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The effect of cavity disinfectants on microleakage of composite restorations in primary teeth

ABSTRACT

Aim The purpose of this study was to determine the effect of different cavity disinfectants on microleakage of Class V resin-based composite restorations in primary teeth.

Materials and methods Standard non-beveled Class V cavities were prepared on 50 human primary anterior teeth with the incisal and cervical margin placed on the enamel. The crowns were randomly divided into 6 groups. Four experimental groups of 10 teeth each, in which cavities were disinfected using the following solutions: 1) *S. persica* extract (Ethanol 1 mg/ml); 2) 1.3% sodium hypochlorite (NaOCl); 3) 0.2% chlorhexidine gluconate (CHX), and 4) No solution applied. Two control groups; 5 teeth each; 5) Negative control: filled cavity and entirely coated with nail varnish and 6) positive control: empty cavity and without nail varnish coating. Each cavity in groups 1-5 were filled with Filtek™ Z350 XT Universal Restorative (3M Espe, St. Paul, USA). All specimens were thermocycled for 500 cycles (5°C/55°C) and prepared for microleakage evaluation using a 2% methylene blue.

Results For the experimental groups; there was no significant difference in dye penetration between the incisal and cervical walls in all groups ($P=0.176$).

However comparing microleakage by location/walls showed a significant difference in dye penetration between the incisal walls ($P=0.014$) and cervical walls ($P=.045$).

Conclusions None of the disinfectant solutions in the experimental groups were able to prevent dye penetration. In comparison to chlorhexidine gluconate and sodium hypochlorite; application of *S. persica* did not increase microleakage and was not detrimental to enamel and dentin adhesion using the restorative technique and materials used in this study.

Keywords Chlorhexidine Gluconate; Dental Materials/ Biomaterials; Microleakage; *Salvadora persica*; Sodium Hypochlorite.

Introduction

The presence of bacteria in dentin after the removal of infected dentin, even after removal of dye-stainable dentin, has been reported [Meiers and Kresin, 1996; El-Housseing and Jamjoum, 2000]. As this may contribute to recurrent caries and restoration failure, disinfectant solutions have been introduced, as alternative means to caries dentin removal, in order to reduce or eliminate bacteria from cavity preparations [Meiers and Kresin, 1996]. The smear layer is an amorphous layer that may harbor bacteria and prevent the cavity from being disinfected [Berutti et al., 1997]. Therefore, removal of the smear layer may act as cavity disinfectant. Phosphoric acid etchant materials have demonstrated antimicrobial activity against some of the bacteria involved in caries [Settembrini, 1997].

Some antibacterial solutions have been studied: chlorhexidine, sodium hypochlorite, and fluoride solutions [Meiers and Kresin, 1996; Grgan et al., 1999]. The results of in vitro studies are controversial regarding the disinfectants' effect on adhesion [Meiers and Kresin, 1996; Cunningham and Meiers, 1997; Tulunoglu et al., 1998; Grgan et al., 1999]. Moreover, there is still not much laboratory data that discusses the adhesion of the new generations of resin-based composite restorations with the use of cavity disinfectants.

Chlorhexidine is one of the most widely used and effective broad spectrum antibacterial or antiseptic agent in the maintenance of plaque control and an aid in gingivitis and it is also used as cavity disinfectant because it continues to kill bacteria for several hours by binding to the aminoacids in the dentin, making it a good antimicrobial agent [Grgan et al., 1999; de Castro et al., 2003]. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigation solution with excellent tissue-dissolving and antimicrobial

abilities and several studies have shown its effectiveness on residual bacteria [Guerreiro-Tanomaru et al., 2011; Xie et al., 2012; Mohammadi et al., 2013]. The toothbrush tree, *Salvadora persica* (*S. persica*), locally known as miswak, is a member of the Salvadoraceae family. *S. persica* extracts possess various biological properties with clinical applications including significant antibacterial [Wolinsky and Sote, 1983; Al-Sohaibani and Murugan, 2012], antifungal [Al-Bagieh et al., 1994] and anti-inflammatory effects [Wolinsky and Sote, 1983]. Furthermore, *S. persica* extracts have been shown to have smear layer removal capability by its acidity [Balto et al., 2012]. Aqueous *S. persica* extract have been showed to remove the smear layer partially, while the alcoholic extract completely [Sofrata et al., 2008; El-Tatari et al., 2011; Balto et al., 2012; Moeintaghavi et al., 2012] which inspired us to use it in this study.

