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Original Article

Effect of chlorhexidine as a vehicle for sodium perborate in non-vital tooth bleaching on microleakage of resin composite

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Abstract

Objective To study the effect of chlorhexidine (CHX) as a vehicle for sodium perborate (SP) on microleakage of resin composite by using glucose filtration.

Materials and methods Thirty six single-rooted teeth were used. The specimens with cavity were prepared and divided into three groups: Group 1, SP mixed with 2% CHX; Group 2, SP mixed with distilled water; Group 3, SP mixed with 30% hydrogen peroxide. With a total conduction of three times, bleaching agent was applied in the cavities for 1 week and rinsed. The cavities were then filled with resin composite. The specimens were thermocycled for 500 cycles, followed by a microleakage determination (glucose filtration). The concentration of leaked glucose was analyzed by spectrophotometer. Dentin surfaces of each group were prepared and observed under SEM.

Results Mean concentrations and standard deviations of leaked glucose (mM) were 1.001 ± 0.147, 1.005 ± 0.093, and 1.304 ± 0.406, in Groups 1, 2, and 3, respectively. Inter-group significant differences were found by a one-way ANOVA ($p < 0.05$). Using the *post-hoc* Tukey HSD's test analysis, some significant differences were found between Groups 1 and 3, together with Groups 2 and 3 ($p < 0.05$). The morphology of dentin surfaces under SEM of Group 1 showed mild erosion similar to that of Group 2. Group 3 showed more eroded dentin.

Conclusion Using CHX as a vehicle for SP in this study did not affect the microleakage of resin composite and dentin. Clinically, CHX may be a good vehicle for non-vital tooth bleaching.

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Key words: *chlorhexidine; glucose filtration; microleakage; non-vital tooth bleaching; sodium perborate*

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Introduction

The walking bleach method is non-vital tooth bleaching which is easy to perform and preserves tooth structure for the anterior teeth. The bleaching agent is put inside the pulp chamber proper. There are many types of bleaching agents, for example hydrogen peroxide (H_2O_2), sodium perborate (SP; $NaBO_3 \cdot 4H_2O$), superoxol (30% H_2O_2), carbamide peroxide ($CO(NH_2)_2H_2O$) (Plotino et al., 2008).

SP mixed with distilled water is used as bleaching agent in walking bleach technique (Holmstrup et al., 1988). Instead of water, 3% H_2O_2 can be used in case of severe discolored tooth (Nutting and Poe, 1963). When the bleaching paste is put inside the pulp chamber proper, the top is sealed with temporary filling. H_2O_2 is released from the chemical reaction of bleaching paste. H_2O_2 can then generate different free radicals, together with reactive but unstable oxygen molecules, which subsequently change to oxygen gas. The leakage of temporary filling caused by pressure from oxygen gas has been claimed during the walking bleach procedure (Hosoya et al., 2000). Consequently, saliva and other microorganisms can penetrate into pulp chamber.

In case of the leakage of temporary filling, chlorhexidine ($C_{22}H_{30}CI_2N_{10}$, CHX) gel has been suggested as a vehicle for the bleaching process, because of its anti-microbial property which helps prevent the bacterial contamination. Additionally, SP mixed with 2% CHX gel did not affect the bleaching efficiency, when compared to those mixed with 30% H_2O_2 or distilled water (de Oliveira et al., 2006). CHX has a unique property known as substantivity, the application of 2% CHX solution for 5 min has continued the anti-microbial substantivity in dentin for up to 4 weeks (Khademi et al., 2006) and remained for up to 12 weeks after application for 10 min (Rosenthal et al., 2004). Moreover, previous study found that 0.2% CHX solution can penetrate up to 500 μm within dentinal tubules for eliminating bacteria (Heling et al., 1992).

After bleaching process, it is a common practice to restore an access cavity with resin composite. Using CHX as a bleaching agent's vehicle may affect the quality of bonding between restorative material and dentin. A recent study on the bleaching process has shown that SP mixed with 2% CHX solution did not significantly affect the bond strength between dentin and resin composite, when compared to that mixed with distilled water (Buranakiattipuntr et al., 2011).

