual protein was evaluated according to the requirements for reprocessed instruments (Guideline German Society of Hospital Hygiene,<sup>27</sup> DIN EN ISO 15883-5,<sup>29</sup> and AAMI TIR 30:2011<sup>30</sup>). They define a residual protein quantity of more than 150 µg per sample as threshold value, while a concentration of > 80 to ≤ 150 µg is classified as critical value, and ≤ 80 µg per sample as reference value. The cleaning guidelines based on international standards suggest a residual protein content of < 6.4 µg protein/cm<sup>2</sup> of the product. The extent of depletion was calculated in percentage (%) based on the original degree of contamination (positive controls).

An additional four test specimens of each abutment type (Type I-IV, total n = 16) were soiled in a second soiling test according to DIN EN ISO 15883-5<sup>29</sup> to test the disinfection efficacy. This particular test soiling consisted of heparinized sheep blood (Fiebig Xebios Diagnostics GmbH, Düsseldorf, Germany, charge 31406500/01) diluted 1:5 with 0.85% NaCl and reactivated with protamine and 1% mucin as well as with the test germ *E. faecium*. The second passage of the test bacterium cultured on brain-heart infusion agar (BHI) was adjusted to 1.5-5.0 × 10<sup>9</sup> CFU/ml (colony forming units) in a dilution solution of 0.85% NaCl and 0.1% tryptone. After centrifugation, the dilution solution was decanted and the volume was supplemented with heparinized sheep blood. Thereafter, the suspension was carefully homogenized with glass beads. For contamination, the test specimens were immersed in the test soiling and then dried for 1 h at

22 ± 2°C in a laminar air flow box. Three test abutments per group were ultrasonically cleaned and disinfected according to the procedure described above. One abutment per product type remained unprocessed and served as a positive control.

The ultrasonically processed test abutments were eluted into tryptic soy broth (TSB). It consists of 3.0% polysorbate 80, 3.0% saponine, 0.1% histidine, and 0.1% cysteine. The elution volume was 10 ml. The test samples were eluted in 10 ml of elution solution in a 15 ml tube on a rotary shaker for 10 min at 300 rpm. Glass beads were added for better recovery of the test soils.

Dilutions were prepared from the positive controls using a dilution solution of 0.1% tryptone and 0.85% sodium chloride and spread to tryptic soy agar (TSA). From each of the processed samples, 1.0 and 0.1 ml of the eluate were plated onto TSA. All samples were incubated at 36 ± 1°C for seven days. The grown colonies were counted visually and the determined germ counts were expressed as the decadal logarithm (lg) of the colony-forming units (CFU). The remaining elution volume was incubated for 7 days at 37°C to allow growth of pre-damaged test bacteria that were not completely killed (enrichment). Over the course of the 7 days, this was visible as turbidity of the sample and assessed with a detected colony count of < 10 (resulting in a logarithmic value of < 1), in case no residual germs could be detected on the culture medium. To evaluate the disinfection efficacy of the three-step cleaning protocol, the microbial load of the test abutments was subtracted from the microbial load of the positive controls, i.e., the reduction factor (RF) was calculated according to the following formula: RF = lg CFU/positive control - lg CFU/test specimens. The criterion for acceptance was a reduction of the test organism (bacteria) by ≥ 5 lg steps.

## RESULTS

All test abutments (type I-IV) passed the qualitative-visual inspection with a magnifying glass at 400 × magnification for cleanliness after being subjected to the three-stage cleaning procedure. The prerequisite for determining the amount of residual protein was thus met for all test specimens.

For the uncleaned one-piece zirconia abutment (positive control type I), a protein concentration of 670.55 µg bovine serum albumine (BSA) was detected in the 1:1.25 diluted eluate by modified OPA analysis, whereas after ultrasonic cleaning, protein was not detectable in the undiluted eluate for any of the three test type I specimens examined, and the depletion was ≥ 99.99% (Table 2). The one-piece titanium test abutments (type IV) exhibited a similar high cleaning efficacy based on the initial BSA of 1492.15 µg for the positive control (Table 2). In both two-piece hybrid abutments (type II, type III), minimal traces of BSA were present in the serum after cleaning. The protein concentration was a maximum of 1.83 µg BSA for lithium disilicate hybrid abutments (type II) (Table 2) and 67.74 µg BSA for zirconia hybrid abutments (type III) (Table 2). However, the extent of depletion was over 95% for type III abutments and over 99% for type II abutments. As a result, the test requirements for cleanliness for all investigated one-piece- and two-piece abutments were successfully met (reference value: ≤ 80 µg residual protein quantity per sample).<sup>31</sup> The limit of detection was 3.9 µg/ml, resulting in a limit of quantification of 19.5 µg/sample.

