

In vitro anti-caries effect of fluoridated hydroxyapatite-coated preformed metal crowns

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Abstract

Aim To synthesise fluoridated hydroxyapatite (FA) crystals directly on preformed metal crowns (PMCs) and evaluate the anti-cariogenic properties in an in vitro model.

Methods FA crystals were grown on etched PMCs and stainless steel discs and characterised by SEM. FA-coated discs allowed fluoride release to be assessed from a known surface area of FA crystals. Discs were divided into four groups ($n = 6/\text{group}$) and exposed to solutions at pH 4–7. Fluoride levels in solution were measured after each exposure. Twelve FA-coated and 12 non-coated PMCs were cemented onto human molars using glass ionomer (GI) or unfilled resin, making four groups of six teeth; FA-coated + GI, FA-coated + resin; non-coated + GI and non-coated + resin. Teeth were exposed to acidified gelatin (pH = 4.3) for 9 weeks.

Results SEM showed FA crystal growth on interior and exterior of the crowns. Average fluoride release from FA-coated discs was 0.16 mg/L/cm^2 at pH < 5.0. Teeth were sectioned through the lesion. Polarised microscopic examination revealed significantly smaller lesions in FA-coated crown groups compared to non-coated crown groups.

Conclusion FA-coated PMCs demonstrated carious lesion preventing effects, i.e. fluoride release and reduction

of demineralisation at crown/tooth interface. FA-coated crowns could be an aesthetic, inexpensive and caries preventive alternative in clinical dentistry.

Keywords Dental caries · Preformed metal crowns · Fluoridated hydroxyapatite

Introduction

The current trend in dental materials has produced highly aesthetic products at increasingly high cost. Preformed metal crowns (PMCs) have become widely used as an inexpensive, durable, easy to place alternative (Seraj et al. 2011) but they are not aesthetic and have no intrinsic anti-caries activity. Thus, an inexpensive full coverage restoration capable of preventing primary and/or recurrent caries would be of benefit for caries-susceptible paediatric and adult patients when used as a temporary, semi-permanent or even a permanent restoration depending on the desired clinical outcome and the socio-economic population being treated. When considering the longer term use of full coverage restorations the crown/tooth margin must be protected as it is a common site of failure as defects can result in compromised adhesion, plaque retention and recurrent caries, with recurrent caries as the leading cause of failure (Sundh and Odman 1997; Hammerle 1994; Morse et al. 2002).

A material that releases fluoride during the caries process may slow the caries lesion development and aid in lesion repair. Remineralisation of both enamel and dentine is dependent on the presence of calcium and phosphate at the site of demineralisation and this process is known to be positively influenced by fluoride (Clarkson 2007; Robinson 2009). This research group has recently developed a

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technology to synthesise enamel-like crystals using a hydrothermal method (Chen et al. 2006; Czajka-Jakubowska et al. 2009). The synthetic fluoridated hydroxyapatite (FA) crystals resemble natural dental enamel in colour, chemical composition, surface morphology and structure. The crystals have a hexagonal shape and form bundles to create a prism-like structure when grown on stainless steel surfaces. Their apatitic structure has been confirmed using X-ray diffraction (XRD) pattern analysis and high-resolution transmission microscopy and the presence of fluoride was confirmed by magic angle spinning nuclear magnetic resonance (MAS-NMR) (Chen et al. 2006).

This study was undertaken to establish whether durable enamel-like FA crystal coatings can be grown on PMCs to make them more aesthetic and impart anti-caries properties to the crowns. Scanning electron microscopy was used to investigate crystal growth on the crowns and a scratch test to determine the strength of the coatings' adhesion to the etched stainless steel substrate. Evidence of an anti-caries effect was investigated by measuring the release of fluoride from the FA-coated PMCs and the effect, *in vitro*, of this FA coating on caries-like lesion development.

Materials and methods

FA crystal synthesis on preformed metal crowns

Crystal synthesis followed protocol developed previously by this research group (Chen et al. 2006; Czajka-Jakubowska et al. 2009).