In order to prevent the microbial contamination caused by microleakage of temporary filling during the bleaching process, using 2% CHX as a vehicle of SP for non-vital tooth bleaching can be considered a beneficial alternative to distilled water. However, there have been few studies on the effect of SP and CHX on the microleakage of resin composite and dentin. Some leakage studies in endodontics include dye penetration (Starkey et al., 1993), radioisotope studies (Haïkel et al., 1999), bacterial penetration (Chailertvanitkul et al., 1996), fluid filtration (Pommel and Camps, 2001). Each technique has its own advantages and disadvantages. The results from studies using different techniques cause some conflicts and arguments, due to different opinions (Wu and Wesselink, 1993).

Because of its small molecular size (MW = 180 Da) and being a nutrient for bacteria, glucose has been selected as the tracer. A new method has then been proposed for the measurement of leakages, by using a quantitative measurement of glucose solution (Xu et al., 2005). A spectrophotometer was used to analyze the quantity of the glucose solution flowing through filling material. If glucose could enter the root canal from the oral cavity, bacteria that might survive root canal preparation and obturation could then multiply and potentially lead to periapical inflammation. This method is believed to be the most clinically relevant practice for the leakage measurement, when compared to the others. Hence, the objective of this study was to

study the effect of CHX as a vehicle for SP on the microleakage of resin composite, by using a glucose filtration method.

Materials and methods

This study was approved by the Ethics Committees of Naresuan University. Thirty six single-rooted premolars, which had been extracted for some orthodontic purposes from 18-to 25-year-old patients, were used. Clinically, all teeth had completed root formation and showed no carious lesion, crack, or restorative materials on crown. The extracted teeth were immediately stored in 0.1% thymol solution at 25°C until use. After the removal of the soft tissues, each tooth was embedded in a cylindrical mold, 14 mm in diameter and 20 mm in height, with self-curing acrylic resin. The crown specimens with a 2-mm-height were prepared by horizontally cutting at cemento-enamel junction (CEJ) and 2-mm above CEJ (Fig. 1A). By using a fissure diamond bur (Jota diamond #012, JOTA AG, Rüthi SG, Switzerland) and a template, a cylindrical cavity with a diameter of 3 mm was prepared through the pulp chamber proper (Fig. 1B). The final diameter of 3 ± 0.05 mm was measured by a digital vernier caliper (500-171, Mitutoya, Kawasaki, Japan) with an accuracy of ± 0.01 mm.

The cavities were rinsed with each 2-mL solution for 1 min, that is, 2.5% NaOCl, 17% EDTA and 2.5% NaOCl, respectively. They were then dried with cotton pellets and paper points. The apical side of specimen was placed on the center of 22 x 22 mm^2 glass cover slip (Fig. 2).

The specimens were randomly divided into three groups (n = 12 for each group). Freshly prepared bleaching agent of SP (Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand) mixed with 2% CHX (Kemcolour International, GIDC

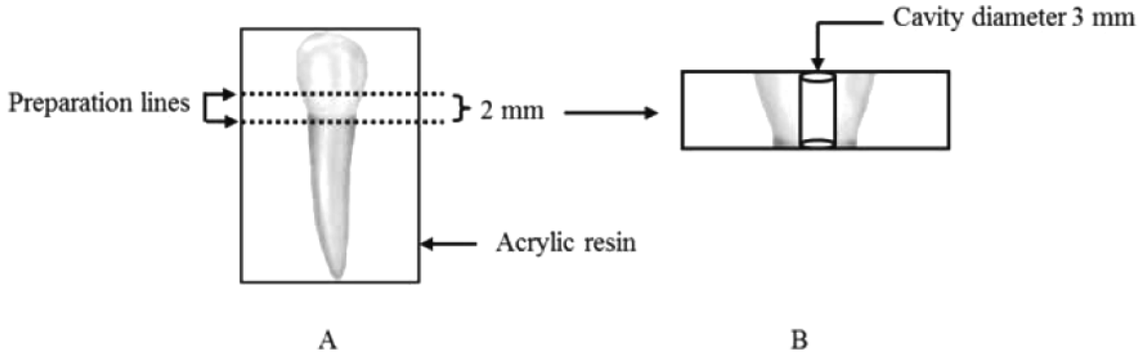

Fig. 1 Specimen preparation: tooth fixed in acrylic resin with preparation lines (A), specimen after cutting with 2 mm thickness and cavity preparation with 3 mm diameter (B).

