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Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules

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ABSTRACT

Background: This study evaluated the antimicrobial effect of a silver diamine fluoride (SDF)/potassium iodide (KI) product (Riva Star) on the viability of intratubular bacteria.
Methods: Forty-five dentine discs prepared from caries-free maxillary premolars were randomly divided into nine groups. Group 1 (negative control) contained non-infected sound dentine discs. The remaining discs were infected with *Streptococcus mutans* suspension and received dentine treatments as follows: Group 2 (positive control), discs were left untreated; Group 3 SDF/KI (Riva Star); Group 4 chlorhexidine (CHX); Group 5 CHX + SDF/KI; Group 6 Carisolv; Group 7 Carisolv + SDF/KI; Group 8 Papacarie, and Group 9 Papacarie + SDF/KI. The discs were then fractured into two halves, stained with fluorescent LIVE/DEAD stain and observed using confocal laser scanning microscopy.
Results: SDF/KI exhibited a potent antibacterial effect, as represented by a significantly higher percentage of dead bacteria, in comparison with Carisolv and Papacarie ($p < 0.05$). The application of SDF/KI following Carisolv and Papacarie chemomechanical caries removel gels significantly reduced the viability of intra-tubular bacteria in these groups.
Conclusions: The use of the silver diamine fluoride/potassium iodide product is effective in reducing the numbers of *S. mutans* in dentinal tubules infected with this organism.

Keywords: Carisolv, CLSM, CMCR, Papacarie, silver diamine fluoride, *Streptococcus mutans*.

Abbreviations and acronyms: BHI = brain heart infusion; CHX = chlorhexidine; CLM = confocal laser scanning microscopy; CMCR = chemomechanical caries removal; KI = potassium iodide; SDF = silver diamine fluoride.

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INTRODUCTION

Chemomechanical caries removal (CMCR) agents can be classified according to their chemistry as sodium hypochlorite (NaOCl)-based or enzyme-based.[1] Currently available CMCR agents are Carisolv (Rubicon Life Science/Medi Team Dentalutveckling AB, Sweden) (NaOCl-based) and Papacarie (Formula & Acao, Brazil) (enzyme-based) gels.[1] A previous confocal laser scanning microscopy study,[2] which evaluated the effect of a five-minute dentine surface treatment with CMCR agents on the viability of intratubular bacteria, reported that the NaOCl-based (Carisolv) CMCR gel exhibited weak antimicrobial activity (~2% dead bacteria); whereas the enzyme-based CMCR (Papacarie) gel had a similar bactericidal effect (~30% dead bacteria) as 2% chlorhexidine (CHX) solution.[2]

Many studies have shown the potent antimicrobial activity of silver salts.[3–7] Hall *et al.*[8] reported that the growth of *Streptococcus mutans* and *Staphylococcus aureas* were effectively inhibited by the application of a 20 ppm solution of silver nitrate.[9] The bactericidal activity is attributed to the high reactivity of silver ions (Ag^+) to the phosphorus components and sulfur-containing proteins of the bacterial cell wall, leading to destruction of the outer bacterial cell membrane and extrusion of the cytoplasm.[10] Furthermore, silver particles can also react with intra-cytoplasmic sulfur-containing enzymes, which consequently inhibit metabolic activities of the bacteria. Silver ions can also bind with the phosphorus-containing DNA molecules.[11] The difference in charges between the bacterial cell wall (negatively charged due to the high concentration of carboxylate groups) and the positively charged Ag^+ might lead to an electrostatic adhesion between the microorganism and the silver particles, preventing the bacterial aggregation process.[10]

Currently, silver diamine fluoride (SDF) salts are seeing a resurgence in use for caries prevention and inhibition of oral biofilm formation.[3,12–14] However,

the topical application of SDF caused a staining of organic material as found in pellicle and active caries, due to the reaction of free silver ions with organic material.[9,15] In 2005, Knight *et al*.[9] introduced a new approach to overcome this problem by applying a saturated solution of potassium iodide (KI) immediately after SDF application. The loose precipitate is then removed by the further addition of KI to minimize the possibility of subsequent staining of an overlying restoration.[9]

Recently, a commercial SDF/KI agent (Riva Star, SDI, Bayswater, Australia), consisting of 30–35% SDF and a saturated solution of KI, has been introduced for the treatment of hypersensitive dentine. It is also likely that the application of SDF/KI, following CMCR may have a synergistic antibacterial effect. Accordingly, the aim of this study was to evaluate the effect of SDF/KI application on the viability of the intra-tubular bacteria following five-minute surface treatment of dentine NaOCl-based (Carisolv) or enzyme-based (Papacarie) CMCR gels. Hence, the null hypothesis tested was that the viability of intratubular bacteria would not be affected by the application of the SDF/KI agent following CMCR.

