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ORIGINAL ARTICLE

The Effect of Chlorhexidine and Dimethyl Sulfoxide on Long-Term Microleakage of Two Different Sealers in Root Canals

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ABSTRACT

Objective: The aim of root canal obturation is to prevent leakage and inhibit microbial invasion. This study aimed to determine the effect of chlorhexidine (CHX) and dimethyl sulfoxide (DMSO) as final irrigants on microleakage of root filling immediately and after 18 months. The hypothesis was that either CHX or DMSO would not affect the immediate or long-term microleakage.

Methods: A total of 120 human third molar root canals were obturated with RealSeal SE or Topseal and gutta percha. Before obturation, the canals were irrigated with saline (control), 2% CHX or 5% DMSO. Microleakage of half of each groups (n=10) was measured after 3 days, and of the other half was measured after 18 months with fluid filtration method.

Results: In immediate measurements, RealSeal SE performed significantly better in CHX-irrigated group (p=0.035; Mann-Whitney test). For both sealers, DMSO had the lowest mean microleakage values, which were also statistically significantly lower than with CHX irrigation within sealers (p<0.009 for Topseal and p=0.04 for RealSeal SE; Mann-Whitney test). With RealSeal SE, the microleakage with CHX was significantly higher than that in controls (p=0.022; Mann-Whitney test).

Conclusion: Neither final irrigant showed statistically significant differences in the immediate microleakage within the two sealers. Irrigation with DMSO caused significantly less microleakage than CHX for both sealers after 18 months.

Keywords: Adhesion, chlorhexidine, dimethyl sulfoxide, microleakage, root canal sealers

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HIGHLIGHTS

- Irrigation with DMSO caused significantly less microleakage than CHX for both sealers after 18 months.
- Neither final irrigant showed statistically significant differences in the immediate microleakage within the two sealers.
- Even though the leakage was small, 97% of the obturations had at least some leakage regardless of the final irrigant used. Material development should concentrate even more to improve the consistency and tightness of the obturation.

INTRODUCTION

An effective coronal seal after root canal treatment is critical in prevention of oral bacteria to causere-infection in the root canal (1). The sealer and its sealing ability play an important role in the prevention of microleakage of the endodontic filling. The quality of the seal depends on sealer's ability to consistently fill the space between the obturation material (e.g., gutta percha) and root canal, but also to fill root canal aberrancies and to penetrate into the lateral canals and dentinal tubules (2-4). Dimensional changes of sealers have also considerable relevance to the sealing ability. Even 1% shrinkage can produce a gap of 1 µm that makes it possible for micro-organisms to occupy and penetrate (5).

Epoxy resin-based sealer materials, such as Topseal (Denstply Sirona, Ballaigues, Switzerland), are assumed to bond with covalent bonds between their epoxide rings and the exposed amino groups in the collagen network (6), and thereby the longevity of the adhesion and optimal seal depends at least partly on the preservation of collagen network (7). Methacrylate-based sealer materials, for example RealSeal (SybronEndo, Glendora, Canada), act like dental adhesives. These materials depend on micro-mechanical interlocking with the collagen matrix for retention (8).

The irrigation solutions may influence the adhesion to radicular dentin. Sodium hypochlorite (NaOCl) as a final irrigant has a negative influence on bonding when epoxy resin-based sealer is

used (9). Chlorhexidine (CHX) has been suggested as an irrigant in endodontic treatment because of its good antimicrobial properties and adhesion into root canal dentin (substantivity) (10-12). CHX also decreases the activity of collagenolytic enzymes, matrix metalloproteinases (MMPs) in radicular dentin (13, 14), and by that could preserve the collagen network that resin-based sealers are possibly bonding to. Numerous studies have shown CHX to be beneficial for long-term adhesive-dentin bond with composite fillings (15, 16), and it may at least moderately improve the immediate (17) and long-term (18-20) root canal post adhesion to root dentin. However, the information of the immediate and long-term effect of CHX on microleakage of resin-based endodontic filling materials is scarce (21, 22).

