journal of prosthodontic research 63 (2019) 271–276

Contents lists available at ScienceDirect

Journal of Prosthodontic Research

journal homepage: www.elsevier.com/locate/jpor

Original article

Effect of different cleansing agents and adhesive resins on bond strength of contaminated zirconia

Kamolporn Wattanasirmkit[a], Taksid Charasseangpaisarn[b,*]

[a] Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[b] College of Dental Medicine, Rangsit University, Pathum Thani, Thailand

ARTICLE INFO

Article history:
Received 14 August 2018
Received in revised form 7 December 2018
Accepted 12 December 2018
Available online 28 January 2019

Keywords:
Zirconia
Adhesive resin
Cleansing agent
Bond strength
Contaminated surface

ABSTRACT

Purpose: This study was observed the effect of cleansing agents and adhesive resins on shear bond strength (SBS), surface morphology and phase transformation of saliva and silicone disclosing medium contaminated zirconia.
Methods: The 110 zirconia specimens size 5 × 5 × 1 mm were fabricated and randomly divided into 5 surface treated groups: Non-contaminated (PC) Saliva and silicone disclosing medium contaminated without cleansing (NC) Surface contaminated and cleansing with Phosphoric acid (PO) Ivoclean (IC) or Hydrofluoric acid (HF). The twenty of each surface treated specimens were selected and bonded with Panavia F2.0 (P) and Superbond C&B (S) for SBS test (n = 10). The data was analyzed by Kruskal–Wallis H and Mann–Whitney U test. The remaining specimens of each surface treated groups were examined by SEM and XRD.
Results: The saliva and silicone disclosing medium contaminated zirconia without cleansing group (PNC) had the lowest SBS when Panavia F2.0 was used for cementation ($p < 0.05$). The SBS of surface cleansing groups (PPO, PIC and PHF) were not different from the non-contaminated group (PPC) ($p > 0.05$). However, there were no difference in SBS among groups when cementation with Superbond C&B (SPC, SNC, SPO, SIC and SHF) ($p > 0.05$). There was no morphologic changing that could be observed by SEM. The XRD showed little phase transformation when surfaces were contaminated and cleaned.
Conclusions: The saliva and silicone disclosing medium contaminated zirconia should be cleaned with Phosphoric acid, Ivoclean or Hydrofluoric acid for 20 s prior to cementation with Panavia F2.0. However, the surface cleansing was not necessary when cementation with Superbond C&B.

1. Introduction

Nowadays, zirconia is widely chosen as an esthetic restorative material. Zirconia has many mechanical properties which are better than other materials such as chemical resistance, high fractural resistance and biocompatibility [1]. The previous studies showed that zirconia restoration should have been bonded with adhesive resin to obtain optimal bond strength. The zirconia surface could be prepared by both mechanical and chemical procedures. From the previous studies, the effective method for zirconia surface preparation is Tribochemical technique followed by silane-coupling agent application. However, the sandblasting is the most popular technique with a lower cost to create mechanical retention. The zirconia could be bonded chemically to adhesive resin with 10-MDP which could be found in some adhesive resin or separate bottles primer as surface priming agents [2–4].

Even though the fabrication technique of zirconia restoration is more accurate than the past, the try in procedure still necessary to assure that restoration is already seated in place before cementation. Many studies show that contamination of zirconia surface with saliva, blood or silicone disclosing medium from the try in procedure could reduce the bond strength of cemented zirconia. The surface cleansing after contamination could be done by both mechanical and chemical methods. The most effective mechanical cleansing method is sandblasting with alumina oxide particles but these may create the crack on the surface of restoration. Thus, many chemical cleansing agents were recommended to recover the bond strength after surface contamination such as hydrofluoric acid, phosphoric acid and sodium hydroxide solution [5–8].

