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Durability of resin bonding to zirconia ceramic after contamination and the use of various cleaning methods

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ABSTRACT

Objectives. The aim of the study was to evaluate the influence of contamination and different cleaning methods on the tensile bond strength with a phosphate monomer containing luting resin to zirconia ceramic.

Methods. After the contamination with saliva or silicone disclosing agent, 228 polished and airborne-particle abraded zirconia discs were ultrasonically cleaned with 99% isopropanol. In a second step, the specimens were either treated with argon-oxygen plasma, air plasma, enzymatic cleaning agent or did not undergo an additional cleaning process. Uncontaminated zirconia specimens were used as the control group. X-ray photoelectron spectroscopy (XPS) was used for chemical analysis of the bonding surfaces of specimens. Plexiglas tubes filled with composite resin were bonded to zirconia specimens with a phosphate monomer containing luting resin. Tensile bond strength (TBS) was tested after 3 days or 150 days water storage with 37,500 thermal cycles.

Results. XPS revealed a decrease of the carbon/oxygen ratio after plasma treatment and an increase after treatment with an enzymatic cleaning agent in all groups. All contaminated specimens showed high and durable TBS after cleaning with a combination of isopropanol and a non-thermal atmospheric plasma. After the cleaning with enzymatic cleaning agent the TBS was significantly reduced in all groups after 150 days thermal cycling.

Significance. The combination of isopropanol and plasma cleaning was effective in removing salvia and disclosing agent contamination. Enzymatic clearing agent was not able to remove contamination effectively and had a negative impact on the TBS of non-contaminated specimens.

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1. Introduction

Over the past years, zirconia ceramic gained importance as a core material for the fabrication of dental restorations [1,2]. Due to its esthetic, biocompatible and mechanical characteristics it is used for various types of restorations including full-coverage crowns, fixed partial prostheses, veneers, endodontic posts and implant abutments. Phosphate monomer containing resin systems allow adhesive bonding of zirconia restorations with dentin and enamel without the limitations of conventional cements [3–5].

However, the bonding process is very sensitive to moisture and contamination of the bonding surface, which can lead to a significant reduction of the bond strength in a clinical situation. Unfortunately, a contamination with silicone indicator, saliva or blood during the preceding try-in procedure is very difficult to avoid [6–12]. Due to this problem, several cleaning methods have been tested in previous studies in order to remove the contamination and provide strong resin bond. Methods of rinsing the contaminated surfaces with water, cleaning it ultrasonically in isopropanol or a combination of both methods resulted in a minor or no cleaning effect and therefore, are not recommended [7,10,11,13]. Another method of covering the surface with phosphoric acid gel showed a good efficiency in removing saliva, however was not effective in the removal of silicone residuals [6,7,10,11,13]. Also airborne-particle abrasion with Al_2O_3 was used and showed a good cleaning effect [7,10]. Yet due to the acquisition costs for an air-abrasion device or the additional time and labor required dentists might delegate the treatment with air-abrasion to dental technicians [14].

A method without any negative side effects and with an equally efficiency in removing contaminates and ensuring a strong resin bond to zirconia ceramic would be advantageous. A possible alternative might be found in the utilization of plasma gas.

Plasma is a fully or partially ionized gas and is defined as one of the four fundamental states of matter. When a plasma atmosphere interacts with various types of materials, the material surfaces can be affected in different ways: The removal of organic materials, cross-linking via activated species of inert gases, ablation, and surface chemical restructuring. These properties are all summarized under the term plasma-surface modification (PSM) and are nowadays standard in many large-scale industrial manufacturing processes due to their cost-effectiveness and reliability [15,16].

In the recent years PSM found increased use in biomedical material science and engineering. The antibacterial properties of non-thermal plasma are already used to sterilize chirurgical instruments, disinfect carious dentin and stop dental bacteria [17–29]. The idea of using PSM to improve adhesive bonding of dental restorations has become a major point of interest as well. It has been proven that a non-thermal atmospheric plasma treatment can create a more hydrophilic surface on materials such as dentin, enamel, composite and ceramics, which could lead to better compound with bonding resin [30–33]. Bonding experiments have already shown an increase of adhesive strength using PSM on ceramic surfaces [34,35]. In addition to the surface activating properties, the cleaning ability of plasma has recently become the focus of research. The cleaning properties of plasma are based on the reactivity of the ionized gas. The highly reactive particles of the plasma are able to split large molecule chains into smaller particles, dissolving debris by chemical reduction. Stable molecular structures, such as those found in ceramics, are not affected [16,34]. A previous study showed that non-thermal low pressure plasma has a moderate cleaning effect on saliva and silicone contamination, but is less efficient than alumina particle air-abrasion [36]. However, a combination of isopropanol and plasma cleaning has not been evaluated yet.

