Original Article

The Effect of Preprocedural Mouthrinses against COVID-19 before Acid Etching on the Microleakage of a Resin-Based Sealant in Permanent Molars: An *In Vitro* Experimental Study

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ABSTRACT

Aim: To determine whether rinsing with preprocedural mouthrinses against coronavirus disease before acid etching affects resin-based sealant microleakage. **Materials and Methods:** A presented *in vitro* experimental study was performed on 15 extracted permanent third molars. Samples were randomly allocated into five groups: Group 1—distilled water (control); Group 2—1% hydrogen peroxide; Group 3—1.5% hydrogen peroxide; Group 4—0.5% povidone-iodine; and Group 5—1% povidone-iodine. After the teeth were immersed in the assigned mouth rinses for 60 s, they were sealed with Concise™ white sealant. Subsequently, the teeth were thermocycled for 500 cycles, immersed in 2% methylene blue solution for 24 h, and sectioned with two parallel cuts in the buccolingual direction. Sixty surfaces (12 surfaces in each group) were examined for microleakage under a 40× light microscope and scored as described by Zyskind *et al*. Welch's one-way analysis of variance test and the Games–Howell test were used to analyze the results at a significance level of $P < 0.05$ for all tests. **Results:** The intergroup comparisons indicated that the 0.5% povidone-iodine group and the 1% povidone-iodine group had significantly higher microleakage compared with the control group. The 1% and 1.5% hydrogen peroxide groups demonstrated no significant difference in mean microleakage scores compared with the control group. There was no significant difference between the povidone groups and the hydrogen peroxide groups. **Conclusions:** Preprocedural rinsing with 0.5% and 1% povidone-iodine before acid etching caused higher microleakage of resin-based sealant, while hydrogen peroxide rinsing gave comparable microleakage compared with the control group.

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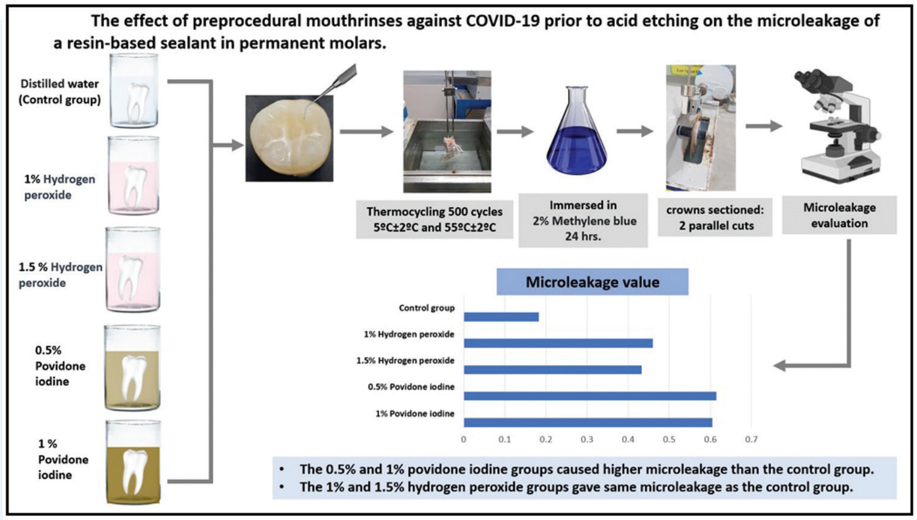
Graphical Abstract



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Introduction

Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be transmitted by the infected secretions or droplets from a contaminated person.[1] Salivary glands can act as a virus reservoir, which can be found in patients' saliva droplets, especially in the early infection period.[2] In dentistry, dentists and their teams are exposed to a very high quantity of contaminated spray from dental instruments.[3,4] Therefore, dentists are among the most at-risk healthcare providers due to the higher risk of being exposed to infected aerosols.[3,5]

