Received: 15 September 2021 | Revised: 17 November 2021 | Accepted: 5 December 2021

DOI: 10.1002/jemt.24028

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

How does antiseptic mouthwashes against SARS-COV-2 affect the bond strength of universal adhesive to enamel?

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Review Editor: adi salomon

Abstract

This study compares the effect of different mouthwashes that have been recommended during the Coronavirus disease 2019 (COVID-19) pandemic on shear bond strength (SBS) of universal adhesive to enamel in regards to self-etch (SE) and etch-and-rinse (ER) modes. Flat enamel surfaces were obtained from 100 sound human maxillary central incisors. They were randomly allocated to five groups according to the different mouthwashes (no mouthwash/control [Ctrl], 0.2% chlorhexidine 1.5% hydrogen peroxide [H_2O_2], 0.2% povidone-iodine [PVP-I], Listerine [L]), and adhesive application modes (ER and SE) ($n = 10$). After the application of a universal adhesive (single bond universal), composite resin (Filtek Z250) was bonded by a cylinder-shaped mold (height: 2 mm, diameter: 2.4 mm). They were subjected to SBS test using a universal testing machine (AGS-X, Shimadzu Corp.) (crosshead speed: 1 mm/min). The resin–enamel interfaces were observed with a scanning electron microscope (SEM). The semiquantitative chemical microanalyses were performed with energy-dispersive spectroscopy (EDS). The data were statistically analyzed by two-way analysis of variance and Bonferroni test ($p < .05$). In SE mode, Group Ctrl revealed significantly higher SBS than all mouthwash groups ($p < .05$). In ER mode, Group Ctrl showed significantly higher SBS than H_2O_2 and PVP-I groups ($p < .05$). ER mode caused significantly higher SBS than SE mode in all mouthwash groups ($p < .05$). The SEM observations highlighted that Group Ctrl had a regular and intact hybrid layer with resin tag formation while the H_2O_2 and PVP-I groups exhibited a thin hybrid layer in both modes. EDS analysis indicated that in SE mode, all mouthwash groups presented increased O content compared to Group Ctrl. H_2O_2 and PVP-I that were suggested for preprocedural use during the COVID-19 pandemic, reduced the enamel bond strength of the universal adhesive in ER mode.

KEYWORDS

chlorhexidine, hydrogen peroxide, mouthwash, povidone-iodine, shear bond strength

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), which causes respiratory disease resulting from SARS-CoV-2 infection, has become a major global public health problem since the beginning of 2020 (Phelan, Katz, & Gostin, 2020). The literature determines an approximate incubation period of one to 14 days for SARS-CoV-2 (Chan et al., 2020). Clinical symptoms of COVID-19 may include sore throat and/or reduction/loss of smell or taste, cough, fever, shortness of breath, and muscle pain. However, the disease can be asymptomatic but still infectious

(Guan et al., 2020). SARS-CoV-2 is highly contagious since it can be transmitted by direct contact with an infected person or through the spread of aerosol droplets (To et al., 2020). The virus can be transmitted to nasal and oral mucosa and eyes by a contaminated hand (Zhang, Zhang, & Wang, 2020).

The aerosols and droplets generated during dental procedures increase the risk of SARS-CoV-2 infection. Furthermore, saliva-contaminated surfaces could lead to potential cross-infection (Van Doremalen et al., 2020). For dental practices and hospitals, strict and effective infection control protocols are needed due to cross-infection risk. The droplets originating from dental treatment can exist for several hours in the air and, ultimately, be a potential source of multiple transmissions (Ge, Yang, Xia, Fu, & Zhang, 2020). Thus, it is necessary to control the viral load in the saliva and respiratory secretions. The risk of transmission or infection might be reduced by lowering the number of infectious virus particles with the use of oral antisepsis (Del Rio & Malani, 2020). Therefore, many associations have advised the use of a pre-operative mouthwash. The New Zealand Dental Association recommends using preprocedural mouthwash of 1% hydrogen peroxide (H_2O_2), 0.2% chlorhexidine (CHX), 0.2% povidone-iodine (PVP-I), or Listerine for 30 s before the dental procedures. Furthermore, the American Dental Association recommends using 0.2% PVP-I since the use of 0.2% PVP-I for 30 s can decrease the infectivity of the SARS-CoV-2 virus to below detectable levels. Additionally, PVP-I can reduce the viral load in the saliva and prevent SARS-CoV-2 attachment to oral and nasopharyngeal tissues (American Dental Association, 2020). H_2O_2 and PVP-I are the most recommended mouthwashes by other dental organizations, such as the Scottish Dental Clinical Effectiveness Programme and the International Federation of Endodontic Association (Jamal et al., 2020). In recent studies, 30 s use of Listerine (Johnson & Johnson, New Brunswick, New Jersey, USA), an antiseptic mouthwash that contains phenolic compounds, also reduced the viral load of human coronavirus to more than 99.9% (Meister et al., 2020; Meyers et al., 2021). CHX is a broad-spectrum antiseptic mouthwash, which is commonly suggested before operative procedures. CHX increases the cell wall permeability, acting against gram-positive and gram-negative bacteria, aerobes, anaerobes, and fungus by causing its lysis (Banakar, Lankarani, Jafarpour, Banakar, & Mohammad, 2020). CHX was claimed to have little effect against coronaviruses when compared with other mouthwashes (Peng et al., 2020). However, a previous study suggests that CHX might be a simple and safe option to prevent COVID-19 and reduce disease spread (Huang & Huang, 2021).

