Effect of Dentine Conditioning with Silver Diamine Fluoride on Wettability of Root Canal Sealers

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Objective: *To test the null hypothesis that dentine treatment with silver diamine fluoride (SDF), a potent antimicrobial agent, following use of proteolytic and chelating agents does not influence the wettability of an epoxy resin (AH Plus, Dentsply Sirona, Charlotte, NC, USA) and a tricalcium silicate sealer (BioRoot RCS, Septodont, Saint-Maur-des-Fossés, France).*
Methods: *Seventy-two intraradicular dentine specimens were divided into six groups based on the final irrigation solutions used: 2.5% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA) (NaOCl-EDTA) (group 1); NaOCl-EDTA-NaOCl (group 2); NaOCl-EDTA followed by 3.8% SDF, NaOCl-EDTA-SDF (group 3); NaOCl-EDTA-NaOCl-SDF (group 4); SDF (group 5) and saline (group 6). After irrigation, the specimens were divided into subgroups according to the sealer used, AH Plus or BioRoot RCS. Contact angles were measured using a contact angle analyser. The data were analysed using an independent t test, one-way analysis of variance (ANOVA) and Tamhane T2 post hoc test, with the level of significance set at $P < 0.05$.*
Results: *In the epoxy resin sealer group, dentine surfaces treated with only SDF showed the lowest contact angle. This was significantly less than the groups in which NaOCl was used as the final irrigant ($P < 0.05$). In the tricalcium silicate–based sealer group, the groups treated with SDF showed significantly greater contact angles when compared to the control group ($P < 0.05$).*
Conclusion: *It was concluded that SDF conditioning of dentine favours the wettability of epoxy resin sealer but is detrimental to the wettability of tricalcium silicate sealer.*
Key words: *bioceramic sealer, contact angle, epoxy resin sealer, ethylenediaminetetraacetic acid, silver diamine fluoride, sodium hypochlorite*
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Root canal disinfection aims to eliminate debris, microorganisms and toxins from the root canal system to facilitate healing of the periradicular tissues. This is primarily achieved using various root canal irrigation solutions. The currently recommended irrigation regimen involves a sequence of proteolytic irrigants such as sodium hypochlorite (NaOCl) and demineralising/chelating agents such as ethylenediaminetetraacetic acid (EDTA). To further improve the antimicrobial effect of this sequence, some studies recommend final rinsing with sodium hypochlorite or antiseptics such as chlorhexidine[1,2]. Despite this, it has been shown that biofilms can recover on irrigated dentine[3,4]. Thus, practitioners are constantly searching for alternative and supplementary irrigants to be used after NaOCl-EDTA.

Silver diamine fluoride (SDF), a potent antimicrobial agent, is a Food and Drug Administration (FDA)-approved treatment for preventing dental caries. The original formulation, i.e., 38% w/v Ag $(NH_3)F_2$, consists of 24.4% to 28.8% (w/v) silver, 5.0% to 5.9% fluoride and around 8% ammonia[5]. SDF has been shown to eradicate biofilm, prevent collagenolytic activity in dentine and

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restore dentine microhardness[5,6]. Its antimicrobial action is attributed to silver and fluoride ions which can interfere with microbial cell division, cellular respiration, metabolism, biofilm formation and adhesion[7]; however, this formulation causes blackish discolouration of the teeth. As such, a 1:10 diluted version of SDF has been proposed as a root canal disinfectant[8]. The diluted SDF solution has demonstrated good penetrability[8], acceptable biocompatibility[9], bactericidal action[7,8,10] and fungicidal action[11] as well as substantivity[12]. Thus, 3.8% SDF can be considered the last solution to be used within the root canal to maximise canal disinfection.