Crystal synthesis

9.36 g ethylenediaminetetraacetic acid calcium disodium salt (EDTA-Ca-Na₂) was mixed with 2.07 g of NaH₂PO₄·H₂O along with 90 ml distilled water. The pH was adjusted to 6.0. NaF was dissolved in 10 ml of water and the pH was adjusted to 7.0. The NaF was added to the EDTA solution and the entire solution was stirred continuously. PMCs (3M ESPE Unitek, MN, US) were etched for 24 h in H₂SO₄ and placed in solution. The solution and crowns were autoclaved at 121 °C at 2 atm for 10 h. This process was then repeated on stainless steel discs, 15 mm diameter and 0.5 mm thick (Ted Pella, CA, US) for the fluoride release experiments.

Scanning Electron Microscopy

Morphological characteristics such as alignment, size and shape of the crystals along with surface coverage of the crowns were evaluated using scanning electron microscopy (SEM). SEM analysis was conducted on a Phillips

XL30FEG Scanning Electron Microscope (FEI company, OR, US) operated at 10 kV (Resolution: 2.0 nm at 30 kV, 5.0 nm at 1 kV). The crystal-coated crowns were coated with Au/Pd film to prevent specimen charging.

Acid exposure and fluoride release measurement

Sixteen stainless steel discs were coated with FA crystals using the above method. The coated discs were divided into four groups of four discs and placed as a group into 5 ml of buffered lactic acid at pH values of 7.0 (group 1), 6.0 (group 2), 4.5 (group 3) and 4.0 (group 4). They were subjected to an oscillating pH for 24 h to more closely resemble the oral pH fluctuations. The discs were exposed to the desired pH for 30 min then removed and placed into a buffered solution at a pH of 7.0. At this point the fluoride concentration in the 5-ml acidic solution was measured using a fluoride electrode. The discs were left in the neutral solution for 3.5 h before they were returned to a new acidic solution at their respective pH. This process was repeated 4 times over a 24-h period at increments of 0, 4, 8 and 24 h, and then once a day over the next 10 days.

Crown application to extracted teeth and acid exposure

Twenty-four caries-free extracted human molars were randomly divided evenly into four groups. Group 1 was a FA-coated PMC and group 2 was a non-coated PMC, both cemented using a glass ionomer (GI) (GC Fuji Plus, G.C. Corp. Tokyo, Japan) to the teeth. Group 3 was a FA-coated PMC and group 4 was a non-coated PMC, both cemented by a resin bonding agent (Panavia 21, Kuraray Medical Co. Okayama, Japan). The crowns of each molar were contoured to expose approximately 1 mm of cervical enamel of each tooth. The caries induction model followed protocol previously developed (Wei and Wefel 1976; Feagin et al. 1985; Clarkson et al. 1986). The crowned molars were placed in wells containing 10 ml of acidified gelatin covering the entire crown. The acidified gelatin (NO. G-2500, Sigma-Aldrich, MO, US) was prepared as a 10 % solution using PBS buffer (Gibco, Invitrogen Co. CA, US). Lactic acid was added to adjust the pH to 4.3. Two grams of thymol was added before the gel mixture was autoclaved at 121 °C at 2 atm for 30 min. The teeth remained in the acidified gelatin for 9 weeks with the gelatin changed biweekly and the pH monitored regularly.

Fluoride diffusion measurement

FA-coated PMC were placed on three caries-free extracted human molars. Each crowned molar was placed in 15 ml of acidified gelatin prepared in the same manner as described above and remained in the gelatin for 9 weeks. At the end

of the 9 weeks gelatin samples were taken and analysed for fluoride. For each FA-coated PMC, 2 × 5 ml samples of gelatin were taken at 1 and 5 cm from the crowned molar. The gelatin samples were dissolved in de-ionised water with the addition of 2 ml of lactic acid and fluoride concentrations were measured with a fluoride electrode.

Lesion measurement

After 9 weeks the teeth were removed from the acidified gelatin and sectioned on a diamond saw buccal-lingually through the induced lesion. The tooth sections were prepared by hand on a high-grit sand paper until they were between 120 and 200 µm. Examination of the lesion depths was carried out using a polarised-light microscope (Leica DMLP, Wetzlar, Germany). Under polarised-light sections were imbibed in water and the difference in birefringence of sound enamel to demineralised enamel was observed (Clarkson et al. 1986). The depth of the lesion was measured at three points along a traverse drawn along the base of the lesion using the graticule of the microscope eyepiece. Differences in lesion depth between the groups were calculated using an unpaired *t* test (Prism 4, Graphpad Software Inc. La Jolla, CA).

