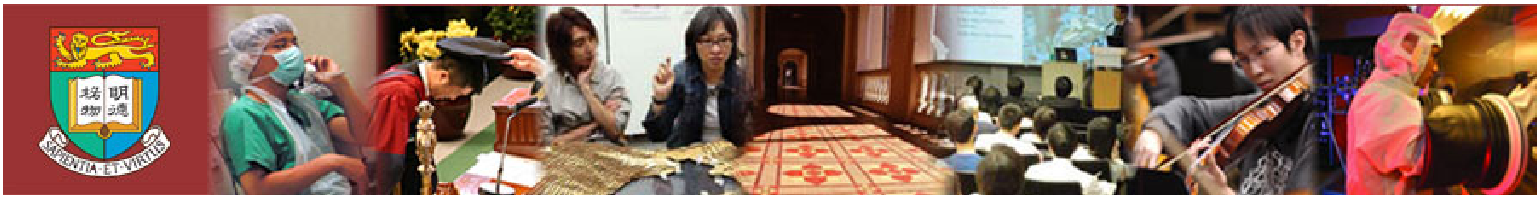

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Prevention of secondary caries by silver diamine fluoride

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Keywords:	silver diamine fluoride, secondary caries, glass ionomer, resin composite
Abstract:	Purpose: This study aimed to investigate the use of 38% silver diamine fluoride (SDF) as a condition for the prevention of secondary caries in glass ionomer cement (GIC) and composite resin (CR) restoration. Methods: Six extracted human sound premolars were collected. Four cavities (4×2×2 mm3) were prepared on each premolar and then allocated to the following restoration groups: Group 1 - SDF conditioning and GIC restoration; Group 2 - GIC restoration; Group 3 - SDF conditioning and CR restoration; and Group 4 - CR restoration. After thermal cycling and sterilization, the teeth were soaked in a 5% sucrose solution with Streptococcus mutans and Lactobacillus acidophilus for 28 days. Micro-computed tomography was used to study the demineralisation. The outer lesion depth (OLD) and wall lesion depth (WLD) of the tooth-restoration interface were measured. The OLD and WLD were directly related to the extend of secondary caries. Two-way ANOVA was used to analyse the effects of SDF conditioning and restorative materials on OLD. Results: The OLD (mean ± SD μm) in Groups 1 through 4 were 156 ± 45, 235 ± 33, 153 ± 20 and 232 ± 24, respectively. The OLD was less in restorations with SDF conditioning ($p < 0.001$) than those without SDF conditioning. No interaction effect on OLD was found between the restorative materials and SDF conditioning ($p = 0.062$). The WLD was detected only in Groups 3 and 4. Clinical significance: Conditioning with 38% SDF can increase resistance of the GIC and CR restorations to secondary caries.

Prevention of secondary caries by silver diamine fluoride

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Running title: Prevention of secondary caries by SDF

Key words: silver diamine fluoride; secondary caries; glass ionomer, resin composite

Abstract

Purpose: This study aimed to investigate the use of 38% silver diamine fluoride (SDF) as a condition for the prevention of secondary caries in glass ionomer cement (GIC) and composite resin (CR) restoration. **Methods:** Six extracted human sound premolars were collected. Four cavities (4×2×2 mm^3) were prepared on each premolar and then allocated to the following restoration groups: Group 1 - SDF conditioning and GIC restoration; Group 2 - GIC restoration; Group 3 - SDF conditioning and CR restoration; and Group 4 - CR restoration. After thermal cycling and sterilization, the teeth were soaked in a *5% sucrose solution with Streptococcus mutans and Lactobacillus acidophilus* for 28 days. Micro-computed tomography was used to study the demineralisation. The outer lesion depth (OLD) and wall lesion depth (WLD) of the tooth-restoration interface were measured. The OLD and WLD were directly related to the extend of secondary caries. Two-way ANOVA was used to analyse the effects of SDF conditioning and restorative materials on OLD. **Results:** The OLD (mean ± SD µm) in Groups 1 through 4 were 156 ± 45, 235 ± 33, 153 ± 20 and 232 ± 24, respectively. The OLD was less in restorations with SDF conditioning ($p < 0.001$) than those without SDF conditioning. No interaction effect on OLD was found between the restorative materials and SDF conditioning ($p = 0.062$). The WLD was detected only in Groups 3 and 4. **Clinical significance:** Conditioning with 38% SDF can increase resistance of the GIC and CR restorations to secondary caries.