MATERIALS AND METHODS

Dentine discs preparation

Forty-five caries-free maxillary premolars stored in 0.5% chloramine T solution at 4 °C were used in this study within six months of extraction. All premolars were observed under light stereomicroscopy and teeth exhibiting cracks were excluded. The study was approved by the local Institutional Review Board (IRB ref no: UW 11-355) for the collection and use of human teeth. The preparation, infection and observation methods used in the current study followed the non-invasive protocol for comparing the antibacterial effectiveness of different disinfectants used in radicular dentine.[16,17] This method has recently been modified for evaluation of the antibacterial effect of CMCR agents in coronal dentine.[2]

Two millimetre thick dentine discs were prepared using a slow-speed water-cooled diamond saw (ISOMET, Buehler Ltd, Lake Bluff, IL, USA). The discs were cleaned with 5.25% sodium hypochlorite and 6% citric acid solutions for 4 minutes in an ultrasonic cleaner to remove the smear layer. Each disc was stabilized at the bottom of a centrifugation 50 mL tube (CentriStar, Corning Inc., MA, USA) using a resin composite material (Filtek Z250, 3M ESPE, MN, USA, Batch #N347713). The spaces between the disc and the wall of the centrifugation tube were carefully blocked out with the composite restorative material. The disc stabilization procedure was performed inside an ultraviolet (UV) cabinet (Spectroline, Model CL-150, Spectronics Corporation, NY, USA) under shortwave UV light (254 nm). The specimens were further exposed to UV light for 1 hour to kill any possible contaminating bacteria following stabilization and then hermetically sealed to prevent contamination.

Infection of dentine discs with *Streptococcus mutans*

In the current study, the coronal dentine discs were infected following the protocol by Ma *et al*.,[16] except that *S. mutans* was used instead of *Enterococcus faecalis*.[2] The *S. mutans* used was previously isolated from soft human caries lesions and suspended in brain heart infusion (BHI) broth (CM1136, Oxoid Ltd, Hampshire, UK). The bacterial suspension was standardized to [3 x 10^6 colony-forming unit (CFU)/mL] using a spectrophotometer (DU 530, Life Science UV/Vis, Beckman, IN, USA). Five hundred microlitres of the bacterial suspension was added to each tube and centrifuged at 1400 g, 2000 g, 3600 g and 5600 g respectively for 5 minutes each. Each centrifugation cycle was repeated twice, with the replacement of bacterial suspension with a fresh 500 μL of the solution at the end of each cycle. Bacterial recovery was obtained by incubating the tubes for 24 hours at 37 °C prior to removal of the discs from the tubes for the following treatment and staining procedures.

Dentine discs treatment

The infected dentine discs were randomly divided into nine groups according to the treatment regimen (five discs per group). Group 1 (negative control group) contained five non-infected sound dentine discs; Group 2 (positive control group), the discs were infected and left untreated; Group 3, SDF/KI (Riva Star), was applied on the dentine surface following the manufacturer's recommendations without any pretreatment (Table 1); Group 4 chlorhexidine (CHX), the discs were treated with 2% CHX solution for 5 minutes (Table 1); Group 5, CHX + SDF/KI, the discs were treated with 2% CHX, followed by SDF/KI; Group 6, A NaOCl-based (Carisolv) gel, the discs were treated with the NaOCl-based gel for 5 minutes (Table 1); Group 7, the NaOCl-based gel + SDF/KI, the discs were treated with the NaOCl-based gel, followed by SDF/KI; Group 8, An enzyme-based gel (Papacarie), the discs were treated with enzyme-based gel for 5 minutes (Table 1); Group 9, the enzyme-based gel + SDF/KI, the discs were treated by enzyme-based gel, followed by SDF/KI. According to recent published data, the