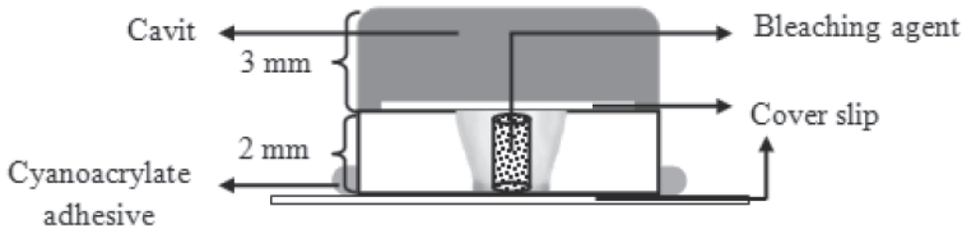

Fig. 2 Specimen preparation for bleaching application. Bleaching agent was applied in the cylindrical cavity. The top surface of cavity was covered with a glass cover slip and sealed with the temporary material.

Industrial Estate, Ankleshwar, India) for Group 1, with distilled water for Group 2, and with 30% H_2O_2 (Sigma-Aldrich®, St. Louis, MO, USA) for Group 3 (20 mg/10 μL for each specimen). Bleaching paste was applied in the prepared cavity of each specimen. The top surface of cavity was covered with a 12-mm-diameter glass cover slip (Electron microscopy Sciences, Hatfield, PA, USA), and sealed with a 3-mm-thick temporary material (Cavit-W, ESPE, Seefeld, Germany) (Rotstein, 2001) (Fig. 2). All specimens were stored at 37°C and 100% humidity for one week. The cavities were rinsed with 20 mL of distilled water. The mentioned procedures were conducted in this manner for a total of three times. The cavities were finally irrigated with 20 mL of distilled water, dried with cotton pellets, and filled with resin composite (Filtek™ Z350, ESPE, Seefeld, Germany) by using a total-etching adhesive system (Adper™ Single Bond 2 total-etch adhesive, ESPE, Seefeld, Germany), according to the manufacturers' instructions. The resin composite filling was qualitatively evaluated by taking radiographic images in bucco-lingual and mesio-distal views. If radiolucency in the filling material was seen in any image, the specimens were excluded. All specimens were thermocycled for 500 cycles in 5 ± 1°C and 55 ± 1°C (Rossomando and Wendt Jr, 1995). The dwell time was 60 s, with the 2-s-between-bath-time.

This glucose filtration leakage model was modified from those previously reported (Puapichartdumrong et al., 2003; Xu et al., 2005). Briefly, the self-curing acrylic resin model comprised two chambers, in which the specimen could be put between. Each chamber was fixed together with four screws. The apical side of chamber was filled with 1.1 mL of distilled water, whereas the coronal side was circulated with 1 M glucose solution (1.7 mL/min) by using the peristaltic pump (BT-1001F, Baoding Longer Precision Pump, Hebei, China) (Fig. 3). In pilot study, the sealing ability of silicone O-rings was evaluated by insertion of a plastic disc instead of the specimen, no glucose solution was found to leak from the coronal chamber into the apical chamber.

All specimens were coated with nail varnish twice on both sides, except around the tested areas (5 mm in diameter), and then left for 2 h to fully dry. The specimens were then inserted between the two