Scratch test

The stylus scratch method was used to determine the scratch resistance and practical adhesion strength of the FA crystal coating to the stainless steel substrate. A diamond stylus was drawn across the coated samples with a constant speed and force. The damage from the stylus was microscopically assessed as a function of the applied force. Testing was performed using the recommendations and procedures from ASTM standards (G171, C1624, D7187). The critical force (*L_c*) at which damage to the coating

occurs was measured. Four measurements were performed for each sample.

Results

Figure 1a shows images of the PMC before and after FA crystal coating. SEM images show the FA-coated PMC surface in low (Fig. 1b) and high (Fig. 1c) magnification. The crowns were exposed twice to the hydrothermal conditions and exhibit a disordered crystal arrangement. All surfaces were fully coated by the crystals leaving no exposure of the underlying stainless steel substrate.

The depths of the acid gelatin-induced lesions in the four different groups of crowned teeth are shown in Fig. 2a, b. The lesions were measured in enamel on each tooth section on the buccal and lingual surfaces of the tooth. Figure 3 depicts representative polarised-light photomicrographs of the lesions with FA and non-FA-coated crowns. The measurements showed a significant difference in the size of the lesions between the FA-coated versus the non-FA-coated groups. The GI cemented crowns showed a smaller mean lesion depth for the FA-coated crowns ($34.44 \pm 6.8 \mu\text{m}$) compared to the non-FA-coated crowns ($99.72 \pm 8.85 \mu\text{m}$) ($p < 0.01$) (Fig. 2a). The resin cemented crown groups showed a smaller mean lesion depth for the FA-coated crowns ($49.72 \pm 10.18 \mu\text{m}$) compared to the non-FA-coated crowns ($90.82 \pm 9.75 \mu\text{m}$) ($p < 0.05$) (Fig. 2b). No statistical differences were seen in lesion depths between the GI or resin cemented control or experimental groups.

Table 1 shows the fluoride release under varying pH and times from the FA-coated discs. The first measurement for all groups independent of pH showed considerable fluoride release. However, after the initial 30 min it was shown that fluoride release occurred at pH < 5.0 but not at pH 7.0 or

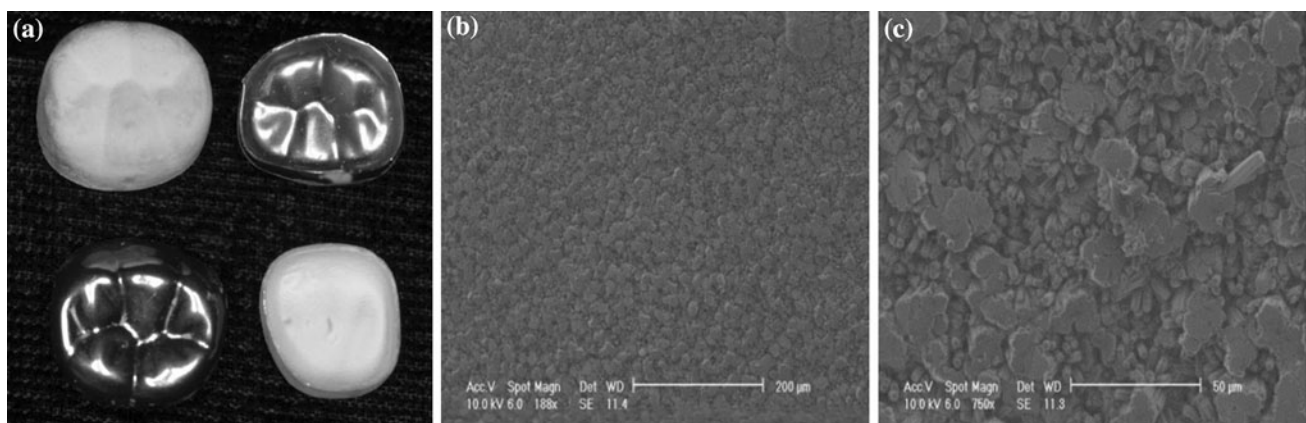


Fig. 1 Images of the preformed metal crown (PMC) before and after crystal coating (a), and scanning electron microscopy images of the surface of the fluorapatite crystal-coated PMCs at low (b) and high magnification (c)