INTRODUCTION

Secondary caries has been considered a major reason for failure of direct restorations (1, 2). A Dental Practice-based Research Network practices in the USA reported that secondary caries was the most common reason for repairing or replacing existing restorations (3). Another Study reported that approximately half of all restorative dentistry is in the form of restoration replacements, with 40% of replacements are attributed to secondary caries (4). This fact has prompted the development of restorative materials that promise anticariogenic properties, such as glass ionomer cement. Glass ionomer cement releases fluoride to promote remineralisation. However, studies found the antibacterial effect of fluoride released is limited (5) and is inadequate to prevent secondary caries development (6).

Streptococcus mutans is important for the initiation and progression of caries. Lactobacillus acidophilus was frequently found in high numbers in both superficial and deep carious lesions. S. mutans and L. acidophilus are often considered the two most important cariogenic bacteria associated with dentine caries (7). Studies demonstrated that silver diamine fluoride (SDF) can inhibit the growth of these 2 cariogenic bacteria (7, 8). SDF is a topical fluoride solution in arresting caries, although it is cleared by the US Food and Drug Administration as an anti-hypersensitivity agent. A review concluded that SDF is a safe, effective, efficient, and equitable caries-preventive agent that appears to meet the World Health Organization Millennium Goals and fulfil the US Institute of Medicine's criteria for 21st-century medical care (9). Studies reported clinical success with SDF in arresting dental caries (10, 11), and laboratory studies also found that SDF has an intense antibacterial effect on cariogenic biofilm and hinders caries progression (12, 13). It was reported that SDF did not adversely affect the bond strength of resin composite to non-carious dentine (14). SDF-treated carious dentine also represented a biologically acceptable pulpal response (15). Therefore, SDF may be useful to prevent secondary caries of dental restorations. However, a search in PubMed and China National Knowledge Infrastructure (CNKI) found that no study in English and Chinese reported the effect of SDF in prevention of secondary caries of direct restorations. Therefore, the purpose of this laboratory study is to investigate the effects of SDF conditioning on the prevention of secondary caries formation around direct composite resin and glass ionomer cement restorations. The null hypothesis is that SDF conditioning has no effect on secondary caries prevention in glass ionomer cement and composite resin restoration.

METHODS

Materials selection and specimen preparation

This study received approval from the Institutional Review Board (the University of Hong Kong) under process number IRB UW13-555 and was conducted in full accordance with the Declaration of Helsinki of the World Medical Association. All participants received dental treatment at the Faculty of Dentistry of the University of Hong Kong and provided written informed consent. The written consents were obtained from the parents/guardians of the teenagers who are under 18 years old. Consent procedure was approved by Institutional Review Board (the University of Hong Kong).

From our pervious and pilot studies we could expected the mean lesion depth of test group was 150μm. We wanted to detect a difference of at least 100μm. Assuming the common standard deviation was 60 μm and with power at 0.80 and α=0.05, the sample size was at least 6 in each group. Six extracted human premolars, intact and without visible defects, were collected with patient's consent from teenagers who require orthodontic treatment. After removal of calculus (if any) and soft tissue and thorough cleaning, four round cavities of a similar size (4×2×2 mm^3) were prepared on each tooth. The cavities were prepared by a carbide bur (FG 330, SS White, Lakewood, NJ, USA) under copious water-cooling. The four cavities of each tooth were cleaned by 10% polyacrylic acid and allocated to the following four restoration groups:

Group 1: the cavity was conditioned with SDF for 3 min, followed by glass ionomer cement restoration.

Group 2: the cavity was bulk filled with glass ionomer cement.

Group 3: the cavity was conditioned with SDF for 3 min. The exposed surface was treated with a single-step bonding agent. The bonding agent was applied to the prepared tooth and rubbed for 20s. It was gently air dried for 5s before lighted cured for 10s. Subsequently, the prepared tooth was filled by composite resin using layering technique.

Group 4: the exposed surface was treated with single-step bonding agent (procedures was mentioned above), and then the cavity was filled with composite resin.

The flow chart of the study is shown in Figure 1. The glass ionomer cement used in this study was Ketac-Molar (3M ESPE, St. Paul, MN, USA). The composite resin was Filtek Z250 (3M ESPE, St. Paul, MN, USA). The bonding agent was Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA), and the SDF was Saforide 38% (Toyo Seiyaku Kasei, Osaka, Japan). SDF was topically applied to the specimens using a micro-brush (Micro applicator - regular, Premium Plus International Ltd., Hong Kong, China). The cavities were gently blown dry with a 3-in-1 syringe before restoration.