A further problem of contamination is the fixation of proteins by the use of current alcohol- or aldehyde-containing cleaning and disinfecting agents. In addition to the moderate cleaning-effect of saliva-contaminated zirconia with isopropanol, direct parallels can also be drawn to medical instrument reprocessing. The treatment of saliva- or blood-containing instruments with a highly alcoholic solution leads to denaturation and fixation of the protein residues. These denaturized residues are very difficult to remove and might provide a residual biofilm for bacteria and viruses. According to the present state, non-fixing detergents are therefore recommended for manual reprocessing. The purification ability of these agents is based on a combination of enzymes that are able to dissolve and degrade organic and inorganic substances [10,37–39].

Due to a comparable form of contamination, non-fixing cleaners for manual instrument preparation might also be used in the cleaning of dental restorations prior to adhesive bonding.

This laboratory study examined the plasma treatment and enzymatic cleaning as possible alternatives to alumina particle air-abrasion of saliva and silicone contaminated zirconia ceramic. The null hypothesis of this study was that the additional used cleaning methods have no influence on the surface contamination and do not affect the resin bonding to the zirconia ceramic and its durability.

2. Materials and methods

2.1. *Specimen preparation*

In this study 228 disk-shaped cylindrical 3-mol yttria stabilized zirconia ceramic specimens (ICE Zirkon Translucent; Zirkonzahn, Gais, Italy) with a diameter of 8 mm and a height of 3.4 mm were used. The bonding surfaces of all disks were wet polished with 600-grit abrasive silicon carbide paper and abraded with 50 μm sized Al_2O_3 airborne-particles with a pressure of 0.1 MPa at a distance of 10 mm. Before alumina particle air-abrasion the bonding surfaces were marked in color with a permanent marker [40]. The surfaces were air-abraded until the color was completely removed. Afterwards the specimens were ultrasonically cleaned in 99% isopropanol for 3 min and dried using a blast of oil-free air. The specimens were randomly distributed into 12 groups according to contamination and cleaning methods with 19 specimens each. Three groups of contamination and four subgroups of cleaning were distinguished as follows (Table 2, Fig. 1):