Table 1. Materials used in the study

Material	Composition	Batch	Manufacturer	Application
Riva Star	• **Step 1 (Silver capsule):** 30–35% silver fluoride and > 60% ammonia solution • **Step 2 (Green capsule):** Saturated KI solution	292792	SDI, Bayswater, Australia	• A layer of SDF solution from the silver capsule was topically applied on the dentine surface • Then a generous amount of KI solution from green capsule was immediately applied on the SDF-treated surface, until the formed creamy white colour turned clear
2% chlorhexidine	1,1'-Hexamethylenebis[5-(P-chloro-phenyl) biguanide]) Dihydrochloride) (C_{22} H_{30} Cl_2 $N_{10.}$ 2Hcl)	65H0778	Sigma Chemical Company, Mo, USA	The solution was passively applied on the dentine surface for 5 min, then thoroughly rinsed with deionized water for 1 min
Carisolv	• **Compartment A:** carboxy-methylcellulose-based gels, and amino acids (glutamic, leucine and lysine) • **Compartment B:** 0.5% NaOCl in the other	1206	Rubicon Life Science AB/ Medi Team Dentalutveckling AB, Gothenburg, Sweden	Same as chlorhexidine
Papacarie	Papain enzyme, chloramine, toluidine blue, salts, preservatives, a thickener, stabilizers and deionized water	23002	Formula & Acao, Brazil	Same as chlorhexidine

Abbreviations: NaOCl = sodium hypochlorite; SDF: silver diamine fluoride, KI: potassium iodide.

mean caries excavation time of CMCR methods is between 4–6 minutes;[1] therefore, a 5-minute exposure time to each group was used. According to the protocol by Ma *et al.*,[16] the untreated surface of each disc (opposite side to treated surface) was covered with two layers of nail varnish to ensure bacteria were maintained within the dentinal tubules. Each dentine disc was then fractured longitudinally into two halves and stained with fluorescent LIVE/DEAD Baclight Bacterial Viability Stain (Molecular Probes, Eugene, OR, USA).

Confocal laser scanning microscopy evaluation

Observations were performed using confocal laser scanning microscopy (CLSM) (Fluoview 1000, Olympus, PA, USA) at five randomly selected sites for each disc. The CLSM software was operated using a Multiline Argon laser (488 nm wave length) for the first channel and Helium-Neon (gas) [HeNe (G)] laser (543 nm wave length) for the second. The observations were performed using a X20 objective lens at a resolution of 512 x 512 pixels. The depth of each scan was 10 μm (0.5 μm step size, 20 slices/scan), followed by three-dimensional reconstruction of the 20 scans by the attached (FV 10-ASW, V 1.7a, Olympus, PA, USA) software. Each 3D-reconstructed photograph was then analysed using BiolmageL software (V2, developed by Dr de Paz L, Malmö, Sweden) to calculate the red/green fluorescence (dead/live) volume percentage, using an established colour segmentation method for characterization of the viability and physiological activity of biofilms.[18]

Statistical analysis

Initially, the mean biomass area percentage of each specimen was calculated using the same software, and then subjected to one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference *post hoc* multiple comparison tests. This was performed to exclude any group that did not show sufficient bacterial infiltration and consequently would bias the results. The distribution of the percentages of both live and dead bacteria was then checked by Kolmogorov–Smirnov test, while the equality of variance assumptions were checked with the modified Levene test. Based on the results of the previous tests, the percentages of live/dead were statistically analysed using an appropriate statistical method. (The data were statistically analysed using SPSS™ Software,V.20, IBM, NY, USA).

RESULTS

It was noticed that the production of a white precipitate after application of the potassium iodide solution was slightly delayed in the NaOCl-based gel + SDF/KI group, when compared with the other SDF/KI groups.