Thermocycling

All the restored teeth were covered by acid-resistant nail varnish (Clarins, Paris, France), except for a zone approximately 1 mm wide around the restoration. To mimic aged restoration, the restored teeth were thermocycled in 55 ± 5°C, and 10 ± 5°C distilled water baths for 500 cycles with a 32-second dwell time in each bath and a 14-second interval between baths (1). The teeth were then sterilized by autoclave before cariogenic bacterial challenge (16).

Cariogenic bacterial challenge

The microorganisms used for cariogenic challenge were *Streptococcus mutans* American Type Culture Collection 35668 and *Lactobacillus acidophilus* American Type Culture Collection 9224 (7). The bacteria were grown in blood agar plates to obtain isolated colonies (37°C for 24 hours, anaerobically). Then, the grown colonies were transferred to tubes containing a brain–heart infusion with 5% sucrose. Subsequently, bacterial cell pellets were harvested after 24 hours and re-suspended in brain–heart infusion to a cell density of McFarland 2 (6×10^8 CFU/mL). Each tooth was soaked into a tube containing brain–heart infusion + 5% sucrose and 5.0 ml of the inoculum broth of each bacterium. The teeth were maintained in this bacterial solution at 37°C for 28 days anaerobically; the medium was refreshed every 48 hours. During the incubation period, the test was performed daily to check for contaminant (8).

Lesion assessment and data collection

The teeth were scanned by a SkyScan 1172 X-ray micro-computed tomography (SkyScan, Antwerp, Belgium) for lesion depth assessment. The X-ray source was operated at a voltage of 100 kV and a current of 80 lA. The highest spatial resolution of 9 local maxima (lm) was used for the scanning. The signal-to-noise ratio was 5, and a 1 mm aluminium filter was used to cut off the softest X-rays. SkyScan 1172 has a self-calibrating computed tomography imaging system. Briefly, calibration with 20 and 250 micron thick AI foils* showed that both thicknesses could be measured accurately simultaneously. The thickness calibration with 20 micron thick AI foil was found to be stable over the range of magnifications or x 40 and higher, or pixel sizes 6.8 microns and lower (*embedded aluminium foil thickness phantom (embedded set of 4 aluminium foils of 20, 50, 125, 250 microns nominal thickness (+/- 10% tolerance), item no. SP-4001). Scanning results of each tooth were reconstructed using the reconstruction software NRecon (SkyScan Company, Antwerp, Belgium). The reconstructed 3-dimenional images were viewed and processed using the data-analysing software CTAn (SkyScan Company, Antwerp, Belgium). From the reconstructed 3-dimenional image of each specimen, cross-sectional images in each tooth were located (17). Approximately one hundreds images were obtained for each restoration, from these lesion images, five images were selected by systematic random sampling. Greyscale values of the sound enamel in the image were estimated from the image profile. Image area with a greyscale value of more than 95% of the sound enamel was defined as sound enamel (17). Special image analysis software (Image J, National Institutes of Health, MD, USA) with plot profile was used to determine demineralized enamel in terms of different greyscale values. The method of lesion assessment on the restoration-tooth interface was adapted from Hsu et al. (1) by assessing the outer lesion depth (the deepest point of the lesion from the tooth surface) and wall lesion (from the inner border of the outer lesion adjacent to the restoration to the tooth (Figure 2a). Starting and ending points of the outer lesion were determined according to corresponding grey value (Figure 2b&c). For each group, the outer lesion depth and wall lesion (to a depth of 500 μm) were assessed using special image analysis software (Image J; National Institutes of Health, USA).

Statistical analysis

The experiment was a randomized complete block with factorial treatment structure (2×2 factorial combination with 6 tooth blocks). The primary outcome measured was outer lesion depth. Therefore, randomized block analysis of variance (ANOVA) with 2 fixed factors and random block was performed to compare the effects of restorative materials and SDF (as two predicting variables) on secondary caries formation. The computer software SPSS Statistics - V20.0 (IBM Corporation, Armonk, USA) was used for statistical analysis, and the level of statistical significance for all tests was set at 0.05.