Preprocedural mouthrinses can reduce the SARS-CoV-2 viral load and minimize the cross-infection risk when providing dental procedures.[6] Chlorhexidine is an antimicrobial mouthrinse, which has bactericidal and virucidal effects.[7,8] Nevertheless, some studies stated that chlorhexidine cannot effectively reduce the SARS-CoV-2 viral load.[9,10] The adverse effects of chlorhexidine are tooth pigmentation and change in taste sensation.[7,11,12] Besides, cetylpyridinium chloride (CPC) is recently used in medical mouthwashes and antiseptic products at 0.02%–0.075% concentration.[7] It acts by interfering with the lipid components on the surface of bacteria, disrupting the integrity of the viral envelope.[7,8,13] Limited research has demonstrated that CPC exhibits virucidal activity against SARS-CoV-2.[14-16] Tooth pigmentation and change in taste sensation are also adverse effects of CPC.[7] The oxidizing mouthwashes, which contain povidone-iodine and hydrogen peroxide, are recommended for reducing the viral load in the oral mucosa and salivary glands, including transmission potency.[9] Moreover, World Health Organization[17] and several international guidelines[9,12,18] for dental procedures during the COVID-19 pandemic have recommended the use of hydrogen peroxide and povidone-iodine for preprocedural mouthwashes. In addition, povidone-iodine and hydrogen peroxide mouthrinses were widely used to study the results of reducing COVID-19 infection.[8,19,20] Several studies recommended povidone-iodine, a broad-spectrum microbicide, which can inactivate fungi, bacteria, and several types of viruses.[21,22] Studies demonstrated that povidone-iodine gargle and mouthrinse products at 1%[8,10,22-24] and 0.5%[7,10,25,26] concentration reduced SARS-CoV-2 levels. It has the highest virucidal activity among other several antiseptics followed by hydrogen peroxide.[23,27,28] Hydrogen peroxide is also known as a bactericidal oxidizer and is effective against viral infections. Several studies recommended using 1%[29,30] and 1.5%[17,23] hydrogen peroxide mouthrinses that were effective as a virucidal agent against COVID-19.

Sealants are a physical barrier that can protect from bacterial access into the pits and fissures. Compared with the nonuse of sealants[31] or the application of fluoride varnishes,[32] sealants are effective in preventing and arresting pit and fissure carious lesions in primary and permanent molars. The poor marginal sealing of the sealant is the most important factor that fails sealant therapy.[33,34] Sealant microleakage leads to bacteria and fluid penetration, which is a cause of caries progression under a sealant.[35]

In May 2023, the World Health Organization announced that COVID-19 has become a persistent health challenge and no longer qualifies as a public health emergency of international concern.[36] However, preprocedural mouthrinses still play an important role in reducing bacterial and viral infection and their use should not be abandoned.

To the best of our knowledge, there is no report concerning the impact of the preprocedural use of these mouthrinses against COVID-19 on resin-sealant microleakage. Consequently, the purpose of this study was to determine whether rinsing with preprocedural mouthrinses against COVID-19 before acid etching affects the microleakage of a resin-based sealant.

Materials and Methods

Sample size calculation

The sample size calculation was derived from a pilot study using the standard deviation and the number of intervention and control groups in this study. The calculation used one-way analysis of variance (ANOVA) by nQuery Advisor program version 6.01 with 0.05 significance level (α) and 80% power. The calculated sample size was at least 11 surfaces per group. The final sample size was 60 surfaces (5 groups of 12 surfaces each). This study protocol was reviewed and certified for exemption by the Institutional Review Board due to unidentifiable and anonymized data.

Mouthrinses preparation

The mouthrinses used in this study are presented in Table 1.