Microleakage of oral fluids and bacteria between the cavity wall and restoration has been associated with postoperative sensitivity, recurrent caries, pulpal irritation and pulpal pathosis [Trowbridge, 1987; Shidu, 1992], and is a primary cause of restoration failure [Bauer and Henson, 1984]. Some studies have shown that microleakage permits the penetration of oral fluids that can be used by residual bacteria as a nutritional requirement and bacterial port of entry [Meiers and Kresin, 1996; Settembrini, 1997], which could explain the mechanism responsible for recurrent caries. A major goal in restorative dentistry is the control of marginal leakage, which may occur because of dimensional changes or lack of adaptation of the restorative material to the prepared cavity [Bullard et al., 1988]. The use of disinfectant solutions is an alternative procedure to reduce or eliminate bacteria from cavity preparations. *In vitro* evaluations remain an essential method for an initial screening of dental materials and might act as a predictable indicator of *in vivo* microleakage [Raskin et al., 2003].

To date, no published studies have been carried out to investigate and compare the microleakage of the new generation resins composite restorations in primary teeth after application of *S. persica* in comparison to chlorhexidine gluconate and sodium hypochlorite. Therefore, the purpose of this study was to determine the *in vitro* effect of three cavity disinfectants (*S. persica*, chlorhexidine gluconate and sodium hypochlorite) on microleakage of Class V resin-based composite restorations in primary teeth.

Materials and methods

Fifty extracted human primary anterior teeth with mild to moderate caries were collected and stored in saline and each tooth was sectioned at the cement-enamel junction (CEJ) using low-speed carborundum discs under water spray. Standard non-beveled Class V

cavities (3 mm wide, 2 mm long and 1 mm deep) were prepared on the 50 crowns using metal templates with the incisal and cervical margin placed on the enamel using burs 229 (SS White Burs, Inc., Lakewood, New Jersey) with water-cooling. The bur's length was used as a guide for the cavity depth and the burs was replaced every 5 preparations. The crowns were randomly divided into 6 groups. Groups 1-4 were composed of 10 crowns each, and cavities were irrigated using the following disinfectant solutions.

- 1) *S. persica* extract (Ethanol 1 mg/ml).
- 2) 1.3% sodium hypochlorite (NaOCl).
- 3) 0.2% chlorhexidine gluconate (CHX).
- 4) No solution applied (control group).

Final rinse was carried out with 2 ml physiological saline to avoid long-term action of the respective disinfectant solutions. Five specimens (Group 5) with a filled cavity and entirely coated with nail varnish served as negative control and 5 specimens with an empty cavity and without nail varnish coating served as positive control (Group 6). *S. persica* extract was prepared from fresh ground roots of *S. persica* with 10% water in ethanol then underwent freeze-drying to ensure that the remaining solvent was completely removed. The extract was suspended in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml. This was the stock preparation and was kept in a freezer at -20° C. Working concentrations of 1 mg/ml were prepared in sterile physiological saline at a pH of 7.4. Each cavity in groups 1-5 was treated with Scotch bond™ Etchant and Adper™ Single Bond 2 Adhesive and filled with Filtek™ Z350 XT Universal Restorative, a visible light-activated composite (3M™ ESPE™, St. Paul, MN) according to the manufacturer's instructions. Composite was leveled even with the contour of the tooth and covered with a transparent cellulose acetate strip before light curing. Composite was finished and polished immediately in one direction using graded Sof-Lex discs (3M™ ESPE™, St. Paul, USA) according to the manufacturer's instructions.