RESULTS

The outer lesion depths (mean ± SD µm) in Group 1 to 4 were 156 ± 45, 235 ± 33, 153 ± 20 and 232 ± 24, respectively (Figure 3). A statistically significant difference was detected between Groups 1 and 2 and Groups 3 and 4, respectively. Different restorative materials (glass ionomer cement or composite resin) have no significant effect on outer lesion depth ($p = 0.797$). However, outer lesion depth was reduced in restorations with SDF conditioning ($p < 0.001$). Randomized block ANOVA with 2 fixed factors showed that there is no interaction effect on outer lesion depth SDF conditioning and the restorative material (glass ionomer cement or composite resin) ($p = 0.963$). Different sample did not have a significant impact on outer lesion depths ($p = 0.811$). Wall lesion was observed in two restorations in both Groups 3 and 4 (composite resin groups) (Fig 2d), but not in Groups 1 and 2 (glass ionomer cement groups).

DISCUSSION

The study sought first to examine if 38% SDF conditioning could prevent the glass ionomer cement and composite resin restoration from secondary caries. Based on the results of this study, the null hypothesis was rejected. The clinical implication is that SDF can be recommended and incorporated into restorative therapy for the prevention of secondary caries.

A randomized block ANOVA with 2 fixed and random block was performed due to correlation between restorations in the same tooth. The method of assessment of secondary caries was adapted from a previous study (1). Four cavities were prepared on the same premolar. They were allocated to the four experimental groups. This minimised variation of the mineral content of the teeth used (13). We used thermocycling treatment to mimic aging process of the restoration (1). The cariogenic bacterial challenge was carried out using two major cariogenic bacteria. The experimental duration in this study was 28 days (2). This period of time mimicked the clinical situation of cariogenic challenge and allowed the developing caries on smooth surface coronal restoration. These in vitro conditions were different from in vivo conditions and the results should be interpreted with caution.

Conditioning with polyacrylic acid was recommended prior to glass ionomer cement application (18). Phosphoric acid conditioning has been reported would not influence micro-shear bond strength of etch-and-rinse bonding system adversely (19). In this laboratory study, we aimed to standardise the sample cavities and used polyacrylic acid to remove the smear layer before SDF application. This might prevent any unknown effect of SDF with smear layer on dentine. However, dentists in their clinical practice do not use polyacrylic acid before resin composite restorations.

Wall lesion and outer lesion depth were used to evaluate the inhibitory effect of secondary caries. Wall lesion refers to the inner border of the outer carious lesion adjacent to the restoration to the tooth. Ozer and Thylstrup reported no caries lesion was present along cavity wall unless large voids or gaps existed (20). They also found wall lesion was associated with gap size between tooth and restoration. In our study, we detected wall lesion in the composite resin groups but not in the glass ionomer cement groups. This indicated that interface between the tooth and the composite resin was less resistant than the glass ionomer cement. This concurred with the finding of a previous study (1). Composite resins shrink when they polymerised. The shrinkage tends to cause contraction away from the walls and floor of the prepared tooth, towards the more rigid surface layer, thus jeopardizing fit (21). Outer lesion depth is the length from the deepest point of the lesion to the tooth surface. It is a commonly used parameter to evaluate the integrity of tooth restoration interface (1). We found that the restorative material was a significant factor for development of the wall lesion. Not all specimens had wall lesion developed. Therefore, assessment using outer lesion depth was more predictable than using wall lesion.

Glass ionomer cement containing calcium and fluoride reacts with poly-acid to produce a gel of hydrated silica. This is an acid–base reaction. Two mechanisms have been proposed by which fluoride may be released from a glass-ionomer into an aqueous environment (22). The first mechanism is a short-term reaction that involves rapid dissolution from the outer surface into a solution. The second is more gradual and results in the sustained diffusion of ions through bulk cement. However, a study reported that the release of an initial high amount of fluoride from glass ionomer cement rapidly decreased after 1 to 3 days and subsequently plateaued to a nearly constant level (23). Another study found that the concentration of fluoride released significantly decreased to a very low level which was about 1 to 4 ppm after 60 days (24). This could be one of the main reasons for the no significant difference in the outer lesion depths of glass ionomer cement and composite resin restorations.