The trend of the fluorescence patterns of live and dead bacteria after different dentine treatments is

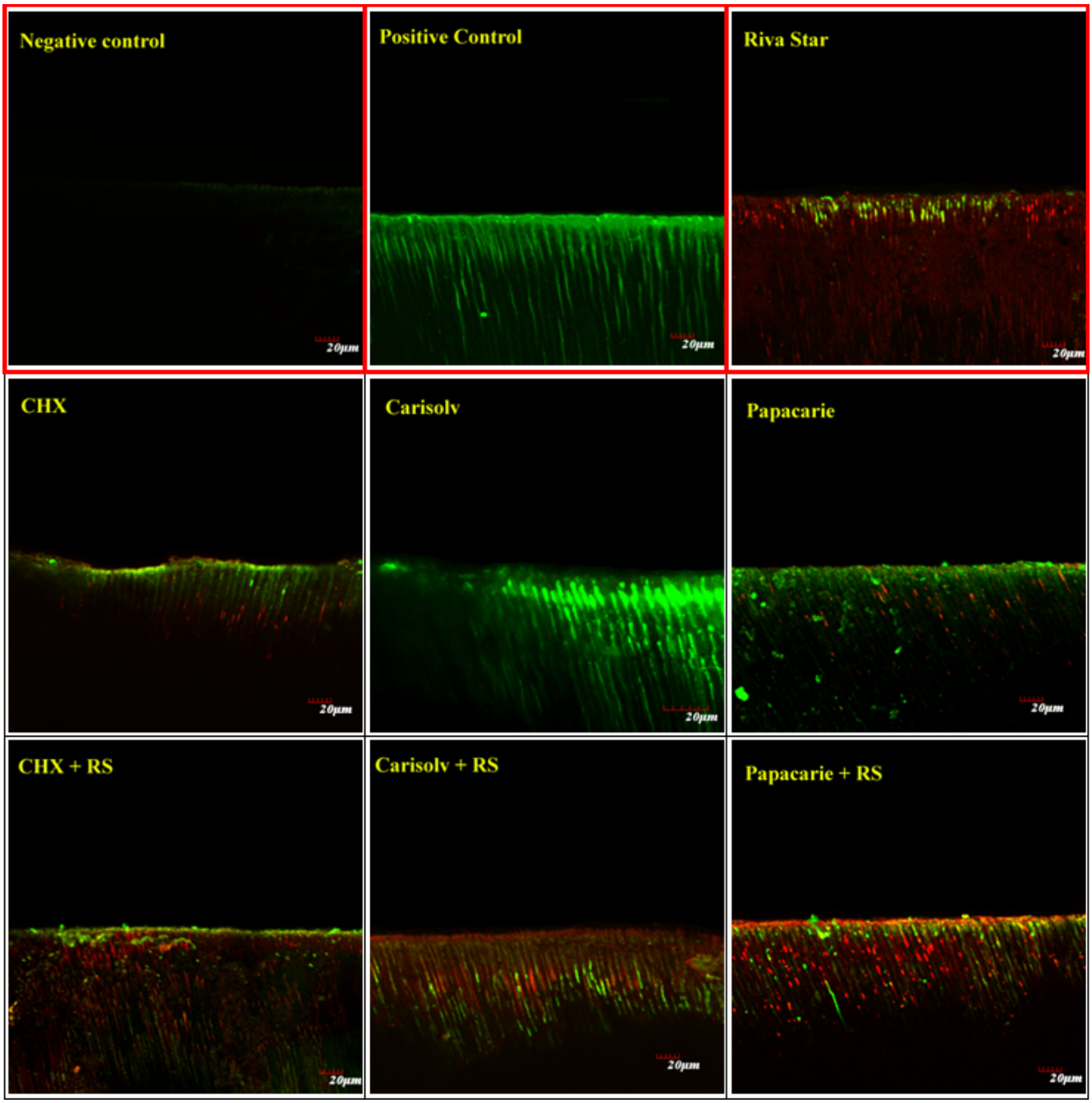

Fig. 1 Confocal laser scanning microscopy images of *Streptococcus*-infected dentinal tubules of coronal dentine following different dentine treatments. The red regions represent the fluorescence from dead bacteria, while the green regions represent the fluorescence from live bacteria. RS: Riva Star; CHX: 2% chlorhexidine solution.

