

1 **Prevention of secondary caries using silver diamine fluoride treatment and casein**  
2 **phosphopeptide-amorphous calcium phosphate modified glass-ionomer cement**

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10 Running title: Secondary caries prevention using silver diamine fluoride and CPP-ACP  
11 modified glass-ionomer

12 Keywords: caries, silver diamine fluoride, CPP-ACP, glass-ionomer cement

13 **Abstract**

14 **Objective:** To study the effect of silver diamine fluoride (SDF) treatment and incorporating  
15 casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) into a glass-ionomer cement  
16 (GIC) to prevent secondary caries.

17 **Method:** A cervical cavity was prepared on 32 premolars for the following restoration groups:  
18 group 1, conventional GIC restoration; group 2, SDF (38%) treatment and conventional GIC  
19 restoration; group 3, CPP-ACP (3%) modified GIC; and group 4, SDF treatment and CPP-ACP  
20 modified GIC. The restored teeth were thermal-cycled before undergoing a multi-species  
21 cariogenic biofilm challenge. The restored teeth were examined by micro-computed  
22 tomography (micro-CT), scanning electron microscopy with energy dispersive X-ray  
23 spectroscopy (SEM/EDX) and Fourier transform infrared (FTIR) spectroscopy. Data were  
24 analyzed by two-way ANOVA.

25 **Results:** Micro-CT determined outer lesion depths for groups 1-4 were:  $123 \pm 6 \mu\text{m}$ ,  $87 \pm 7 \mu\text{m}$ ,  
26  $79 \pm 3 \mu\text{m}$  and  $68 \pm 5 \mu\text{m}$  respectively. An interaction effect on the outer lesion depth was found  
27 between the restorative materials and SDF treatment ( $p < 0.001$ ). Both SDF treatment and  
28 modification with CPP-ACP had a significant effect on outer lesion depth ( $p < 0.001$ ).  
29 SEM/EDX showed an increase of calcium and phosphorus at the root dentine adjacent to the  
30 restoration in groups 3 and 4 (CPP-ACP modified GIC). FTIR revealed that SDF treatment and  
31 CPP-ACP modified GIC had a significant effect on amide I-to-hydrogen phosphate ratio on  
32 the material-root interface ( $p = 0.001$ ).

33 **Conclusion:** SDF treatment and incorporation of CPP-ACP into GIC restorative material can  
34 prevent secondary root caries development.

35 **Clinical significance:** The results provide useful information to dentists in formulating clinical  
36 management protocols and material when treating root caries.

37

## 38 **1 Introduction**

39 Secondary (recurrent) caries has been considered to be the most common factor for failure of  
40 direct restorations [1]. A study reported that more than 25% of replacements of silver amalgam  
41 and resin composite restorations were attributed to secondary caries [2]. The use of alternative  
42 restorative materials possessing potential anti-cariogenic capabilities such as glass-ionomer  
43 cements (GICs) has often been suggested [3]. GIC has the ability to enhance remineralisation  
44 through its release of fluoride ions; and inhibit secondary caries *in vivo* and *in vitro* [4].  
45 However, some researchers found that fluoride leached from GIC was limited and insufficient  
46 to prevent the development of secondary caries [2, 3]. Therefore, new remineralising agents  
47 have been introduced to supplement and enhance fluoride to restore tooth minerals when  
48 demineralization has occurred [5].

49

50 Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a bioactive agent derived  
51 from bovine milk protein, casein, calcium and phosphate [6]. Casein is the principal  
52 phosphoprotein in bovine milk, predominantly as calcium phosphate stabilised micellular  
53 complexes [5]. Studies have demonstrated CPP-ACP can prevent enamel demineralisation and  
54 promote enamel subsurface lesion remineralisation in animal and human *in situ* studies [7-9].  
55 CPP-ACP has been regarded as a calcium phosphate reservoir, buffering the activities of free  
56 calcium and phosphate ions [5]. The anticariogenic activity of CPP-ACP has been ascribed to  
57 its ability to concentrate amorphous calcium phosphate at the tooth surface and within the  
58 biofilm, thus consequently helping to maintain a state of supersaturation with regard to tooth  
59 mineral [9]. In addition, it was demonstrated that CPP-ACP was stable in the presence of  
60 fluoride and interacted with fluoride ions to yield amorphous calcium fluoride phosphate  
61 (ACFP) [8]. The ACFP complex, which was stabilized by CPP at the tooth surface, can provide  
62 the necessary elements to promote remineralisation via fluorapatite formation [5]. This makes

63 CPP-ACP a promising addition to restorative materials and dental products. CPP-ACP could  
64 be incorporated into GIC, and a study showed it became more resistant to acid challenge [10].  
65 It also has been suggested that CPP-ACP modified GIC is able to enhance calcium and  
66 phosphate ion release [11], while there were no significant adverse effects on the mechanical  
67 properties of the restorative material [12].