All restored crowns were stored in distilled water at 23 °C±1°C for 48 hours, then thermocycled for 500 cycles (5°C/55°C) with a dwell time of 30 seconds and a transfer time of 10 seconds. Marginal microleakage was evaluated using a conventional dye penetration method. The crowns were sealed with sticky wax and resin composite and the entire crowns coated with 2 layers of nail varnish except for the location of the restorations and 1 mm around the restoration margins. The specimens were immersed in a 2% methylene blue solution and stored at room temperature for 24 hours. After storage the specimens were rinsed with distilled water and air dried. After embedding in acrylic resin, bucco-lingual sections in the center of each cavity were obtained from each specimen through cutting in a bucco-lingual direction with a diamond saw (IsoMet®, Lake Bluff, USA) mounted in a cutting machine under

| | Level | CHX | No Solution | NaOCl | S. persica | Negative Control | Positive Control |
|-----------|----------|--------------|-------------|--------------|--------------|------------------|------------------|
| Mean & SD | Incisal | 1.20 (.834) | 2.15 (.933) | 2.05 (1.050) | 2.00 (1.124) | 0.00 (0.00) | 3.00 (0.00) |
| | Cervical | 1.55 (1.191) | 2.30 (.865) | 2.50 (.761) | 2.10 (1.071) | 0.00 (0.00) | 3.00 (0.00) |
| Median | Incisal | 1.00 | 2.00 | 2.50 | 2.50 | 0.00 | 3.00 |
| | Cervical | 1.50 | 2.50 | 3.00 | 2.50 | 0.00 | 3.00 |

TABLE 1 Mean, standard deviation, median and range scores for the experimental and control groups according to the location.

water cooling. Dye penetration was examined under a stereomicroscope (SMZ 1000, Nikon Instruments Inc., Japan) at x40 magnification. Scoring of dye penetration was examined by two examiners who were unaware of the groupings of the specimens. All scorings were taken from the junction of the tooth-restoration interface to the point of termination of the dye and recorded according to ISO specification (ISO/TS, 2003): 0 = No penetration; 1 = Penetration into the enamel part of the cavity wall; 2 = Penetration into the dentin part of the cavity wall but not including the pulpal floor of the cavity; and 3 = Penetration including the pulpal floor of the cavity. Both sections of each tooth were examined, and the worst scores for both the incisal and cervical margins were used for data analysis.

The reliability of the inter-examiner scorings was verified by using kappa test. The score data for microleakage were analysed using Kruskal-Wallis analysis with subsequent pair-wise comparisons of the individual groups. All statistical analysis were set with a significance level of $p < 0.05$.

Results

The inter-examiner reproducibility was good for both observers with kappa values equal to .740 which indicates substantial agreement for the four experimental groups (S. persica, sodium hypochlorite,

chlorhexidine gluconate or no solution applied). Microleakage mean score and standard deviation are shown for all groups in Table 1. All the experimental groups showed microleakage at the incisal and cervical walls. The negative controls showed no evidence of dye penetration whereas the dye completely penetrated the positive control cavities. Figure 1 shows examples of microleakage for all groups.

For the experimental groups, there was no significant difference in dye penetration between the incisal and cervical walls in each group ($P = 0.176$) (Fig. 1). However, comparing microleakage by location/walls showed a significant difference in dye penetration between the incisal location ($P = 0.014$) and cervical location ($P = .045$); (Fig. 1). Microleakage was higher at the cervical wall/margin than at the incisal one.