There has been a tendency to use one product for multiple purposes. Universal adhesives are novel adhesive systems that were launched on the market with the ability of bonding to various substrates (Perdigão, Araujo, Ramos, Gomes, & Pizzolotto, 2021). These multipurpose adhesives also provide the opportunity to choose which etching strategy to use. Universal adhesives are produced to bond to tooth structures via the etch-and-rinse (ER), self-etch (SE), and selective-etch strategies (da Rosa, Piva, & da Silva, 2015).

Some studies evaluate the effect of CHX, PVP-I, and Listerine on shear bond strength (SBS) to enamel (Catalbas, Ercan, Erdemir, Gelgor, & Zorba, 2008; Singh et al., 2018). However, there are limited data on the effect of recently recommended pre-operational mouthwashes on bond strength to enamel. Thus, this study aims to compare the effect of different mouthwashes that have been recommended during the COVID-19 pandemic to decrease the viral load on SBS of universal adhesive to enamel in regards to SE and ER application modes.

The research study proposes two null hypotheses:

1. Antiseptic mouthwashes will not influence the SBS of universal adhesive to enamel.
2. The application mode will not affect the SBS of universal adhesive to enamel.

2 | MATERIALS AND METHODS

The local ethics committee approved this in vitro study (2021/121).

2.1 | Specimen size calculation

The specimen size calculation was performed based on the estimated effect size between groups, according to the literature (Sai et al., 2018). This study required 10 specimens for each group to obtain a medium effect size ($d = 0.5$), setting 95% power, and 5% Type 1 error rate.

2.2 | Specimen preparation and restorative procedures

The chemical compositions and manufacturers of restorative materials and mouthwashes used in this study are presented in Table 1. One hundred extracted, sound, human maxillary anterior central incisors were used for the SBS test and kept in saline solution. After removing the root from 2 mm below the cementoenamel junction, the teeth were embedded in cold cured acrylic resin (Integra, BGD, İstanbul, Turkey). The labial enamel surfaces of each tooth were ground with 400- and 600-grit silicon carbide paper under water cooling respectively to achieve a standard smear layer using a polishing machine (Minitech 233, Presi Grenoble, France). Next, the enamel specimens were examined with a stereomicroscope (SMZ 1000, Nikon, Japan) for any cracks or exposed dentin. Then, the teeth were randomly allocated into five groups according to mouthwashes:

1. Group Control (Ctrl): No mouthwash application.
2. Group CHX: The specimens were rinsed with 0.2% CHX (Werax, SDD, İzmir, Turkey) for 30 s.
3. Group H_2O_2: The specimens were rinsed with 1.5% H_2O_2 (Naturel Hydrogen Peroxide, Naturel Medical Pharma, Istanbul, Turkey) for 30 s (Banakar et al., 2020).

TABLE 1 The chemical compositions and manufacturers of restorative materials and mouthwashes used in this study

	Manufacturer	Composition
Werax chlorhexidine	SDD, İzmir, Turkey	2% Chlorhexidine solution
Naturel hydrogen peroxide	Naturel Medical Pharma, Istanbul, Turkey	3% Hydrogen peroxide, 0.03% stabilizer, 96.97% solvent
Listerine cool mint antiseptic mouthwash	Johnson & Johnson, Istanbul, Turkey	Thymol, eucalyptol, methyl salicylate, menthol, water, sorbitol solution, 30% alcohol, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate, green dye, mint essence
Batticon	ADEKA, Istanbul, Turkey	10% Povidone-iodine solution, 1.5% emulsifier, 0.5% stabilizer, 0.5% pH adjuster, 87.829% solvent
FiltekZ250 Lot:NA23939	3M ESPE (St. Paul, MN)	Bis-GMA, UDMA, Bis-EMA, TEGDMA, ZrO_2–SiO_2 (60%vol)
Single Bond Universal Lot: 81129D	3M ESPE	MDP phosphate monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiators, and silane
ScotchbondTM Universal Etchant Lot:0123	3M ESPE	32 wt% phosphoric acid, 60% water, 5% synthetic amorphous silica

4. Group PVP-I: The specimens were rinsed with 0.2% PVP-I (Batticon, Adeka, Samsun, Turkey) for 30 s (Jamal et al., 2020).
5. Group L: The specimens were rinsed with Listerine (Johnson & Johnson) for 30 s.

All mouthwashes were applied to the enamel surfaces for 30 s. There were not any diluted commercial forms of 1.5% H_2O_2, 0.2% PVP-I, and 0.2% CHX. Therefore, the mouthwashes were prepared in specified concentrations by diluting them with sterile water (Chopra, Sivaraman, Radhakrishnan, Balakrishnan, & Narayana, 2021). Besides, the specimens were not rinsed off with water before the application of adhesive systems.