Clinical studies demonstrated that SDF at 38% prevented and arrested coronal (enamel) caries in preschool children (10) and root (dentine) caries in elders (25). Laboratory studies have found that SDF has an intense antibacterial effect on cariogenic biofilm (7, 8). It also possesses a potent inhibitory effect on the activity of matrix metallo-proteinases (26) and cysteine cathepsins (27). SDF treatment can increase the mineral density of enamel carious lesions (17) and the micro-hardness of dentine carious lesions (28). The mechanism can be explained from two perspectives (9). First, silver has been demonstrated to have an antibacterial effect and prevent biofilm formation. It could interact with sulfhydryl groups of proteins and with DNA (29), thereby altering hydrogen bonding and inhibiting respiration, DNA unwinding, cell-wall synthesis, and cell division (12). Moreover, silver ions can interact with a reactive side chain of the dentine degradation collagenase to inactive their catalytic functions (13). Second, fluoride plays a crucial role in the remineralisation process; calcium fluoride is an important product that is produced when fluoride is deposited onto the tooth surface. Calcium fluoride can act as a temporary fluoride reservoir and can release fluoride ions at a low pH (30). The fluoride ion released facilitates formation of fluoroapatite and make the tooth surface more resistant to acid dissolution. Fluoride enhances enamel remineralisation, increasing the speed of the remineralisation process and the mineral content of early carious lesions. The incorporation of fluoride also makes the deposited mineral less acid-soluble (31). This synergistic effect of silver and fluoride ion could be the reason behind the promising caries-arresting effect of SDF.

The results of this study showed that the restorations with SDF conditioning were more resistant to development secondary caries during a cariogenic challenge. SDF at 38% contains a relatively high concentration of fluoride ions (44,800 ppm) and silver ions (253,870 ppm) (32). 10% silver nitrate has showed to greatly enhance the concentration of fluoride released from glass ionomer cements and a resin modified glass ionomer cement (33). This large amount of fluoride and silver ions might alter the micro-environment around the restoration and retarded the caries process. This study found that the SDF condition can also apply to composite restoration. Quock et al (14) reported that SDF does not adversely affect the bond strength of composite resin. SDF is not known to produce pulpal damage (34). Gotjamanos reported a favourable response in primary teeth treated with SDF, including the formation of reparative dentine (15). A major concern with the use of SDF is aesthetics because SDF stains caries lesions with a dark coloration (34). In this study, a stained margin of the restoration was also found after SDF treatment. Therefore, care should be taken when treating patients with a high demand for aesthetics. Studies have tried to use chemicals like potassium iodide (35) or nano-silver particles (36) to improve the anaesthetics outcome, which still need further investigation. Another concern is the discolouration caused by SDF. Clinicians might mis-diagnose the stained restoration margins as arrested or even secondary caries. It is important that clinicians should use adjunctive tools such as intra-oral dental radiography before making final diagnosis.

CONCLUSION

In this laboratory study, conditioning with SDF at 38% increased the resistance of the glass ionomer cement and composite resin restorations to secondary caries. SDF at 38% can be incorporated into restorative therapy to improve the success rate of direct restorations.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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Reviewer #1 comments	Author' response
The abstract can be improved markedly. The abstract should state the design/model clearly, including what is meant by a cariogenic environment.	Done. Details of the cariogenic challenge is added. Line 26-27, marked in red.
It should clearly state the primary outcome measure. The values of OLD and WLD need to explain better. A reader, especially a non-specialist reader of IDJ, will have no idea what is a meaningful number. How might these values vary?	Done. The explanation of OLD and WLD is added. line 29-30, marked in red.
How does one define failure in this model in terms a clinician could grasp. As with the main body of the paper, this should be described as an in vitro preliminary study and results interpreted with greater caution.	Done. Due to the limitation of words of the abstract, detailed explanation is added in the main text. Line 188-197, marked in red.
The literature review could be improved by addressing what is known about preventing recurrent tooth decay around restorations. It seems as if the primary focus of the literature is on improving bonding but there also is literature on the role of fluoride and perhaps antimicrobials. Some of the discussion about silver diamine fluoride is not relevant to the purpose of the paper.	Done. The discussion about of silver diamine fluoride is shortened.
The key question the authors need to address is "What are the gaps in the literature about preventing recurrent decay around restoration margins?"	Done. The key question is added. Line 63-66, marked in blue.
The methods section needs to include a section that describes the purpose and design of the study and any hypotheses. Currently the description of the treatments is mixed with the design.	Done. The purpose of the study and hypothesis are added. Line 67-71, marked in red.
The in vitro model needs to be stated more clearly with appropriate discussion of is reliability and validity.	Done. The reliability and validity is discussed in line 188-197, marked in red.
The primary outcome measure needs to be specified.	Done. The primary outcome is specified in line 163, marked in red.
When the placement of the restorations is discussed, it is not sufficient to say the manufacturers' instructions were followed. The paper should be complete enough that another investigator could replicate it from the information given in the paper alone.	Done. The procedure of the restorations is added. Line 97-99, marked in red.
The results should be described as preliminary. This is a valuable but limited study. Please see the comments about the abstract for additional concerns about the presentation of the results and their interpretation.	The limitation of the study is discussed in line 196-197, marked in red.