Dimethyl sulfoxide (DMSO) is a solvent that has long history in industry and pharmacology (23). It has been used as a penetration enhancer for medical subjects since 1960 (23). DMSO delivers both hydrophilic and lipophilic medications through skin (23). In human medication up to 50% DMSO concentrations, and in veterinary medicine as high as 90% concentrations are used (23). To date, DMSO has been very little studied in dental applications. However, recent studies suggest that DMSO improves both immediate and long-term adhesive bond strength in dentin (24, 25). The increase in immediate bond strength may relate to DMSO's ability to reduce dentin surface free energy, and improve wettability (26) and adhesive penetration (27). The preservation of bond strength durability may at least partially be due to inhibition of MMPs by DMSO (27).

To date, removing smear layer with ethylenediaminetetraacetic acid (EDTA) as final irrigant has been accepted to improve the seal (28), while the effect of other irrigants frequently used after EDTA, such as CHX or CHX-based irrigants, have not been extensively studied. DMSO's effect on sealability has not been examined at all. Therefore, the aim of this study was to determine the effect of CHX and DMSO as final irrigant on microleakage of root canal obturation with two different sealers immediately and after 18 months. The hypothesis was that neither CHX nor DMSO would affect the immediate or long-term microleakage with any sealer.

MATERIALS AND METHODS

A total of 120 human third molars extracted as a part of normal treatment in the University Student Health Care Centre in Tampere and Oulu, Finland, were used for the study with the patients' consent and approval from the Ethical Committee, Faculty of Medicine, University of Oulu. Teeth were stored in 0.2% sodium azide at 4°C until used. Only molars with one straight separate root were selected. Crowns were removed at the cemento-enamel junction, and the selected root was separated with a diamond disk. The length of the roots was adjusted to 9-10 mm. The study protocol is presented in Figure 1. Root canals were prepared with Profile rotary NiTi-instruments (Dentsply Maillefer, Ballaigues, Switzerland) to size 40/0.04. Apical 1 mm was left unprepared to prevent apical extrusion of irrigants and sealer. Between each file size, the roots were irrigated with 3% NaOCl (ChlorCid, Ultradent, Salt Lake City, UT, USA). After the preparation, the canals were irrigated with 2 ml of 3% NaOCl followed by 2 ml of 18% EDTA (Ultradent) to remove the smear layer. The roots were randomly divided to three groups (40 roots for each group). Before obturation, the canals of the experimental groups were irrigated either with sterile saline (control), 2% CHX

