Original Article

The effect of irrigation solutions with or without sodium ascorbate on the microleakage of class II composite restorations

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ABSTRACT

To compare the effects of four different irrigation solutions [sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), chlorhexidine (CHX), and sterile saline] on the microleakage of class II composite restorations. The effects of the four irrigation solutions individually and in combination with sodium ascorbate on the microleakage of class II composite restorations were also investigated. A total of 180 permanent molar teeth were used, and class II cavities (3 mm wide × 1.5 mm deep) with gingival margins ended 1 mm below the cementoenamel junction were prepared and divided randomly into nine groups (n = 20 in each group). The four different irrigation solutions with or without sodium ascorbate were used and applied in this study. All of the groups were restored using Filtek P60. The specimens were thermocycled between 5°C and 55°C with 30-second dwell times for 5000 cycles. The samples were then immersed in 0.5% methylene blue dye for 24 h and sectioned longitudinally. Dye penetration at the occlusal and gingival margins was quantified by stereomicroscopy at ×15 magnification. Use of the NaOCl solution caused greater microleakage than the other irrigation solutions on the gingival surfaces, whereas use of sodium ascorbate reduced the microleakage. When NaOCl is used as the canal irrigation solution, subsequent use of sodium ascorbate may reduce the microleakage.

Key words: Irrigation Solution, Microleakage, Sodium Ascorbate

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INTRODUCTION

The most important cause of long-term endodontic treatment failure is the presence of microorganisms.[1] Although the bacterial population in root canals is decreased by the mechanical preparation, due to the complex anatomical structure of the root canal system, complete success is not possible without the use of antibacterial drugs and irrigants.[2]

The use of various irrigation solutions including sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), chlorhexidine (CHX), and sterile saline is preferred for root canal therapy. NaOCl is the most commonly used irrigating solution in endodontics because its mechanism of action causes biosynthetic alterations in cellular metabolism and phospholipid destruction, the formation of chloramines that interfere with cellular metabolism, oxidative action with irreversible enzymatic inactivation in bacteria, and lipid and fatty acid degradation.[3] Disodium salt EDTA is used to enlarge the root canals, remove the smear layer, and prepare the dentinal walls to facilitate better adhesion of the filling materials. EDTA is generally accepted as the most effective chelating agent in endodontics.[4] CHX has been suggested as an irrigating solution or intracanal dressing in endodontic therapy. Irrigation with 2% CHX has been shown to prevent microbial activity with residual effects for 48 h.[5] CHX is an excellent root canal irrigant for patients who are allergic to NaOCl.[6] Saline solution consists of sodium chloride, or salt, in sterile water. Normal saline

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solution is 0.9% sodium chloride, although this is only one possible concentration. Other concentrations may be used for different purposes. Saline solution provides moderate antibacterial activity in root canals and has higher biocompatibility than the other irrigation solutions.[7]

Composite resins are the most advanced aesthetic dental filling materials currently used in dentistry.[8] Recent studies reported that root canal irrigation solutions used during root canal treatment may cause structural changes in the enamel and dentin, can affect the adhesion of composite restorations, and may cause microleakage that adversely affects the clinical success of dental filling.[9,10] Microleakage is defined as the seepage of fluids, debris, and microorganisms along the interface between a restoration and the walls of a cavity.[11]

Sodium ascorbate (SA), a water-soluble salt, contains ascorbic acid (vitamin C) combined with sodium. SA is used as an antioxidant in many products and is also used as the source of sodium in mineral drinks and baby foods.[12]

In this study, the effects of the different irrigation solutions (NaOCl, EDTA, CHX, and sterile saline), individually and in combination with SA, on the microleakage of Class II composite restorations located in the dentin were investigated.