68

69 The cariogenic biofilm of secondary caries is similar or identical to that of primary caries, and  
70 comprised mainly of *Streptococci*, *Lactobacilli* and *Actinomyces naselundii* [13]. Silver  
71 diamine fluoride (SDF) can inhibit the formation of multi-species cariogenic biofilms on  
72 dentine surfaces [14]. SDF is an inexpensive topical fluoride medicament commonly used for  
73 preventing and arresting caries, and has been approved for clinical use by the United States  
74 Food and Drug Administration [15]. Clinical trials showed that SDF was effective in  
75 preventing and arresting caries [16, 17]. Laboratory studies have also demonstrated that SDF  
76 has a strong antibacterial effect on cariogenic biofilms and hampered caries progression [14,  
77 18, 19]. Another study concluded that SDF did not adversely affect the bond strength of  
78 restorations to dentine using resin-based adhesives [20]. SDF can be used to treat carious  
79 lesions without excavation of the superficial infected layers and thus it minimizes the risk of  
80 pulp exposure [21]. A laboratory study reported that prior treatment with SDF can increase  
81 resistance of GIC and composite restorations to secondary caries [3]. Another laboratory study  
82 found that CPP-ACP modified GIC restoration had an improved anticariogenic potential  
83 compared to a control GIC [10]. However, it is unknown whether the combination of SDF  
84 treatment in conjunction with the placement of a CPP-ACP modified GIC restorative material  
85 can enhance the preventive effects on secondary caries development. Therefore, the aim of this  
86 study was to investigate the effects of SDF treatment on the prevention of secondary root  
87 dentine caries development around conventional GIC and CPP-ACP modified GIC restorations.

88 The null hypotheses tested were that: (1) SDF treatment before GIC restoration has no effect  
89 on secondary caries prevention; (2) incorporating CPP-ACP into GIC has no effect in  
90 prevention of secondary caries; and (3) there is no interaction effect on secondary caries  
91 prevention between SDF treatment and incorporating CPP-ACP into GIC restorative material.

92

## 93 **2 Methods**

### 94 2.1 Specimen preparation

95 This study was approved by the local Institutional Review Board: IRB number UW 12-221.  
96 Extracted mature (closed apex) human premolars, without visible defects, were collected with  
97 the patients' consent. The teeth were stored in a 0.1% thymol solution. After thorough cleaning,  
98 a box-shaped cavity (4 x 2 x 2 mm) located across the cemento-enamel junction was prepared  
99 on each premolar. The cavities were prepared by means of a high-speed handpiece with a  
100 tungsten carbide bur (FG 330; SS White, Lakewood, NJ, USA) under copious water-cooling.  
101 The teeth were then sterilised by autoclaving [22]. After conditioning with 10% polyacrylic  
102 acid [3], the teeth were randomly allocated to the following treatment groups: Group 1  
103 (conventional GIC), the cavities were filled with a conventional GIC, Group 2 (conventional  
104 GIC + SDF), the cavities were treated with SDF for 3 minutes, and then were filled with  
105 conventional GIC, Group 3 (CPP-ACP modified GIC): the cavities were filled with GIC  
106 containing 3% w/w CPP-ACP, and Group 4 (CPP-ACP modified GIC + SDF), the cavities  
107 were treated with SDF for 3 minutes, and then were filled with GIC containing 3% w/w CPP-  
108 ACP. The operator wore sterile gloves and used sterile hand instruments. All restored teeth  
109 were then stored at 37°C and 100% humidity for 24 hours. The conventional GIC used in this  
110 study was a conventional self-curing glass-ionomer cement (Fuji VII, GC Corp, Tokyo, Japan).  
111 The CPP-ACP modified GIC used was Fuji VII EP (GC Corp, Tokyo, Japan). The 38% SDF  
112 solution was from the silver capsule of Riva Star (SDI, Bayswater, Australia). It was topically

113 applied for 3 min to the cavities using a micro-brush provided by the manufacturer. Before  
114 restoration, the cavities were blown dry gently by a 3-in-1 syringe.

115

116 Results of a previous study suggested an outer lesion depth of approximately 120  $\mu\text{m}$  in the  
117 dentine at the material-root junction would develop after cariogenic challenge [3]. The common  
118 standard deviation ( $\sigma$ ) was set at 20  $\mu\text{m}$ , the type I error ( $\alpha$ ) was 0.05 and the power was  
119 0.90. It required at least 6 samples to detect a difference of 40  $\mu\text{m}$  between groups. This study  
120 used 32 teeth with 8 samples in each group. The flowchart of this study was shown in Figure  
121 1.

122

## 123 2.2 Thermocycling

124 The restoration surfaces were polished with sterile sand-paper discs to ensure there was no  
125 excess material over the cavity margins. All teeth were coated with an acid-resistant nail  
126 varnish (Clarins, Paris, France), leaving approximately a 1-mm window around the cavity  
127 margins. To mimic the aging process, all groups were thermocycled for 1,500 cycles between  
128  $55 \pm 5 \text{ }^\circ\text{C}$  and  $10 \pm 5 \text{ }^\circ\text{C}$  deionized water baths with a 32-second dwell time in each bath and a  
129 14-second interval between baths [1]. The teeth were then immersed in 70% ethanol for 1  
130 minute, followed by drying in air for 20 seconds before cariogenic biofilm challenge [23].

131

## 132 2.3 Cariogenic biofilm challenge

133 Four common species of cariogenic bacteria-*Streptococcus mutans* ATCC (American Type  
134 Culture Collection) 35668, *Streptococcus sobrinus* ATCC 33478, *Lactobacillus rhamnosus*  
135 ATCC 10863 and *Actinomyces naselundii* ATCC 12014 were used in this study [24]. The  
136 bacteria were cultured in blood agar plates at  $37 \text{ }^\circ\text{C}$  anaerobically for 2 days. Then, isolated  
137 colonies were transferred to tubes containing brain-heart infusion (BHI) broth with 5% sucrose

138 and incubated for another 24 hours (37 °C, anaerobically). Subsequently, the bacterial cell  
139 pellets were harvested and re-suspended in BHI broth with 5% sucrose to a cell density of  
140 McFarland 2 ( $6 \times 10^8$  cells/mL) [3]. A 500  $\mu$ L aliquot of each bacteria culture was mixed and  
141 inoculated on each tooth sitting in a well of a 12-well plate. The teeth were stored in an  
142 anaerobic chamber at 37 °C for 7 days. The medium was refreshed daily [18] and contaminants  
143 were checked by performing Gram stain tests of the used media.