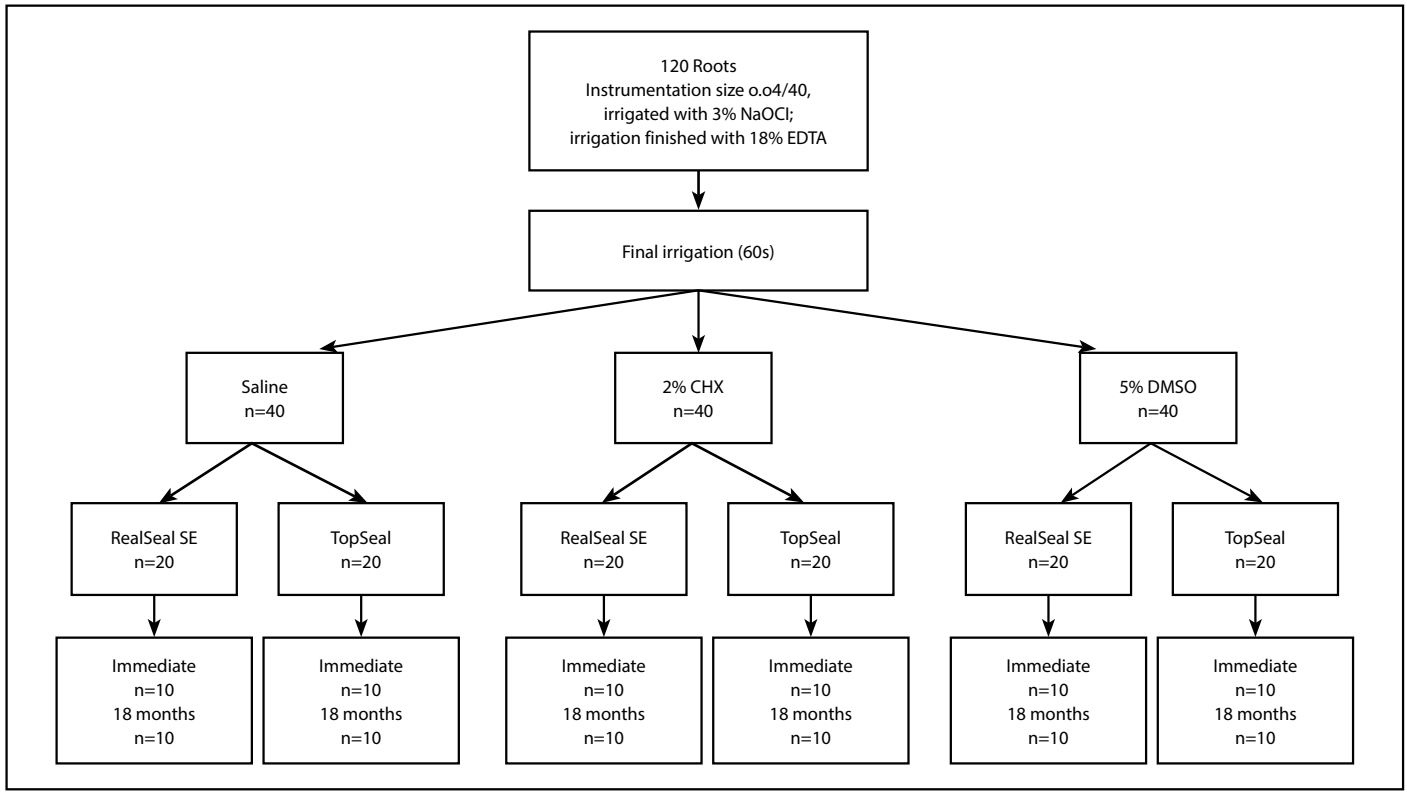

Figure 1. The flow chart describing the study protocol

TABLE 1. The materials used in the study

	Composition	Delivery/mixture	Instructions for use
RealSeal	Uretane dimethacrylate (UDMA), polyethylene glycol dimethacrylate (PEGDMA), ethoxylated bisphenol-A dimethacrylate (EBPADMA), bisphenol glycidyl dimethacrylate (BISGMA), barioum borosilicate glass, barium sulfate, silica, calcium hydroxide, bismuth oxychloride with amines.	Paste/paste automix	Dry canal with paper points, apply with cones, light cure 40 s
Topseal	Component A: bisphenol-A-diglycidyl ether, bisphenol-F epoxy resin, calcium tungstate, iron oxide, ZrO_2, silica Component B: adamantine amine, dibenzyl diamines, tricyclodecane-diamine, calcium tungstate, ZrO_2, silica, silicon oil.	Paste/paste automix	Dry canal with paper points. Apply sealer into canal with automix syringe and with the cone
Artificial saliva	0.7 mmol/l $CaCl_2$ *$2H_2O$, 0.2 mmo/l $MgCl_2$ *$6H_2O$, 4.0 mmol/l KH_2PO_4, 20 mmol/l Hepes buffer, 0.02% Na-azide to prevent microbial growth.		