Discussion

In the present study, application of S. persica in comparison to chlorhexidine gluconate and sodium hypochlorite did not increase microleakage and was not detrimental to enamel and dentin adhesion using the restorative technique and materials used in this study. However, compared to the other cavity disinfectants, S. persica has various biological properties such as significant antimicrobial activity, antifungal and anti-inflammatory effects, as well as ability to remove the

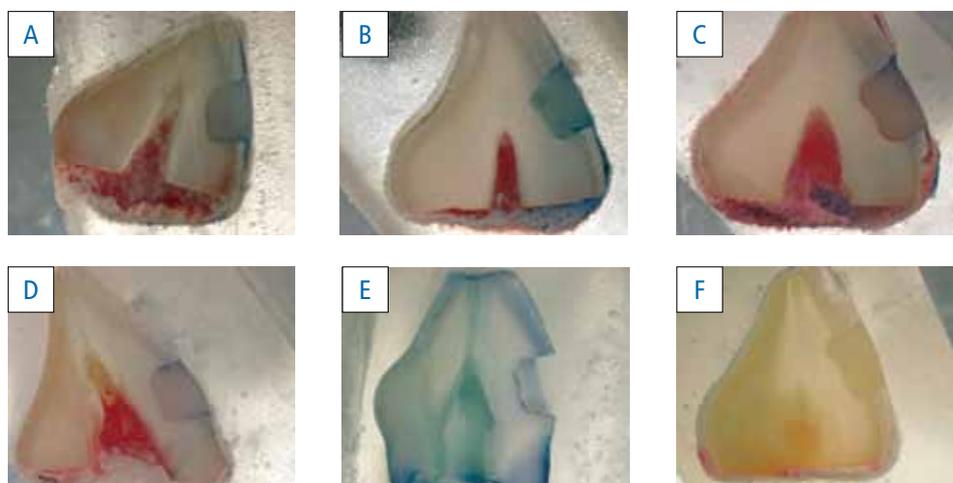


FIG. 1 Example of microleakage for all groups.

- A S. persica
- B NaOCl
- C CHX
- D No solution applied
- E Negative control
- F Positive control

smear layer by its acidity [Wolinsky and Sote, 1983; Al-Bagieh et al., 1994; Balto et al., 2012; Al-Sohaibani and Murugan, 2012] may be beneficial before restoring cavities in primary teeth. The antimicrobial and cleaning effects of *S. persica* may be attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadoreia, salvadorine, saponins, tannins, vitamin C, silica and resin [Darout et al., 2000]. A concentration of 1 mg/ml of *S. persica* extract was used in this study, due to lack of its toxicity when used in a low concentration has been confirmed by several investigators [Abu-ALSamh and Al-Nazhan, 1997; Rajabalian et al., 2009; Ibrahim and El-Gengaihi, 2012]. The ability of *S. persica* to remove the smear layer may be due to the neutral pH of the extract (pH=7.4), as etching at neutral pH is equal if not more efficient than with the agents at low pH in exposing collagen fibrils on dentin surfaces [Blomlöf and LindsKog, 1995].

Results of *in vitro* studies in permanent and primary teeth are controversial regarding the effect of cavity disinfectants on adhesion [Meiers and Kresin, 1996; Tulunoglu et al., 1998; Gürkan et al., 1999]. In the present study, no difference in microleakage was found between NaOCl, CHX, *S. persica* and control where no disinfectant applied. None of the aforementioned disinfectants did increase microleakage or was detrimental to enamel and dentin adhesion of the restorative technique and materials used in this study compared to control where no disinfectant was applied.

It has been reported that chlorhexidine-containing cavity disinfectant increased microleakage scores when used prior to application of dentin adhesive systems and there might have been some negative interaction between the cavity disinfectants and dentin bonding agents [Tulunoglu et al., 1998]. The authors concluded that the use of cavity disinfectants with composite resin restorations appeared to be material specific regarding the interactions with various dentin bonding systems and the ability to seal dentin [Tulunoglu et al., 1998]. Also, an investigation on the effect of chlorhexidine-containing cavity disinfectant on bond strength of chlorhexidine as cavity disinfectant on primary tooth dentin reported that 2% chlorhexidine had an adverse effect on the adhesive system when used prior to etching [Vieira and da Silva 2003]. In contrast, a study in permanent teeth evaluated the effect of two dentin disinfectants on the dentin sealing ability of two dentin bonding systems, the results showed that the use of cavity disinfectants after tooth preparation and before the application of a dentin bonding agent could help to reduce the potential for residual caries [Meiers and Kresin, 1996]. In the present study we followed the same sequence for disinfectant application. Other studies applied the disinfectant after etching and did not report any decrease in the adhesion of the adhesive system in bond strength testing [Perdigao et al., 1994;