144

#### 145 2.4 Assessment of outer lesion depth

146 The extent of demineralisation of dentine at the material-root junction which represents  
147 secondary caries was assessed by measuring outer lesion depth [3]. To assess the outer lesion  
148 depth, the teeth (n=8 per group) were scanned non-destructively under water [25] using a  
149 SkyScan 1076 micro-CT (SkyScan, Antwerp, Belgium). A 1 mm thick aluminium filter was  
150 used to remove low-energy radiation. Scanning was performed with a spatial resolution of 8  
151  $\mu$ m at a voltage of 80 kV and a current of 100  $\mu$ A. NRecon reconstruction software (SkyScan,  
152 Antwerp, Belgium) was used to reconstruct the scan results of each tooth. After reconstruction,  
153 images of teeth were viewed by the software CTAn (SkyScan, Antwerp, Belgium). Cross-  
154 sectional images displaying lesion area of each tooth were identified from the reconstructed 3-  
155 D images. Ten images were selected by random sampling from those lesion images [26] and  
156 the outer lesion depth was measured using image analysis software (Image J; National Institutes  
157 of Health, USA).

158

#### 159 2.5 Elemental analysis

160 Each sample was sectioned along the long axis of the tooth, midway across the restoration  
161 using a water-cooled copper disc [27]. One half of the sample was used for elemental analysis  
162 with scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX:

163 Hitachi S-4800 FEG Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan), and the other  
164 half was used for Fourier transform infrared (FTIR) spectroscopy (UMA 500, Bio-Rad  
165 Laboratories, Hercules, CA, USA). The objective of SEM/EDX analysis was to evaluate  
166 changes in mineral content along the material-root junction. The cross-section surfaces of the  
167 restored teeth were treated with 1% acetic acid for 5 seconds and washed ultrasonically using  
168 deionized water to remove the smear layer [28]. The prepared teeth were examined under a  
169 scanning electron microscope under operating conditions of 5 kV. Lines with 500  $\mu\text{m}$  length  
170 [28] and 50  $\mu\text{m}$  away from the root surfaces with analysis commencing at the restoration-root  
171 junction. The lines were analyzed by means of line-scan in terms of phosphorus, calcium,  
172 fluoride and silver ion levels.

173

## 174 2.6 Structural evaluation of dentine

175 The potential changes in the chemical structure of dentine on the restored root surface adjacent  
176 to the restoration were analyzed by FTIR spectroscopy with the infrared radiation ranging from  
177 650 to 4000  $\text{cm}^{-1}$  in wavelength number [19]. Spectra of the demineralised dentine along the  
178 material-root junction (n=8 per group) were obtained by the average acquisition of data at the  
179 spatial resolution achieved with a 50 x 50  $\mu\text{m}$  aperture. The organic matrix of dentine is mainly  
180 composed of type I collagen, and the mineral fraction is composed of hydroxyapatite. The  
181 amide I band in the FTIR spectrum is representative of the secondary structure of collagen, and  
182 the  $\text{HPO}_4^{2-}$  band is representative of mineral [24]. The ratio (amide I:  $\text{HPO}_4^{2-}$ ) of the areas of  
183 absorbance of collagen amide I peak between 1585 and 1720  $\text{cm}^{-1}$  to the phosphate  $\text{HPO}_4^{2-}$   
184 peak between 900 and 1200  $\text{cm}^{-1}$  was an indicator of the extent of demineralisation of dentine  
185 due to the activity of cariogenic biofilm [19].

186

## 187 2.7 Statistical analysis



188 The experiment was a 2 x 2 factorial (GIC with or without CPP-ACP and presence or absence  
189 of SDF treatment) combination design. Therefore, data from the outer lesion depth and amide  
190 I:  $\text{HPO}_4^{2-}$  were subjected to two-way ANOVA test. All of the analyses were conducted using  
191 IBM SPSS Version 20.0 software (IBM Corporation, Armonk, New York, USA), and the level  
192 of significance was set at 5%.

193

### 194 **3 Results**

195 Representative images of micro-CT from the experimental groups are displayed in Figure 2.  
196 The mean outer lesion depth ( $\pm$ SD) of secondary caries in groups 1 to 4 were  $123 \pm 6 \mu\text{m}$ ,  $87$   
197  $\pm 7 \mu\text{m}$ ,  $79 \pm 3 \mu\text{m}$  and  $68 \pm 5 \mu\text{m}$  respectively. An interaction effect on outer lesion depth was  
198 found between incorporating CPP-ACP into the GIC with and SDF treatment ( $p < 0.001$ )  
199 (Figure 3). SDF treatment (without SDF vs SDF) and incorporating CPP-ACP into GIC  
200 (conventional GIC vs CPP-ACP modified GIC) showed a significant effect on outer lesion  
201 depth ( $p < 0.001$ ). Outer lesion depth was reduced in groups with SDF treatment and  
202 incorporating CPP-ACP into GIC restoration compared with all other groups.

203

204 The results of SEM/EDX revealed that after culture for 1 week with the multi-species biofilm,  
205 specimens with the CPP-ACP modified GIC (groups 3 and 4) showed an increase of calcium  
206 and phosphorus in the root dentine adjacent to the GIC restoration (Figure 4c and 4d); whilst  
207 levels of calcium and phosphorus were not noticeably different at the material-root junction  
208 compared to the lesion body in specimens with the conventional GIC (groups 1 and 2) (Figure  
209 4a and 4b). The intensity of fluoride and silver ions was inconspicuous in the four groups.