MATERIALS AND METHODS

A total of 180 extracted, caries-free human third molar teeth were used in this study. Tissue debris was removed from the teeth, and the teeth were stored in distilled water solution. The teeth were divided randomly into nine groups, and class II cavities with gingival margins that ended 1 mm below the cementoenamel junction were prepared using diamond burs (806314110534014, Medin, Joint Stock Company, Vlachovicka, Czech Republic) under water cooling and a caliper was used to confirm the measurements. The irrigation solutions were applied to the cavities for a period of 60 seconds and washed for 10 seconds. SA was then applied to the cavities for 10 min [Table 1]. The teeth were etched with 35% phosphoric acid for a period of 15 seconds, washed for 10 seconds according to the manufacturer's instructions, and then dried with sterile cotton palettes. Adper Single Bond (3M ESPE, St. Paul, USA) was applied and light-cured for 10 seconds. The cavities were restored with Filtek P60 (3M ESPE, St. Paul, USA) composite, using the incremental technique in which the light is applied to each layer for 20 seconds. The finishing and polishing procedures were completed using diamond finishing burs and polishing disks (Soflex, 3M Dental Products, St. Paul, USA).

The teeth were then subjected to a thermal cycling regimen of 5000 cycles between 5°C and 55°C using water baths. The teeth were then dried superficially and the roots were embedded in chemically activated acrylic (polymethyl methacrylate) resin, while the exposed crown and root structure was covered with two coats of nail varnish and a 1-mm window was left around the cavity margins. The samples were then immersed in 2% methylene blue solution for 24 h. Any surface-adhered dye was carefully rinsed away with tap water. To measure the vertical extent of the microleakage, the teeth were bisected longitudinally in the mesiodistal direction by using a low-speed diamond saw and the occlusal and gingival regions of the restorations were scored. Dye penetrations were evaluated according to the scale suggested by Lucena-Martin *et al.*[13] [Figure 1] using an optical stereomicroscope at ×15 final magnification.

Study design

A total of 180 extracted wisdom teeth were randomly distributed into nine groups and examined under experimental conditions as mentioned above.

Sample size and power

The G*Power program was used to calculate the sample size and power. A total of 162 (at least 18 in each group) teeth was calculated to be needed for the study with an $f = 0.40$ domain width to achieve 95% power, with an $\alpha = 0.05$ Type I and $\beta = 0.05$ Type II error rate. An extra 10% (two teeth) was added to each group with the aim of compensating for possible data losses, and the study was conducted using a total of 180 teeth (20 in each group).

Table 1: Study groups

Group	Irrigation solution	*n*
1	Sterile saline	20
2	NaOCl (5%)	20
3	EDTA (17%)	20
4	CHX	20
5	Sterile saline+SA (20%)	20
6	NaOCl (5%)+ SA (20%)	20
7	EDTA (17%)+SA (20%)	20
8	CHX+SA (20%)	20
9	No solution is applied (+) control	20

EDTA: Ethylenediaminetetraacetic acid, NaOCL: Sodium hypochlorite, CHX: Chlorhexidine, SA: Sodium ascorbate

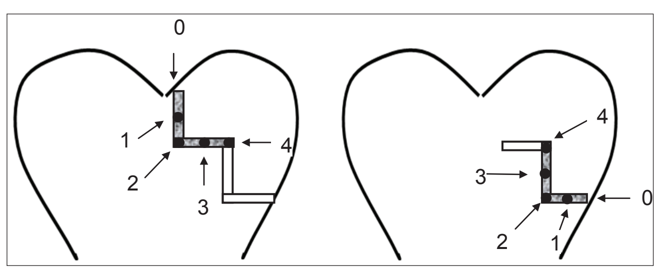

Figure 1: Evaluation of microleakage scores

Statistical analysis

Because the microleakage scores were obtained from teeth that did not fit the normal distribution, descriptive statistics of the microleakage scores are shown with the smallest, largest, and median values [interquartile range (IQR)]. Kruskal–Wallis nonparametric analysis of variance (ANOVA) was used to compare the effect of the irrigation solutions into the gingival and occlusal surfaces. When differences were found using the Kruskal–Wallis nonparametric ANOVA, a Bonferroni-corrected Mann–Whitney test was used to make *post-hoc* bilateral comparisons to determine the differences between the groups. The Mann–Whitney test was used to evaluate the differences between microleakage scores on the occlusal and gingival surfaces. Microsoft Excel 2003 and SPSS for Windows Ver. 15 software programs were used to conduct the statistical analysis and calculations. A level of $P \leq 0.05$ was adopted as an indicator of statistically significant differences.