Some studies show that phosphoric acid had a negative effect on cleansing the zirconia surface due to significantly reducing in bond strength when compared to non-contaminated surface. However, some studies show that phosphoric acid reduces the

* Corresponding author at: 52/347 Muang-Ake, Phaholyothin Road, Lak-Hok, Muang Pathum Thani, 12000, Thailand.
E-mail address: taksid.c@rsu.ac.th (T. Charasseangpaisarn).

https://doi.org/10.1016/j.jpor.2018.12.007
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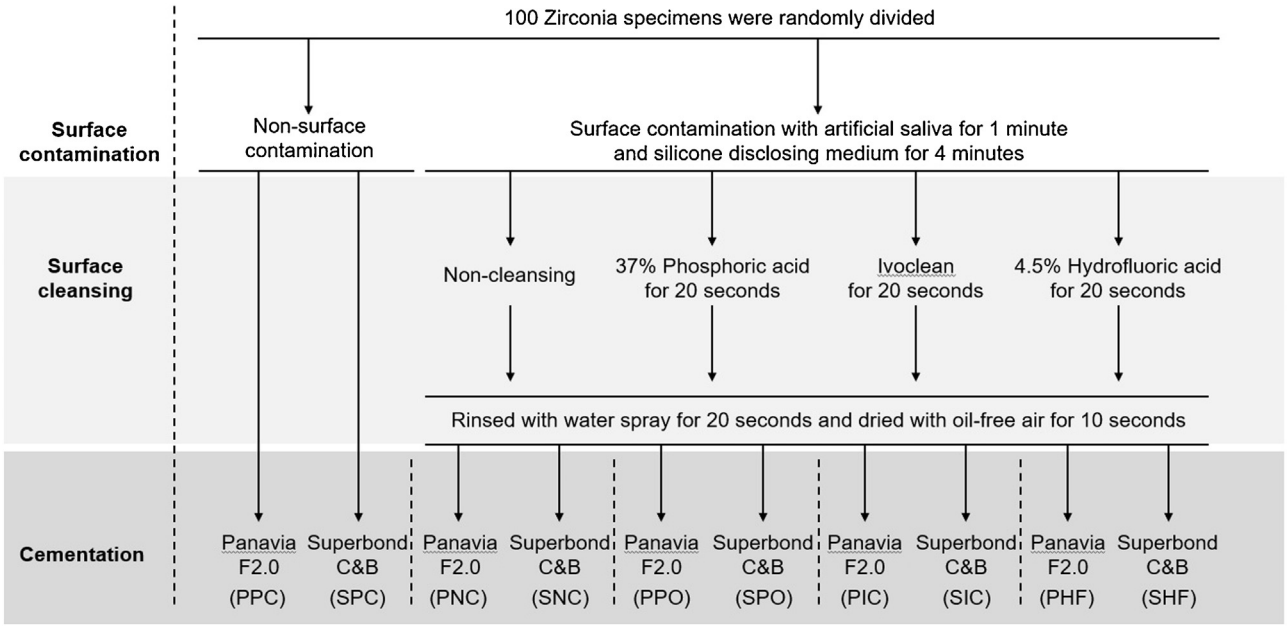

Fig. 1. The surface treatment methods in each experimental group.

bond strength but not significantly different from non-contaminated surfaces [6,7,9]. Hydrofluoric acid is recommended for cleansing the ceramic restoration and it could create the surface roughness of the silica-based restoration. The study of Sriamporn et al. found that zirconia's surface could be etched with hydrofluoric acid in some specific conditions, creating microscopic change and may be beneficial for mechanical bonding [10]. Ivoclean, alkaline base cleansing agent with zirconia oxide particles, is recently recommended for cleansing the contaminated surface of restoration without significantly morphologic changing [11,12].

Zirconia oxide is commonly in the monoclinic phase at room temperature, thus yttrium oxide is added to maintain the tetragonal phase which called "Yttria-stabilized tetragonal zirconia polycrystal" or "Y-TZP". When the crack initiates at the surface of Y-TZP, the stress of the crack tip will cause phase transformation from tetragonal to monoclinic. From the above situation, the volumetric expansion is occurred and will compress the crack tip to inhibit crack propagation, as this is called "Transformation toughening". However, applying the chemical agents to the surface of zirconia may cause significant change in the phase of zirconia and leading to worsening properties of zirconia restoration due to low temperature degradation [13].