210

211 The mean values ( $\pm$ SD) of amide I:  $\text{HPO}_4^{2-}$  of root dentine adjacent to the restoration in groups  
212 1 to 4 were  $0.69 \pm 0.06$ ,  $0.49 \pm 0.05$ ,  $0.47 \pm 0.05$  and  $0.39 \pm 0.04$  respectively. Figure 5 shows

213 typical FTIR spectra of material-root junctions of the four groups. Two-way ANOVA analysis  
214 showed that there was an interaction effect on amide I:  $\text{HPO}_4^{2-}$  between the 2 factors (SDF  
215 treatment and CPP-ACP incorporation) ( $p=0.001$ ) (Figure 6). The 2 factors had significant  
216 effects on amide I:  $\text{HPO}_4^{2-}$  ratios ( $p<0.001$ ).

217

#### 218 **4 Discussion**

219 This study sought first to investigate whether secondary root dentine caries could be prevented  
220 by SDF treatment combined with the placement of a CPP-ACP modified GIC restorative  
221 material. Based on the results of the study, the three null hypotheses were rejected. The results  
222 showed that SDF treatment before GIC restoration and incorporation of CPP-ACP into the GIC  
223 (CPP-ACP modified GIC) could increase resistance to the development of secondary caries. In  
224 addition, prevention of secondary caries was more substantial when the SDF treatment and  
225 CPP-ACP modified GIC was used for restoring the cavities. While this laboratory model could  
226 offer useful information to potentially predict clinical outcomes, it is noteworthy that the  
227 laboratory conditions differ from the *in vivo* situation in which secondary caries may develop.  
228 The results cannot be extrapolated directly to the clinical situation and therefore should be  
229 interpreted with caution.

230

231 The GIC restorative material was believed to be likely to develop surface crazing and cracks if  
232 sterilization with ethylene oxide was used due to severe dehydration, hence this method was  
233 not used. In this laboratory study, autoclaving was used to sterilize the teeth. Autoclaving is a  
234 cost-effective and chemically safe technique for tooth sterilization [22], and has been  
235 successfully used in previous laboratory studies [3, 22]. Some investigators have raised the  
236 concern that the high temperature and pressure may affect the physical properties of a tooth.  
237 Autoclaving was shown to weaken the hydroxyapatite / collagen structure but it did not

238 chemically destroy to any major degree, since the heat and pressure can affect the ionic bond  
239 between hydroxyapatite and collagen but the molecular structure of the collagen remained  
240 relatively unaffected [22].

241

242 *Streptococci, Lactobacilli* and *Actinomyces* are involved in the early phase of bacterial invasion  
243 in caries development [14]. *Streptococcus mutans* and *Streptococcus sobrinus* are two of the  
244 most important pathogens to initiate caries, since they are highly acidogenic and can produce  
245 carboxylic acids that dissolve dental hard tissues [19]. *Actinomyces naselundii* is regarded to  
246 be associated with root dentine caries and has the capacity to invade dentinal tubules [14].  
247 *Lactobacillus rhamnosus* is one of the most abundant bacteria which can be found in both  
248 superficial and deep carious lesions [14]. The microbiology of secondary caries and primary  
249 caries is similar [13]. For the above-mentioned reasons, these four common cariogenic species  
250 were selected in this study to form a multi-species cariogenic biofilm to create the carious  
251 lesions.

252

253 Wall lesion and outer lesion depth have been frequently used to appraise the inhibitory effects  
254 on secondary caries [3]. The term “wall lesion” was used to describe the part of a carious lesion  
255 or artificial caries-like lesion which is in contact with a restoration [1]. Some investigators  
256 reported that wall lesions were related to the size of the gap between the cavity wall and the  
257 restoration [29]. However, this term is ill-defined and has been used indiscriminately [13]. In  
258 addition, it is doubtful whether an entity like a wall lesion exists per se [13]. Therefore, wall  
259 lesion was not determined in this study. The results of this study also found no recognizable  
260 wall lesion in the specimens (data not shown). Outer lesion depth, which is a common  
261 parameter to estimate the integrity of the tooth-restoration interface was assessed in this study

262 [3]. In the current study, the measurement of the outer lesion depth was the located at the  
263 deepest point of the carious lesion to the tooth surface [3].

264

265 Micro-CT is a non-destructive technique to estimate the outer lesion depth. It carries benefits  
266 with regard to less manipulation of specimens and can be a substitute for traditional polarized  
267 light microscopy and transverse microradiography in laboratory studies [30]. The results of  
268 micro-CT revealed that the CPP-ACP modified GIC had a significant effect on secondary  
269 caries prevention. The findings from FTIR were consistent with those from the micro-CT. FTIR  
270 is an easy way to identify the presence of molecular functional groups (namely, amide I and  
271  $\text{HPO}_4^{2-}$ ) and thus was used in this study. However, the instrument must be repetitively  
272 calibrated, because the analog connection between the monochromater position and the  
273 recording device is subject to misalignment and wear.