The discussion can be improved by staying focused on the key question that is stated initially. "How does this study add to our knowledge about (a) preventing recurrent decay at restoration margins? and (b) how does it add to the methods in this area? Its sometimes moves into clinical discussion which goes beyond the limited findings in this study. The figures are nicely done and are appropriate. The labeling on figure 3 can be improved by explaining how outer lesion depth relates to the abbreviations used for the measure in the results. Also, the type of test and results should be included in the figure. Ideally the figure can be read without reference to the text. The references appear to be carefully cited without errors. The number and nature of the references will probably change as the introduction and discussion are rewrittten. The capitalization in reference 2 is not consistent with the other references. Reference 33 contains a typo-spacing.	The clinical discussion is deleted and the discussion is now stayed focus on the laboratory study. Discussion has been modified and focused more on the current study, in line 207-221, marked in red. The methods added to the area were mentioned in line 188-197, marked in red. The clinical discussion is deleted. Agree and done. Interpretations, type of test and results of figure 3 have been added. Done.
Reviewer #2 comments	
Reviewer's report The present study is of clinical relevance. The subject of secondary caries under restorations is indeed the main reason of failure of restorations. The idea of applying SDF as a conditioner before applying the restorative material is interesting and might be feasible. In the present study the question is presented in a clear way. I do have a few comments: 1. An additional figure- presenting the results of Wall Lesion Depth (WLD) should be presented' since this stresses out the difference between GIC and Composite restorations and their different reaction to SDF. 2. This should be stressed out also in the discussion. GIC and Composite materials react differently with tooth structure, and therefore a different result might be expected. 3. There is a spelling mistake in the discussion: (page 12 row 5 and 6) should be: "aesthetics".	Thank you. Done. Please see Fig 2d. The interpretation of the result between GIC and composite materials has been added to line 224-234, marked in green. Done.