Then, each group was divided into two subgroups according to the application mode of the universal adhesive ($n = 10$):

1. *ER mode*: 37% phosphoric acid gel (Scothbond Universal Etchant, 3M ESPE, St. Paul, MN) was applied on the enamel surfaces for 15 s, rinsed with water for 10 s, and dried with cotton pellets. Single Bond Universal (3M ESPE) was applied and rubbed for 20 s with a micro brush and light-cured for 10 s with a light-emitting diode (LED) light-curing unit (LCU) (1,000 mW/cm^2) (Valo, Ultradent, South Jordan, UT).
2. *SE mode*: Single bond universal was applied with no prior acid etching, as mentioned for the ER groups.

Following the adhesive procedures, a microhybrid composite resin (Filtek Z250, 3M ESPE) was bonded by a cylinder-shaped Teflon mold (2 mm height, 2.4 mm diameter) according to ISO 29022 (ISO-Standards, 2013), and light-cured for 20 s with LED LCU. The light intensity was checked with a radiometer (Demetron LED Radiometer, Kerr Corp., Orange, CA). All the restorative procedures were done according to the manufacturer's recommendations. All restorative procedures were done by a single operator who was blinded to the mouthwashes used in this study.

2.3 | SBS test

Before the SBS testing procedures, all specimens were stored in distilled water at room temperature for 24 hr. Then, the specimens were subjected to a notch-edge SBS test using a universal testing machine (AGS-X, Shimadzu Corp., Japan) with a crosshead speed of 1 mm/min. The testing load was directly applied on the tooth–restoration interface until the fracture. The diameter of the bonded composite was the same with a notched-edge crosshead (Sabatini, 2013). SBS values were calculated by dividing the failure load (*N*) by the adhesion area (mm^2), converted to MPa. An operator, who was unaware of the mouthwashes used in this study, performed all the SBS tests.

2.4 | Failure mode analysis

Failure mode analysis was performed with a stereomicroscope under ×15 magnification. The failure mode was recorded as an “adhesive” failure if the fracture occurred along the junction of the resin composite and the enamel. The failure mode was recorded as a “cohesive” failure if the fracture occurred in the resin composite or enamel. The failure mode was recorded as a “mixed” failure if the fracture occurred in the resin composite or enamel. An operator, who was unaware of the mouthwashes used in this study, performed all the failure mode analyses.

2.5 | Scanning electron microscopy analysis

One specimen from each group was prepared for scanning electron microscope (SEM) (Carl Zeiss Evo LS 10, Germany) analysis of the enamel–restoration interfaces. The surfaces were ground and an adhesive system and composite resin were applied to the enamel surfaces as stated above. Specimens were kept in distilled water for 24 hr and then cross-sectioned through the bonded interface on the sagittal plane using the diamond saw. The sectioned surfaces were submerged in 6 N HCL for 30 s to demineralize the dentin, then rinsed with water for 5 min. Then, they were submerged in 3% NaOCl for 10 min and rinsed with water for 5 min. A desiccator was used to dehydrate the specimens for 12 hr (Perdigão, Lopes, & Gomes, 2008). The specimens were gold-sputter coated to evaluate the resin–enamel interface at an accelerating voltage of 10 kV in a secondary mode. The micromorphology of representative surfaces was achieved at ×1,000 and ×2,500 magnification. An operator, who was unaware of the mouthwashes used in this study, performed all the SEM analyses.

2.6 | Energy-dispersive X-ray spectroscopy analysis

The semiquantitative chemical microanalyses were performed with energy-dispersive spectroscopy (EDS) to evaluate the effect of mouthwash application or mouthwash application after acid etching on enamel. Two specimens were prepared from each mouthwash group for EDS analysis. All mouthwashes were applied to the enamel surfaces as previously mentioned. Next, half of the specimens were etched with 37% phosphoric acid gel for 15 s. The surfaces were dehydrated in a desiccator for 12 hr. Then, all specimens were gold-sputter coated and the analysis was performed along the entire length of the flattened surface. The EDS module of the SEM (Thermo Fischer Scientific, Phenom XL, Waltham, Massachusetts, USA) was used because it can determine elements from boron (B) to americium (Am) due to the ultrathin silicon nitride X-ray window. A thermoelectrically cooled (LN2 free) silicon drift detector was used, and an energy resolution below 137 eV at Mn Kα was achieved with a 10 eV/ch processing capability of 2,048 channels and 300,000 counts/s. Thus, elemental analysis with a high certainty was achieved for the basic element percentages (C, O, F, P, Ca, I). An operator, who was unaware of the mouthwashes used in this study, performed all the EDS analyses.

2.7 | Statistical analysis

Statistical analysis was conducted with SPSS 22.0 for Windows (SPSS Inc., Chicago, IL). Normality was measured by the Shapiro–Wilk test and variance homogeneity was measured by Levene's test. Since there was normality and variance homogeneity, a two-way analysis of variance was made to compare differences within and between groups. All pairwise comparisons were performed with the Bonferroni test. The statistical significance was considered at a confidence level of .05 for all analyses.