274

275 The anticariogenic potential of incorporating CPP-ACP into GIC can be elucidated as follows.  
276 Firstly, CPP-ACP may work as a calcium and phosphate reservoir [7]. CPP has the capacity to  
277 stabilize calcium and phosphate on the tooth surface, which thereby maintains high  
278 concentration gradients of inorganic calcium and phosphate ions to promote remineralisation  
279 of hard tissues [5]. Secondly, CPP-ACP inhibits the adhesion of cariogenic bacteria to tooth  
280 surfaces stimulating the formation of a non-cariogenic plaque [6]. With the high extracellular  
281 free calcium ion concentrations, CPP-ACP may encompass bactericidal or bacteriostatic effects  
282 on cariogenic bacteria. In addition, the presence of CPP-ACP can retard formation of the  
283 cariogenic biofilm [6]. Thirdly, CPP-ACP reacts with fluoride ions to yield an amorphous  
284 calcium fluoride phosphate (ACFP) and it provides all the essential elements for  
285 remineralisation of dentine [8]. The soluble calcium, phosphate and fluoride ions facilitate  
286 remineralisation with fluorapatite which is more resistant to demineralisation. It is indicated

287 that incorporation of CPP-ACP into GIC significantly increases the release of calcium,  
288 phosphate and fluoride ions in an environment that has either an acidic or neutral pH [10]. In  
289 this study, the cariogenic bacteria produced carboxylic acids to generate an acidic environment,  
290 which enabled more release of free calcium, phosphate and fluoride ions from CPP-ACP  
291 modified GIC than from the conventional GIC. This is the reason for the increase in calcium  
292 and phosphorus at the root dentine adjacent to the restoration in groups 3 and 4 as shown in the  
293 SEM/EDX results.

294

295 The results of this study also illustrated that GIC restorations with SDF treatment were more  
296 resistant to the cariogenic biofilm challenge, thereby preventing secondary caries formation.  
297 Clinical studies showed that SDF at a concentration of 38% arrested coronal caries in preschool  
298 children [16] and prevented root caries in elders [31]. The possible mode of action of SDF can  
299 be attributed to the following aspects. SDF used in this study contains a relatively high  
300 concentration of silver and fluoride ions. Laboratory studies have found that silver ions possess  
301 an intense antibacterial property on cariogenic biofilms [14, 18, 19]. Moreover, it has been  
302 suggested that fluoride ions appear to inhibit the formation of cariogenic biofilms, due to  
303 binding to bacterial cellular components [14]. SDF can react with hydroxyapatite to form silver  
304 phosphate and calcium fluoride [26]. Calcium fluoride may act as a pH-regulated slow-release  
305 fluoride reservoir on the tooth surface [3], thus enhancing mineral remineralisation of dental  
306 hard tissues. Type I collagen accounts for approximately 90% of the organic component in  
307 dentine. SDF was shown to have an inhibitory effect on the action of matrix metalloproteinase  
308 [32] and cysteine cathepsins [33], therefore protecting the collagen matrix from degradation.  
309 In addition, other studies have found that SDF is effective in inhibition of demineralisation [18,  
310 26]. This could explain why SDF treatment had a significant effect on amide I:  $\text{HPO}_4^{2-}$  of  
311 material-root junction.

312

313 **Conclusion**

314 In this laboratory study, SDF treatment and incorporating CPP-ACP into GIC had a synergistic  
315 effect in preventing the development of secondary root dentine caries. The results may provide  
316 useful information to dentists in formulating clinical protocols and dental material selection in  
317 clinical practice, particularly when root caries is present.

318

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322

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406 **Figure 1 Flow chart of the study**