- Hydrogen peroxide mouthrinses were prepared by the Faculty of Dentistry, Mahidol University, Bangkok, Thailand, at 1% and 1.5% concentrations.
- Povidone-iodine mouthrinses were prepared from a commercially available solution (70 mg/mL) in two concentrations by a single operator.
    - 0.5% povidone-iodine mouthrinse: Dilute 2 mL commercially available povidone-iodine solution (70 mg/mL) in 26 mL sterile water.
    - 1% povidone-iodine mouthrinse: Dilute 5 mL commercially available povidone-iodine solution (70 mg/mL) in 30 mL sterile water.

Table 1: Preprocedural mouthrinses against COVID-19 used in this study

Materials		Composition	Manufacturing company
1	1% hydrogen peroxide mouthrinse	1% hydrogen peroxide, strawberry flavoring agent	Faculty of Dentistry, Mahidol University, Bangkok, Thailand
2	1.5% hydrogen peroxide mouthrinse	1.5% hydrogen peroxide, strawberry flavoring agent	Faculty of Dentistry, Mahidol University
3	Povidone-iodine mouthrinse	Povidone-iodine 70 mg/mL	Betadine, Thai Meiji, Pharmaceutical Co., Ltd., Bangkok, Thailand

Specimen preparation

Fifteen extracted permanent third molars were stored in a 0.1% thymol solution at room temperature for no longer than 2 months. Teeth with dental caries, a crack line, fluorosis, or restoration were excluded from this study. Calculus and soft tissue remnants were removed using an ultrasonic scaler and a pumice slurry with a rubber cup.

The teeth were allocated into five groups by simple random sampling using the lottery method. Each group received different mouthrinse treatments: Group 1 (Control group): immersed in distilled water. Group 2: immersed in 1% hydrogen peroxide. Group 3: immersed in 1.5% hydrogen peroxide. Group 4: immersed in 0.5% povidone-iodine. Group 5: immersed in 1% povidone-iodine, for 60 s in all groups.

After the teeth were immersed in their assigned mouthrinses, the occlusal surfaces were cleaned by pumice slurry for 15 s, washed and dried with a triple syringe for 15 s, etched with 37% phosphoric acid etchant gel (Scotchbond Multi-purpose Etchant, 3M™ ESPE™, Saint Paul, MN, USA) for 15 s, the acid was completely rinsed off, and dried. Concise™ white sealant (3M™ ESPE™) was applied by a Dycal carrier and explorer and light cured for 20 s. The light intensity was controlled within 1200 mw/cm^3 ± 10%. The teeth were treated by a single, blinded operator. Moreover, all interfaces were checked for any bubbles, porosities, or bonding defects under a light microscope at 40× magnification (Nikon® Model Eclipse E400 POL, Tokyo, Japan). Teeth with leakage or sealant defects were excluded. The remaining teeth were stored in distilled water for 24 h.

Thermocycling and dye immersion

The teeth were thermocycled in water for 500 cycles at a temperature of 5°C ± 2°C and 55°C ± 2°C with a

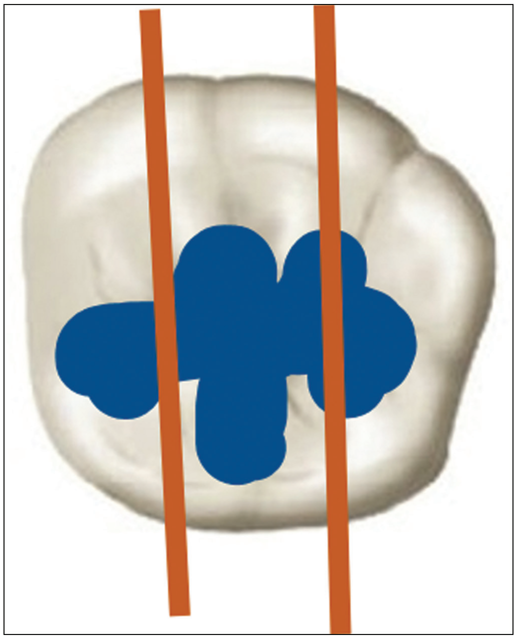

Figure 1: The sealed tooth was sectioned with two parallel cuts in the buccolingual direction and four surfaces from each tooth were used to determine the microleakage scores