On the other hand, irrigating sequences alter the compositional and surface characteristics of dentine, which influence the interactions between it and root canal sealers[13]. Specifically, it has been shown that irrigants influence the wettability and adhesion of root canal sealers[14,15]. The relative surface free energy, also known as the wetting ability of a material on the dentine surface, influences the material's spreadability, adaptation and adhesion to dentine[14,16]. Surface free energy is measured and expressed in terms of the contact angle between a liquid and a solid surface. The contact angle and surface free energy are inversely related. Therefore, if the value of the contact angle is low, the surface free energy (wettability) will be greater[17]. Close adaptation of the root canal filling to the dentine is considered to provide a durable seal that can entomb bacteria and prevent microbial recolonisation[18]. Although the prospect of using SDF as a root canal disinfectant appears promising, little is known about its impact on the wettability of root canal sealers. Thus, the present study aimed to assess the wettability of an epoxy resin and tricalcium silicate sealer on dentine surfaces treated with diluted SDF following the use of a proteolytic and chelating agent. The null hypothesis tested was that dentine treatment with 3.8% SDF following the use of a proteolytic and chelating agent does not influence the wettability of an epoxy resin and a tricalcium silicate sealer.

Materials and methods

Study design and approval

The in vitro study was initiated after obtaining ethical clearance from the Institutional Ethics Committee (reference no. 21030).

Sample size calculation

In reference to the article by Gandhi et al[15], and with 5% alpha error, 80% power of the study and a clinically significant difference of fifteen units, the required sample in each group for this study was determined to be six using PASS 11.0.7 (NCSS, LCC, Kaysville, UT, USA).

Tooth selection and preparation

Prior to the experiment, 36 premolars with single root canals extracted for orthodontic treatment were cleaned and stored in 0.2% sodium azide (Sigma Aldrich, St Louis, MO, USA) at 4^{0}C until use. The teeth included had a mature apex and no cracks or apical resorption. Teeth with root canal filling and calcified canals were excluded from the study.

Specimen preparation

A low-speed diamond disc (Diamond Disk DA0001 4A, Toboom, Shanghai, China) under water coolant was used to decoronate the teeth at the cementoenamel junction and for apical third resection. The radicular sections were then split longitudinally in a buccolingual direction into 72 halves, then a 600-grit silicon carbide paper under distilled water was used to polish these sections for 15 seconds to produce a uniform smooth surface for analysis and standardise the thickness of the smear layer. The dimension of the dentine disc was maintained at around 5 × 5 × 2 mm (width × length × thickness).

Experimental groups

The dentine discs (n = 72) were randomly divided into six groups and the dentine specimens were immersed in the irrigating solution corresponding to 2 ml per sample as described below. In groups 3, 4 and 5, the dentine specimens were saturated with 3.8% SDF and spread on the surface using a microtip brush (Denmax International, Bengaluru, India). The details of the irrigants and sealer used in the study are shown in Table 1. The groups were as follows:

- Group 1: 2.5% NaOCl for 1 minute followed by 17% EDTA for 1 minute (NaOCl-EDTA);
- Group 2: 2.5% NaOCl for 1 minute, followed by 17% EDTA for 1 minute, then a final flush of 2.5% NaOCl for 1 minute (NaOCl-EDTA-NaOCl);
- Group 3: 2.5% NaOCl for 1 minute, followed by 17% EDTA for 1 minute, then application of 3.8% SDF for 3 minutes (NaOCl-EDTA-SDF);
- Group 4: 2.5% NaOCl for 1 minutes, followed by 17%

Table 1 Details of the materials used in the present study.

Material	Abbreviation	Manufacturer	Composition and Usage
Sodium hypochlorite	NaOCl	Vishal Dentocare Pvt, Ahmedabad, India (lot no. VM-122)	The samples were immersed in 2.5% NaOCl for the specified time
Ethylenediaminetetra-acetic acid	EDTA	B.N Laboratories, Mangalore, India (batch no. 23)	The samples were immersed in 17% EDTA for the specified time
Silver diamine fluoride	SDF	Saforide RC, Bee Brand Medico Dental, Osaka, Japan) (manufacturing no. 912 GA)	3.8% SDF $[Ag(NH_3)F_2]$ was applied on the samples using a microtip brush for the specified time
Epoxy resin–based sealer	ER	AH Plus (Dentsply, Sirona, Charlotte, NC, USA) (lot no. 2204000468)	Paste A (epoxide paste): diepoxide, calcium tungstate, zirconium oxide, silica, iron oxide pigment; Paste B (amine paste): Dibenzydiamine, aminoadamante, trycyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil. 1:1 ratio of both pastes was mixed on a paper pad to a homogenous consistency
Tricalcium silicate sealer	C3S	BioRoot RCS (Septodont, Saint Maur-des-Fosses, France) (lot no. B28766)	Powder: tricalcium silicate, zirconium oxide and povidone; liquid: aqueous solution of calcium chloride and polycarboxylate. One scoop of powder is mixed with 5 drops of liquid to form a smooth paste.