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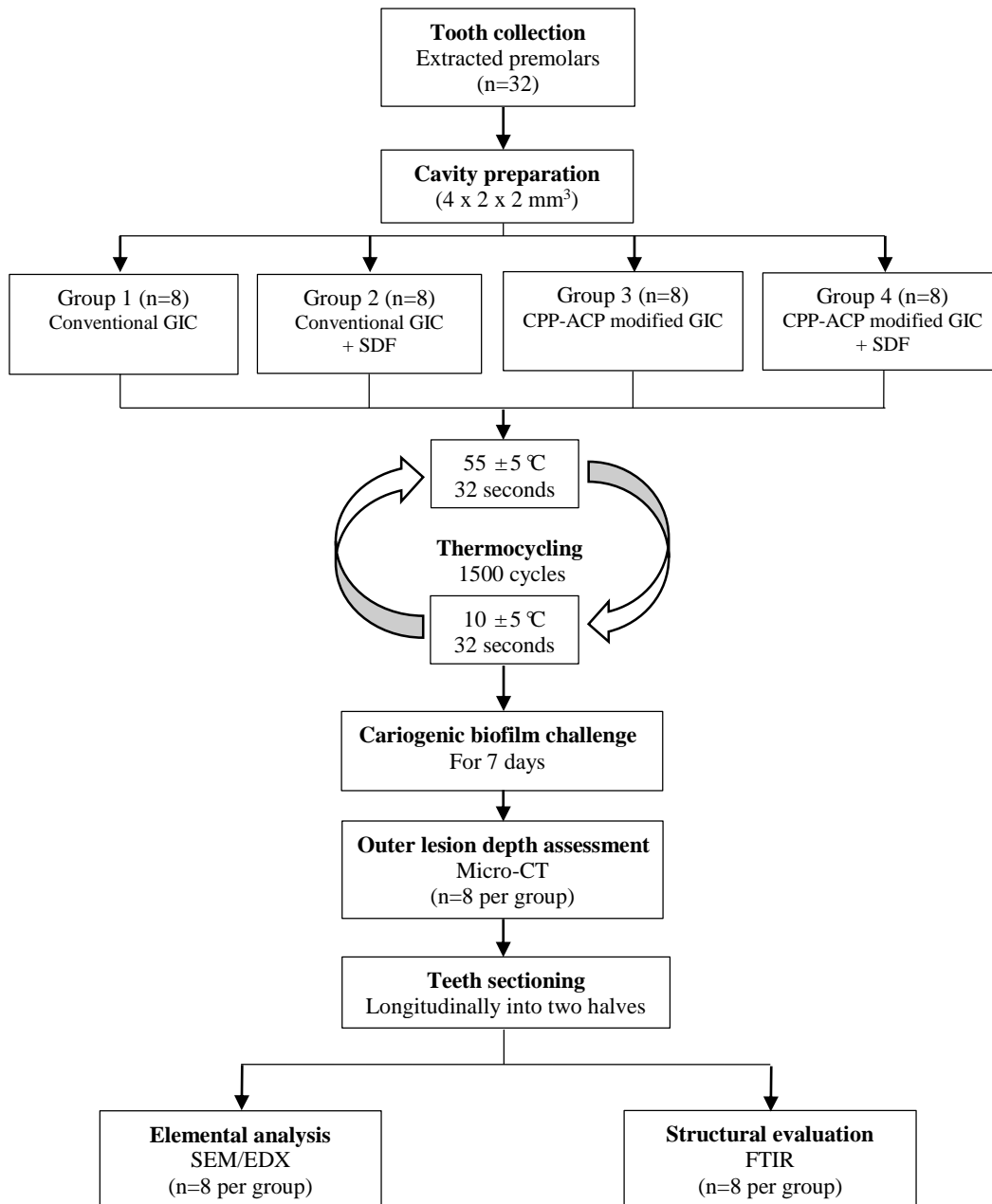
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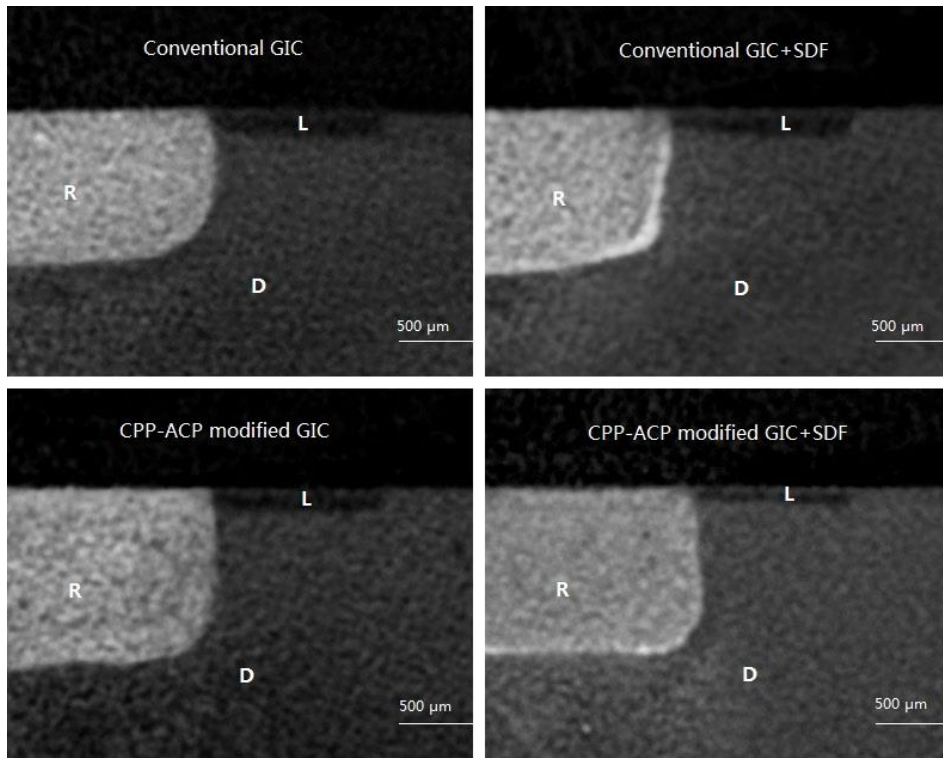
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440 **Figure 2 Micro-CT images of the 4 groups**

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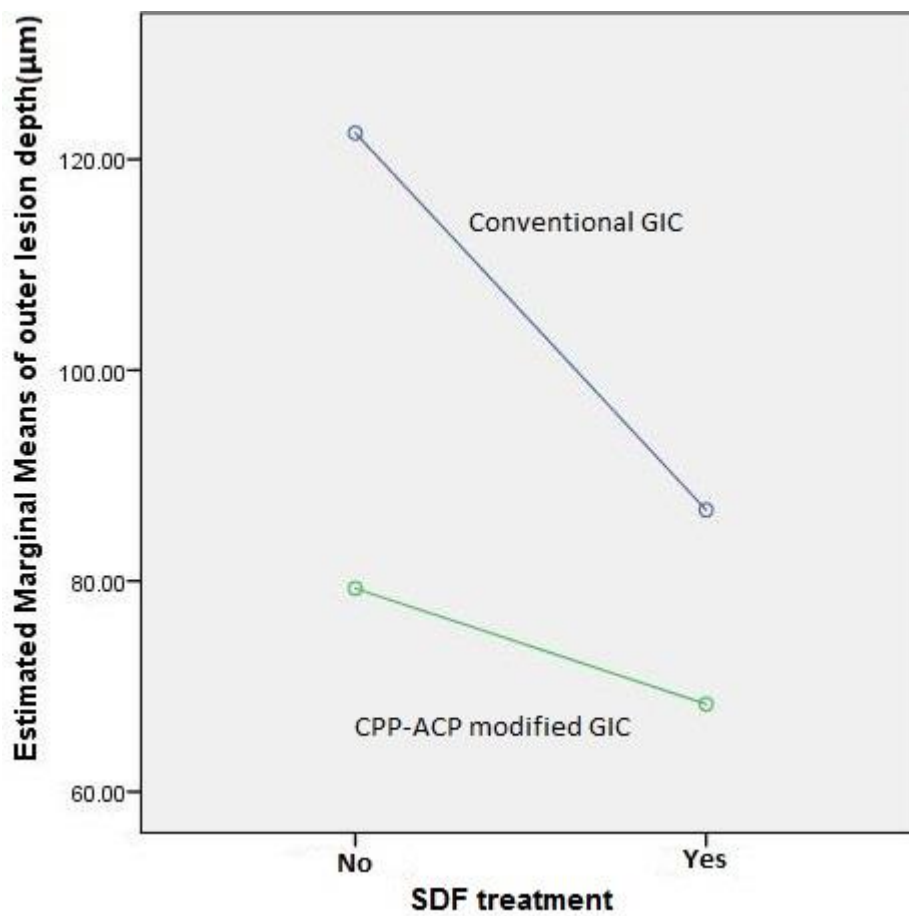