RESULTS

Gingival surface results

When the microleakage scores observed on the gingival surface in five different groups were examined statistically, the median microleakage score differed from the others in at least one group ($\chi^2 = 13.292$; $P = 0.010$). Bonferroni correction *post-hoc* pairwise comparison was used to determine differences between groups. We observed statistically significant differences between NaOCl and the control ($Z = 2.866$; $P = 0.004$), and EDTA ($Z = 3.082$; $P = 0.002$) and CHX ($Z = 2.797$; $P = 0.005$) groups, but there were no significant differences between the other groups. These results show that NaOCl irrigation solution causes more microleakage than all of the other irrigation solutions [Figure 2].

When the microleakage scores of the groups that used SA were examined, we found that the median microleakage score of at least one group was different from that of the others ($\chi^2 = 20.759$, $P < 0.001$). Bonferroni correction *post-hoc* pairwise comparison was used to determine differences between groups.

Statistically significant differences were observed between the control and EDTA + SA ($Z = 3.161$; $P = 0.003$), and the NaOCl + SA and EDTA + SA ($Z = 3.683$; $P = 0.001$) groups. Microleakage scores were similar between the other groups. The median gingival microleakage score of the EDTA + SA group was significantly lower than that of the NaOCl + SA group [Figure 3].

Occlusal surface results

When the microleakage scores observed on the occlusal surface in five different groups were examined statistically, the median microleakage scores of the groups were similar ($\chi^2 = 8.847$; $P = 0.065$) [Figure 4].

When the microleakage scores of the groups that used SA were examined, we found that the median microleakage scores differed between the groups ($\chi^2 = 28.596$; $P < 0.001$). Bonferroni correction *post-hoc* pairwise comparisons were used with the aim of determining the differences between the groups.

Statistically significant differences were found between the control and EDTA + SA ($Z = 4{,}668$; $P < 0.001$), control and sterile saline + SA ($Z = 3.636$; $P = 0.001$), and control and CHX + SA ($Z = 3.884$; $P < 0.001$) groups, and there were no significant differences between the other groups [Figure 5]. The differences between the NaOCl and NaOCl + SA groups, and the NaOCl + SA and sterile saline + SA groups were not statistically significant because of the Bonferroni correction.

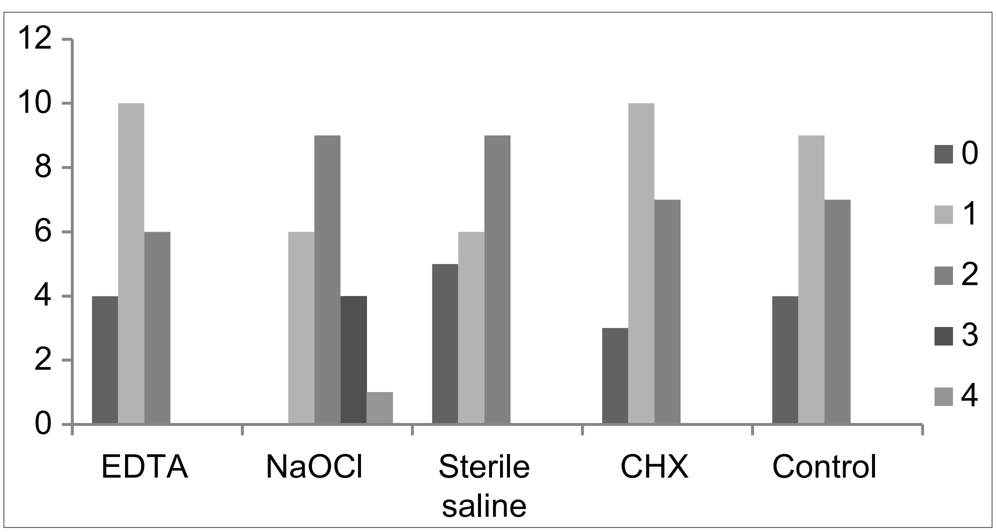

Figure 2: Microleakage scores on the gingival surfaces in the groups in which SA was not applied