shown in Fig. 1. The red regions represent fluorescence from dead bacteria, while the green regions represent fluorescence from live bacteria. The CLSM micrographs of the negative and positive controls and SDF/KI groups represent the baseline observations of this study (Fig. 1). The CLSM micrographs revealed that most of the intra-tubular bacteria were alive after a 5-minute dentine treatment with a NaOCl-based gel and in the positive control (infected non-treated) group. Conversely, a high volume of dead bacteria was observed in the SDF/KI group, which indicated that the SDF/KI agent exhibited a strong antimicrobial activity. All CLSM micrographs of the non-infected sound discs (negative control group) showed no bacteria inside the dentinal tubules, with a faint green dentine background fluorescence (Fig. 1). This observation was confirmed by the calculation of the biomass area percentage, which revealed that the negative control (non-infected) group exhibited a biomass percentage of less than 0.25%, which was significantly lower

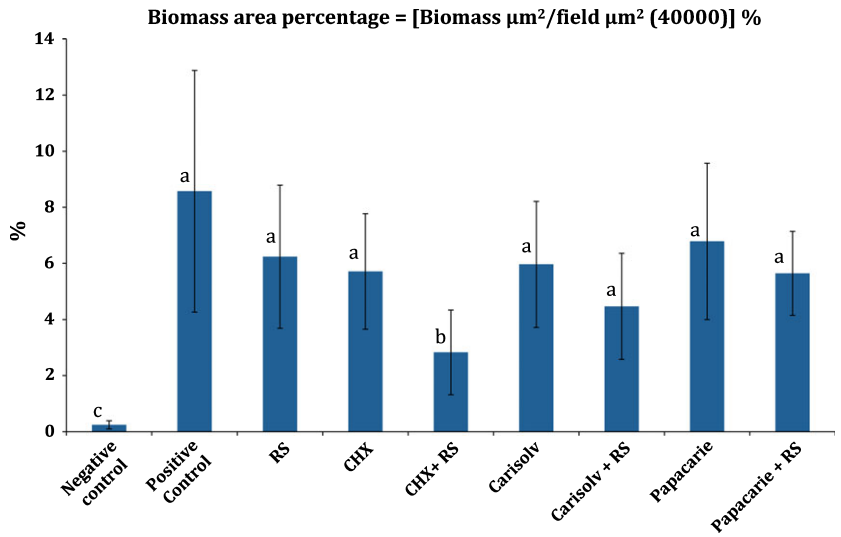

Fig. 2 The mean biomass area percentages of different dentine treatment groups calculated using BiolmageL software. RS: Riva Star; CHX: 2% chlorhexidine solution. Groups identified by different superscripts were significantly different at $p < 0.05$.

than the other groups ($p < 0.05$) (Fig. 2). Hence, this group was not included in the statistical analysis.

Kolmogorov–Smirnov test showed that the calculated percentages for all groups followed a normal distribution pattern ($p > 0.05$); however, the Levene test showed the assumption of equality of variance was not valid ($p < 0.05$). This was attributed to the low variation in both the positive control and the NaOCl-based gel groups. Therefore, ANOVA test was not the ideal test for the current study; hence, multiple independent sample *t*-tests were performed, preceded by a Levene test for each pair, and when the assumption of equal variance was not fulfilled ($p < 0.05$), the corrected calculated *p*-value (SPSS™ Software, V. 20, IBM, NY, USA) was used (Table 2). The results of *t*-tests and mean percentages of dead and live bacteria following different dentine treatments are shown in Table 2. No significant difference in the mean percentages of dead bacteria was observed between the positive control (0.0 ± 0.0%) and the NaOCl-based gel (0.4 ± 0.7%) groups ($p > 0.05$) (Table 2). SDF/KI exhibited potent bactericidal effect, as represented by a significantly higher percentage of dead bacteria (56.1 ± 13.0%) ($p < 0.05$), in comparison with the 5-minute surface treatment with NaOCl-based gel (0.4 ± 0.7%) and enzyme-based gel (19.5 ± 6.6%) (Table 2).