The purpose of this study was to determine the effect of three different cleansing agents and two adhesive resins on shear bond strength of surface contaminated zirconia to composite resin. Furthermore, the changing of surface morphology and phase of zirconia at the surface when applying the cleansing agents was also investigated in this study.

2. Materials and methods

2.1. Specimen preparation

The 110 square shaped zirconia specimens (VITA YZ-HT, Vita Zahnfabrik, Bad Säckingen, Germany) size $5 \times 5 \times 1$ mm were fabricated and fully sintered by VITA ZYRCOMAT 6000 MS (Vita Zahnfabrik) following the manufacturer's instruction. The specimens were randomly divided as follows: 100 specimens for 10 groups of shear bond strength (SBS) test, 5 specimens for morphologic changing observation with Scanning electron microscope (SEM) followed by elemental changing with Energy Dispersive X-ray Spectroscope (EDS) and lastly, 5 specimens for zirconia phase transformation with X-ray Diffraction (XRD). The 100 zirconia specimens, for SBS test, were invested in the center of PVC tube with self-curing acrylic resin (Unifast Trad, GC, Tokyo, Japan).

The 100-cylindrical shaped composite resin specimens (Filtek Z350, 3M ESPE, St. Paul, MN, USA), 3 mm diameter and 4 mm height, were fabricated by silicone mold and covered with a glass slide. The composite was added with an incremental layering technique and curing with LED light curing unit (Elipar S10, 3M ESPE) for 20 s in each increment.

2.2. Surface preparation and SBS test

The 100 invested zirconia specimens were randomly divided into 5 surface preparation procedures, 20 specimens per procedures, as follows; the non-surface contamination group (Group PPC and SPC); Surface contamination with artificial saliva for 1 min followed by silicone disclosing medium under 1 kg pressure for 4 min then rinsed with water spray for 10 s and gently dried (Group PNC and SNC); Surface contamination and cleansing with 37% phosphoric acid for 20 s, water spray and gently dried (Group PPO and SPO); Surface contamination and cleansing with Ivoclean for 20 s, water spray and gently dried (Group PIC and SIC); Surface contamination and cleansing with 4.5% hydrofluoric acid for 20 s, water spray and gently dried (Group PHF and SHF). After the surface were treated as above methods, the bonding procedures were immediately carried out.

The surface treated specimens were randomly divided into 2 subgroups according to types of adhesive resins (n = 10) as Panavia F2.0 and Superbond C&B (Fig. 1). The specimens which subjected to bond with Superbond C&B were primed with Superbond Universal Ceramic Primer, MDP containing surface priming agent, for 20 s and dried with oil-free air prior to cementation. The adhesive resins were mixed following manufacturer's instruction and bonded to composite specimens under 1 kg pressure for 8 min with Durometer. In groups, which were bonded with Panavia F2.0, the resin was light-cured with an LED light curing unit for 40 s in

each side. Then, the bonded specimens were kept in 37 °C distilled water in an incubator (Contherm1200, Contherm, Wellington, New Zealand) for 24 h prior to the SBS test. The SBS test was done by a universal testing machine (EZ-S, Shimadzu, Kyoto, Japan) at cross head speed 1 mm/min. The data was analyzed by Mann–Whitney U test for effect of 'Adhesive resin' and Kruskal–Wallis H test for effect of 'Surface treatment'. Then the Kruskal–Wallis H test was performed to identified different among groups of surface treatment and post-hoc test was done by Mann–Whitney U test at 95% confidence level with SPSS 22.0 (IBM, New York, NY, USA).