(Consepsis, Ultradent) or with 5% DMSO (Sigma-Aldrich, St Louis, MO, USA) for 60 s, and then dried with paper points. The roots were further randomly divided into two groups (20 in each). The first group was obturated using RealSeal SE with RealSeal points and Accessory points M (SybronEndo). The second group was obturated using Topseal with gutta percha points and gutta percha auxiliary points C (Dentsply Sirona). The obturations were performed using lateral condensation technique with one master point and 1-3 accessory points according to each manufacturer's protocols (Table 1). The roots were stored in artificial saliva in 37°C (Table 1) for 3 days to allow complete setting of the sealers. The roots that were aged for 18 months were equally stored in artificial saliva in 37°C for 18 months. Artificial saliva was used to ensure the presence of Ca^{2+}–and Zn^{2+}–ions required for the function of dentin endogenous enzymes (29). Artificial saliva has been frequently used when studying the durability of the adhesive hybrid layers (30). The microleakage of half (n=10) of each group was measured after 3 days, and of the other half was measured after 18 months with fluid filtration method (Flodec, De Marco Engi-neering, Geneva, Switzerland).

Fluid filtration test

Microleakage was measured using a fluid filtration method as described by Bouillaguet et al. (31) (Fig. 2). The apical part of the root was glued with cyanoacrylate glue (Flex Gel, LOCTITE Super Glue, Henkel, Düsseldorf, Germany) into a silicone tube connected to the device recording the fluid flow (Flodec). The tube was filled with distilled water under constant hydrostatic pressure of 10 psi (6.89 kPa) (32, 33). The system was tested before every measurement session with one negative control root (covered with three layers of nail varnish) to ensure the proper function of the equipment. If any tube leakage occurred (observed as extremely strong leakage) during the testing, the measurement was stopped, the leaks were sealed, and the measurement was repeated. The water pressure was applied to each root for 30 min and the fluid flow was recorded constantly with 3 s time interval (Fig. 2).

Statistical analysis

Repeated-measures ANOVA, using immediate and 18-month leakage values as within-subject factors, and final irrigant (saline, CHX, and DMSO) and sealers (Topseal and RealSeal SE) as between-subject factors, was first performed. Because time together with irrigant in within-subject tests (time*irrigant, $p<0.029$) and both irrigant ($p=0.002$) and sealer ($p<0.001$) in between-subject tests showed statistically significant effect, and because Kolmogorov-Smirnov and Shapiro-Wilk tests demonstrated that all groups did not follow the normal distribution, non-parametric methods were chosen for comparison between the groups. Kruskal-Wallis and Mann-Whitney tests were used to analyze the significance of the differences in microleakage between the groups at each time point. Wilcoxon signed ranks test was used to analyze the significance of the differences between the immediate and 18-month-aged samples within the groups. Statistical analyzes were performed with SPSS 22.0 for Windows (SPSS Inc.,Chicago, IL, USA).

RESULTS

Negative control did not show leakage in any testing period. When the effect of irrigant regardless of the sealer was analyzed, saline had the highest immediate leakage rate that was significantly higher than that with DMSO ($p=0.032$; Mann-Whitney test). However, the 18-month leakage was significantly lower than the immediate leakage ($p=0.027$; related-samples Wilcoxon signed ranks test). Since DMSO had a similar low rate of leakage at both time points, 18-month saline controls did not differ from the respective DMSO samples (Fig. 2a). CHX immedi-ate values did not differ from the other irrigants, and no change was observed when the immediate and 18-month CHX sam-ples were compared. However, the 18-month leakage with CHX was significantly higher than