Fig. 2 Mean depths of the acid gel-induced lesions. Teeth were sectioned through the lesions. Lesions into enamel were measured on each tooth section at two sites, the buccal and lingual surfaces. Fluorapatite (FA)-coated and non-coated crowns were compared using glass ionomer (GI) and resin cement

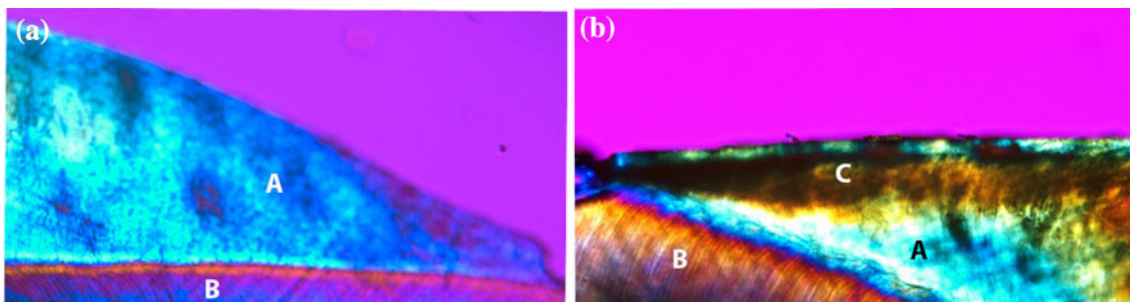
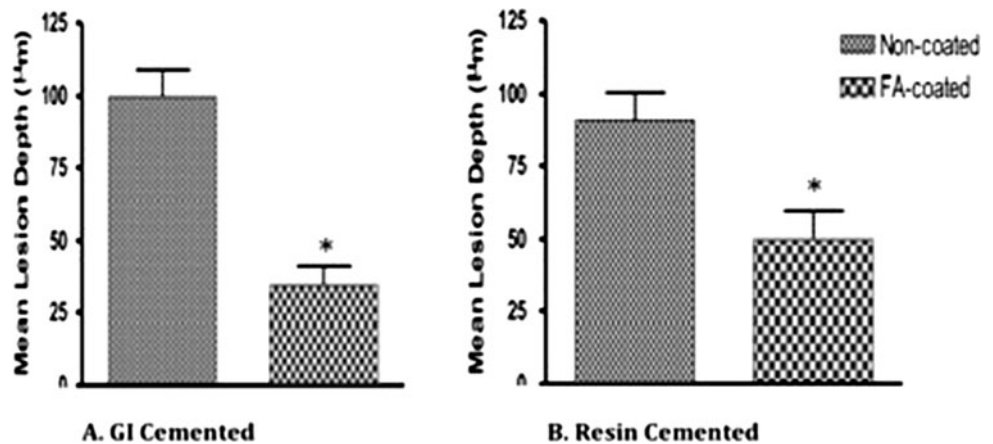


Fig. 3 Polarised microscope photomicrographs of tooth sections at the crown margins from samples of preformed metal crowned teeth after exposure to acidified gelatin for 9 weeks. FA-coated

crown + GI cement (a), non-FA-coated crown + GI cement (b). Sound enamel (A), dentine (B), caries-like lesion (C)

Table 1 Total fluoride release (mg/L) from FA crystals after 30 min exposure to lactic acid at varying pH

Time of reading	Group 1	Group 2	Group 3	Group 4
0.5 h	3.2	2.34	3.2	3.37
4.5 h	0	0	0.62	0.26
24.5 h	0	0	0.39	0.78
Day 5	NM	NM	0.56	1.01
Day 10	NM	NM	0.79	0.50

NM not measured

Group 1: FA-coated discs at pH 7.0, Group 2: FA-coated discs at pH 6.0, Group 3: FA-coated discs at pH 5.0, Group 4: FA-coated discs at pH 4.0

Table 2 Measurements of total diffused fluoride in acidified gelatin after being released from FA-coated preformed metal crowns (PMC)

FA-coated PMC	Distance from source (cm)	Fluoride (mg/L)
1	1	0.718
1	5	0.366
2	1	0.317
2	5	0.151
3	1	0.457
3	5	0.311

6.0. Therefore, the fluoride release measurements over the 10-day experimental period were carried out at pH 4.5 and 4.0 to ensure fluoride release at the acidified gel pH of 4.3. The average fluoride release of groups 3 and 4 was 0.16 ± 0.24 mg/L/cm² per acid exposure.