2.3. Mode of failure analysis

After the SBS test, the fracture surface of the specimens, both zirconia and composite resin, were observed with stereomicroscope (SZ 61, Olympus, Tokyo, Japan) under 30× magnificent level. The mode of failure was classified as follows:

- *Adhesive failure*: The failure which occurred between 'Zirconia and Adhesive resin' (Adhesive ZR) or 'Adhesive resin and Composite resin' (Adhesive RC).
- *Cohesive failure*: The failure which occurred in 'Composite resin' (Cohesive C) or 'Adhesive resin' (Cohesive R).
- *Mixed failure*: The failure which occurred both adhesively and cohesively by at least 25% of the surface area (Mixed).

2.4. Surface morphologic examination, surface element and crystalline analysis

One specimen in each surface treatment group were observed with SEM (JSM-6480LV, JEOL, Dearborn Rd, MA, USA) under 20,000× magnificent level for morphologic changing and average grain size was measured follow ASTM E112. The surface element of specimens was analyzed with EDS (INCA x-sight, Oxford instrument, High Wycombe, UK) at 3 random points (whole examined area, in zirconia crystal, between grain boundary). The rest of the specimens in each surface treatment group were observed with XRD (D8, Bruker, Mannhiem, Germany) for crystalline changing from tetragonal phase to monoclinic phase.

3. Results

The normal distribution of data was performed by Kolmogorov–Smirnov test and found that the SPO group was not normal distribution ($p < 0.05$), then the non-parametric test was chosen in this study. The Kruskal–Wallis H test of 'Surface treatment' show there was no significant differences among groups ($p > 0.05$). The Mann–Whitney U test of 'Adhesive resin' show there was significant differences between Panavia F2.0 and Superbond C&B ($p < 0.05$) which the data showed that Superbond C&B had significantly higher SBS than Panavia F2.0. Due to the adhesive resin had an effect on SBS, the data was separately analyzed by Kruskal–Wallis H for each adhesive resin. The results (Table 1) found that there was no significant different between surface treated group of Superbond C&B ($p > 0.05$), the SBS of SPC, SNC, SPO, SIC and SHF were not significant differences. However, due to Kruskal–Wallis H test there was significant differences between surface treated group of Panavia F2.0 ($p < 0.05$), the post-hoc test was done by Mann–Whitney U test to identify the different between groups and found that the PNC showed significantly lower SBS than the others ($p < 0.05$). While there are no significant differences between PPC, PPO, PIC and PHF ($p > 0.05$) as showed in Table 3.

The mode of failure showed the groups that bonded with Panavia F2.0 were almost adhesive failure between zirconia surface and adhesive resin (Adhesive ZR). However, the groups that bonded with Superbond C&B had a majority of mixture failure (Mixed) or adhesive failure between composite resin and adhesive resin (Adhesive RC).

Table 1. The statistic results of Kruskal–Wallis H test of surface treatment classified by adhesive resin.

	Panavia F2.0	Superbond C&B
Chi-square	14.097	1.232
Df	4	4
Asymp. Sig.	0.007	0.873

4. Discussion

The surface contamination during intraoral try-in procedure with artificial saliva and silicone disclosing medium were proved to decrease in bond strength of the restorations. Previous studies showed that after zirconia surface was contaminated with the saliva, the phospholipid in saliva could bond and occupy the outer oxide layer of zirconia then there was little remaining oxide layer that 10-MDP could be bonded. This could reduce in bond strength of zirconia restoration if the contaminant was not removed. The silicone disclosing medium was also could be reduced in bond strength due to the remnants that limited the bond area of the restoration [11,14–16].

Previous studies have reported the different cleansing method, both mechanical and chemical cleansing, to improve bond strength of contaminated surfaces [17–20]. In this study, the effect of chemical cleansing agents (37% Phosphoric acid, Ivoclean and 4.5% Hydrofluoric acid) were observed on the SBS between zirconia and composite resin that bonded with 2 types of adhesive resin (Superbond C&B and Panavia F2.0).