Ersin et al., 2009]. Furthermore, there are also some studies which showed that applying chlorhexidine after an acid-etching procedure increased bond strength in permanent teeth [Cunningham and Meiers, 1997; Pappas et al., 2005].

In the present study, Class V cavities were selected because they do not have any macro-mechanical undercut, so that the sealing ability of composite restorations was evaluated just based on the bonding effect [Ritter et al., 2009]. In addition, it has a high C-factor value and it is relatively easy to restore, which minimises interoperator variability [Ritter et al., 2009]. In this study we did not bevel the margins of Class V cavities. A study tested effect of beveling cavo-surface margin of Class V preparations on microleakage of composite resin restorations reported no difference in microleakage when margins were beveled [Bagheri and Ghavamnasiri, 2008]. On the other hand, a study compared the effectiveness of self-etch and total-etch adhesive systems in bonding to the beveled and nonbeveled margins of primary and permanent teeth and it concluded that total-etch adhesive and beveled margins resulted in the least microleakage and that margin beveling had a greater effect in minimising microleakage than the type of adhesive used [Swanson et al., 2008]. In the present study, cavities were prepared using burs 229. It has been reported that cavity preparation with burs produces a thick and compact smear layer; such that the prismatic structure of enamel can not be clearly seen [De Munck et al., 2002]. However, in the present study, it was expected that application of *S. persica* would remove the smear layer, as it has shown to have smear layer removal capability by its acidity [Balto et al., 2012]. In this study, the dynamic environment of the oral cavity was simulated by exposing the composite restored teeth to thermal changes via thermocycling before testing for microleakage. For the study, 500 thermal cycles between 5 and 55°C were applied.

Dye penetration testing is one of the main methods of assessing microleakage in which the sample is subjected to a dye marker such as methylene blue, basic fuchsin silver nitrate, and rarely India ink [Heintze, 2013]. We used a 2% methylene blue solution which is a commonly used method due to its easy manipulation, better penetration with respect to other solutions due to its size and it is also inexpensive [Söderholm et al.; 1991, Roulet et al., 1994; Alani and Toh, 1997]. The area of methylene blue is calculated to be approximately 0.52 square nanometers, smaller than the average size of bacteria. As bacteria have a diameter of 0.3-1.5 microns, this technique cannot distinguish between too narrow and sufficiently wide gaps to allow bacteria passage [Almeida et al., 2003]. It has been reported that the use of the methylene blue tracer led to higher microleakage scores than other microscopic evaluations [Gale and Darvell, 1999; Almeida et al., 2003]. In the

present study, after immersing the composite-restored teeth in the dye for 24 hours, two blinded examiners evaluated the level of microleakage between each restoration and the cavity wall. To increase evaluation reliability, the two cut surfaces of each tooth were used for microleakage assessment and the worst scores for both the incisal and cervical margins were used for data analysis. Often the evaluation of penetration scores is performed on one or more specimen cuts and by optical microscopic observation. It has been reported that the use of several (for example, three) sections of one tooth may avoid under-estimation of *in vitro* microleakage [Raskin et al., 2003]. However, in the present study; it was not possible to make several sections, as the mesio-distal cavity size in primary anterior teeth was small. The dye penetration technique is mainly qualitative and to some extent, a quantitative method of evaluation to show the pattern of dye penetration and can indicate where the penetration occurs [Alani and Toh, 1997]. This two-dimensional evaluation method may be less sensitive than three-dimensional evaluation [Lyroutdia et al., 2000]. However, three-dimensional evaluation may not be available in each research laboratory.