The application of SDF/KI following NaOCl-based and enzyme-based gel significantly reduced the viability of intratubular bacteria in these groups: NaOCl-based gel vs NaOCl-based gel + SDF/KI (0.4 ± 0.7% vs 27.7 ± 13.5%) and enzyme-based gel vs enzyme-based gel + SDF/KI (19.5 ± 6.6% vs 36.9 ± 4.4%) ($p < 0.05$) (Table 2). Furthermore, no significant difference in the percentage of dead bacteria was found between a 5-minute surface treatment of dentine with CHX (19 ± 10.8%) and enzyme-based gel (19.5 ± 6.6%) ($p > 0.05$).

Table 2. Independent sample *t*-test results comparing the viability of intratubular bacteria among the different dentine treatments groups

Group	p-values								% Live bacteria	% Dead bacteria	SD†
	Positive control	Riva Star (RS)	CHX	CHX + RS	Carisolv	Carisolv + RS	Papacarie	Papacarie + RS			
Positive control									**100.0**	**0.0**	0.0
RS	0.001*								**43.9**	**56.1**	13.0
CHX	0.017*	0.001*							**81.0**	**19.0**	10.8
CHX + RS	0.011*	0.274	0.059						**57.2**	**42.8**	21.6
Carisolv	0.4	0.001*	0.005*	0.012*					**99.6**	**0.4**	0.7
Carisolv + RS	0.01*	0.01*	0.295	0.222	0.011*				**72.3**	**27.7**	13.5
Papacarie	0.03*	0.001*	0.929	0.073	0.003*	0.259			**80.5**	**19.5**	6.6
Papacarie + RS	0.001*	0.027*	0.009*	0.582	0.001*	0.183	0.001*		**63.1**	**36.9**	4.4

Insignificant p-values ($p > 0.05$) are highlighted in yellow and * significant ($p < 0.05$).
† Standard deviation of both live and dead bacteria is exactly the same. Each group consists of 5 discs (n = 5) and observations were performed at five randomly selected sites of each disc; 25 observation site/group.

DISCUSSION

The non-invasive infection protocol used in the current study has been validated in previous studies for use in both radicular[16,17] and coronal dentine.[2] Unlike previous studies performed in radicular dentine, which used *Enterococcus faecalis* to infect the dentine; *S. mutans* was used in the present study to infect the coronal dentine. *S. mutans* has a similar diameter (~ 0.7–0.9 μm) as *E. faecalis*[19] and is considered one of the principal contributors of coronal caries.[2,20] However, the method used in our study relied on centrifuging *S. mutans* into the tubules of dentine discs. These bacteria were derived from a planktonic culture and therefore would be considered to be more susceptible to antimicrobial agents, which is a limitation of our study.

The 2% CHX was selected as a control in the current study, as it has been regarded as the gold standard antimicrobial agent for toileting cavities prior to the application of restorative materials.[21,22] Furthermore, findings from a recent laboratory study, which showed that enzyme-based gel had the same antibacterial effect as the 2% CHX solution,[2] was confirmed in the current study (Table 2) (Fig. 1). The observed low fluorescence rate from the sound non-infected dentine (Figs. 1 and 2) is in accordance with the CLSM observation by Banerjee *et al.*[23] NaOCl-based gel showed the lowest antibacterial activity among all groups. This could be attributed to the viscosity of the gel, which limits its penetration into the dentinal tubules.

The delayed appearance of the white 'AgI' precipitate after a 5-minute surface treatment of dentine with NaOCl-based gel is most likely attributed to the reaction between silver salts in solution and NaOCl, in the formation of silver chloride and silver oxide. Both are precipitated and thus reduce the number of free silver ions to react with potassium iodide.

Several medical studies, particularly those from the orthopedic[24–26] and wound healing[27,28] fields, have considered silver-containing medications as potent alternatives to antibiotics. These studies demonstrated the lethal actions of silver ions on different bacterial species and their inhibitory effects on biofilm formation.[29] In dentistry, silver-containing medicaments have been widely used in the field of caries prevention due to its bactericidal effects on most of the cariogenic bacterial species, as well as their antibiofilm activities.[3–7]