## DISCUSSION

Recent *in vitro* studies have detected contaminants on the surfaces of titanium and zirconia CAD-CAM customized implant abutments from various manufacturers.<sup>8-10</sup> All tested specimens exhibited process-related roughening, micro wear deposits, and organic and inorganic debris upon delivery. These contaminants can be caused by machining residues, coolant or chemical washing protocols after industrial milling as a remnant of surface processing in centralized production. The fabrication of CAD-CAM abutments in the dental laboratory is basically subject to the same sources of contamination. In addition, the risk of remaining blasting media, excess adhesive, hand grease, polishing agents and rubber residues must be taken into account.<sup>9,33</sup> Soft tissues surrounding implants are hypovascular and hypocellular scar tissues with a considerably lower immunologic capacity than periodontal tissues around teeth.<sup>12,34</sup> If not removed, these particulate contaminants can have

**Table 2.** Results of quantitative protein analysis based on modified o-phthalaldehyde spectrophotometric method (OPA) for: type I abutments (one-piece zirconia abutments); type II abutments (two-piece lithium disilicate abutments); type III abutments (two-piece zirconia abutments); type IV abutments (one-piece titanium abutments)

	Dilution factor	Extinction at 340 nm (OPA)	Protein concentration [ $\mu\text{g}$ BSA/TS]	Extend of depletion [%]	Criterion [ $\leq 80 \mu\text{g}$ residual protein TS]
<b>Type I sample</b>					
PC-I	1:1.25	0.293	670.55	-	-
Test I-1	1.0	0.000	b.d.	$\geq 99.99$	Pass
Test I-2	1.0	-0.001	b.d.	$\geq 99.99$	Pass
Test I-3	1.0	0.000	b.d.	$\geq 99.99$	Pass
<b>Type II sample</b>					
PC-II	1:1.25	0.344	787.27	-	-
Test II-1	1.0	0.001	1.83	$\geq 99.73$	Pass
Test II-2	1.0	0.001	1.83	$\geq 99.73$	Pass
Test II-3	1.0	-0.001	b.d.	$\geq 99.99$	Pass
<b>Type III sample</b>					
PC-III	1:1.25	0.652	1492.15	-	-
Test III-1	1.0	0.037	67.74	95.46	Pass
Test III-2	1.0	-0.001	b.d.	$\geq 99.99$	Pass
Test III-3	1.0	0.034	62.25	95.83	Pass
<b>Type IV sample</b>					
PC-IV	1:1.25	0.625	1492.15	-	-
Test IV-1	1.0	-0.002	b.d.	$\geq 99.99$	Pass
Test IV-2	1.0	-0.003	b.d.	$\geq 99.99$	Pass
Test IV-3	1.0	0.000	b.d.	$\geq 99.99$	Pass

Three test specimens (Test X-1,2,3) were subjected to ultrasonic cleaning once. The fourth test specimen remained uncleaned as positive control. BSA = Bovine Serum Albumin, b.d. = below detection, PC = positive control, TS = test sample.

a potentially adverse effect and may trigger inflammatory responses of the peri-implant tissues.<sup>24,28,35</sup> Titanium particles have been shown to trigger acute inflammation via increased interleukin (IL)-1  $\beta$  secretion and IL-1-associated signaling by promoting the NALP3 inflammasome, which is a temporal enzyme of PGE2 synthesis, and RANL/RANKL that differentiates osteoclasts.<sup>36,37</sup> In an effort of surface cleaning, the impact of different cleaning, disinfection and sterilization methods for implant abutments is therefore comprehensively discussed.<sup>25,26,38,39</sup> However, a solid clinical relationship between abutment cleanliness and peri-implant bone preservation has not yet been demonstrated.<sup>40</sup> It is worth noting that both European and North American health regulations have approved only sterilization or high-level ultrasound disinfection procedures for cleaning semi-crit-

ical medical devices such as CAD-CAM implant abutments.<sup>13,14</sup> Despite the existence of these regulatory guidelines for adequate cleaning or sterilization, there does not appear to be a consistent application.<sup>21</sup> Sterilization by autoclaving involves a physical process that uses a combination of appropriate heating and pressure to remove or destroy all viable forms of microorganisms, including bacterial spores. Some concerns have been raised about the use of resin cements with regard to their hydrothermal aging resistance during autoclaving, as a detrimental effect on bond strength (degradation) has been reported.<sup>41</sup> A recent systematic review on the bond strength of resin based cements, however, showed that thermo-artificial aging has minimal effect if the adhesive surface was pretreated, such as sandblasted and/or coated with acid monomers.<sup>42</sup> More recent *in vitro* results on