dwell time of 30 s[34,37] (Thermocycling machine, Model TC400, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand). After thermocycling, the surfaces of the teeth were coated with two layers of nail varnish by leaving 1 mm around the peripheral area of the sealant. Moreover, the roots were covered by sticky wax preventing the dye from penetrating the teeth.[38] The teeth were immersed in 2% methylene blue solution for 24 h to allow dye penetration into the space between the sealants and tooth surfaces.[39,40] The teeth were rinsed with distilled water and let dry. Two parallel cuts were made in the buccolingual direction [Figure 1] through the mesial and distal pit of the crowns using a slow-speed saw (Accutom-50, Struers, Ballerup, Denmark), whereas copious water spray was applied. After sectioning, four surfaces per tooth were evaluated under a polarizing light microscope (Nikon® Model Eclipse E400 POL) at 40× magnification and photographed (Nikon Coolpix 900, Tokyo, Japan).[33,39]

Microleakage evaluation

The images were evaluated with Image Pro® Plus (Media Cybernetics, Rockville, MD). The system described in Zyskind *et al*.[41] was used to score the dye penetration for each surface [Figure 2].

For the intra-examiner reliability, 20% (12 surfaces) of the samples were randomly selected and re-examined by the same examiner under the same conditions. The intra-examiner reliability was tested with the intra-class correlation coefficient.

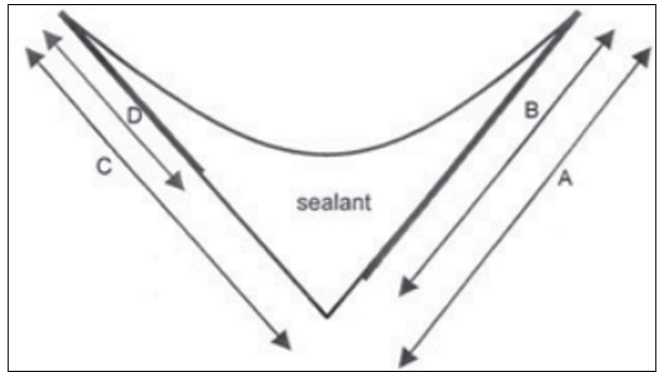

Figure 2: The system in Zyskind *et al*[41] (adapted from Rirattanapong *et al.* 2013).[39] (A and C) The length of sealant–tooth interface (mm). (B and D) the length of dye penetration (mm). Scoring for microleakage = B + D/A + C

Statistical analysis

The microleakage score data were calculated and analyzed for normality using the Shapiro–Wilk test. The homogeneity of variance was tested with the Levene statistic test. Welch's one-way ANOVA test was used to compare the mean microleakage value among the five groups. The Games–Howell test was used for intergroup comparisons. The significance level was set at 0.05 for all tests. The Statistical Package for Social Sciences (SPSS), version 21.0 for Windows (IBM, Armonk, NY, USA) was used for statistical analysis.

Results

The mean microleakage score and standard deviation in each group of mouthrinses are presented in Table 2. The dye penetration images in each group are seen in Figure 3.

The intra-class correlation coefficient demonstrated that the intra-examiner reliability in scoring was 0.997. A significant difference in mean microleakage score was found among groups ($P < 0.001$). The multiple comparisons revealed that the 0.5% povidone-iodine group and the 1% povidone-iodine group had significantly higher microleakage compared with the control group ($P = 0.002$ and 0.003, respectively). The 1% and 1.5% hydrogen peroxide groups demonstrated no significant difference in mean microleakage score compared with the control group ($P = 0.127$ and 0.274, respectively). Moreover, there was no significant difference between the povidone groups and the hydrogen peroxide groups ($P > 0.05$).