EDTA for 1 minute, 2.5% NaOCl for 1 minute, and then a final application of 3.8% SDF for 3 minutes (NaOCl-EDTA-NaOCl-SDF);
- Group 5: 3.8% SDF application for 3 minutes (SDF);
- Group 6: Saline application for 3 minutes.

The treatment of the dentine discs was performed by a single operator. Following irrigation, the dentine discs were submerged in deionised water for around 2 minutes to remove the test solutions. The specimens were then dried with absorbent paper to remove any excess water without drying the specimen. Following this, the twelve specimens in each group were randomly divided into two subgroups with six samples each, based on the sealer tested. Both epoxy resin (ER) sealer (AH Plus, Dentsply Sirona, Charlotte, NC, USA) and tricalcium silicate sealer (C3S) sealer (BioRoot RCS, Septodont, Saint-Maur-des-Fossés, France) were manipulated according to the manufacturer's instructions (Table 1).

Contact angle measurement

The specimens were stabilised on a glass slab using cyanoacrylate glue (Feviquik, Pidilite, India). Following this, 0.2 ml AH Plus and BioRoot RCS sealer was placed over the tooth specimen. A calibrated micropipette (Eppendorf, Hamburg, Germany) was used to control the volume of sealer. The image of the droplets was captured using contact angle analyser (Holmarc contact angle meter, Opto-Mechatronics, Kochi, India), then the static contact angle made by the sealers on the intraradicular dentine after 1 minute was evaluated using Contact Angle Meter software version 5.0.0.0 (Holmarc Opto-Mechatronics, Kochi, India.)[15]. All the measurements in duplicate were taken at a room temperature of approximately 22^{0}C.

Statistical analysis

The contact angle values obtained were tabulated and subjected to statistical testing using statistical software (SPSS version 20.0.0, IBM, Armonk, NY USA). The normality of the data was tested using a Shapiro-Wilk test. Since the values were normally distributed, an independent *t* test was used to compare the contact angle of two root canal sealers specific to each irrigation strategy. A one-way analysis of variance (ANOVA) and a Tamhane T2 post hoc test were used to determine the level of significance for each root canal sealer in relation to various irrigation protocols evaluated. The level of statistical significance was set at $P < 0.05$.

Results

ER sealer

Treatment with SDF alone (group 5) resulted in the lowest contact angle for AH Plus. This was significantly less than dentine treated with NaOCl-EDTA-NaOCl (group 2) ($P = 0.03$), or the former followed by SDF (group 4) ($P = 0.008$) (Table 2 and Fig 1). Group 3 (NaOCl-EDTA-SDF) demonstrated a significantly lower contact angle than group 2 ($P = 0.000$) and group 4 (P = 0.000). Groups in which NaOCl was used after EDTA (groups 2 and 4)

Table 2 Wetting angle of ER sealer and tricalcium silicate sealer on intraradicular dentine after treating the dentine surface with various final irrigation regimen.

Group	Contact angle, ⁰ (mean ± standard deviation)	
	ER sealer	C3S sealer
Group 1: NaOCl-EDTA	36.8 ± 4.8^{AB}	40.1 ± 4.3^{a}
Group 2: NaOCl-EDTA-NaOCl	36.6 ± 1.9^{A}	57.7 ± 8.0^{ab}
Group 3: NaOCl-EDTA-SDF	27.5 ± 1.6^{B}	53.7 ± 4.8^{b}
Group 4: NaOCl-EDTA-NaOCl-SDF	38.9 ± 1.8^{A}	47.3 ± 3.6^{ab}
Group 5: SDF	26.9 ± 3.6^{B}	58.9 ± 5.6^{b}
Group 6: Saline	35.5 ± 4.5^{A}	47.5 ± 2.9^{ab}

A,BWithin the column means the mean value of the contact angle of ER sealer without a common superscript indicated a significant difference (*P* < 0.05) between the groups.

a,bWithin the column means the mean value of the contact angle of C3S sealer without a common superscript indicated a significant difference (*P* < 0.05) between the groups.