**Table 3.** Results of disinfection test for: type I abutments (one-piece zirconia abutments); type II abutments (two-piece lithium disilicate abutments); type III abutments (two-piece zirconia abutments); type IV abutments (one-piece titanium abutments)

	Holding time	CFU/TS	Enrichment*	log/TS	RF	Criterion
Type I sample						
PC-I	-	$1.45 \times 10^8$	-	8.19	-	-
Test I-1		< 10	+	< 1.00	> 7.19	Pass
Test I-2	5 min.	< 10	+	< 1.00	> 7.19	Pass
Test I-3		0	-	0.00	$\geq 8.19$	Pass
Type II sample						
PC-II	-	$4.4 \times 10^8$	-	8.64	-	-
Test II-1	5 min.	0	-	0.00	$\geq 8.64$	Pass
Test II-2		0	-	0.00	$\geq 8.64$	Pass
Test II-3		0	-	0.00	$\geq 8.64$	Pass
Type III sample						
PC-III	-	$1.47 \times 10^8$	-	8.17	-	-
Test III-1	5 min.	0	-	0.00	$\geq 8.17$	Pass
Test III-2		0	-	0.00	$\geq 8.17$	Pass
Test III-3		0	-	0.00	$\geq 8.17$	Pass
Type IV sample						
PC-IV	-	$1.33 \times 10^8$	-	8.12	-	-
Test IV-1	5 min.	0	-	0.00	$\geq 8.12$	Pass
Test IV-2		0	-	0.00	$\geq 8.12$	Pass
Test IV-3		0	-	0.00	$\geq 8.12$	Pass

Three test specimens (Test X-1,2,3) were subjected to ultrasonic cleaning once. The fourth test specimen remained uncleaned as positive control. CFU = colony forming units, log = logarithm [of colony forming units], TS = test sample, RF = reduction factor = (initial contamination [lg] - residual contamination [lg], PC = positive control, + = Turbidity due to bacteria growth in sub cultivation (enrichment), - = lack of turbidity due to lack of bacterial growth, \* = Incubation of the eluate 7d at  $36 \pm 1^\circ\text{C}$ .

the structural integrity and bond strength between zirconia frameworks and titanium-based abutments after autoclave sterilization have also shown no negative influence on bond strength.<sup>18,43</sup>

While sterilization has the ability to effectively eliminate microbial surface contamination to achieve aseptic and sterile settings, particulate debris on CAD-CAM abutments cannot be removed by this process alone. However, ultrasonic cleaning with high-frequency waves in approved disinfectants is validated and effective in the mechanical removal of foreign bodies and microbiota from the surface of abutments.<sup>10,39</sup> *In vitro* results employing a three-stage ultrasonic cleaning procedure confirm generally good cell viability on titanium specimens and improved cell attachment and reduced inflammatory response of human gingival fibroblasts (HGF) on zirconia sur-

faces.<sup>28,44</sup> Ultrasonic devices are widely available and employed in clinics and dental laboratories and can be conveniently used for abutment hygiene.<sup>45,46</sup>

While the effectiveness of the three-step ultrasonic cleaning protocol for removing particulate contamination from CAD-CAM-fabricated surfaces has already been conclusively demonstrated,<sup>9,10</sup> the present study aimed to evaluate the microbiological disinfection efficacy of this regimen for CAD-CAM implant abutments. The present data show that an antibacterial cleaning solution with chlorhexidine gluconate and cetrimide as active ingredients, followed by ethyl alcohol and medically pure water in sonication treatment, resulted in effective bacterial decontamination. It revealed a decrease in residual protein of less than 80  $\mu\text{g}$  and a reduction in microbiota of more than 7 lg levels of colony-forming units