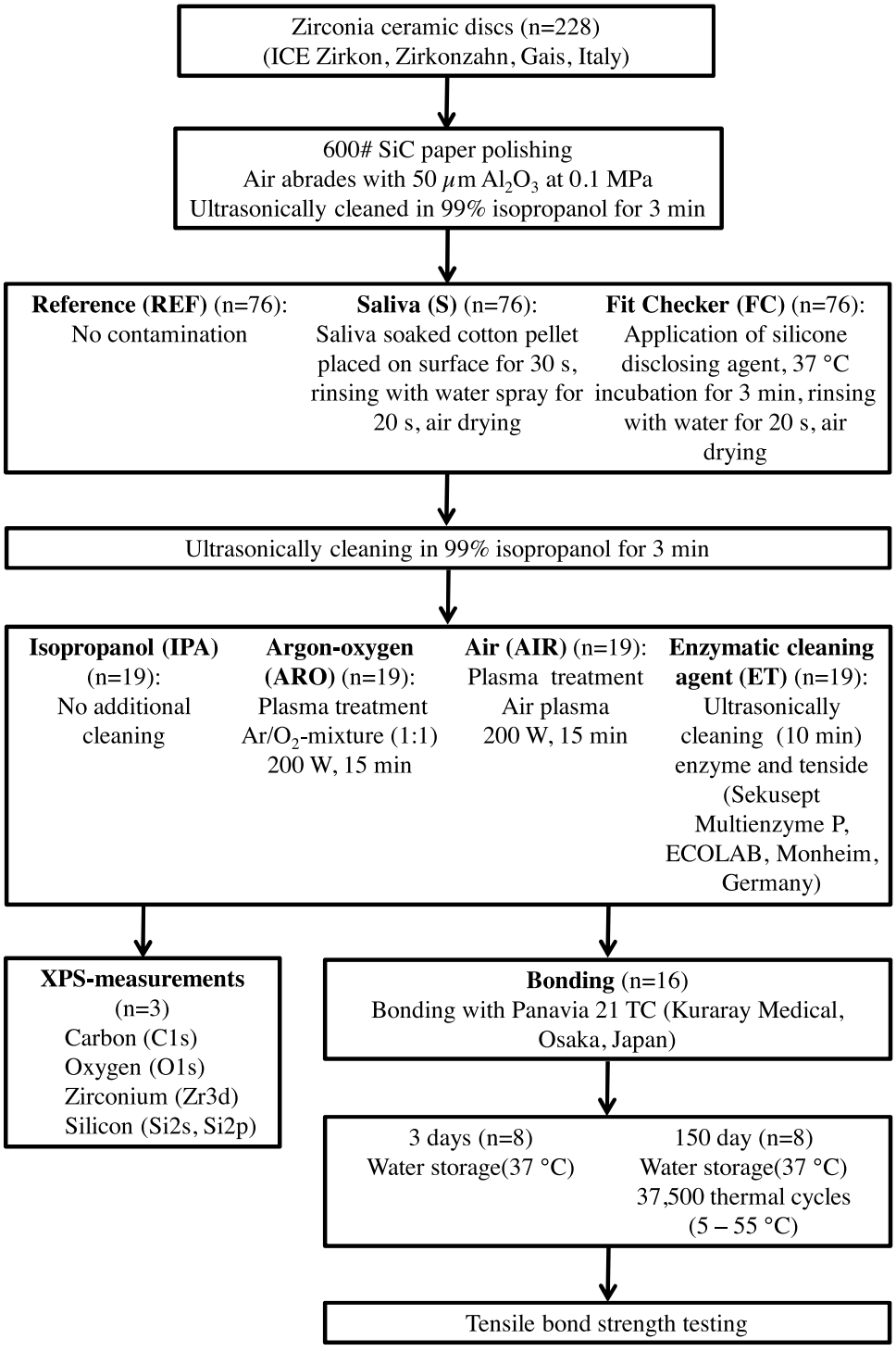

Fig. 1 – Study design for the experiment.

- **REF** (Reference): The specimens were not contaminated and served as reference groups.
- **FC** (Fit Checker): The specimens were pressed into freshly mixed silicone indicator (Fit Checker white advanced, GC Germany GmbH, Bad Homburg, Germany) and placed into a 37 °C incubator for three minutes to imitate clinical conditions. Afterwards the silicone was removed and specimens were rinsed with water spray for 20 s and dried using a blast of oil-free air.
- **S** (Saliva): Cotton pellets soaked with saliva were placed on the specimens for 30 s. Afterwards specimens were rinsed with water spray for 20 s and dried with oil-free air. Saliva was collected from one healthy male donor who had refrained from eating and drinking 1.5 h prior to the collec-

Table 1 – List of materials and the corresponding composition and batch number used in this study.

Material	Company	Composition	Batch No.
GC Fit Checker white advanced Base	GC Germany GmbH, Bad Homburg, Germany	Silicon dioxide: 30–60%, Vinyldimethylpolysiloxane: 30–50%, Polyether compound: 30–60%, Methylhydrogen dimethylpolysiloxane: 5–15%	1407291, 1610121
GC Fit Checker white advanced Catalyst	GC Germany GmbH, Bad Homburg, Germany	Vinyldimethylpolysiloxane: 50–90%, Silicon dioxide: 10–30%, Titanium dioxide: 1%	1407291, 1610121
Clearfil Core New Bond Base	Kuraray Medical, Osaka, Japan	Bis-GMA, TEGDMA, DMA	000041
Clearfil Core New Bond Catalyst	Kuraray Medical, Osaka, Japan	Barium sulfate, silica containing resin composite	000041
Panavia 21 TC Base	Kuraray Medical, Osaka, Japan	Ba–B–Si-glass powder, sodium fluoride bisphenol	000019, 000024
Panavia 21 TC Catalyst	Kuraray Medical, Osaka, Japan	A polyethoxy dimethacylate, 10 MDP, hydrophilic and hydrophobic dimethacylates, self-cure initiators	000019, 000024
Oxyguard II	Kuraray Medical, Osaka, Japan	BPEDMA, MDP, DMA, chemical initiators Glycerol (50–70%), polyethyleneglycol, catalysts, accelerators, dyes, others	000019, 000024
Sekusept MultiEnzyme P	Ecolab Deutschland GmbH, Monheim am Rhein, Germany	Proteases, amylase, lipase, surfactants, corrosion inhibitors, solvents, additives, emulsifiers, perfume	4376AP0911

Bis-GMA, bisphenol-A-diglycidylmethacrylate; BPEDMA, bisphenol-A-polyethoxy dimethacrylate; DMA, aliphatic dimethacry-late; MDP, 10-methacryloyloxy-decyl dihydrogenphosphate; TC, tooth color; TEGDMA, triethyleneglycol dimethacrylate. According to the information provided by the manufacturers.

tion procedure. All experiments were performed using fresh saliva collected on the same occasion.