TABLE 2 The mean SBS values and ±*SD* of all tested groups in MPa ($n = 10$)

Groups	ER	SE	p
Group control	31.48 ± 7.45a	26.43 ± 4.35a	.015
Group CHX	28.30 ± 6.03a,c A	17.67 ± 4.37b B	<.001
Group H_2O_2	21.40 ± 3.67b A	3.77 ± 1.37c B	<.001
Group PVP-I	24.04 ± 5.01a,b A	8.70 ± 1.92cd B	<.001
Group L	29.61 ± 4.78b,c A	9.99 ± 2.90d B	<.001
	<0.001	<0.001	

Abbreviation: CHA, chlorhexidine; ER, etch-and-rinse; H_2O_2, hydrogen peroxide; PVP-I, povidone-iodine; SBS, shear bond strength; SE, self-etch. Different small letters on the same column and capital letters on the same row indicate significant differences between mouthrinses ($p < .05$).

3 | RESULTS

3.1 | SBS test

Table 2 presents the mean SBS values with standard deviations of all the tested groups.

In SE mode, Group Ctrl displayed significantly higher SBS than all mouthwash groups. Group CHX had significantly higher SBS than other mouthwash groups ($p < .05$). However, there were no significant differences in SBS between Groups H_2O_2 and PVP-I and Groups L and PVP-I ($p > .05$).

In ER mode, Group Ctrl had significantly higher SBS than Groups H_2O_2 and PVP-I ($p < .05$) while it had similar SBS to Groups CHX and L ($p > .05$). Furthermore, Group PVP-I exhibited similar SBS to Group H_2O_2, Group CHX, and Group L ($p > .05$).

ER mode caused significantly higher SBS than SE mode, regardless of the preoperational antiseptic mouthwash used ($p < .05$).

3.2 | Failure mode analysis

Figure 1 presents the stacked column chart illustrating the frequency distribution of the failure mode analysis of the all tested groups. The predominant failure mode was the adhesive type for all groups. The second most frequently observed failure mode was the mixed type. Cohesive failures were detected for Groups Ctrl + ER, CHX + SE, CHX + ER, PVP-I + SE, and H_2O_2 + ER.

3.3 | SEM analysis

Figure 2 presents the representative SEM images of all tested groups. Hybrid layers were detected for all groups. For Group Ctrl + ER, more resin tags were observed with an intact hybrid layer while for Group Ctrl + SE, the hybrid layer was visible with few resin tags and gap formation (Figure 2a,b). In both application modes, Group CHX exhibited a regular and intact hybrid layer appearance (Figure 2c,d). Additionally, shorter resin tags were visible for Group CHX + ER (Figure 2c). In

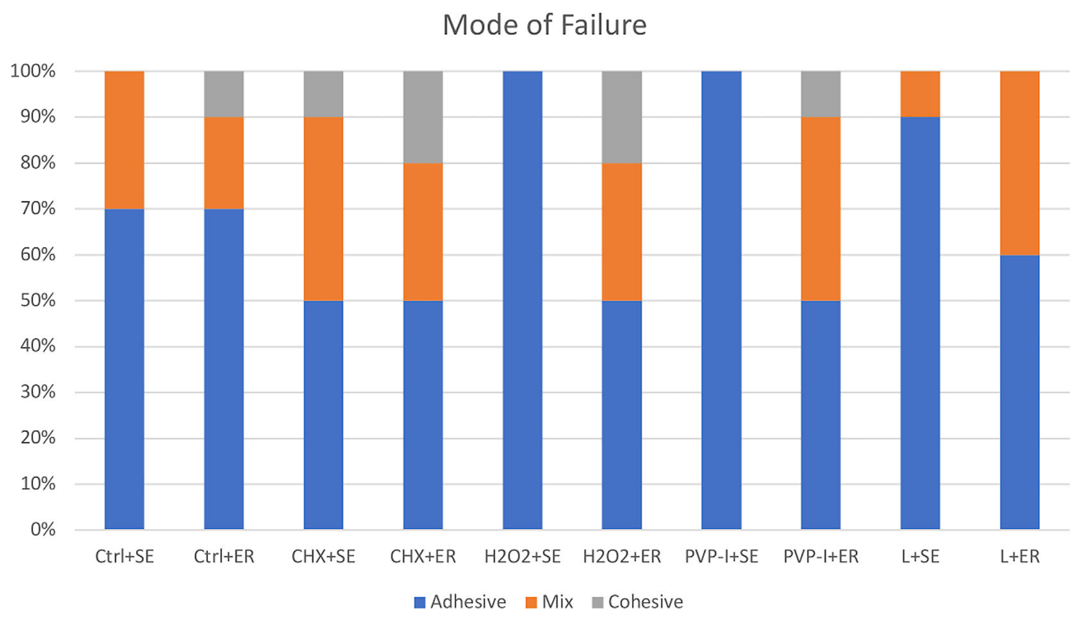

FIGURE 1 The stacked column chart illustrating the frequency distribution of the failure mode analysis of all tested groups

both application modes, Group H_2O_2 had thin hybrid layer appearances. Fewer and shorter resin tags were observed for Group H_2O_2 + ER whereas no resin tag formation was detected for Group H_2O_2 + SE (Figure 2e,f). In both application modes, Group PVP-I had a thin hybrid layer appearance with less clear resin tag formation (Figure 2g,h). Furthermore, more prevalent gap formation was seen for Group PVP-I + SE (Figure 2h). Group L + ER demonstrated intact and regular hybrid layer appearance while Group L + SE had a hybrid layer with gap formation (Figure 2i,j).