Discussion

This study demonstrated the effect of preprocedural mouthrinses against COVID-19 on the microleakage of

Table 2: Mean microleakage scores of the resin-based sealant pretreated with five different mouth rinses before acid etching

	Treatment				
	Control (Distilled water) 60 s ($N = 12$)	1% H_2O_2 60 s ($N = 12$)	1.5% H_2O_2 60 s ($N = 12$)	0.5% PI 60 s ($N = 12$)	1% PI 60 s ($N = 12$)
Microleakage score: Mean ± SD	0.1825 ± 0.1371^{a}	0.460 ± 0.3469^{ab}	0.4333 ± 0.3901^{ab}	$.6142 \pm 0.2816^{b}$	0.605 ± 0.2966^{b}

Same lowercase letter indicates no significant difference between each treatment group ($P > 0.05$)

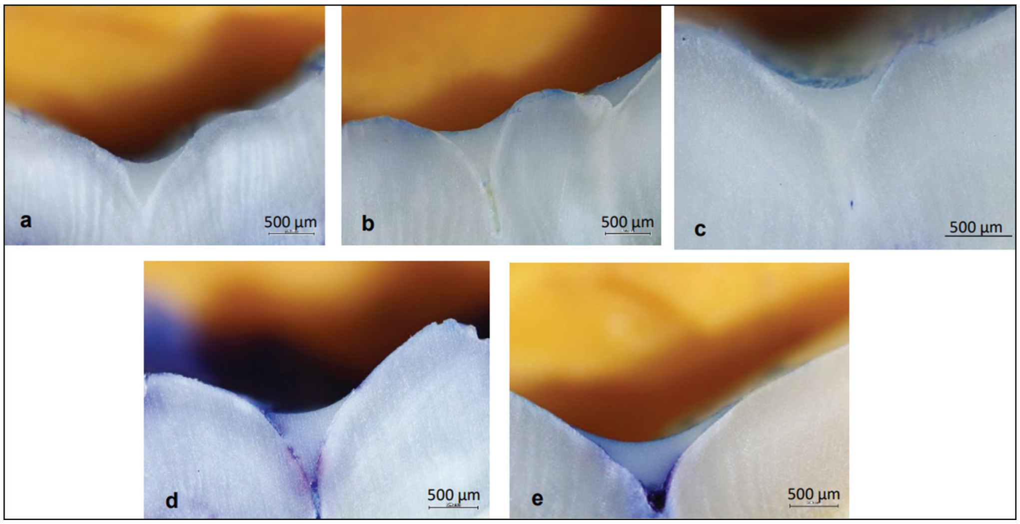

Figure 3: Dye penetration: Images obtained using a light microscope at 40×. (A) Group 1:control group (distilled water). (B) Group 2: 1% hydrogen peroxide. (C) Group 3: 1.5% hydrogen peroxide. (D) Group 4: 1% povidone-iodine. (E) Group 5: 1.5% povidone-iodine

a resin-based sealant in permanent teeth. Significantly increased resin-based sealant microleakage was found when the molars were pretreated with 0.5% and 1% povidone-iodine mouthrinses before acid etching. The enamel interface that was preprocedurally rinsed with povidone-iodine likely had less resin tag formation.[42] Moreover, the pH of the 0.5% and 1% povidone-iodine used in this study was 2.85 and 2.63, respectively. One study quantified the mineral loss on the enamel surface after using 7.5% w/v povidone-iodine for 24 h compared with the control group.[43] The mineral loss was seen as a white spot-like lesion on the enamel.[43]