Each group of contamination was subjected to four subgroups of cleaning (N = 19):

- **IPA** (Isopropanol): The specimens were cleaned ultrasonically in 99% isopropanol for 3 min and then dried using a blast of oil-free air. No additional cleaning method was used on the specimens, serving as a reference towards the other methods.
- **AIR** (Air): The specimens were cleaned ultrasonically in 99% isopropanol for 3 min and then dried using a blast of oil-free air. Followed by the plasma treatment with air gas for 15 min.
- **ARO** (Argon-oxygen mixture): The specimens were cleaned ultrasonically in 99% isopropanol for 3 min and then dried using a blast of oil-free air. Followed by the plasma treatment with a 1:1 argon-oxygen mixture gas for 15 min.
- **ET** (Enzyme and surfactant containing cleaning agent): The specimens were cleaned 10 min in an ultrasonic bath filled with an enzymatic detergent (Sekusept Multienzyme P, Ecolab Deutschland GmbH, Monheim am Rhein, Germany) with a concentration of 1.5%. Afterwards the specimens were rinsed with water spray for 20 s and dried with a blast of oil-free air. Finally, the specimens were cleaned ultrasonically in 99% isopropanol for 3 min and then dried using a blast of oil-free air.

All plasma treated specimens were first cleaned ultrasonically in 99% isopropanol for 3 min and dried using a blast of oil-free air, before they were placed in the plasma chamber for a final cleaning process. This was implemented, because a previous study showed, that plasma cleaning alone was not effective enough against these particular contaminations [36]. A low vacuum non-thermal-plasma chamber (Femto PCCE Zahntechnik, diener electronic GmbH und Co. KG, Ebhausen, Germany) was used for the plasma treatment. It is equipped with a high-frequency generator producing an asymmetric capacitively coupled radio-frequency (rf) discharge of up to 200 W and a frequency of 100 kHz. The 19 specimens of every group were placed centrally on the specimen tray and were treated simultaneously. The plasma treatment with a duration of 15 min was carried out with a rf power of 200 W, a target temperature of 70 °C and a working pressure of 50 Pa. The groups were bonded directly after the plasma treatment.

2.2. *X-ray photoemission spectroscopy (XPS) measurement*

Three specimens of each test group were analyzed with XPS to quantify the contamination and the effectiveness of the cleaning methods. All measurements were done using an Omicron Full Lab (Omicron NanoTechnology GmbH, Taunusstein, Germany) equipped with an Al Kα X-ray source providing an excitation energy of 1486.6 eV and a VSW 125 hemispherical analyzer with five channeltrons set to constant analyzer energy (CAE) mode. Survey scans with a pass energy setting of 100 eV allowed for adequate quantitative analysis. To examine the surface composition of the test samples wide spectrum scans of the carbon (C1 s), oxygen (O1 s), zirconium (Zr3d), and silicon (Si2s, Si2p) peaks were taken.

Table 2 – Group codes, contamination and cleaning treatments of all groups of the study.

Groups	Contamination	Cleaning
REF-IPA	No contamination	Isopropanol only
REF-ARO	No contamination	Argon-oxygen plasma
REF-AIR	No contamination	Air plasma
REF-ET	No contamination	Sekusept MultiEnzyme P
S-IPA	Saliva	Isopropanol only
S-ARO	Saliva	Argon-oxygen plasma
S-AIR	Saliva	Air plasma
S-ET	Saliva	Sekusept MultiEnzyme P
FC-IPA	Fit Checker	Isopropanol only
FC-ARO	Fit Checker	Argon-oxygen plasma
FC-AIR	Fit Checker	Air plasma
FC-ET	Fit Checker	Sekusept MultiEnzyme P