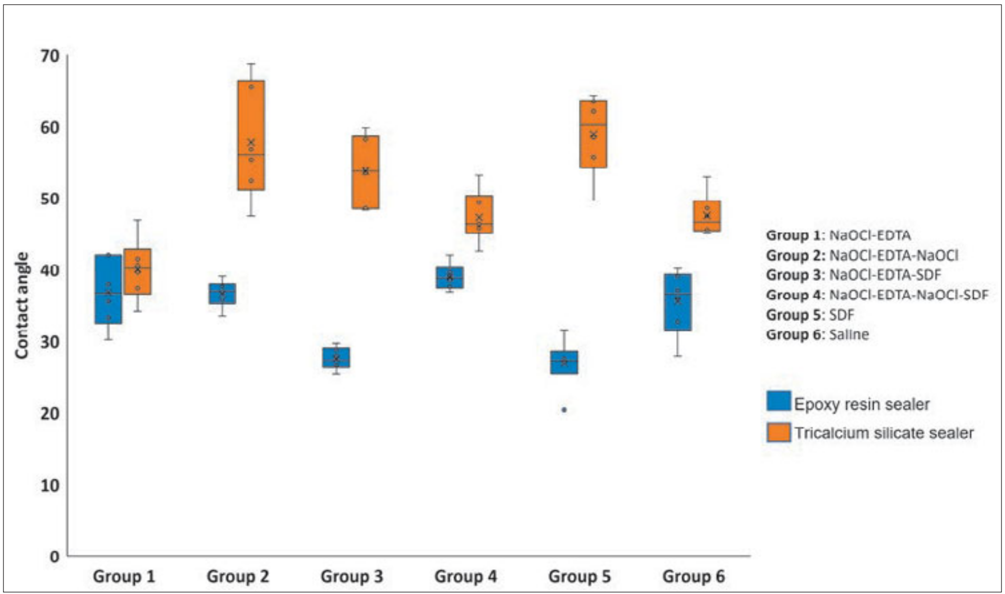

Fig 1 Contact angle measurement of root canal sealer tested following various irrigation protocols.

showed poor wetting characteristics in the ER sealer group.

C3S sealer

Dentine treated with NaOCl-EDTA (group 1) showed the smallest contact angle of all the groups. It was significantly lower than group 3 (NaOCl-EDTA-SDF) (*P* = 0.027) and group 5 (3.8% SDF only) (*P* = 0.006) (Fig 1 and Table 2). The application of SDF resulted in an increase in the contact angle of BioRoot RCS sealer.

ER vs C3S sealer

The contact angles made by AH Plus sealer were smaller than those made by BioRoot RCS sealer in all groups. The difference was significant in all groups except group 1.

Discussion

This study assessed the effect of SDF on the wettability of an ER and C3S sealer and found that SDF treatment of dentine improved the wettability of the former but was detrimental to that of the latter; thus, the null hypothesis was rejected. In the ER sealer group, dentine treated with NaOCl after the traditional irrigation approach using NaOCl and EDTA (groups 2 and 4) showed the least wettability regardless of final use of SDF. It is now well known that AH Plus, the ER sealer used in this study, chemically bonds to the amide groups in dentinal collagen. It has been demonstrated that NaOCl-treated dentine exhibits a reduction in the intensity of collagen amide peaks and an increase in the apatite/collagen ratio, confirming degradation of the collagen matrix[19]. Ramirez-Bommer et al[20] showed that the presence of mineralised matrix offers some protection against this degradation. They found that NaOCl-EDTA-NaOCl increased collagen depletion on dentine surfaces approximately fourfold compared with NaOCl alone[20]. Thus, the excessive degradation of collagen by NaOCl after the removal of the protective hydroxyapatite by EDTA could be the reason for the poor spreading of the ER sealer.