Superbond C&B showed significantly higher SBS than Panavia F2.0. Monomer base of Superbond C&B is poly-methyl methacrylate (PMMA) linear chain which is has smaller molecules than Bis-GMA molecule of Panavia F 2.0 (Table 2). Penetrating ability of small molecules along irregularity surface of bonding interface could be easier than bigger molecules and form strong mechanical bond. When considering with mode of failure, the groups that bonded with Superbond C&B found a major cohesive failure in adhesive resin or mixed failure that means the bond strength between zirconia and adhesive resin is more than fractural strength of adhesive resin. In contrast of the groups that bonded with Panavia F2.0, adhesive failure between zirconia and adhesive resin was major which meant lower bond strength between zirconia and adhesive resin when comparing to bond strength between adhesive resin and composite resin. Due to Panavia F2.0 was containing 10-MDP in both ED primer and adhesive resin, thus the surface priming agent was not necessary. In the contrary of Superbond C&B which not contained 10-MDP, the Superbond Universal Ceramic Primer was suggested to application on zirconia surface prior to bonding procedure due to the primer contained 10-MDP and silane coupling agent.

In this study, the zirconia surface contaminated with artificial saliva and silicone disclosing medium could significantly reduce in SBS when bonded with Panavia F2.0. This may cause from contamination which residual organic substance from saliva and disclosing medium remained on the zirconia surface [16,21]. However, the SBS was recovered, and it was not significantly different from non-contaminated surface when the surfaces were cleansed with 37% Phosphoric acid, Ivoclean and 4.5% hydrofluoric acid for 20 s. These results were in agreement with many previous studies that showed the cleansing agents could be cleaned the organic substance on saliva contaminated surface and partially cleaned the silicone disclosing medium [6,22]. In contrast of some

Table 2. The details of materials using in the study.

Materials	Compositions	Lot/batch no; manufacturer
Superbond C&B	Powder: polymethyl methacrylate (PMMA)	RR1; Sun Medical, Chiba, Japan
	Liquid: 4-methacryloyloxyethyl trimellitate anhydride (4-META)/methyl methacrylate (MMA)	RR1; Sun Medical
	Catalyst V: partially oxidized tri-*n*-butyl borane (TBB), others	RR1; Sun Medical
Panavia F2.0	Paste A: 10-methacryloyloxy-decyl dihydrogen phosphate (MDP), hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated silica filler, silanated colloidal silica, camphorquinone, benzoyl peroxide	000033; Kuraray Dental, Tokyo, Japan
	Paste B: sodium fluoride, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, diethanol-*p*-toluidine, catalysts, pigments, others	000033; Kuraray Dental
Superbond Universal Ceramic Primer	Liquid A: 10-MDP, MMA	RR1; Sun Medical
	Liquid B: silane coupling agents, MMA	RR1; Sun Medical
Artificial saliva	Sodium carboxymethylcellulouse, potassium chloride, magnesium chloride, calcium chloride, Di-potassium hydrogen phosphate, potassium dihydrogen phosphate	Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
Fit tester	Silica containing silicone	092E77; Tokuyama Dental, Tokyo, Japan

Table 3. The mean, SD and mode of failure in each group.

Group	Mean ± SD (MPa)	Mode of failure (n = 10)				
		Adhesive		Mixed	Cohesive	
		ZR	RC		C	R
PPC	4.38 ± 0.59^{a}	10	0	0	0	0
PNC	3.33 ± 0.42^{b}	9	0	0	1	0
PPO	4.19 ± 1.04^{a}	10	0	0	0	0
PIC	4.44 ± 1.07^{a}	10	0	0	0	0
PHF	4.55 ± 0.62^{a}	10	0	0	0	0
SPC	30.33 ± 2.59^{A}	1	4	3	2	0
SNC	31.53 ± 4.33^{A}	0	5	4	1	0
SPO	30.77 ± 2.96^{A}	0	5	4	0	1
SIC	30.87 ± 3.15^{A}	2	3	3	0	2
SHF	30.87 ± 3.27^{A}	0	2	6	1	1