Comparisons of gingival and occlusal surfaces

When the median microleakage scores observed on the gingival and occlusal surfaces were examined in the control group, the score on the gingival surface was 2 (IQR = 1), while that on the occlusal surface was 0 (IQR = 1). The median microleakage scores were the same in the control,

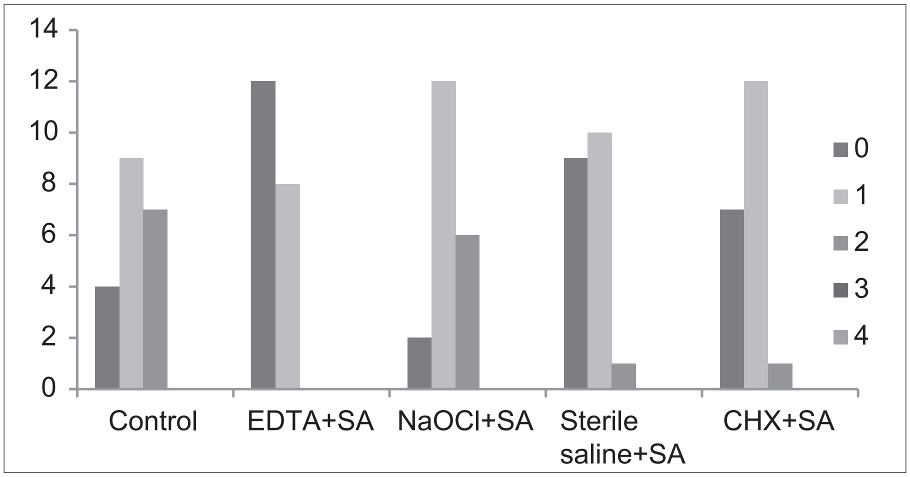

Figure 3: Microleakage scores on the gingival surfaces in the groups in which SA was applied

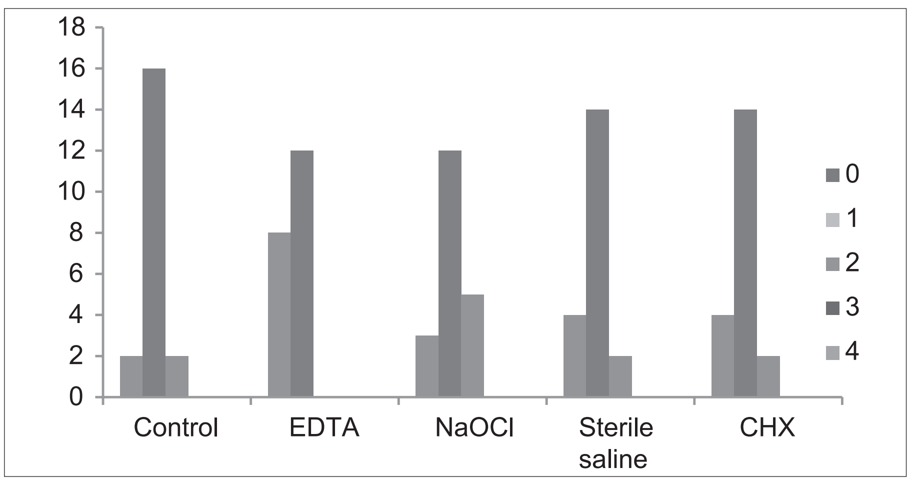

Figure 4: Microleakage scores on the occlusal surfaces in the groups in which SA was not applied