Interestingly, in groups where SDF was applied to dentine samples not treated with NaOCl as the final irrigant, wetting of the ER sealer was significantly greater. SDF is known to interact with hydroxyapatite, resulting in the formation of silver phosphate and calcium fluoride[21]. Additionally, silver ions can be reduced by proteins (collagen), resulting in the metallic silver–collagen complex[22]. Therefore, it could be speculated that the silver–collagen complex stabilises the dentine collagen and this could have encouraged better interaction with the AH Plus sealer.

On the other hand, tricalcium silicate–based sealers have been proposed to bond to the dentine surface through a process called biomineralisation, whereby nucleation of carbonated apatite occurs at the sealer–dentine interface[23]. The reaction between SDF and hydroxyapatite that leads to the formation of silver phosphate and calcium fluoride precipitate[21] may render the calcium and phosphate ions unavailable for the biomineralisation process. This may have resulted in the poor spreading and adaptation of the tricalcium silicate sealer after SDF application (groups 3 and 5).

The wettability of the tricalcium silicate sealer was significantly enhanced by using the commonly recommended final irrigation protocol for removing the smear layer, NaOCl followed by EDTA (group 1). Removal of the smear layer has been shown to improve the micromechanical interactions of the calcium silicate–based sealer with dentinal tubules and thus provided better sealer–dentine interaction in the short term[24]; however, final EDTA rinsing is detrimental to the adhesion and biomineralisation of tricalcium silicate–based materials[25,26] and this may impact the sealer–dentine interface in the long term. Similar to the ER sealer group, the contact angle of the tricalcium silicate sealer on the dentine surface with NaOCl rinsing (group 2) was relatively higher, though not statistically significant. The reason for this needs to be investigated further.

Regardless of the final irrigation protocol used, BioRoot RCS sealer showed poor wettability compared to AH Plus sealer. This could be attributed to the higher surface tension, intermolecular attraction and increased viscosity of the tricalcium silicate sealer. Although these results are in accordance with others[15,27,28], they are contrary to the findings of Ha et al[14]. The bioceramic sealers used in the study by Ha et al14 were all supplied in paste form rather than BioRoot RCS, which is available in powder and liquid form for controlled hydration. This, as well as the difference in methodology, could have contributed to the contrasting results. In the present study, the wettability of the root canal sealer was measured by the contact angle made by the sealer on a flat radicular dentine surface, whereas Ha et al[14] evaluated the wetting properties of the root canal sealers by testing the materials as solids and measuring the contact angles of two probe liquids on their surfaces.

Contrary to the study by de Assis et al[29], the control group (group 6), i.e., the group where the smear layer was left intact, showed no significant influence on the wettability of both sealers assessed. This could be attributed to variation in the surface roughness of the dentine disc prepared and the thickness of the smear layer produced, which in turn can influence the surface free energy of the dentine surface.

A limitation of this study was that mechanical instrumentation of the root canals was not performed; rather, the surface was polished with fine sandpaper discs to achieve standardised surfaces between the experimental groups. Additionally, the dentine specimens were dipped in irrigating solutions, unlike in a clinical situation where only one surface is exposed to the irrigants. As a result, outcomes may have been overestimated. Nonetheless, this study provides valuable evidence on the effect of SDF on the wettability of sealers. Additionally, studies on the chemical interaction of SDF with bioactive materials such as tricalcium silicates is an important area for future research.

Conclusion

The results of the present study demonstrated that the wettability of ER-based and tricalcium silicate sealers was influenced by the final irrigation protocol used. SDF was shown to improve the wettability of AH Plus sealer, whereas it had a detrimental effect on the wettability of BioRoot RCS. Additionally, both sealers tested in this study exhibited poor wetting characteristics when NaOCl was used as the final irrigant.

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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Surmayee SINGH, Sajan Daniel GEORGE, Prasanna NEELAKANTAN and Manuel S THOMAS contributed to the study design, manuscript draft and revision; Drs Rajat KUNDRA and Swithin HANOSH contributed to the experimental work, data collection and manuscript revision.

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