Figure 1 Flow chart of the study

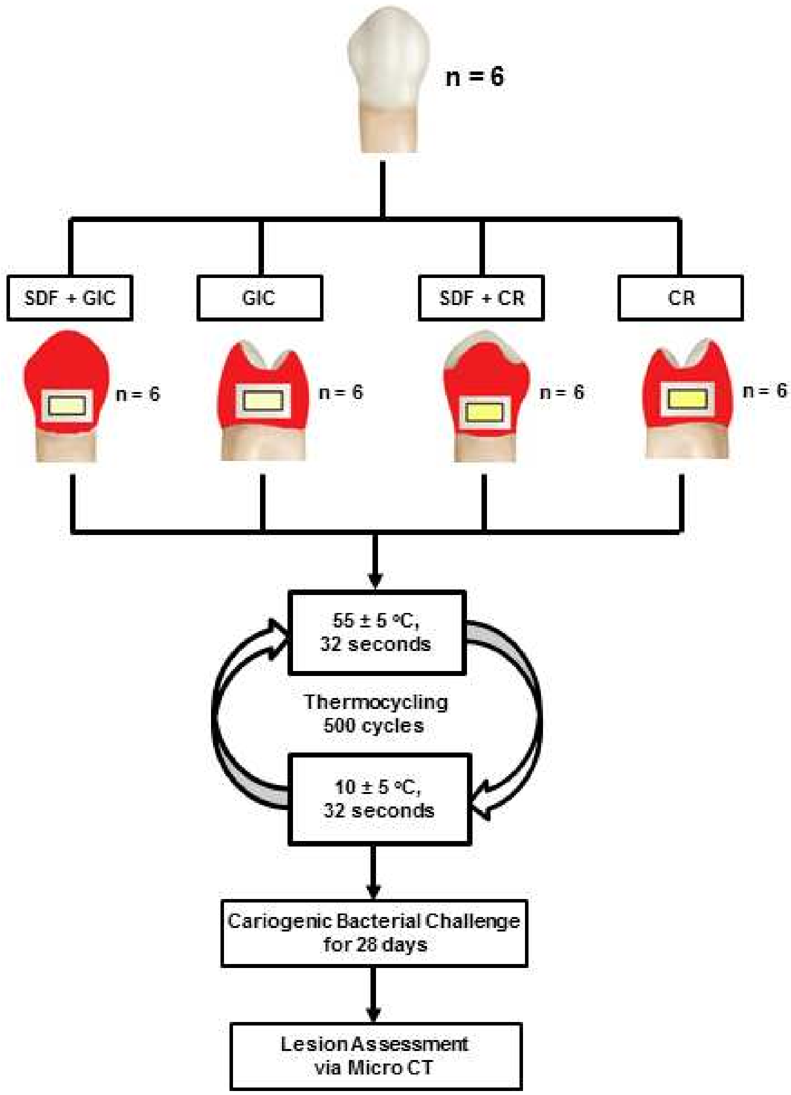
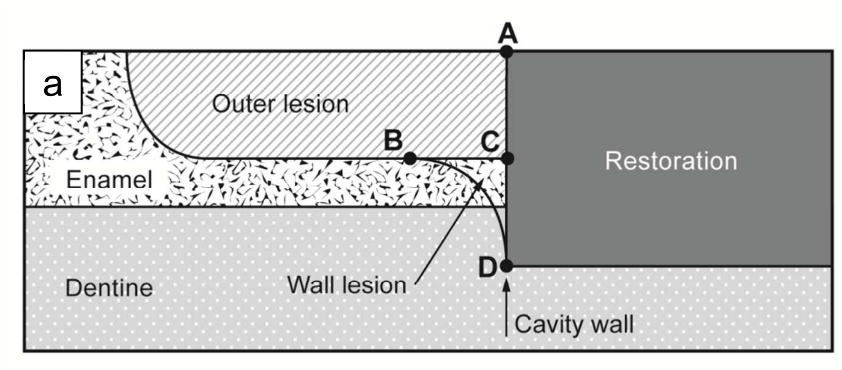
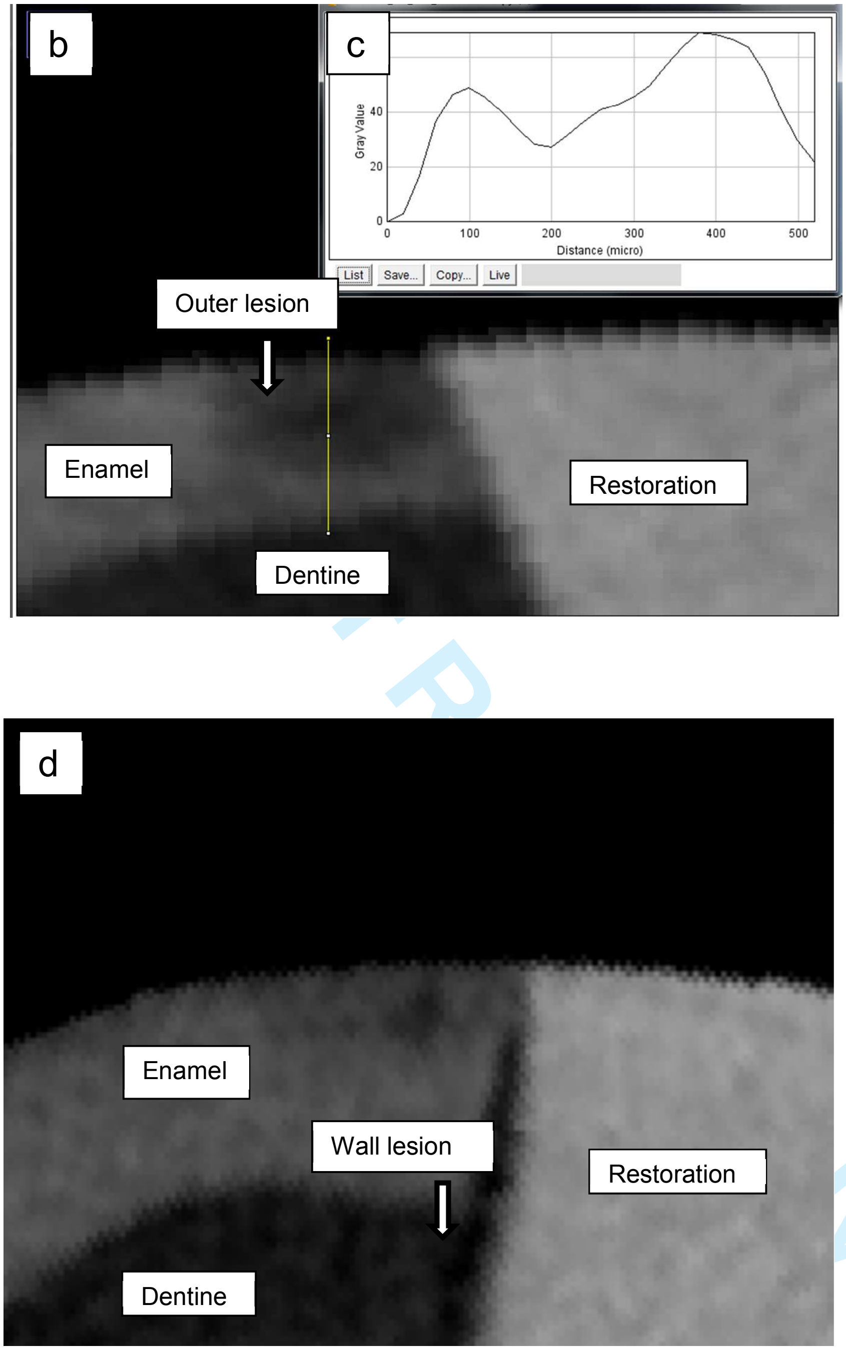