2.3. *Tensile bond strength testing*

Batch numbers and composition of the materials used are shown in Table 1. Plexiglas tubes with a length of 15.5 mm and an inner diameter of 3.2 mm were filled with self-curing resin composite (Clearfil DC Core New Bond, Kuraray Medical, Osaka, Japan). After seven minutes from mixing begin, the filled tubes were bonded with a phosphate monomer containing self-curing luting resin (Panavia 21 TC, Kuraray Medical, Osaka, Japan) to the zirconia bonding surface using an alignment apparatus under a load of 750 g. The apparatus ensured that the tube axis was perpendicular to the surface [41]. After excess resin was removed using a foam pellet, an air blocking gel (Oxyguard II, Kuraray Medical, Osaka, Japan) was applied around the bonding margins. The bonded specimens were stored in a 37 °C incubator for 10 min still placed in the alignment apparatus to ensure complete curing of the resin. The bonded specimens were split into two subgroups, which were stored in 37 °C water for 3 days or for 150 days with additional 37,500 thermal cycles between 5 and 55 °C with a dwell time of 30 s for an artificial aging process [7,10]. After different storage conditions, tensile bond strength (TBS) was measured with a universal testing apparatus (Zwick Z010/TN2A, Zwick Roell, Ulm, Germany) using tensile force at a crosshead speed of 2 mm/min using a chain loop alignment which facilitated a moment-free axial application [41,42].

The statistical analyses of the results showed that not all groups were distributed normally using the Shapiro-Wilk test. This led to the use of the Kruskal–Wallis test, giving the probability value followed by the Mann–Whitney-U test performing multiple pairwise comparisons of groups. Significance level α was determined at 0.05 and was corrected by using the Bonferroni-Holm method.

2.4. *Failure mode*

After tensile bond strength testing, a light microscope (Wild M 420 Heerbrugg, Germany) with 25× magnification was used to determine the portions of the failure modes of the debonded specimens. The failure modes were classified into one of the following: A: adhesive failure at zirconia ceramic surface; C: cohesive failure in the luting resin composite or in the tube filling resin composite. In addition, representative specimens were examined at high magnification using a scanning electron microscope (SEM, XL 30 CP, Philips, Kassel, Germany) with an acceleration voltage of 15 keV after sputtering (Leica EM QSG 100, Wetzlar, Germany) a gold alloy conductive layer of 15 nm.

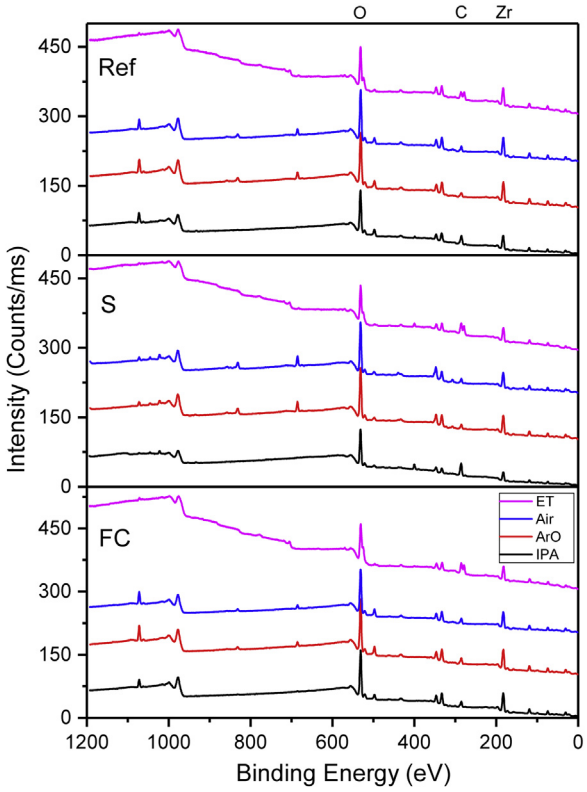

Fig. 2 – XPS wide spectrum analyses of all groups. Mean results of each test group were used. Curves of the subgroups were shifted by 100 counts/ms each.

3. Results

3.1. *XPS*

Mean wide spectrum XPS curves of all tested groups are shown in Fig. 2. Besides the examined peaks of C 1s (carbon), O 1s (oxygen) and Zr 3d (zirconia), elemental peaks of Y 3d (yttrium), Na 1s (sodium) and Al 2p (alumina) were measured on all specimens. Additionally, minor F 1s (fluorine) peaks were measured in the plasma treated groups, which also showed slightly higher Al 2p peaks. In the groups S, S-ARO, S-AIR and FC minimal peaks of Zn 2p3/2 (zink) were measured.

Elemental ratios obtained from the XPS line intensities corrected by the respective XPS sensitivity factors of carbon/oxygen (C/O), carbon/zirconia (C/Zr) and oxygen/zirconia (O/Zr) atoms in the test groups are shown in Table 3. After plasma treatment, the C/O and C/Zr ratios decreased indicating a lower concentration of carbon on the surface compared to the groups only treated with isopropanol. O/Zr ratios showed a small decrease for plasma treated groups with saliva and no contamination and a small increase for silicone detergent contamination. The argon-oxygen and air plasma treated groups showed similar values in the C/O, C/Zr and O/Zr ratios.