Class V cavities in primary teeth may be located either in enamel/dentin or cementum/dentin [Ritter et al., 2009]. Studies have shown that microleakage was greater on the cervical wall localised in dentin or cementum than on the occlusal/incisal wall localized in enamel [Corona et al., 2003; Baygin et al., 2012]. These findings are in agreement with our results, which showed higher dye penetration at the cervical wall for all the samples in the experimental groups tested despite that both incisal and cervical walls were located in enamel. One likely reason might be the thinner enamel of the cervical walls which may result in compromised bonding of composite to the cavity wall due to lack of enough enamel for bonding. The enamel thickness in primary teeth is less than that in permanent teeth and enamel thickness in cervical area is lower than the coronal margin and therefore, in Class V restorations, the maximum bond is with dentin [El-Housseiny and Farsi, 2002]. On the other hand; some studies reported no significant differences in microleakage degree at the cementum/dentin and enamel/dentin margins [El-Housseiny and Farsi, 2002; Ernst et al., 2008; Ferreira and Vieira, 2008; Lien and Vanderwalle, 2010; Poureslami et al., 2012].

The present study has some limitations. Despite all efforts to standardise the size of Class V cavities, there was a slight variation in size of the cavities between the specimens. Also, the dye penetration technique is a two-dimensional evaluation method, which may be less sensitive than three-dimensional one. We used methylene blue, which has a small particle size and its permeability may lead to overestimate the relevance of infiltration. In addition, in the present study carious primary teeth which may have invisible cracks were used and not sound teeth as in another study [Poureslami et

al., 2012]. In summary, the results of this study inspire conduction of more *in vitro* and *in vivo* studies to evaluate the interaction between different restorative materials and different cavity disinfectants.

Conclusion

Within the limitations of this *in vitro* study, none of the cavity disinfectants in the experimental groups were able to prevent microleakage completely. Higher microleakage was observed along the cervical margin than along the incisal margin for all specimens in the experimental groups. In comparison to chlorhexidine gluconate and sodium hypochlorite; application of *S. persica* did not increase microleakage and was not detrimental to enamel and dentin adhesion using the restorative technique and materials used in this study of Class V in primary teeth.

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References

- Abu-ALSamh D, Saad Al-Nazhan. *In vitro* study of the cytotoxicity of the Miswak ethanolic extract. *Saudi Dent J* 1997; 9: 125-132.
- Alani AH, Toh CG. Detection of microleakage around dental restorations: a review. *Oper Dent* 1997; 22: 173-185.
- Al-Bagieh NH, Idowu A, Salako NO Effect of aqueous extract of miswak on the *in vitro* growth of *Candida albicans*. *Microbios Letters* 1994; 80: 107-113.
- Almeida JB, Platt JA, Oshida Y, Moore BK, Cochran MA, Eckert GJ. Three different methods to evaluate microleakage of packable composites in Class II restorations. *Oper Dent* 2003; 28: 453-460.
- Al-Sohaibani S, Murugan K. Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of streptococcus mutans: *in vitro* and molecular docking studies. *Biofouling* 2012; 28:29-38.
- Bagheri M, Ghavamnasiri M. Effect of cavosurface margin configuration of Class V cavity preparations on microleakage of composite resin restorations. *J Contemp Dent Pract* 2008; 9:1-8.
- Balto H, Ghandourah B, Al-Sulaiman H. The efficacy of *Salvadora persica* extract in the elimination of intracanal smear layer: SEM study. *Saudi Dent J* 2012; 24: 71-77.
- Bauer JG and Henson JL. Microleakage: a measure of the performance of direct filling materials. *Oper Dent* 1984; 9: 2-9.
- Baygin O, Korkmaz F, Arslan I. Effects of different types of adhesive systems on the microleakage of compomer restorations in Class V cavities prepared by Er, Cr: YSGG laser in primary teeth. *Dent Mater J* 2012; 31: 206–214.
- Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. *J Endod* 1997; 23: 725–727.
- Blomlöf J, Lindskog S. Root surface texture and early cell and tissue colonization after different etching modalities. *Eur J Oral Sci* 1995; 103:17-24.
- Bullard RH, Leinfelder KF and Russell CM. Effect of coefficient of thermal expansion on microleakage. *J Am Dent Assoc* 1988; 116: 871-874.