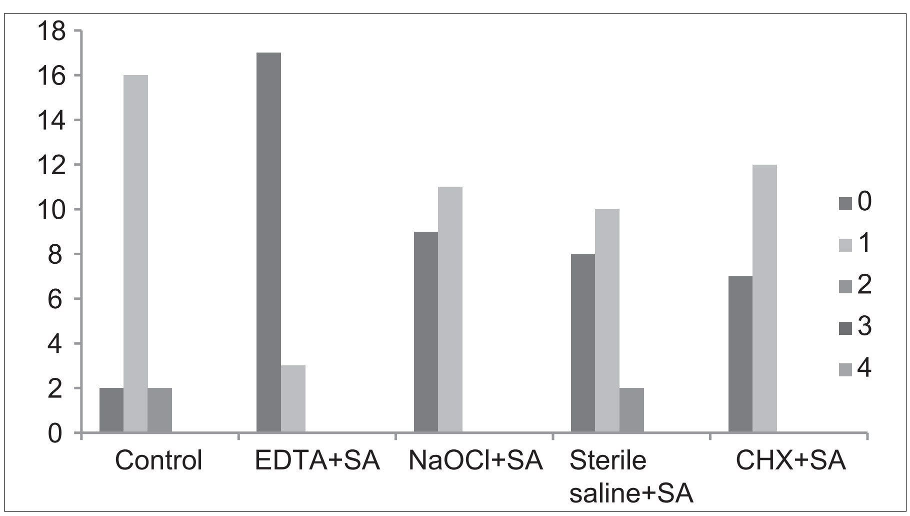

Figure 5: Microleakage scores on the occlusal surfaces number in the groups in which SA was applied

sterile saline, and CHX groups in both the gingival and occlusal surfaces. Additionally, in the EDTA group, the median microleakage score on the gingival surface was statistically higher than the median microleakage score on the occlusal surface (Z = 3.162; P = 0.002). Similarly, in the NaOCl group, the median microleakage score on the gingival surface was statistically higher than the median score of microleakage on the occlusal surface (Z = 3.626; P < 0.001). In the CHX + SA group, the median microleakage score on the gingival surface was 1 (IQR = 1) while that on the occlusal surface was 0 (IQR = 1). The median microleakage scores of the gingival and occlusal surfaces were observed to be statistically the same in the control, CHX + SA, and sterile saline + SA groups. In the EDTA + SA (Z = 2.236; P = 0.025) and the NaOCl + SA groups, the median microleakage score on the gingival surface was statistically higher than that on the occlusal surface (Z = 3.606; P < 0.001).

DISCUSSION

NaOCl remains the most widely recommended irrigating solution for endodontic therapy because of its capacity to dissolve necrotic tissue remnants.[14-16] It is a strong biological oxidant and deproteinization agent that undergoes destruction as sodium chloride and oxygen. Oxygen has been shown to reduce bond strength. It also negatively affects polymerization.[17,18] Reactive residual free radicals and adhesive vinyl free radicals formed during the implementation of light are available on the dentin surfaces irrigated with NaOCl. Limited or late polymerization may occur as a result.[16]

A few studies have examined the effect of irrigants on endodontic dentin permeability and adhesion.[19] The vast majority of these studies have reported that NaOCl reduces bonding to dentin.[16]

Moosavi *et al*.[9] reported that irrigation with NaOCl during root canal therapy has a negative effect on the microleakage of resin composite restorations. Extended restoration time did not compensate for the negative effect of NaOCl. Shinohara *et al*.[10] evaluated the effect of three different adhesive systems on the microleakage of class V restorations after the use of NaOCl. As a result, even when adhesive systems were used, use of NaOCl still increased the microleakage.

In our study, when the microleakage scores between groups were examined on gingival surfaces, statistically significant differences were seen between the NaOCl and control groups and the other groups. When the occlusal surface microleakage scores between the groups were examined statistically, no significant differences between the NaOCl group and other groups were detected. When the results were evaluated on the gingival surface, the teeth on which the NaOCl irrigation solution was applied had greater microleakage levels than all of the other groups.

The use of EDTA helps smear layer removal by forming calcium complexes with calcium formations in the root canal dentin or smear layer.[1] Various concentrations and combinations of EDTA are used in root canal treatment.[4] The effectiveness of these solutions depends on the root canal length; material penetration depth; dentin hardness; and material application time, concentration, and pH. Despite the fact that EDTA is usually used at concentrations of 10-17%, the most preferred concentration is 17%.[20,21]

Jahromi *et al*.[22] investigated the effect of three irrigation solutions [doxycycline, citric acid, and Tween 80 (MTAD); citric acid; and EDTA/NaOCl] on the coronal microleakage of root canals. They stated that use of MTAD, citric acid, and EDTA/NaOCl all resulted in less microleakage compared with normal saline, which was likely to be due to various factors, including their ability to remove the smear layer. In our study, when the gingival and occlusal surface microleakage scores were compared, no significant difference was seen between the EDTA and control groups.