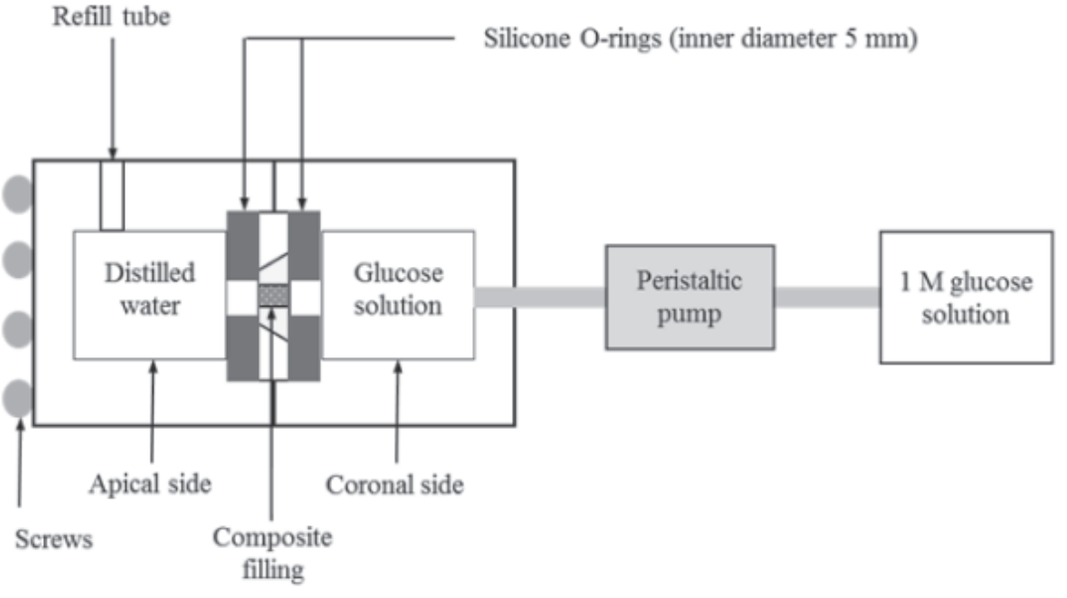

Fig. 3 The glucose filtration model.

chambers and sealed with a 4-mm-thick silicone O-ring (inner diameter = 5 mm). The whole sample from the apical side of chamber was taken after 80 min. The concentrations of glucose solution were analyzed with a glucose kit (Sigma-Aldrich®, St. Louis, MO, USA). The kit was composed of glucose oxidase/peroxidase reagent and o-dianisidine reagent. Two reagents were mixed in a specific ratio for each test (assay reagent). Absorbance of the obtained pink color's intensity from the test was measured at 540 nm by using a spectrophotometer (Genesys G10-S, Thermo, MA, USA) and compared with a standard curve. To obtain a standard curve of the absorbance of the mixed and known solutions, a blank reagent (deionized water) and three glucose standards, with concentrations of 0.1, 0.2 and 0.3 mM, were prepared. The reaction was started by adding 2-mL assay reagent from the glucose kit to the first test tube, and left 30-60 s before starting the next tube. The appropriate time for an obvious reaction at 37°C was 30 min. The reaction was then stopped by adding 2 mL of 12 N sulfuric acid with a 30- to 60-s time interval. All test tubes were finally brought to measure the absorbance against the blank reagent at 540 nm. If the measured absorbance of the sample was higher than the standard curve, the sample was diluted with deionized water and re-evaluated.

For scanning electron microscopic observations, four specimens of each group and normal dentin were prepared and observed under a scanning electron microscope (SEM; LEO1455vp, LEO Electron Microscopy Ltd, Cambridge, UK). The specimens were fixed with 2% glutaraldehyde in phosphate buffered solution (PBS) (pH 7.4) for 20 min (primary fixation) and secondary fixation with 2% osmium tetroxide in PBS for 20 min. The specimens were dehydrated through ascending concentrations (50, 70, 80, 95; twice each, and 100; three times) of ethanol (2 min of each), and then were dried by three immersions for 5 min each in 100% alcohol/hexamethyldisilazane (HMDS) in a ratio of 2:1, 1:1 and 1:2, respectively. After that, all specimens were immersed in 100% HMDS solution, placed in a fume hood and left overnight to allow HMDS solution to completely evaporate. Finally, the

Table 1 Mean concentration of leaked glucose solution in mM, SD

Groups	Mean	SD
1. Sodium perborate + 2% chlorhexidine	1.001[a]	0.147
2. Sodium perborate + distilled water	1.005[a]	0.093
3. Sodium perborate + 30% hydrogen peroxide	1.304[b]	0.406

Means identified by the different superscript letters are significant difference ($p < 0.05$).

Table 2 The one-way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig. 2 3
Between Groups	.726	2	.363	5.595	.008
Within Groups	2.142	33	.065		4
Total	2.868	35			5