The same superscript meant no significant difference between groups at 95% confidence level (Capital and small alphabet were separated analysis).

studies which found that surface cleansing with phosphoric acid could significantly reduce bond strength of zirconia. The authors discussed that reducing in bond strength was caused from the phosphate of the phosphoric acid to be bonded to the oxide layer of zirconia and interrupted the chemical bond of 10-MDP [9,21]. When the SBS was correlated to the mode of failure in each group of Panavia F2.0, the results showed that the surface contamination was affected directly to the bonding between zirconia surface and adhesive resin due to the mode of failure was still occurred on the same area with decreasing in SBS value. In conclusion from SBS and mode of failure observation, the acidic (37% Phosphoric acid and 4.5% hydrofluoric acid) or alkaline (Ivoclean) cleansing agents could be used to recover the SBS of saliva and silicone disclosing medium contaminated zirconia not different from non-contaminated surface.

However, the SBS among the groups which bonded with Superbond C&B were not significantly different. The result might cause from the characteristic of the cement that have a lower viscosity and could penetrated to the roughness of the zirconia's surface. Furthermore, the application of Superbond Universal Ceramic Primer was used prior to cementation and could improve the surface wettability due to the containing silane coupling agents containing. This supports previous studies which observed that when the bonding which had low viscosity was applied to the contaminated zirconia prior to cementation, the SBS is not significant different from the non-contaminated surface [8,23]. The SBS was correlated to the mode of failure in each group of Superbond C&B which showed cohesive failure in adhesive resin and mixed failure which meant the bonding between zirconia and adhesive resin is higher in bond strength and was not affected by saliva and silicone disclosing medium contamination. Thus, the result may imply that mechanical retention had more effect on the bond strength of restoration than chemical retention and this could not have been substituted. However, the long-term observation on the leakage and aging condition, which may affect to decreasing the bonding durability of zirconia and adhesive resin, should be performed in further studies.

The SEM with 20,000× magnificent of 5 surface treated groups showed no significantly difference between groups were observed (Fig. 2). This was supported with the study of Smielak and Klimek who found that the surface morphology of zirconia from SEM observation has no change even through 5% hydrofluoric acid was applied for 15 min [24]. Furthermore, the EDS investigation (Table 4) showed that most elements on the zirconia surface was zirconium and oxygen but the phosphorus element, composition of phosphate from phospholipid in saliva, could not be observed in every group. This meant either the amount of phosphate was very low when compared to the investigated surface or there were no phosphate remaining on the surface. This result was in contrast of Phark et al. who found that phosphorus on the zirconia surface which was cleansed with phosphoric acid was significantly increased when compared to the others. However, the phosphorus in saliva and blood contaminated zirconia was not significantly different from the non-contaminated group [6]. From the results of this study, it could be implied that the bonding energy of phosphate to zirconia oxide is very low and could be eliminated by rinsing with the water.

Also, from EDS observation, the carbon which is composed in artificial saliva was observed in group NC, PO, IC and HF, except PC. Due to there was no either carbon or silicon element on the zirconia surface before contamination (PC) but after contamination (NC, PO, IC and HF), the carbon and silicon could be found on the surface that meant organic substance from saliva and silicone disclosing medium were remained on the zirconia surface and could not be completely elimination even the cleansing agents were applied. From previous studies which observed the carbon/oxygen to oxygen/zirconia ratio with X-ray Photoelectron Spectroscopy (XPS) found the decreasing in carbon concentration when the surface was contaminated with saliva and was cleaned with phosphoric acid or Ivoclean comparing to non-cleansing surface. Furthermore, the silicon from silicone disclosing medium was also partially removed when cleansing with cleansing agents. The silicon in PO and IC could be detected in low concentration but could not be detected in NC and HF group that may cause from the very low concentration of silicon or there was no remaining silicon on the surface. However, the bond strength was recovered not different from the non-contaminated surface [9,16,21]. The limitation of this study was that the EDS could not show the exact amount of the element on the investigated surface. In a further study, the XPS should be performed to confirm the results from this study.