When using hydrogen peroxide mouthrinses, the microleakage values were higher than the control group, however, the difference was not significant. This might be due to short resin tags on the connection interface.[42] Furthermore, the hydrogen peroxide groups might have residual oxygen ions on the tooth surfaces.[42,44] Some studies have shown that free radicals alter the minerals and proteins of the enamel surface, which reduces adhesion.[45] However, the phosphoric acid in the etch and rinse system may reduce the residual oxygen, which can hamper resin polymerization.[42,46] Moreover, the pH of the 1% and 1.5% hydrogen peroxide in this study was 5.28 and 5.19, respectively. In this study, the pH of povidone-iodine and hydrogen peroxide groups show acid properties. Consequently, they might cause demineralization of the enamel surface after rinsing. An *in vitro* study found that caries or demineralized areas located in fissures and extending to adjacent enamel surfaces had significantly more microleakage than sound enamel.[47,48] In addition, an *in vitro* study found that etched enamel, after rinsing with povidone-iodine and hydrogen peroxide, exhibited a lesser etching depth and shallower porosities compared to the control group.[45] The scanning electron microscopic images of the enamel surface revealed a honeycomb appearance and a limited quantity of shallow porosities on the etched enamel surface in both groups.[45] It might affect the adhesion of sealant, which causes microleakage in the povidone-iodine and hydrogen peroxide groups.

Although there were different concentrations in the povidone-iodine mouthrinse or the hydrogen peroxide mouthrinse, the different concentrations were very small and the pH values were similar in each type of mouthrinse. Thus, there was no significant difference in microleakage between the two different concentrations in each type of mouthrinse.

The determination of the sample size for this study was derived from a pilot study, using the standard deviation and considering the number of participants in the intervention and control groups because there is no previous report that evaluated the effect of preprocedural mouthrines against COVID-19 on microleakage of a resin-based sealant.

Microleakage can be evaluated using clinical and laboratory methods. However, laboratory studies are widely and easily used. Various laboratory methods are used to evaluate the microleakage in dental restorations, such as radioactive isotopes,[49,50] dye penetration,[38,51-53] confocal laser scanning microscopy,[35,54] optical coherence tomography,[55] and glucose leakage method.[56] Dye penetration has many advantages over the other techniques. It is a highly feasible and easy method with no reaction chemical.[57,58] Many different dye solutions are used, such as 0.5% basic fuchsin,[52] 50% silver nitrate,[53] and 2% methylene blue.[38] Additionally, the size of the dye particles is smaller than that of bacteria and comparable with the size of bacterial endotoxin.[58] In our study, 2% methylene blue was used to estimate the microleakage estimation because it is easily visualized and provides a good contrast with the surrounding tissue in the image.[38,59] For microleakage scoring, this study used the system in Zyskind *et al.*[41] due to the suggestion that evaluating the percentage of dye penetration between the enamel and sealant interface would be more accurate than using numerical or dichotomous scales.[37,60]

The limitation of an *in vitro* study is that it cannot imitate the oral environment due to lacking several factors, such as saliva, pH cycling, and occlusal force. Furthermore, *in vivo* studies should be performed to evaluate the preprocedural mouthrinses effect on microleakage or the retention of resin-sealant. Different antiseptic mouthrinses might affect the resin-sealant procedure or properties of resin restoration so they should also be investigated.

Conclusion

Preprocedural rinsing with 0.5% and 1% povidone-iodine before acid etching caused higher resin-based sealant microleakage, whereas hydrogen peroxide rinsing presented comparable microleakage compared with the control group.

Acknowledgements

Nil.

Financial support and sponsorship

No funds were received.

Conflicts of interest

There are no conflicts of interest.

Authors contributions

All authors contributed to the study concepts, design, and manuscript review. SU: Literature search, Experimental studies, Data acquisition, Statistical analysis. SU and VY: Manuscript preparation and editing.

Ethical policy and Institutional Review Board statement

This study protocol was reviewed and certified of exemption by Institutional Review Board, Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, Bangkok, Thailand (Research code: MU-DT/PY-IRB 2022/032.0706).

Patient declaration of consent

Not applicable.

Data availability statement

The data used to support the findings of this study are included within the article.

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