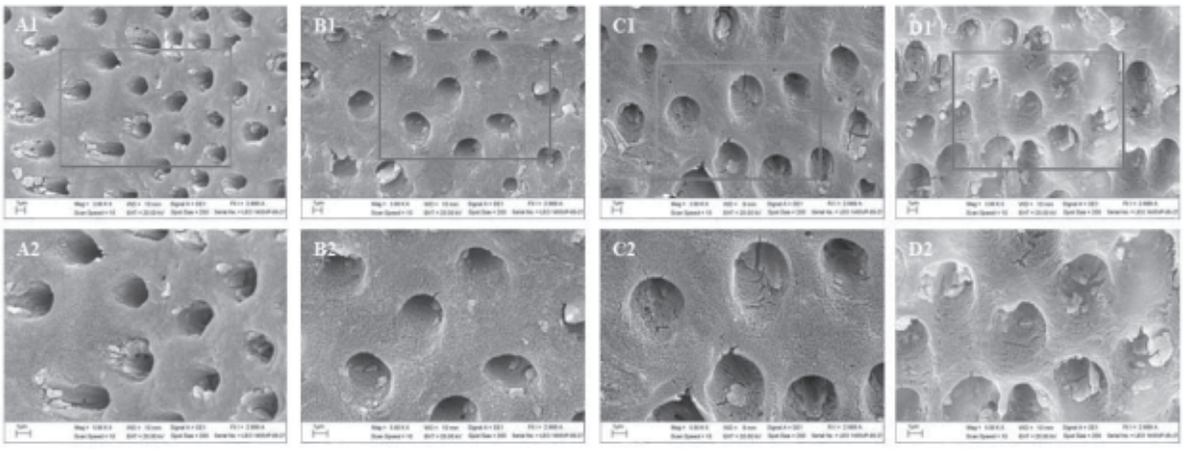

Fig. 4 SEM micrographs: (A1 (3000x), A2 (5000x)) Normal dentin showed intact dentinal tubules, some dentinal tubules were partially occluded with debris. (B1 (3000x), B2 (5000x)) Bleached dentin with SP + 2% CHX (Group 1) showed mild erosion of intertubular dentin, the dentinal tubules were slightly wider. (C1 (3000x), C2 (5000x)) Bleached dentin with SP + distilled water (Group 2) showed similar dentin changes as Group 1. (D1 (3000x), D2 (5000x)) Bleached dentin with SP + 30% H_2O_2 (Group 3) showed severe erosion of intertubular dentin, the dentinal tubules were wider than the other groups.

specimens were coated with gold using the SPI-Module sputter coated (Structure Probe Inc., West Chester, PA, USA).

Statistical analysis

Mean concentrations of the leaked glucose solution were calculated by using a one-way ANOVA. At a significant level of $p < 0.05$, inter-group differences were analyzed by using the Turkey HSD.

Results

Mean concentrations of the leaked glucose solution and their standard deviations are shown in Table 1. The greatest and the least mean concentrations of the leaked glucose solution was seen in Group 3 (1.304 ± 0.406 mM) and Group 1 (1.001 ± 0.147 mM), respectively. One way analysis of variance of the study groups was shown in Table 2, there was a statistic difference among groups. Inter-group analyses disclosed some significant differences ($p < 0.05$) between Groups 1 and 3 and between Groups 2 and 3, but not between Groups 1 and 2.

The results of SEM observations, normal dentin surface showed intact dentinal tubules (Fig. 4: A1, A2). The morphology of surfaces bleached with SP ± 2% CHX and SP + distilled water (Groups 1 and 2) showed mild erosion of dentinal tubules (Fig. 4: B1, B2 & C1, C2), in Group 3 bleached with SP + 30% H_2O_2 showed severe erosion of dentinal tubules (Fig. 4: D1, D2).

Discussion

Almost all anterior teeth underwent extraction procedures belonged to the elderly and had been diagnosed as periodontitis. Some dentinal changes due to senility or secondary dentinogenesis were also reported to affect the adhesion capacity of resin composite (Mjör, 2009). Instead of the anterior teeth which were generally treated with non-vital tooth bleaching, single-rooted premolars were then used in this study to control some confounding factors of dentinal changes following aging processes.

By means of thermocycling procedures, an in *vivo* situation of artificial aging processes was simulated (Rossomando and Wendt Jr, 1995), but without absolute conclusions on the dwell times. Short dwell times (10 or 15 s) had no effect on the microleakage of glass ionomer cements or resin composite, which were non-metallic materials (Doerr et al., 1996). However, the marginal leakage values of both were significantly affected by increasing the dwell times of thermocycling in both 500 and 1,000 cycles (Cenci et al., 2008). Therefore, this study used the thermocycling regimen comprising the dwell times of 500 cycles and 60 s to simulate some clinical functions in the microleakage test.

This glucose filtration leakage model was modified from the models elsewhere (Puapichartdumrong et al., 2003; Xu et al., 2005). The applicably quantitative measurement and the shortened time for evaluating each specimen were some advantages of this modification. In addition, glucose tracer was considered to be more clinically relevant than the other ones (Xu et al., 2005). However, the disadvantage of this method was the high cost of glucose examination which due to the expensive assay kits for analysis the concentration of leaked glucose solution.