3.4 | EDS analysis

The representative semiquantitative chemical microanalyses and distribution of elements for all tested groups are presented in Table 3 and Figure 3. Fluoride and iodine contents were similar for all groups. In SE mode, all mouthwash groups presented an increased O content compared to Group Ctrl. In ER mode, the O content of most mouthwashes was similar to Group Ctrl. However, a decrease in the O content was observed for Group PVP-I.

4 | DISCUSSION

The patients may feel uncertainty and fear about visiting dental offices during the COVID-19 outbreak. This can cause several negative economic impacts on the dental clinics and also negatively impact the dental health of the population (González-Olmo, Delgado-Ramos, Ortega-Martínez, Romero-Maroto, & Carrillo-Díaz, 2021). However, presymptomatic and asymptomatic patients present risks to dentists, allied health staff, and other patients (Palla & Callahan, 2021). The excessive viral load found in the saliva, oropharynx, and nasopharynx is the cause of the transmission risk in dental care (Meyers et al., 2021). Thus, many organizations and specialty associations have offered guidelines and advice to reduce the transmission of SARS-CoV-2 from patients to dentists and vice versa. Some associations have advised using different preprocedural mouthwashes to minimize the viral load in the oral cavity and aerosols that originate during dental procedures (Banakar et al., 2020). Using these mouthwashes before and during the operative procedures can affect adhesive procedures as well (Catalbas et al., 2008; Singh et al., 2018).

In clinical applications, only adhesive restorations are applied to enamel surfaces in treatments, such as composite lamina construction and diastema closure. In these treatments, to protect the enamel tissue, adhesive systems could be applied without the preparation of the enamel for some clinical situations (Fahl & Ritter, 2021). Thus, this in vitro study compares the effect of different mouthwashes that have been recommended during the COVID-19 pandemic to decrease the viral load on the SBS of universal adhesive to enamel regarding SE and ER application. Based on the results of this study, the first null hypothesis, which states that antiseptic mouthwashes will not influence the SBS of universal adhesive to enamel, is rejected. The second null hypothesis, which states that the application mode will not affect the SBS of universal adhesive to enamel, is also rejected because the ER application mode of the universal adhesive system led to higher SBS than the SE application mode for all groups.

Listerine contains essential oils, which are menthol, thymol, and eucalyptol. Polyphenols have antiseptic, antioxidant, and anti-inflammatory effects. Ethanol is also present in Listerine antiseptic mouthwash (Sabatini, 2013). Pelio et al. determined that alcohol-containing mouthwashes caused no morphological, ultrastructural, or biochemical change in the hydroxyapatite of human enamel (Pelino, Passero, Martin, & Charles, 2018). A recent study noted that using CHX once might result in the suppression of COVID-19 transmission.