- › Corona SA, Borsatto MC, Pecora JD, De SA Rocha RA, Ramos TS, Palma-Dibb RG. Assessing microleakage of different Class V restorations after Er: YAG laser and bur preparation. *J Oral Rehabil* 2003; 30: 1008-1014.
- › Cunningham MP, Meiers JC. The effect of dentin disinfectants on shear bond strength of resin modified glass ionomer materials. *Quintessence Int* 1997;28: 545-551.
- › Darout IA, Christy AA, skaug NS, Egeberg PK. Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Indian J Pharmacology* 2000; 32: 11-14.
- › de Castro FL, de Andrade MF, Duarte Júnior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. *J Adhes Dent* 2003; 5: 129-138.
- › De Munck J, Van Meerbeek B, Yudhira R, Lambrechts P, Vanherle G. Micro-tensile bond strength of two adhesives to Erbium: YAG-lased vs. bur-cut enamel and dentin. *Eur J Oral Sci* 2002; 110: 322-329.
- › El-Housseing AA, Jamjoum H. The effect of caries detector dyes and cavity cleansing agent on composite resin bonding to enamel and dentin. *J Clin Pediatr Dent* 2000; 21: 57-63.
- › El-Housseiny AA, Farsi N. Sealing ability of a single bond adhesive in primary teeth. An in vivo study. *Int J Paediatr Dent* 2002; 12: 265-270.
- › El-Tatari A, de Soet JJ, de Gee AJ, Abou Shelib M, van Amerongen WE. Influence of *Salvadora persica* (miskwak) extract on physical and antimicrobial properties of glass ionomer cement. *Eur Arch Paediatr Dent* 2011; 12: 22-25.
- › Ernst C, Galler P, Willershausen B, Haller B. Marginal integrity of Class V restorations: SEM versus dye penetration. *Dent Mater* 2008; 24: 319-327.
- › Ersin NK, Candan U, Aykut A, Eronat C, Belli S. No adverse effect to bonding following caries disinfection with chlorhexidine. *J Dent Child* 2009; 76: 20-27.
- › Ferreira MC, Vieira RS. Marginal leakage in direct and indirect composite resin restorations in primary teeth: an in vitro study. *J Dent* 2008; 36: 322-325.
- › Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* 1999; 27: 105-108.
- › Guerreiro-Tanomaru JM, Morgental RD, Faria-Junior NB, Berbert FL, Tanomaru-Filho M. Antibacterial effectiveness of peracetic acid and conventional endodontic irrigants. *Braz Dent J* 2011; 22: 285-287.
- › Gürkan S, Bolay S, Kiremitci A. Effect of disinfectant application methods on the bond strength of composite to dentin. *J Oral Rehabil* 1999; 26: 836-840.
- › Heintze S. Clinical relevance of tests on bond strength, microleakage and marginal adaptation. *Dental Materials* 2013; 29: 59-84.
- › Ibrahim AY, El-Gengaihi SE. Safety Profile of Meswak Root Extract on Liver, Kidney, Sexual Hormones and Hematological Parameters of Rats. *Not Sci Biol* 2012; 4: 18-23.
- › ISO/TS 11405: 2003; Edition: 2. Dental materials : Testing of adhesion to tooth structure.
- › Lien W, Vanderwalle KS. Physical properties of a new silorane-based restorative system. *Dent Mater* 2010; 26: 337-344.
- › Lyroudia K, Pantelidou O, Mikrogeorgis G, Chatzikallinikidis C, Nikopoulos N, Pitas I. The use of 3D computerized reconstruction for the study of coronal microleakage. *Int Endod J* 2000; 33: 243-247.
- › Meiers JC, Kresin JC. Cavity disinfectants and dentine bonding. *Oper Dent* 1996; 21: 153-159.