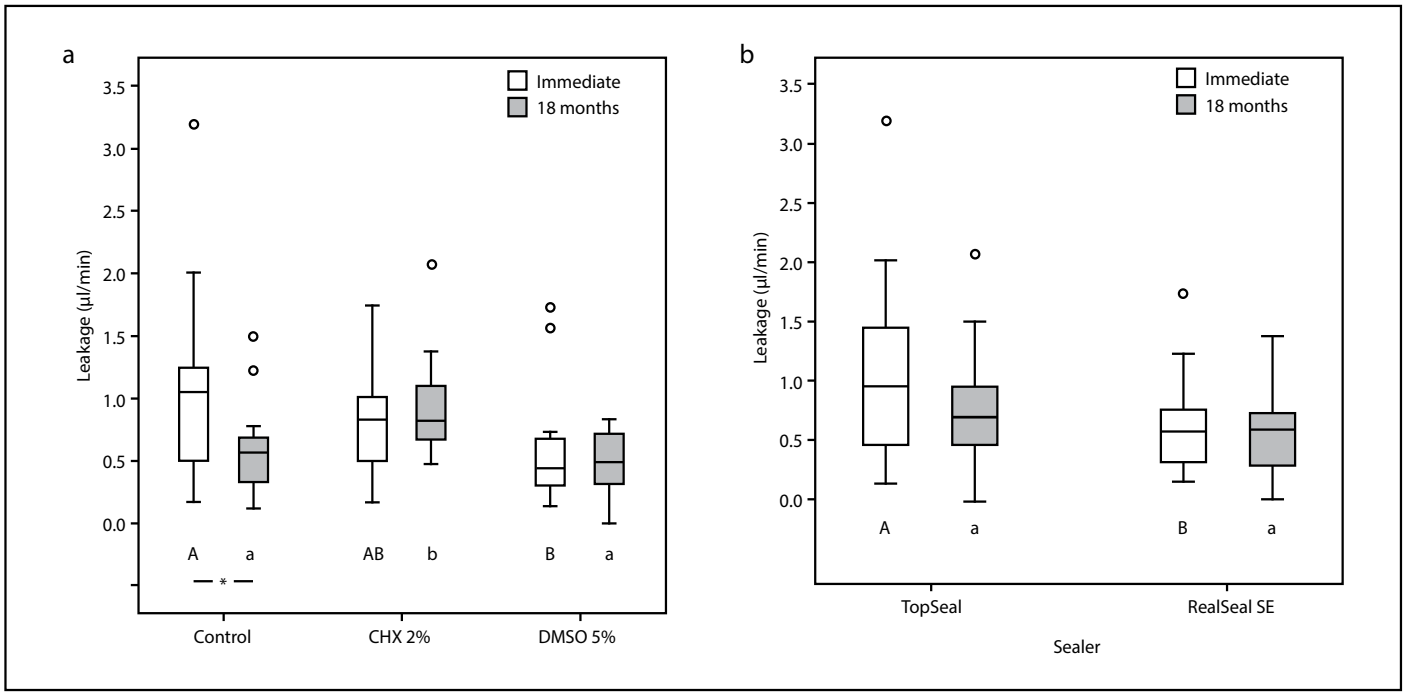

Figure 2. (a) The effect of irrigant regardless of the sealeron the microleakage. Upperand lower case letter sindicate the statistically significant difference between the irrigant satthe immedieate and 18-month measurements, respectively. (b) The effect of the sealerre gardless of the final irrigant. The letters indicating the statistical significance are the same as in Figure 2a. The circle sindicate extreme values identified by the statistical program

TABLE 2. Microleakage (ml/30 min) of the samples. The group mean values with different superscript letter demonstrate statistically significant differences within each time point

		Immediate					
		Mean	SD	Median	Min	Max	95% CI of mean
RealSeal	Control	724.5^{AB}	384.7	798.1	152.0	1222.7	428.8; 1020.2
	CHX	647.3^{A}	464.6	544.4	162.0	1734.5	290.1; 1004.4
	DMSO	448.0^{A}	180.5	405.4	243.5	718.3	309.2; 586.8
TopSeal	Control	1316.7^{B}	879.5	1183.6	185.8	3191.7	687.0; 1945.9
	CHX	1080.3^{B}	300.5	961.2	773.2	1686.8	802.4; 1358.1
	DMSO	687.3^{AB}	571.9	471.0	140.9	1717.5	244.7; 1126.9
		18 months					
		Mean	SD	Median	Min	Max	95% CI of mean
RealSeal	Control	460.8^{A}	215.1	565.1	233.2	746.3	295.5; 626.1
	CHX	747.4^{BC}	264.1	723.3	475.9	1369.9	544.4; 950.5
	DMSO	403.6^{A}	309.0	348.5	0.0	823.2	166.1; 641.1
TopSeal	Control	665.2^{ABC}	411.4	575.0	102.7	1491.7	370.9; 959.6
	CHX	1173.5^{C}	440.9	996.1	762.1	2069.8	765.7; 1581.3
	DMSO	526.3^{AB}	251.7	559.5	6.9	789.7	332.9; 719.8