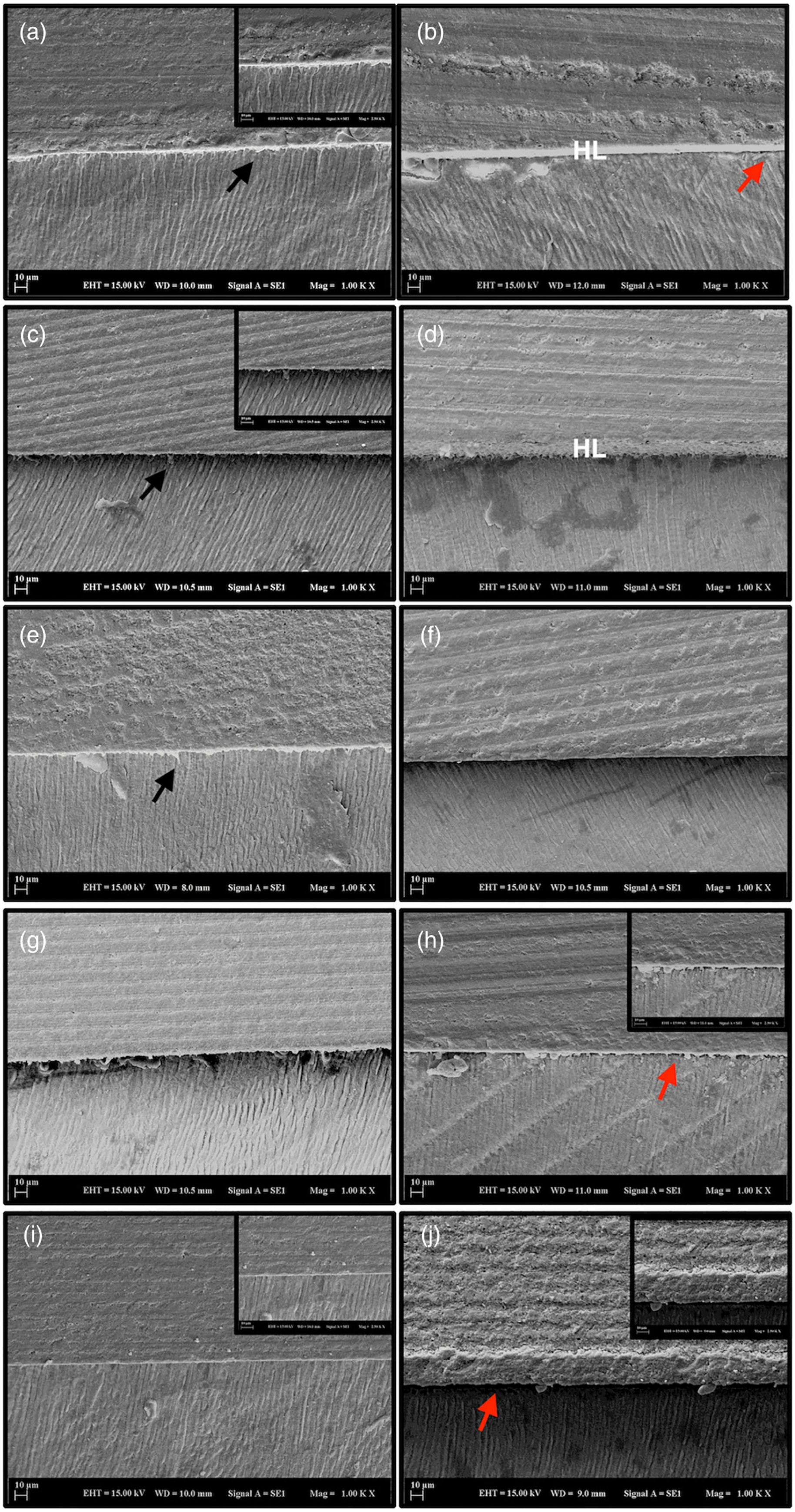

FIGURE 2 Representative scanning electron microscope (SEM) images of all tested groups at ×1,000 (larger images) and ×2,500 (smaller images) magnification. (a) Group Ctrl + ER, (b) Group Ctrl + SE, (c) Group CHX + ER, (d) Group CHX + SE, (e) Group H_2O_2 + ER, (f) Group H_2O_2 + SE, (g) Group PVP-I + ER, (h) Group PVP-I + SE, (i) Group L + ER, and (j) Group L + SE. HL: hybrid layer, black arrows: resin tags, red arrows: gap formations. CHA, chlorhexidine; ER, etch-and-rinse; H_2O_2, hydrogen peroxide; PVP-I, povidone-iodine; SE, self-etch

TABLE 3 The representative semiquantitative chemical microanalyses of all tested groups (%)

	Group Ctrl + SE	Group Ctrl + ER	Group CHX + SE	Group CHX + ER	Group H_2O_2 + SE	Group H_2O_2 + ER	Group PVP-I + SE	Group PVP-I + ER	Group L + SE	Group L + ER
C	6	12	8	12	7	14	11	4	8	33
O	11	22	23	21	13	18	20	8	21	23
F	1	3	1	2	1	1	2	1	2	2
P	32	30	32	30	32	27	30	37	32	20
Ca	45	30	32	31	42	35	33	45	33	20
I	5	4	3	4	5	4	4	5	4	3

Abbreviations: CHA, chlorhexidine; ER, etch-and-rinse; H_2O_2, hydrogen peroxide; PVP-I, povidone-iodine; SE, self-etch.