Table 3 – X-ray photoelectron spectroscopy (XPS) results: Mean ratios of carbon (C), oxygen (O) and zirconia (Zr) elements in groups.

Test group	C/O	C/Zr	O/Zr
REF-IPA	0.72	5.05	7.05
REF-ARO	0.26	1.59	6.07
REF-AIR	0.31	1.89	6.11
REF-ET	0.82	5.93	7.23
S-IPA	1.14	10.46	9.15
S-ARO	0.27	1.71	6.32
S-AIR	0.28	2.11	7.50
S-ET	1.22	10.71	8.78
FC-IPA	0.39	2.20	5.65
FC-ARO	0.35	2.16	6.20
FC-AIR	0.37	2.33	6.34
FC-ET	1.01	9.67	9.55

The treatment with enzymatic cleaning agent increased the C/O and C/Zr ratio in all groups. During XPS-measurements no significant peak for silicon atoms (Si2s, Si2p) appeared in any group.

3.2. *Tensile bond strength*

Median, means and standard deviations of the TBS in MPa of all tested groups are shown in Table 4. The not contaminated reference group (REF-IPA) showed high initial TBS, which remained stable over 150 days water storage including thermal cycling. After three days of water storage only the specimens of S-IPA and S-ET showed significantly lower TBS in both Fit Checker and saliva contaminated groups. Considering the artificially aged and 150 days stored specimens, all groups treated with enzymatic cleaning agent (ET-REF, ET-S, ET-FC) showed significantly lower TBS results, compared with the reference groups or plasma treated groups. The only exception can be seen in the REF-S group, in which six of eight specimens debonded spontaneously before tensile testing. All other tested groups showed durable bond strengths in both types of storage.

3.3. *Failure modes*

The proportional amount of areas assigned to the two failure modes, are shown in Fig. 3 in mean percentage. For the reference groups, Fit Checker groups and the plasma treated saliva groups with a high TBS, the failures were dominantly found to be cohesive, showing residue of luting composite resin or filling composite resin on the ceramic surface. Groups REF-ET and FC-ET showed slightly higher portions of adhesive failures. Particularly after 150 days storage the adhesive failure portion increased significantly. Groups S-IPA and S-ET showed even a dominantly adhesive failure of the resin bonding. Representative scanning electron micrograph (SEM) images of specimens with an exclusively adhesive failure mode, a mixed failure mode and a cohesive failure mode are shown in Fig. 4.

4. Discussion

Previous studies have already confirmed that roughness, hydrophilicity and contamination of the ceramic surface have a major influence on the adhesion of resin bonding [7,10,12,43]. This study confirmed that saliva contamination during the try-in of the ceramic restoration have a negative impact on the tensile bond strength (TBS). XPS measurements proved that a single isopropanol cleaning is not effective for the removal of saliva. Also a standardized water storage and thermal cycling procedure was used to simulate aging, showing the long term influence of contamination on the resin bonding. During artificial aging, most of the saliva contaminated specimens debonded spontaneously.

Contrary to comparable studies [11,36,44] silicone contamination had no relevant impact on the TBS. Also the XPS results showed no significant silicon peaks in these groups. A possible explanation is a different procedure of the silicone paste application, as the silicone covered specimens were stored in an incubator for three minutes to simulate the clinical try-in situation with intraoral temperature. The incubation could have led to a fully polymerization of the silicone that left much less residuals on the ceramic surface. This resulted in a higher TBS than in a comparable study, where the same product was used

Table 4 – Median, means and standard deviation (SD) in MPa of the tensile bond strength (TBS) of test groups (N = 8). Statistically different means ($p \leq 0.05$) are indicated by different superscript upper case letters (within a column), or by subscript lower case letters (within a row, for the same kind of water storage), or by different superscript lower case Greek letters (within a row, comparing 3 and 150 days' storage within the same test group).