with saline or DMSO (p=0.002 and p=0.001, respectively; Mann-Whitney test) (Fig. 2a).

There was no statistically significant difference in leakage between the sealers either immediately or after 18 months (Fig. 2b).

Effect of irrigants within the sealers

Next, the effects of irrigants and sealers were analyzed together. In immediate testing, even though RealSeal SE in general performed better than Topseal in all groups, the difference between the sealers was statistically significant only in CHX-irrigated group (p=0.035; Mann-Whitney test) (Table 2; Fig. 3a). DMSO irrigation produced the lowest mean microleakage for both sealers, followed by CHX and control groups. However, within sealers, the differences were not statistically significant.

When 18-month-aged groups' microleakage was compared to the immediate values, no statistically significant differences were found (Table 2).

After 18 months aging, there were no statistically significant differences between the two sealers within any irrigation group (Table 2; Fig. 3b). For both sealers, DMSO had the lowest mean microleakage values, which were also statistically signif-

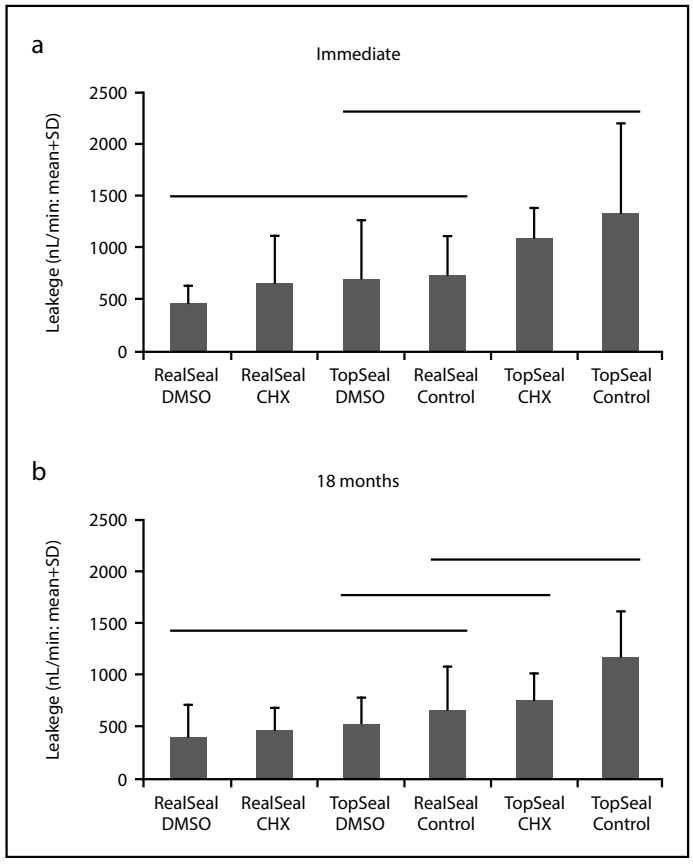

Figure 3. The immediate (a) and 18-month aged (b) micro leakage for each group, presented in ascending order. The bar sconnected with horizontal lines did not show statistically significant differences

icantly lower than with CHX irrigation within sealers ($p<0.009$ for Topseal and $p=0.04$ for RealSeal SE; Mann-Whitney test). Two RealSeal SE samples had practically no leakage at all (less than 0.01 nl/min). For both sealers, final irrigation with CHX had the highest microleakage scores, and with RealSeal SE, the microleakage with CHX was significantly higher than that in controls ($p=0.022$; Mann-Whitney test). The microleakage values between the DMSO-irrigated and controls did not reach statistical significances.