- › Moeintaghavi A, Arab H, Khajekaramodini M, Hosseini R, Danesteh H, Niknami H. In vitro antimicrobial comparison of chlorhexidine, persica mouthwash and miswak extract. *J Contemp Dent Pract* 2012; 13: 147-152.
- › Mohammadi Z, Giardino L, Palazzi F, Shahriari S. Effect of initial irrigation with sodium hypochlorite on residual antibacterial activity of tetraclean. *N Y State Dent J* 2013; 79: 32-36.
- › Pappas M, Burns DR, Moon PC, Coffey JP. Influence of a 3-step tooth disinfection procedure on dentin bond strength. *J Prosthet Dent* 2005; 93: 545-550.
- › Perdigao J, Denehy GE, Swift E. Effects of chlorhexidine on dentin surfaces and shear bond strength. *Am J Dent*. 1994; 7: 81-84.
- › Poureslami H, Sajadi, F, Sharifi M, Ebrahimi S. Marginal Microleakage of Low-shrinkage Composite Silorane in Primary Teeth: An in Vitro Study. *JODDD* 2012; 6: 94-97.
- › Rajabalian S, Mohammadi M, Mozaffari B. Cytotoxicity evaluation of Persica mouthwash on cultured human and mouse cell lines in the presence and absence of fetal calf serum. *Indian J Dent Res* 2009; 20: 169-173.
- › Raskin A, Tassery H, D'Hoore W, Gonthier S, Vreven J, Degrange M, Déjou J. Influence of the number of section on reliability of in vitro microleakage evaluations. *Am J Dent* 2003; 16: 207-210.
- › Ritter AV, Swift EJ, Jr, Heymann HO, Sturdevant JR, Wilder AD Jr. An eight-year clinical evaluation of filled and unfilled one-bottle dental adhesives. *J Am Dent Assoc* 2009; 140: 28–37.
- › Roulet JF. Marginal integrity: clinical significance. *J Dent* 1994; 22: 9-12.
- › Settembrini WA. A comparison of antimicrobial activity of etchants used for a total etch technique. *Oper Dent* 1997; 22: 84-88.
- › Shidu SK. Sealing effectiveness of light cured glass ionomer cement liners. *J Prosthetic Dent* 1992; 68: 891-894.
- › Söderholm KLM. Correlation of in vivo and in vitro performance of adhesive restorative materials. *Dent Mater* 1991; 7: 74-83.
- › Sofrata AH, Claesson RL, Lingström PK, Gustafsson AK. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. *J Periodontol* 2008; 79: 1474-1479.
- › Swanson TK, Feigal RJ, Tantbirojn D, Hodges JS. Effect of adhesive systems and bevel on enamel margin integrity in primary and permanent teeth. *Pediatr Dent* 2008; 30: 134-140.
- › Trowbridge HO. Model systems for determining biological effects of microleakage. *Oper Dent* 1987; 12: 164-172.
- › Tulunoglu O, Ayhan H, Olmez A, Bodur H. The effect of cavity disinfectants on microleakage in dentin bonding systems. *J Clin Pediatr Dent* 1998; 22: 299-305.
- › Vieira S, da Silva IA Jr. Bond strength to primary tooth dentin following disinfection with a chlorhexidine solution: An in vitro study. *Pediatr Dent* 2003; 25: 49-52.
- › Wolinsky L, Sote EO. Inhibiting effect of aqueous extracts of eight Nigerian chewing sticks on bacterial properties favouring plaque formation. *Caries* 1983; 17: 253-257.
- › Xie Q, Johnson BR, Wenckus CS, Fayad MI, Wu CD. Efficacy of berberine, an antimicrobial plant alkaloid, as an endodontic irrigant against a mixed-culture biofilm in an in vitro tooth model. *J Endod.* 2012; 38: 1114-1117.