CHX is an antibacterial solution that has been commonly used in both medicine and dentistry since the 1970s.[1] It affects the metabolic activity of bacteria, is bacteriostatic at low concentrations, and functions as a bactericidal that can irreversibly collapse the cellular content at high concentrations.[23] CHX is especially effective against microorganisms such as *Streptococcus mutans* and is available on the market as solution, toothpaste, mouthwash, gel, and irrigation solution.[20,21]

Nassar *et al*.[24] assessed the adhesion of Epiphany self-etch root canal sealer to dentin treated with different irrigation regimens. They reported that CHX had neither a negative nor a positive influence on bond strength.

Sung *et al*.[19] evaluated the microleakage of class V composite restorations after irrigation of an acid conditioner with various solutions. As a result, they reported that CHX has no effect on the microleakage. Similar to this result, while gingival and occlusal surface microleakage scores between the groups were examined statistically, there was no significant difference between the CHX and control groups in our study.

SA, a sodium salt of ascorbic acid that fixes oxygen, is used to prevent oxidation. Celik *et al*.[1] suggested that if bond strength decreases on NaOCl-treated dentin due to the oxidative effect of NaOCl, the reaction can be reversed using a biocompatible antioxidant such as SA. Ascorbic acid and sodium salts of SA are well-known antioxidants that are capable of reducing the diversity of oxidative components, especially free radicals. In addition, the use of SA has been reported to reduce the microleakage caused by H_2O_2 and NaOCl irrigation solutions.[12]

The real reason for the increased adhesion is the chemical reaction between SA and the surface. In a study, the effect of 10% SA on NaOCl-treated dentin using different self-etching and etch-and-rinse adhesives was studied. In this study, SA was applied for 10 min to eliminate the harmful effects of NaOCl to dentin surface, and increased adhesion grades and statistically significant results were obtained. As a result, the researchers stated that sodium ascorbate application after NaOCl treatment improved the bond strength values.[1]

Kimyai *et al*.[25] compared the effects of the hydrogel and solution forms of SA on the microleakage of composite restorations after a nonvital bleaching procedure using 10% carbamide peroxide. They reported that use of 10% carbamide peroxide significantly increased the microleakage of composite restorations when bonding was performed immediately after nonvital bleaching. The compromised sealing ability of composite restorations is reversed by use of either form (hydrogel and solution) of SA as an antioxidant.

Vongphan *et al*.[12] aimed to determine the microtensile bond strengths of total etching adhesive systems to pulpal chamber wall dentin after treatment with various irrigants. They reported that the use of NaOCl significantly reduced the bond strengths of the total etching adhesives and the application of SA to sodium hypochlorite-treated dentin significantly improved the bond strength.

Celik *et al*.[1] evaluated the effects of SA on the bond strengths of different adhesive systems on NaOCl-treated dentin. For this purpose, they mounted the teeth in a self-curing resin and irrigated the dentin surfaces with NaOCl for 10 min. Half of the specimens were treated with SA for 10 min. They reported that SA application after NaOCl treatment improved the bond strength values.

In our study, we determined that the amount of microleakage on the gingival and occlusal surfaces in groups treated with SA was lower compared with the groups in which SA was not applied, and the use of SA showed the best results on the gingival and occlusal surfaces after EDTA application.

The median microleakage scores were the same in the control, sterile saline, CHX, CHX + SA, and sterile saline + SA groups for the gingival and occlusal surfaces. Additionally, in the EDTA, NaOCl, EDTA + SA, and NaOCl + SA groups, the median microleakage score on the gingival surface was statistically higher than the median microleakage score on the occlusal surface.

CONCLUSION

In our study, the NaOCl irrigation solution caused greater microleakage than any other irrigation solution on both gingival and occlusal surfaces. When the groups with SA were compared with those without SA, significantly less microleakage was observed on both gingival and occlusal surfaces in the groups with SA.

In conclusion, SA application reduces the microleakage on gingival and occlusal surfaces. In addition, SA application is most effective after the use of EDTA irrigation solution on both gingival and occlusal surfaces.

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