DISCUSSION

Regardless of the final irrigant and the sealer used, all but two samples (97% of the samples) had at least some leakage. This is in accordance with several other studies (31, 34, 35). Since the groups with different final irrigation regimen did not show statistically significant differences in the immediate microleakage within the two sealers, the hypothesis that DMSO or CHX irrigation would not affect immediate sealing capacity was accepted. However, final irrigation with DMSO caused significantly less microleakage than CHX irrigation did for both sealers in 18-month-aged samples. Therefore, the hypothesis regarding the effects after aging was partially rejected. CHX demonstrated the worst leakage after 18 months for both sealers. The plausible explanation for this could be the increased wettability caused by DMSO (26, 36).

In general, the 18-month storage did not increase the microleakage. These results support the impression that tight root filling will minimize the leakage, and the leakage will not increase in time. It also indicates that the degradation of the interface between the sealer and dentin does not affect the seal in a way that has been observed with composite adhesives (30). Previous studies have shown CHX to significantly improve the longevity of composite adhesive bonding to dentin (16, 37, 38). It may also at least moderately improve the initial post adhesion to dentin when composite resin cements are used (17). However, in this study with sealers, the final irrigation with CHX resulted with the highest microleakage score in the 18-month-aged samples with both sealers. The finding indicates that the effect of CHX on inhibition of dentin endogenous enzymes is probably not as important with sealers as it is with adhesives. The reason for this can only be speculated at this point. Because EDTA, which was used before the experimental irrigants, is a chelator and exposes the collagen in dentin surface, the degradation of the exposed collagen by endogenous enzymes present also in root dentin (13, 14) is expected. The reason may be the difference between the natures of the interface. Composite adhesive bonding, including self-etch adhesives, relies on the penetration of the primer/adhesive monomers between the exposed collagen fibers and the mechanical interlocking of collagen and polymerized adhesive (39, 40), while the sealers rely on close contact with the dentin surface and dentinal tubule penetration. The importance of the sealer penetration to dentinal tubules may rely on the relief of shrinkage stress of the material (35). Even though RealSeal SE has been claimed to have self-etching properties, which should further expose dentin collagen during the application of the sealer, it might not be aggressive enough to achieve true dentin bonding (41).

A good wettability of sealers to the root canal dentin is prerequisite for a good and tight seal (42). CHX has been indicated to improve wettability of both dentin (43) and gutta percha (44). DMSO is known to increase surface free energy and thus improve wettability (36) also in dentin (26). These effects may have some significance because both RealSeal SE and Topseal with both irrigation agents had slightly (but not significantly) lower immediate microleakage than controls. However, in aged samples, CHX had the highest while DMSO had the lowest microleakage scores. The findings do not indicate that simply increasing the wettability the better seal would be achieved.

CONCLUSION

Although the amount of leakage in this study was very small, we cannot assume that these obturations would have totally inhibited the bacterial invasion in time. CHX had the worst long-term results with (10) both sealers, so the use of CHX as final irrigant (Basrani et al. 2002) should perhaps be reconsidered in clinical setting. Further research should concentrate to develop materials that allow reproducible creation of impermeable root canal obturations. For example, incorporation of DMSO into the sealers could be investigated.

Disclosures

Clinical significance: Even though the leakage was small, 97% of the obturations had at least some leakage regardless of the final irrigant used. Material development should concentrate even more to improve the consistency and tightness of the obturation.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethics Committee Approval: A total of 120 human third molars extracted as a part of normal treatment in the University Student Health Care Centre in Tampere and Oulu, Finland, were used for the study with the patients' consent and approval from the Ethical Committee, Faculty of Medicine, University of Oulu.

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