		Reference		Saliva		Fit Checker	
		3 d	150 d	3 d	150 d	3 d	150 d
Isopropanol only	Median	$40.9^{A}{}_{a}{}^{\alpha}$	$37.5^{A}{}_{a}{}^{\alpha}$	$19.8^{C}{}_{b}{}^{\alpha}$	$0.0^{C}{}_{b}{}^{\beta}$	$39.4^{A}{}_{a}{}^{\alpha}$	$39.6^{A}{}_{a}{}^{\alpha}$
	Mean	40.2	36.7	19.7	1.8	38.6	39.5
	SD	3.3	7.9	3.2	3.3	3.7	5.7
Argon–Oxygen	Median	$45.5^{A}{}_{a}{}^{\alpha}$	$44.6^{A}{}_{a}{}^{\alpha}$	$40.9^{A}{}_{a}{}^{\alpha}$	$39.3^{A}{}_{a}{}^{\alpha}$	$45.7^{A}{}_{a}{}^{\alpha}$	$38.0^{A}{}_{a}{}^{\alpha}$
	Mean	46.3	45.8	40.5	39.0	43.5	39.1
	SD	5.0	6.9	5.7	5.7	7.2	6.6
Air	Median	$46.3^{A}{}_{a}{}^{\alpha}$	$48.8^{A}{}_{a}{}^{\alpha}$	$40.3^{A}{}_{a}{}^{\alpha}$	$41.2^{A}{}_{a}{}^{\alpha}$	$49.9^{A}{}_{a}{}^{\alpha}$	$44.6^{A}{}_{a}{}^{\alpha}$
	Mean	45.2	48.5	40.7	39.9	48.6	43.9
	SD	6.7	7.5	5.6	7.7	10.1	5.7
Sekusept MultiEnzyme P	Median	$41.7^{A}{}_{a}{}^{\alpha}$	$20.5^{B}{}_{ab}{}^{\beta}$	$29.4^{B}{}_{b}{}^{\alpha}$	$13.6^{B}{}_{b}{}^{\beta}$	$40.2^{A}{}_{a}{}^{\alpha}$	$22.6^{B}{}_{a}{}^{\beta}$
	Mean	40.0	19.8	28.9	13.8	40.2	23.3
	SD	4.9	5.3	3.5	3.4	6.4	4.8

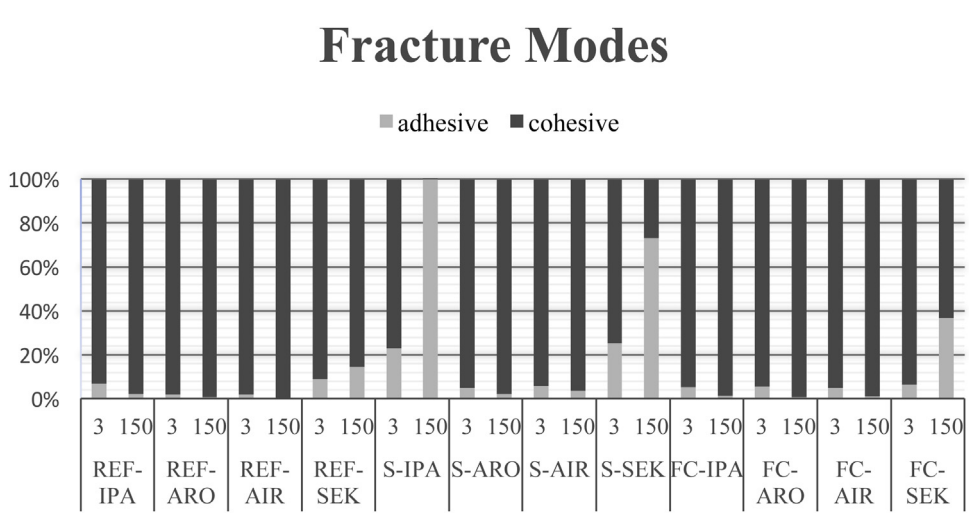

Fig. 3 – Mean percentages of areas assigned to the failure modes observed in the test groups after tensile bond strength testing comparing 3 and 150 days of storage. Adhesive failure: debonded failure at the zirconia ceramic surface. Cohesive failure: debonded failure in the luting resin composite (Panavia 21 TC) or in the tube-filling resin composite (Clearfil Core New Bond).