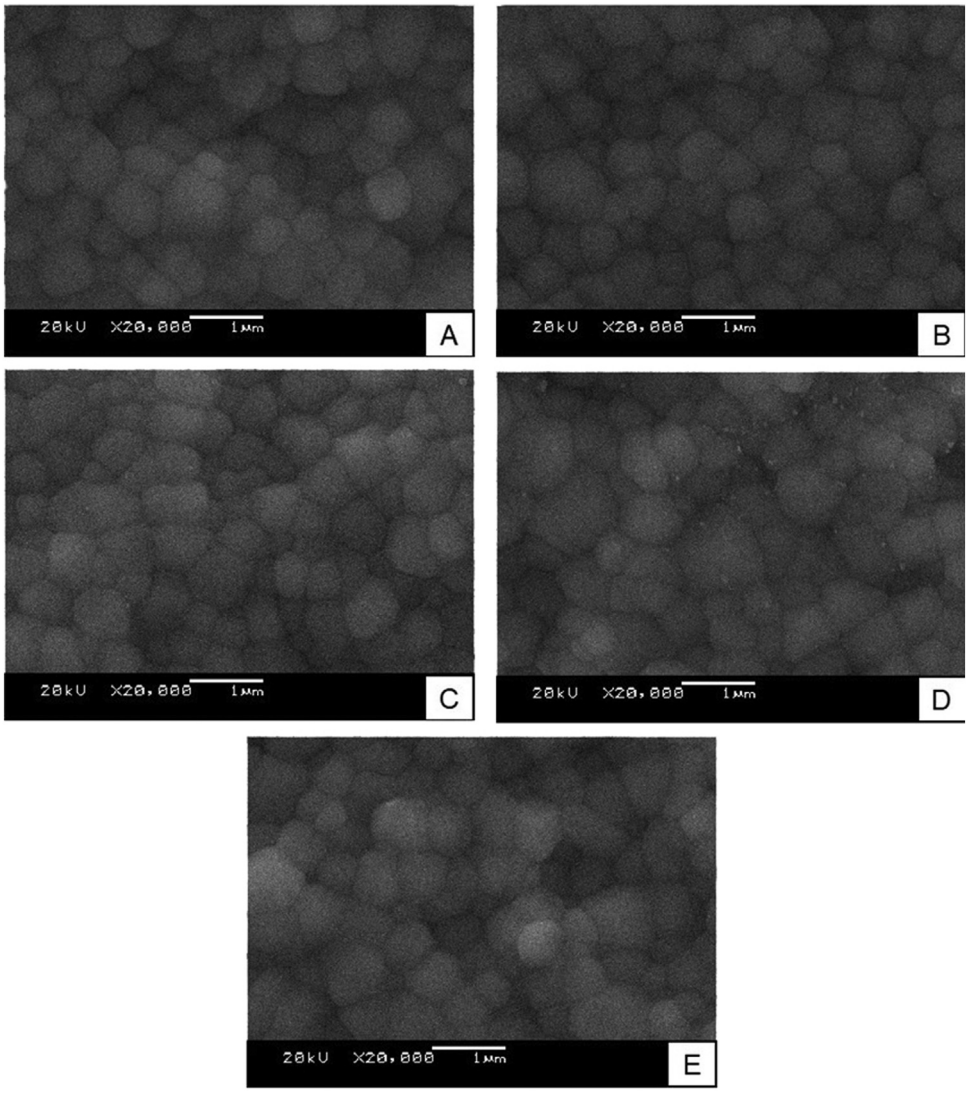

Fig. 2. The SEM showed surface morphology of zirconia at 20,000× magnificent of non-contaminated surface (A), contaminated surface without cleansing (B), contaminated and cleansing with 37% phosphoric acid (C), Ivoclean (D) and 4.5% hydrofluoric acid (E).