(CFU) for all abutment types, exceeding the effect required for cleaning and disinfection efficacy (Table 3). OPA assays failed to detect residual protein quantity for the ultrasonically processed zirconia (type I) and titanium (type IV) monotype test abutments. Only minimal traces of protein residues were measured in the eluates of the two-piece hybrid abutments (type II, type III) after ultrasonic cleaning and disinfection, amounting to 1.83 µg BSA equivalent for lithium disilicate hybrid abutments (type II) and 67.74 µg BSA for zirconia hybrid abutments (type III) (Table 2). The degree of disinfection, as measured by the residual protein concentration, was thus dependent on the manufacturing process of the respective CAD-CAM abutment. Nevertheless, all specimens successfully met the standard reference value of  $\leq 80$  µg residual protein per sample.<sup>31</sup> Since the titanium and ceramic abutments tested had non-porous surfaces, it can be hypothesized that the materials employed did not have a significant influence on the disinfection effect. It can only be assumed that macro- and micro-design of the abutments such as geometry, cavities, surface roughness, micro texturing and polishing had a potential influence on the efficacy of high-frequency microwaves and the disinfection regime. A possible explanation for the marginally inferior cleaning results of the tested hybrid abutments after sonication processing could be the fact that their manufacturing process is predominantly manual and thus minimal residues of polishing agents and hand grease may not be completely removed by ultrasound and cleaning and disinfection. The residual levels of protein found on hybrid abutments in the current study are consistent with the results of previous investigations. An *in vitro* study using scanning electron microscopy (SEM), X-ray dispersion analysis (EDX) and computer-assisted planimetric analysis (CAPM) to evaluate the quality and quantity of process-related surface contamination of various customized implant abutments also concluded that hybrid abutments, in contrast to monotype abutments, exhibited minimal amounts of debris after ultrasonic cleaning.<sup>10</sup> Whether the adhesive gap and bonding material of hybrid abutments have an influence on the removal of protein contamination remains to be investigated. The specific influence of the abutment material and the post-pro-

cessing measures on the cleanliness of the abutment before and after ultrasonic cleaning has yet to be determined in this contest. For the detection of residual protein, the modified OPA assay was chosen because it is considered a suitable method for the quantitative determination of free and terminal amino groups (sensitivity: 0.03 - 1 µg/ml).<sup>47</sup> In practice, this method was proven to be easy to adopt, relatively simple to use, and readily implementable under the given conditions. The normative acceptance criterion for confirming disinfection efficacy with a reduction of test germs by at least 5 lg levels compared to the positive control was clearly exceeded in the present study. Consequently, the null hypothesis can be considered accepted, as the ultrasonic protocol successfully met the test requirements for disinfection of all investigated one- and two-piece implant abutments.

The test soiling of blood and *Enterococcus faecium* proved to have good reproducibility in the evaluation of medical devices.<sup>48</sup> In the present study, the addition of bacterial cultures and blood as test soil allowed the validation of the disinfection efficacy on the tested CAD-CAM implant abutments. Disinfection alone is not able to achieve the necessary reduction of microorganisms, and therefore efficient cleaning, e.g. by ultrasound, is also advised. Since customized abutments were sequentially immersed in three different solutions with ultrasound, the physical effects of ultrasonic cleaning and the effect of antimicrobial solutions were not separately evaluated in the limitation of this study. Thus, further studies will be necessary to assess each effect of antimicrobial solutions and ultrasonic procedure separately. Although the reproducibility of the *in vitro* investigation was ensured by the use of a defined contamination and ultrasonic cleaning protocol, the employment of an artificial soil could be considered a drawback of the present study. Another limitation concerns the relatively small number of samples per subgroup and their limited variety of material and size, which should be expanded in future studies with a larger number and selection of samples.

## CONCLUSION

Based on the present findings, the following conclu-

sions were obtained:

A three-step ultrasonic cleaning and disinfection process is reproducible and complies with the standardized reprocessing and disinfection specifications for custom one- or two-piece CAD-CAM implant abutments made of titanium or ceramic. The cleaning solution with the active ingredients chlorhexidine gluconate and cetrimide, followed by ethyl alcohol and medically pure water in the ultrasonic treatment, leads to effective bacterial decontamination. Ultrasonic reprocessing resulted in a substantial decrease of residual protein of less than 80 µg and a reduction in microbiota of more than 7 lg levels of colony-forming units for all abutment types, exceeding the effect required for disinfection.

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