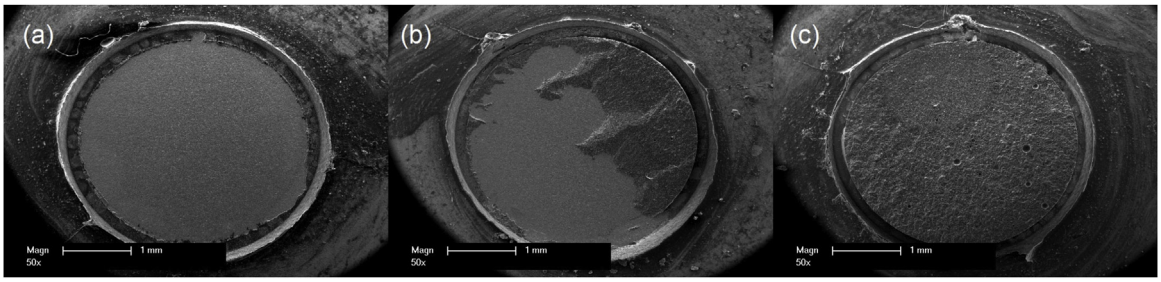

Fig. 4 – Scanning electron micrograph (SEM) images (×50 magnification). (a) example for 100% adhesive failure mode (S-IPA), (b) example for mixed failure mode (S-ET), (c) example for 100% cohesive failure mode (REF-AIR).

[36]. Another explanation might be the use of an updated product compared with older studies. The precursor product (Fit Checker black, GC Germany GmbH, Bad Homburg, Germany) was based on a polysiloxane and therefore underwent a different polymerization process [11,44].

Among cleaning methods, plasma treatment has the additional property of surface activation. Due to the atmosphere of ionized gas new oxygenic polar groups are formed, creating reactive species and functional groups [16,34]. This may increase primary chemical bonding between the zirconia surface and the phosphate functional groups of the resin [45]. Comparing the reference groups (REF-IPA, S-IPA, FC-IPA) to the argon-oxygen and air plasma treated groups (REF-ARO, REF-AIR, S-ARO, S-AIR, FC-ARO, FC-AIR), contrary to C/O and C/Zr ratios, no big difference in the O/Zr ratios can be seen. Therefore, no new oxygenic polar groups where detected on the sample surfaces by XPS-measurements. The decrease of carbon atoms in REF-ARO and REF-AIR, showed by low C/O and C/Zr ratios, proved that high TBS were caused by the removal of naturally occurring debris and dust on the bonding surfaces by plasma cleaning in the reference group.

The cleaning properties of plasma are based on the highly reactive ionized atmosphere that breaks chemical bonds like C—H and C—C. This process is mostly chemical with only a small proportion of mechanically cleaning [16,34]. Therefore, a big advantage of plasma treatment is that there is no measurable degradation of the ceramic-surface-morphology. In contrast, plasma cleaning alone was not powerful enough to remove greater amounts of debris effectively. A previous study showed this problem with saliva and silicone as contamination on a zirconia surface. Plasma treated specimens showed a lower bond strength than the isopropanol ultrasonically cleaned groups [36]. Therefore, a chemical-mechanically cleaning using an ultrasonic bath filled with 99% isopropanol solution followed by a plasma treatment was combined in current study. The XPS measurements showed a lowering of the C/O and C/Zr ratios in the plasma treated groups S-ARO and S-AIR, with a decrease of carbon atoms, which indicates a removal of organic residuals from the ceramic surfaces. Above all, plasma treated groups showed similar bond strengths and adhesive failures mode compared with that of the reference group.

Enzymatic cleaning can be accomplished by using a medical cleaning agent typically applied for manual reprocessing of instruments. The water based cleaning agent used in this study, contains an emulsion of proteases, amylase, lipase, surfactants, corrosion inhibitors, solvents, additives, emulsifiers and perfume. According to the manufacturer, it is capable of

killing bacterial biofilm and removing other kinds of debris by dissolving organic and certain inorganic materials on an instrument's surface. The XPS results showed the highest C/O and C/Zr ratios compared to plasma cleaning and the reference groups. This indicates that the treatment with enzymatic cleaning agent resulted in an increase of the carbon concentration on the ceramic surface. The low TBS results in the 150 days reference and Fit Checker group (REF-ET, FC-ET) can be referred to this high carbon concentration. In contrast, the S-ET group showed a higher TBS compared to S-IPA, proving a moderate cleaning effect of the enzymatic cleaning agent. Taking into account these two observations, it can be assumed that the agent performed a surface cleaning, but also led to a contamination of the ceramic surface by its protein-containing ingredients like proteases, amylase and lipase. This assumption can be consolidated by the results of the facture mode in the reference groups, showing the highest portion of adhesive failure mode in group REF-ET.

5. Conclusions

Taking the results of this study into account, the following conclusions can be drawn:

1 The combination of ultrasonically isopropanol-cleaning and plasma treatment is an effective method for cleaning saliva contaminated zirconia specimens.
2 Plasma cleaning with argon-oxygen and air atmosphere showed a comparable cleaning effect
3 The enzymatic cleaning agent was not able to sufficiently remove saliva and showed indications of contaminating the specimens with its ingredients.

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