An early histological study by Englander *et al.*[15] demonstrated that free silver ions from topical application of silver-containing treatments formed a black precipitate on the superficial layer of dentine. This black discolouration was more commonly found in the caries-affected dentine, due to the reaction between the unreacted silver ions and the partially denatured collagen.[15] To overcome this major drawback, Knight *et al.*[9] introduced the novel technique of immediate application of a saturated KI solution, following topical treatment of dentine with SDF-containing agents. The loose precipitate is then removed by the further addition of potassium iodide to minimize the possibility of subsequent staining of an overlying restoration. Although it has been reported in a subsequent laboratory study[30] that the application of SDF/KI agent had no adverse effects on bonding of conventional GICs to dentine, future studies are needed to evaluate the effect of SDF/KI agent on bonding of other restorative materials, such as RMGICs and resin composites.

Results of the current study were in agreement with the outcomes of previous microbiological studies,[3,9,31] showing the potent antibacterial effects of SDF/KI-containing agents. However, none of the previous studies have evaluated the viability of the intra-tubular bacteria following CMCR and SDF/KI application. The outcomes of the current study also support the conclusions of the study by Besinis *et al.*,[32] which reported that topical application of silver nitrate showed greater antibacterial activity against *S. mutans* than CHX application. In light of the results of the current study, the null hypothesis that the viability of intratubular bacteria would not be affected by the application of SDF/KI agent following CMCR was rejected.

The original experimental SDF/KI solution used in the study by Craig *et al.*[33] consisted of 38% SDF; while the currently available commercial product 'Riva Star' consists of 30–35% silver fluoride and >60% ammonia solution.[34] The difference in SDF composition may have affected the antibacterial efficacy of SDF and the outcomes of this study. In our study, the calculation of intratubular bacterial viability exhibited a relatively high standard deviation, which typically occurs in biological structures such as this. To achieve a more accurate measurement on the percentage of the dead bacteria, conventional culturing methods are required. The culturing method provides a more accurate overall estimation of the antimicrobial activity of the test agents; however, the laboratory results correlate poorly with the clinical situation.[16] By contrast, the methodology used in our study provides a better understanding of the antibacterial effects of different dentine treatments on the intratubular bacteria.

Although the outcomes of the current study demonstrated the enhanced antibacterial efficacy of using SDF/KI agent, following NaOCl-based gel, further laboratory studies and clinical evaluations are needed to support these findings. Finally, it should be mentioned that the manufacturer of Riva Star states that the material should not be used in ante-

rior teeth and patients should be informed of the possible risk of tooth discolouration prior to starting the treatment.

CONCLUSIONS

The use of the silver diamine fluoride/potassium iodide product is an effective approach in reducing the amount of *S. mutans* in dentinal tubules infected with this organism. The application of silver diamine fluoride/potassium iodide product is a reliable method to improve the weak antimicrobial activity of the NaOCl-based gel. However, such application may cause a delayed production of the white precipitate following application of the silver diamine fluoride/potassium iodide product.