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444 R-Restoration; D-Dentine; L-Demineralised outer lesion

445 Fig 3 SDF treatment and restorative materials on outer lesion depth  
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449 **Figure 4 Line-scan images of elemental profile of dentine at the material-root junction**

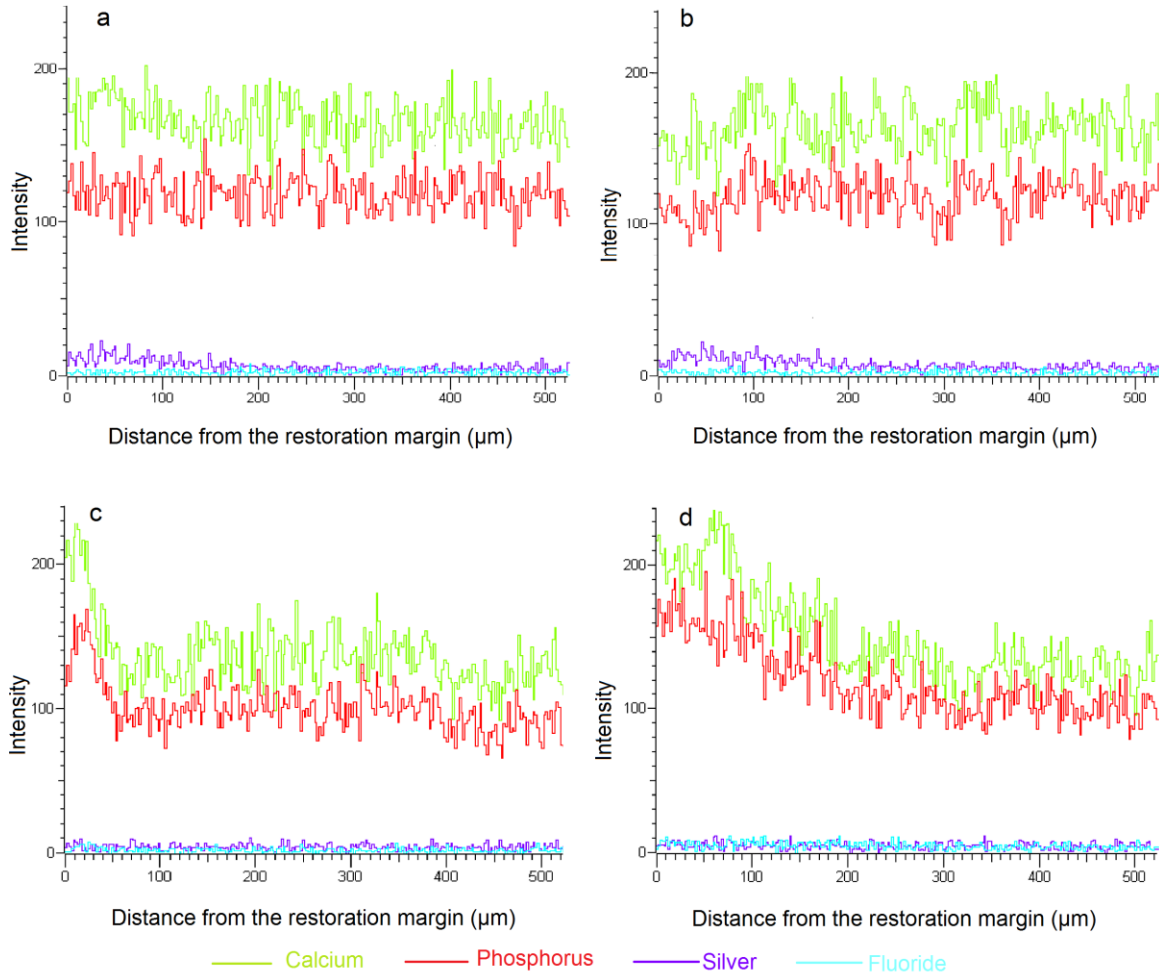
450 (a) Conventional GIC restoration

451 (b) SDF treatment and conventional GIC restoration

452 (c) CPP-ACP modified GIC restoration

453 (d) SDF treatment and CPP-ACP modified GIC restoration.