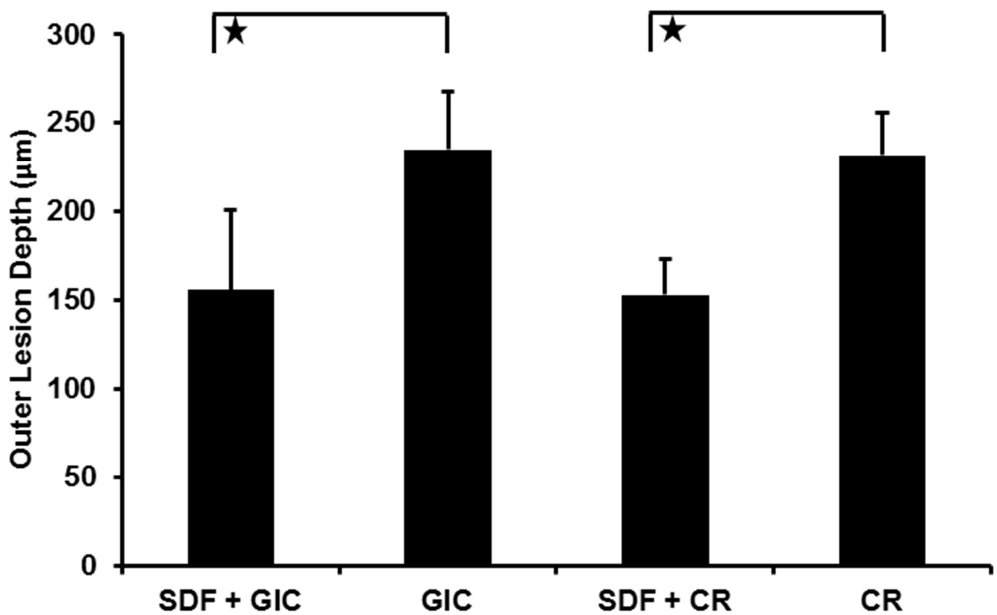
Figure 2 Assessment of demineralization along the restoration margin





a) Diagrammatic illustration of the lesion assessment (modified from Hsu et al., 1998) Outer lesion depth: line AC area; wall lesion: area BCD
b) Micro-CT image of the restoration margin after cariogenic biofilm challenge.
c) Grey-value profile along the path (yellow line in b). The starting and ending points of the demineralised lesion were determined according to grey value.
d) Wall lesion was presented in two restorations between composite resin and tooth

Figure 3 Outer lesion depth (mean ± SD μm) of different restoration groups (n=6)



Randomised block analysis of variance (ANOVA) with 2 fixed factors and random block was performed to compare the effects of silver diamine fluoride (SDF) and restorative materials (as 2 predicting variables) on outer lesion depth. A statistically significant difference was detected between Groups SDF+GIC (glass ionomer cement) and Group GIC, Groups SDF+CR (composite resin) and Group CR, respectively. Different restorative materials (glass ionomer cement or composite resin) have no significant effect on outer lesion depth ($p = 0.797$). However, outer lesion depth was reduced in restorations with SDF conditioning ($p < 0.001$).