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REFERENCES

1. Hamama H, Yiu C, Burrow M. Current update of chemomechanical caries removal methods. Aust Dent J 2014;59:446–456.
2. Hamama H, Yiu C, Burrow M. Viability of intratubular bacteria after chemomechanical caries removal. J Endod 2014;40:1972–1976.
3. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodide-treated demineralized dentin. Quintessence Int 2009;40:155–161.
4. Comar LP, Wiegand A, Moron BM, *et al.* In situ effect of sodium fluoride or titanium tetrafluoride varnish and solution on carious demineralization of enamel. Eur J Oral Sci 2012;120:342–348.
5. Hosoya Y, Watanabe E, Tadokoro K, Inoue T, Miyazaki M, Tay FR. Effects of ammonium hexafluorosilicate application on demineralized enamel and dentin of primary teeth. J Oral Sci 2012;54:267–272.
6. Shibata S, Suge T, Kimura T, Ishikawa K, Matsuo T. Antibacterial activity of ammonium hexafluorosilicate solution with antimicrobial agents for the prevention of dentin caries. Am J Dent 2012;25:31–34.
7. Mei ML, Chu CH, Low KH, Che CM, Lo EC. Caries arresting effect of silver diammine fluoride on dentine carious lesion with S. mutans and L. acidophilus dual-species cariogenic biofilm. Med Oral Patol Oral Cir Bucal 2013;18:e824–831.
8. Hall RE, Bender G, Marquis RE. Inhibitory and cidal antimicrobial actions of electrically generated silver ions. J Oral Maxillofac Surg 1987;45:779–784.
9. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans. Aust Dent J 2005;50:242–245.
10. Morones JR, Elechiguerra JL, Camacho A, *et al.* The bactericidal effect of silver nanoparticles. Nanotechnology 2005;16:2346–2353.
11. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res 2000;52:662–668.
12. de Almeida Lde F, Cavalcanti YW, Valenca AM. In vitro antibacterial activity of silver diammine fluoride in different concentrations. Acta Odontol Latinoam 2011;24:127–131.
13. Peng JJ, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: a review. J Dent 2012;40:531–541.
14. Shah S, Bhaskar V, Venkataraghavan K, Choudhary P, Ganesh M, Trivedi K. Efficacy of silver diammine fluoride as an antibacterial as well as antiplaque agent compared to fluoride varnish and acidulated phosphate fluoride gel: an in vivo study. Indian J Dent Res 2013;24:575–581.
15. Englander HR, James VE, Massler M. Histologic effects of silver nitrate of human dentin and pulp. J Am Dent Assoc 1958;57:621–630.
16. Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod 2011;37:1380–1385.
17. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. J Endod 2012;38:948–953.
18. Chavez de Paz LE. Image analysis software based on color segmentation for characterization of viability and physiological activity of biofilms. Appl Environ Microbiol 2009;75:1734–1739.
19. Samaranayake LP. Essential microbiology for dentistry. 4th edn. Edinburgh: Churchill Livingstone Elsevier, 2012.
20. Ryan KJ, Ray CG, Sherris JC. Sherris medical microbiology: an introduction to infectious diseases. 4th edn. New York: McGraw-Hill Medical Publishing Division, 2004:273–296.
21. Mobarak EH. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. Oper Dent 2011;36:649–660.
22. Lafuente D. SEM analysis of hybrid layer and bonding interface after chlorhexidine use. Oper Dent 2012;37:172–180.
23. Banerjee A, Sherriff M, Kidd EA, Watson TF. A confocal microscopic study relating the autofluorescence of carious dentine to its microhardness. Br Dent J 1999;187:206–210.
24. Collinge CA, Goll G, Seligson D, Easley KJ. Pin tract infections: silver vs uncoated pins. Orthopedics 1994;17:445–448.
25. Karlov AV, Khlusov IA, Pontak VA, Ignatov VP, Ivin MA, Zinatulina SY. Adhesion of Staphylococcus aureus to implants with different physicochemical characteristics. Bull Exp Biol Med 2002;134:277–280.
26. Zhao L, Wang H, Huo K, *et al.* Antibacterial nano-structured titania coating incorporated with silver nanoparticles. Biomaterials 2011;32:5706–5716.
27. Percival SL, Thomas J, Linton S, Okel T, Corum L, Slone W. The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds. Int Wound J 2012;9:488–493.
28. Sachsenmaier S, Peschel A, Ipach I, Kluba T. Antibacterial potency of VAC GranuFoam Silver® Dressing. Injury 2013;44:1363–1367.
29. Percival SL, Hill KE, Williams DW, Hooper SJ, Thomas DW, Costerton JW. A review of the scientific evidence for biofilms in wounds. Wound Repair Regen 2012;20:647–657.

30. Knight GM, McIntyre JM, Mulyani. The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. Aust Dent J 2006;51:42–45.

31. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS, Gully NJ. Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an in vitro challenge by Streptococcus mutans. Aust Dent J 2007; 52:16–21.

32. Besinis A, De Peralta T, Handy RD. Inhibition of biofilm formation and antibacterial properties of a silver nano-coating on human dentine. Nanotoxicology 2014;8:745–754.

33. Craig GG, Knight GM, McIntyre JM. Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. Aust Dent J 2012;57: 308–311.

34. SDI Ltd. Material Safety Data Sheet (MSDS) of Riva Star. Available at: 'http://www.sdi.com.au/images/stories/MSDS/MSDS_EN/Riva_Star_DS_EN.pdf'. Access date 23 January 2014.

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