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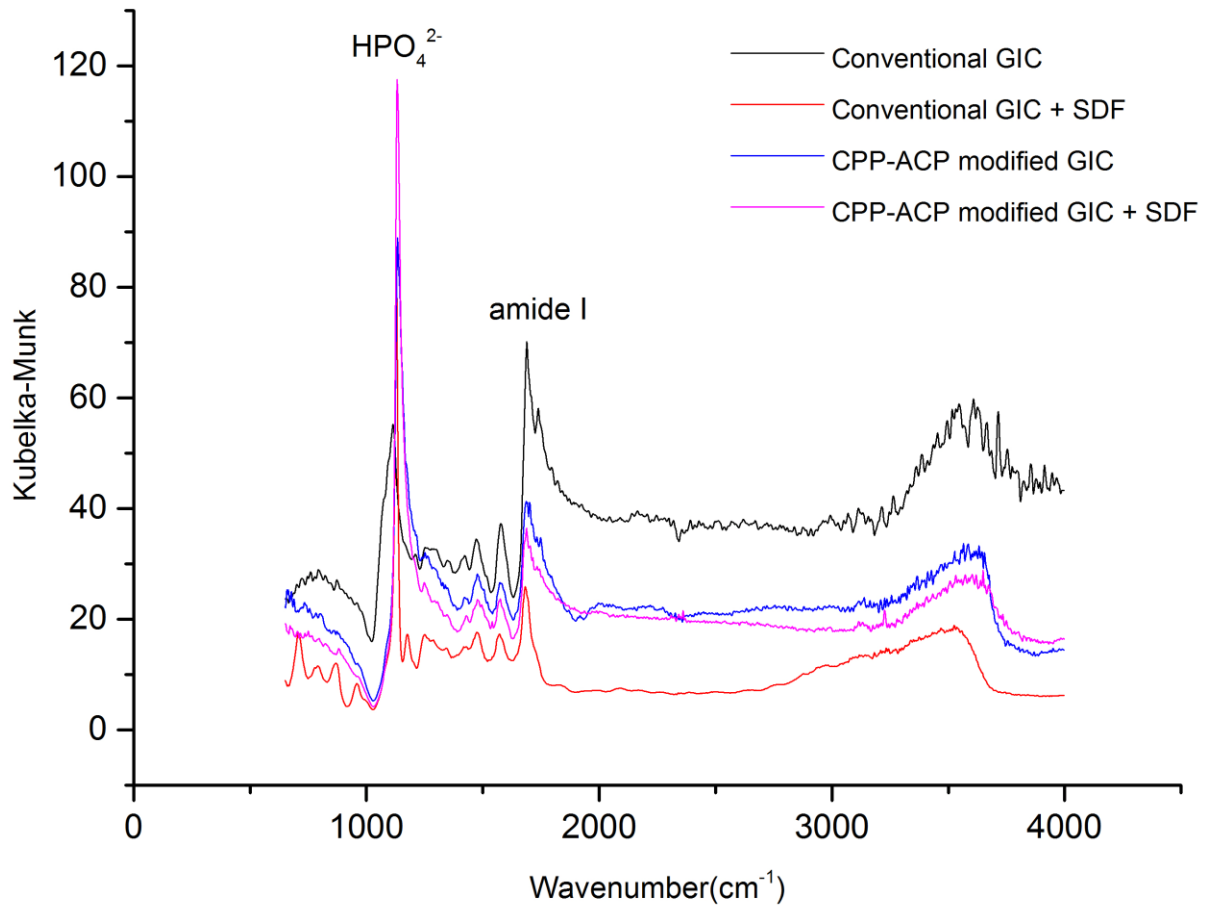


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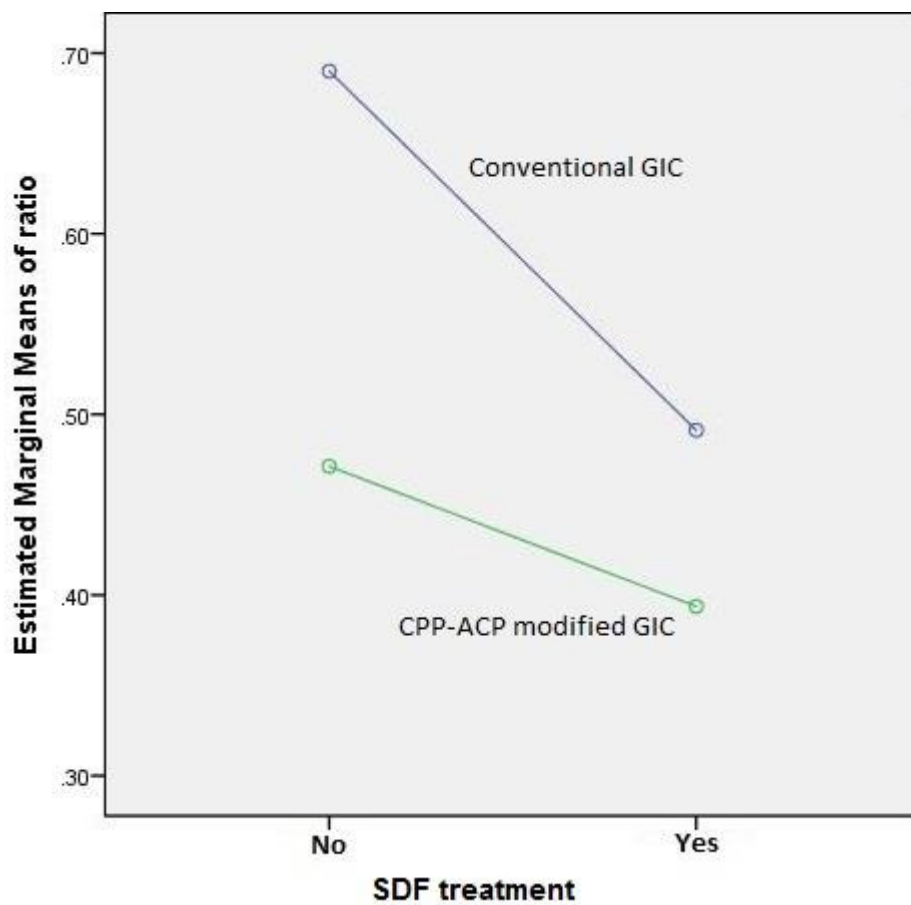
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**Figure 5 FTIR spectra of dentine at the material-root junction**



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461 **Figure 6 SDF treatment and restorative materials on amide I:  $\text{HPO}_4^{2-}$**   
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