This study has shown that bleaching dentin with SP mixed with 30% H_2O_2 as a vehicle resulted in higher glucose leakage than those with 2% CHX or distilled water. The result coincides with a previous research which reported that 30% H_2O_2 mixed with SP significantly increased the microleakage by using the dye penetration method (Ellias and Sajjan, 2002). It may be explained that both organic and inorganic components of dentin are affected by using a high concentration (30%) of H_2O_2 (Lewinstein et al., 1994).

In the present SEM images showed that severe surface alterations were seen in Group 3 which was bleached with SP + 30% H_2O_2 while Groups 1 and 2 which were bleached with SP + 2% CHX and SP + distilled water, respectively, showed similar the slight changes of dentin surface. Our results corresponded to previous study demonstrated that more surface changes, widen dentinal tubules and demineralization of intertubular dentin were seen in dentin bleached with SP + 30% H_2O_2 (Santos et al., 2009). Due to its strong oxidizing action and highly acidic property, H_2O_2 causes some changes in the biomechanical properties of dentin (Marshall Jr et al., 1997). Such alterations might be the results of the denaturation of collagen (Lado et al, 1983) and the exaggerated demineralization of dentin (Chng et al., 2005). Therefore, eroded dentin surface found in Group 3 in this study may affect the bonding ability and results in an increased in microleakage.

Some changes in the mechanical properties of dentin can be the main factors affecting the further adhesion and sealing ability of restorative materials (Chng et al., 2005). Previous studies have demonstrated that bleaching treatments altered the substrate and interfered with the adhesion of composite resin to the tooth structures (Cavalli et al., 2001). In addition, an exposure to the high concentration of H_2O_2 significantly reduced microtensile bond strength (Timpawat et al., 2005) and shear bond strength (Teixeira et al., 2004) between resin composite and dentin. On the other hand, microtensile bond strength was not significantly affected by using 2% CHX solution (Buranakiattipuntr et al., 2011) and distilled water (Timpawat et al., 2005) as vehicle of SP.

When compared to those by controls (distilled water), glucose leakage from bleaching with SP and 2% CHX as a vehicle in this study was not significantly differed. CHX showed no negative effects on the sealing ability between filling materials and dentin (Sung et al., 2004; Darabi and Eftekhari, 2009). Using CHX as an irrigating solution in Class V composite restorations for 10 s (Sung et al., 2004) or as a cavity disinfectant before applying bonding agent in

total-etching system (Darabi and Eftekhari, 2009) did not affect the microleakage between resin composite and dentin. Under a polarized light microscope, the organic components of dentin were not affected by using 2% CHX gel during chemo-mechanical preparation of root canal (Moreira et al., 2009). In a total-etching system, 2% CHX solution applied to dentin either pre- or post-etching procedures did not significantly affect the microtensile bond strength of bonding agent (Soares et al., 2008). Moreover, CHX has been reported to improve some bond strength by the preservation of hybrid layers' durability. It illustrated an inhibition of matrix metallo-proteinase enzymes which deteriorated the layer (Carrilho et al., 2007).

On the contrary, prior to the application of a bonding agent in one-step self-etching system, CHX applied to a cavity reduced bond strength and increased microleakage (Tulunoglu et al., 1998). The residual of CHX molecules may interact with hydrophilic molecules in the bonding agent. The chemical bonds between filling material and dentin were then interfered. Consequently, the sealing ability was affected and microleakage occurred (Tulunoglu et al., 1998). To avoid such negative effects of CHX, it was suggested that the smear layer should be removed by using the phosphoric acid of a total-etching system (Cardoso et al., 2011). The residual of CHX was then removed via this process. Hence, the total-etching systems were utilized in this study to avoid some negative effects of CHX after bleaching.

The residual oxygen from chemical reactions of the bleaching agents (such as SP, carbamide peroxide or H_2O_2) would affect the bond strength (Torneck et al., 1990) and may increase microleakage. Due to this concern, an alcohol-based bonding agent (Adper™ Single bond 2) were used in this study. Some alcohol's interactions with residual oxygen preventing the loss of bond strength, the alcohol-based bonding agents were suggested to decrease the damaging effects from bleaching agents (Sung et al., 1999).

Conclusions

Using CHX as a vehicle for SP in this study did not affect the microleakage of resin composite and dentin. Clinically, CHX may be a good vehicle for non-vital tooth bleaching.

Acknowledgements

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