However, Yoon et al. indicated that the use of 15 ml 0.12% CHX would be beneficial for providing SARS-CoV-2 suppression for 2 hr (Yoon et al., 2020). In the present study, in SE mode, CHX and Listerine mouthwashes led to significantly lower enamel bond strength compared to no mouthwash application (control). When the SBS values of the two types of mouthwash were compared in SE mode, CHX caused significantly higher bond strength values than Listerine. These data obtained from the SBS values are also compatible with the SEM images showing that in the CHX-treated group, regular and an intact hybrid layer was observed, while in the hybrid layer of the Listerine groups, gap formation was seen. In ER mode, the use of CHX and Listerine mouthwashes had no significant change in enamel bond strength. This finding can be explained by the fact that application of phosphoric acid might remove the residue of CHX and Listerine. Demir et al., who evaluated the effect of the application of two antibacterial mouthwashes (0.2% CHX and 7.5% PVP-I) to etched and unetched enamel on the SBS of an orthodontic composite resin, reported that the application of CHX and PVP-I before acid etching did not lead to any reduction in bond strength (Demir, Malkoc, Sengun, Koyuturk, & Sener, 2005). PVP-I is a broad-spectrum microbicidal, which can inactivate viruses, bacteria, and fungi. PVP-I can inactivate both nonenveloped and enveloped viruses. The antimicrobial mechanism of PVP-I is due to its free or nonbound eluted iodine (I_2). PVP-I is the water-soluble polymer polyvinylpyrrolidone. In an aqueous solution, the element iodine is in two forms, I_2 and hypoiodous acid, which are active in terms of the antimicrobial activity of PVP-I (Eggers, 2019). H_2O_2 is a colorless and odorless liquid that has been used for a century. H_2O_2 is an oxidative agent which makes it effective to SARS-CoV-2 (Sabatini, 2013). Bidra et al. suggest that preprocedural rinsing with diluted PVP-I in the range of 0.5–1.5% may be preferred over H_2O_2 (1.5 and 3.0%) to prevent transmission of SARS-CoV-2 (Bidra et al., 2020). In this study, the results revealed that the preoperative use of H_2O_2 and PVP-I mouthwashes had significantly lower enamel bond strength than no mouthwash application in both the SE and ER mode of the universal adhesive. When the connection interfaces of these groups were examined, a thin hybrid layer was observed with short resin tags in the H_2O_2 groups, and many gaps were observed in the hybrid layer with less resin tag formation in the PVP-I groups. In an in vitro study by Bistey et al., the alteration in human enamel after H_2O_2 treatment was evaluated by FT-IR spectroscopy. Their results revealed that even at low (10%) concentrations, H_2O_2 caused changes in superficial enamel after H_2O_2 treatment. Furthermore, it was highlighted that significant changes with a considerable decrease of phosphate ions occurred at H_2O_2 exposures of longer than 30 min (Bistey, Nagy, Simo, & Hegedus, 2007). However, in this study, no significant decrease in phosphate ions was detected in the SEM–EDX examination since 1.5% H_2O_2 was applied for 30 s, Furthermore, when compared to the control group, there was a slight increase of oxygen ions in the mouthwash groups. The reason for the significantly lower bond strength observed in all mouthwash groups applied in SE mode compared to the control group can be attributed to oxygen ions. In the literature, it was determined that the existence of residual oxygen in enamel pores, jeopardizes the polymerization of resins. However, if bond strength was decreased due to residual oxygen, the phosphoric acid application might decrease this negative effect (Rueggeberg & Margeson, 1990). In this study, it was determined that the ER mode application after all mouthwashes caused significantly higher bond strength than the SE mode for the mouthwash and control groups. Stereomicroscope observations confirmed this finding that the frequency of mixed failure, in addition to cohesive failures, tended to increase for the ER mode. When universal adhesives are employed with the SE mode, their lower aggressiveness decreases their demineralization potential for enamel, hence, leading to improper retentive microporosities (Nagarkar, Theis-Mahon, & Perdigão, 2019). Rosa et al. highlighted that universal adhesive with the SE mode caused significantly lower enamel microshear and μTBS values compared to the ER mode (da Rosa et al., 2015). Previous studies (McLean, Meyers, Guillory, & Vandewalle, 2015; Suda et al., 2018) indicated that phosphoric acid etching significantly increased the SBS of an adhesive system to enamel. It was concluded that phosphoric acid application before universal adhesives resulted in significantly higher bond strength, regardless of whether the enamel was unground or ground (Takeda et al., 2019).

Kutuk et al., who investigated the effect of H_2O_2 (1.5%) and PVP-I (0.2%) mouthwashes on the SBS of universal adhesive to enamel, reported that the highest SBS in the ER application mode was obtained for the control group, followed by Groups PVP-I and H_2O_2, respectively. Furthermore, they indicated that the ER application mode exhibited higher SBS on the enamel for Group H_2O_2 (Kutuk,