Table 4. The percentage of element from EDS and average grain size of each group.

Group	Weight % (± SD)					Average grain size (μm)
	Zr	O	P	C	Si	
PC	76.40 ± 3.93	23.60 ± 3.93	–	–	–	0.83
NC	57.46 ± 5.74	18.29 ± 4.31	–	24.25 ± 6.35	–	0.85
PO	59.45 ± 4.83	22.22 ± 3.61	–	18.02 ± 5.39	0.31 ± 0.49	0.79
IC	58.24 ± 5.04	17.91 ± 3.44	–	23.67 ± 5.66	0.19 ± 0.48	0.83
HF	60.08 ± 5.34	30.39 ± 4.13	–	8.59 ± 6.35	–	0.84

The XRD investigation found that PC has the highest ratio of tetragonal to monoclinic. The surface contamination and surface cleansing with different cleansing agents (NC, PO, IC and HF) could lead to phase transformation of zirconia from tetragonal to monoclinic as shown in Table 5. The XRD comparing graph (Fig. 3) was also showed little elevation of monoclinic phase at about 13 s of NC, PO, IC and HF group but cannot detected in PC group. Furthermore, the tetragonal phase was showed in high peak at about 15 s in all group. The phase transformation from tetragonal to monoclinic in surface treated groups may cause from the force that was applied during the contamination procedures. However, the percent changing was too low, from 1.55 to 1.95%. The average grain is one method that could determine the phase transformation of zirconia. When the zirconia phase was changed from tetragonal to monoclinic, the grain size was increased. Thus, the average grain size of each surface treated groups was determined followed ASTM E112-10 and found that the average grain size ranging from 0.79 to 0.85 μm (Table 4). This could be concluded that there was little changing in phase after surface contamination and cleansing but was not affected to average grain size and SBS in this study. Further studies should be investigated the effects to phase transformation after contamination and cleansing to the mechanical properties of the restoration such as fracture toughness, compressive strength, fatigue loading and etc.

Table 5. Shown percentage of monoclinic to tetragonal phase from XRD.

Group	Monoclinic (%)	Tetragonal (%)
PC	0.40	99.60
NC	1.95	98.05
PO	2.35	97.65
IC	2.02	97.98
HF	1.97	98.03

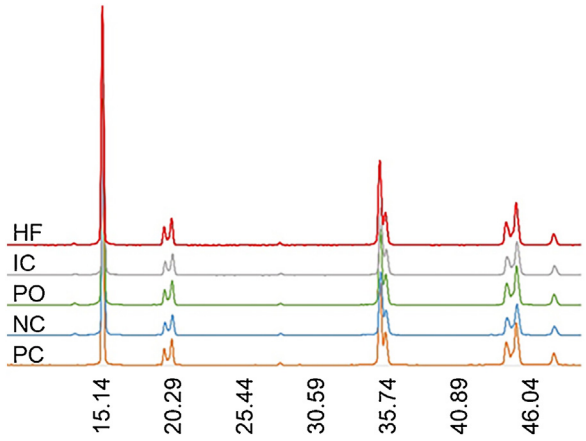

Fig. 3. The XRD graph of each surface treated group show monoclinic and tetragonal phase of zirconia.

5. Conclusion

When the SBS of each group were considered in cooperation with EDS and XRD, the following could be concluded:

1) The saliva and silicone disclosing medium contaminated on zirconia surface could reduce the SBS when bonded to composite resin with Panavia F2.0. Surface cleansing with 37% phosphoric acid, Ivoclean or 4.5% hydrofluoric acid for 20 s is necessary to recover the bond strength of saliva and silicone disclosing medium contaminated zirconia when bonded with Panavia F2.0.
2) The saliva and silicone disclosing medium contaminated on zirconia surface has no effect on the SBS when bonded to composite resin with Superbond C&B. Thus, the surface cleansing after contamination was not necessary.

Acknowledgements

This research was funded by the Research Institute of Rangsit University (Funding no. 47/2559).

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