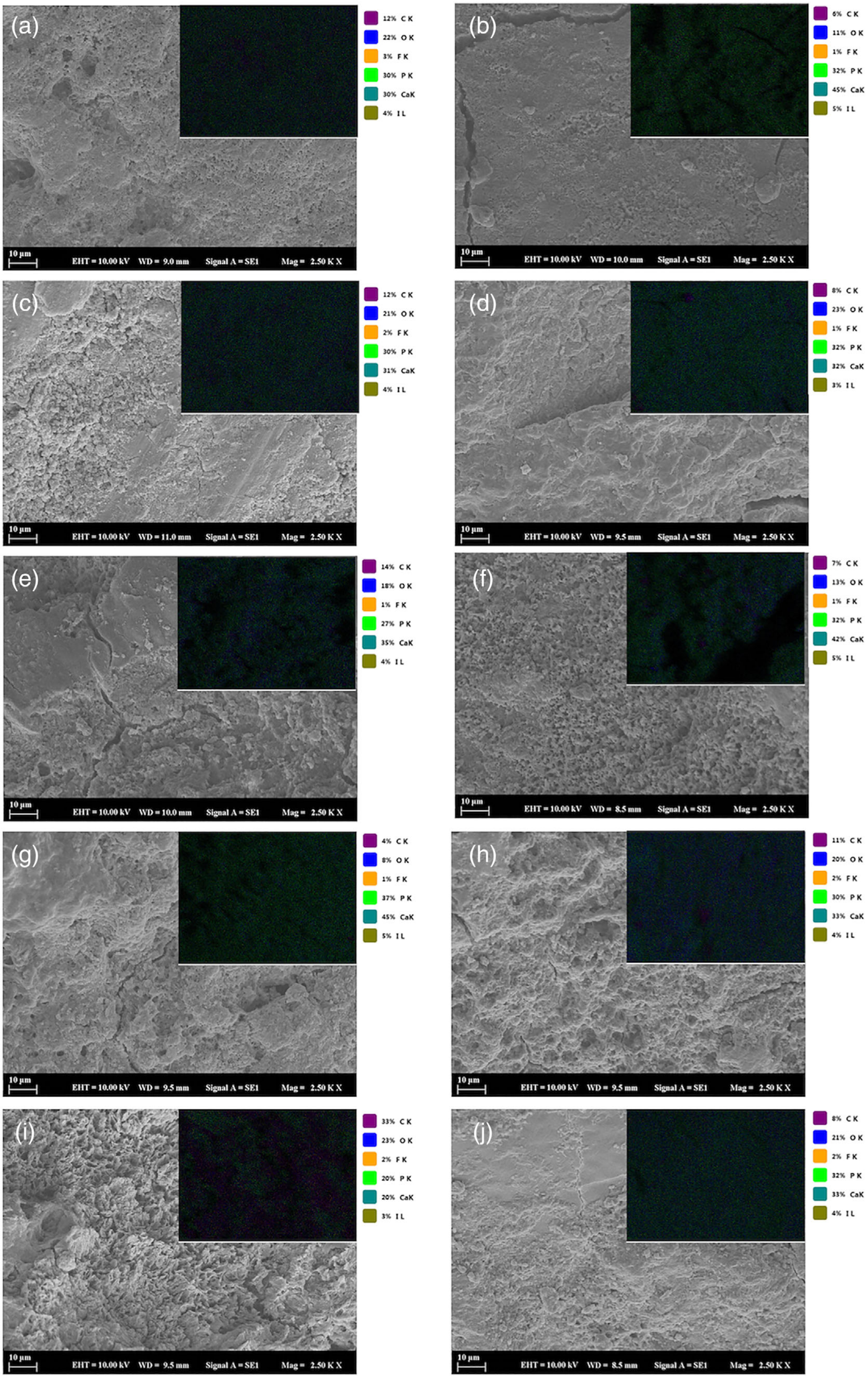

FIGURE 3 The representative semiquantitative chemical microanalyses and distribution of elements for all tested groups at ×2,500 magnification. (a) Group Ctrl + ER, (b) Group Ctrl + SE, (c) Group CHX + ER, (d) Group CHX + SE, (e) Group H_2O_2 + ER, (f) Group H_2O_2 + SE, (g) Group PVP-I + ER, (h) Group PVP-I + SE, (i) Group L + ER, and (j) Group L + SE. CHA, chlorhexidine; ER, etch-and-rinse; H_2O_2, hydrogen peroxide; PVP-I, povidone-iodine; SE, self-etch

Oz, & Yazici, 2021). The divergence in outcomes can be attributed to the differences in the adhesive systems they used in their study. Legler et al. determined the effect of phosphoric acid etching with various etching times in ground enamel. The researchers reported that the application of 37% phosphoric acid for 15 s resulted in approximately 8.0–10.0 μm etch depth (Legler, Retief, & Bradley, 1990). The findings of this study concerning etched enamel can be explained by the fact that the mouthwashes tested might penetrate the enamel to this extent or less.

In further studies, to reduce the damaging effect of these mouthwashes, new methods or materials can be investigated. Furthermore, the depth of the enamel surfaces affected by the mouthwashes can be evaluated. The etching pattern of different adhesive systems after mouthwash application on enamel surfaces can be observed. Additionally, the deterioration of mouthwashes in SBS to the enamel might be evaluated after aging procedures. A limitation of this study is that the washing effect of saliva was not taken into account. The mouthwashes applied to the enamel surfaces may have been altered after saliva contamination. Cleaning the teeth with a fluoride free paste after using a mouthwash might also affect the results. To reach a conclusion, further studies should be performed with different adhesive systems and antiseptic mouthwashes while simulating an oral environment.

5 | CONCLUSION

Despite the limitations of this study, the following conclusions can be drawn:

1. H_2O_2 and PVP-I that were suggested for preprocedural use during the COVID-19 pandemic, reduced the enamel bond strength of the universal adhesive in ER mode.
2. ER mode caused significantly higher SBS than SE mode in all mouthwash groups.
3. The SEM observations highlighted that Group Ctrl had a regular and intact hybrid layer with resin tag formation while the H_2O_2 and PVP-I groups exhibited a thin hybrid layer in both modes.
4. EDS analysis indicated that in SE mode, all mouthwash groups presented increased O content compared to Group Ctrl. While in ER mode, the O content of most mouthwashes was similar to Group Ctrl.

ACKNOWLEDGMENTS

A part of this study has been presented in 10th Cons Euro 2021 and was published in Clinical Oral Investigations vol 25, page 4190 (2021). Many thanks to Assistant Prof Sevilay Karahan for her help with statistical analyses. This study was co-funded by 3 M ESPE. The funders had no role in the design, conduction, evaluation or interpretation of the study, or in writing the manuscript.

CONFLICT OF INTEREST

The authors do not have any financial interest in the companies whose materials are included in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, author initials, upon reasonable request.

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How to cite this article: Özduman, Z. C., Oglakci, B., Doğan, M., Deger, C., & Eliguzeloglu Dalkilic, E. (2022). How does antiseptic mouthwashes against SARS-COV-2 affect the bond strength of universal adhesive to enamel? *Microscopy Research and Technique*, *85*(3), 1199–1208. https://doi.org/10.1002/jemt.24028