Table 2 shows the fluoride concentration in gelatin at two distances from the FA-coated PMC source. The fluoride concentration decreased as the distance increased from the FA-coated PMC. Fluoride concentrations were detectable at the maximum measured distance of 5 cm.

The stylus scratch test was performed for FA-coated stainless steel discs. The mean Lc was 86.11 mN.

Discussion

This study is the first to create a synthetic substance (fluoridated hydroxyapatite) that can be used as a coating for PMCs. The goal of our study was to demonstrate the anti-caries effect of FA-coated crowns. Our results showed significantly smaller enamel lesion depths at the margins of FA-coated crowns versus non-FA-coated crowns in an in vitro caries model. This decrease in demineralisation was a result of the characteristics of the FA coating alone because no

differences were found between groups cemented with GI or resin. This suggests that GI was not a factor in the reduction of the lesion size in either the FA-coated or non-FA-coated groups.

The fluoride release experiment showed a measurable fluoride release from FA surfaces under carious-inducing conditions. The amount of fluoride released was sufficient to decrease demineralisation of enamel as supported by the reduction of carious lesion size in the above experiment over a 9-week trial. High fluoride release initial readings seen at pH 7.0 and 6.0 was hypothesised to be a result of the synthesis process producing a loosely bound ionic fluoride and/or a readily soluble fluoride containing non-apatitic phase. During the initial 0.5-h exposure at any pH the loosely bound fluoride ions were removed and/or any easily soluble fluoride containing non-apatitic phases dissolve contributing to the high fluoride measurements seen only in the first measurement in all groups. After this initial wash, fluoride release from FA crystals was only below its reported critical pH of 5.5.

A benefit of the FA-coated crowns was the proximity of the crystals to the tooth/crown margin, as the crystals were present on the inner and outer surfaces of the crown. This ensured exposure of the FA crystals to the oral environment and allowed the fluoride ions to be released under acidic conditions at the tooth margin. This fluoride release was shown, in this study, to be effective in reducing lesion progression. Further, as the pH rose the essential ions were conserved within the crystal that increased the longevity and effectiveness of the FA crystals. However, the effect of the fluoride release on lesion size was only examined in the enamel at the crown margin. It was hypothesised that other calcified tissue in similar proximity to the FA surfaces, for example, interproximally, which was another highly susceptible site for caries formation, would also benefit from fluoride release under acidic conditions. This could also be inferred from the fluoride diffusion profile in the gelatin where F was detected 5 cm away from its FA-coated PMC source. Therefore, the introduction of the FA-coated PMC might provide a means to introduce fluoride more generally to the oral environment affecting not only the adjacent interproximal area but, perhaps, the enamel/dentine of neighbouring teeth.

The scratch test results showed that the crystals bound strongly to the etched stainless steel surfaces. This would minimise delamination and deformation of the coating under the forces of mastication. The exact amount of manipulation during fitting and seating that the FA-coated crowns could withstand without substantial loss of the FA crystals was not investigated. If delamination of the FA coatings was to occur it would not be possible to recoat the PMCs. However, further investigation into the limits of

manipulation that the FA-coated crowns could withstand is needed.

The FA coating adds a negligible thickness to the crown. SEM measurements showed the thickness of the coatings to range between 50 and 100 μm . At this thickness the preparation of the tooth can remain conservative and the preparation does not need to accommodate the coating as is necessary with a veneered PMC.

In previous experiments using the acid gelatin for an *in vitro* caries model, we had shown caries-like lesions could be produced in weeks at pH = 4.3 (Clarkson et al. 1986). We therefore exposed the FA-coated stainless steel discs to pH values of 4.0 and 4.5 to ensure that the FA crystal coatings would release fluoride within this pH range. The results showed that this was the case. To gain the maximum effect of the fluoride release, in a relatively short time, on the demineralisation around the margins of the crowns, a pH of 4.3 was chosen for the acid gelatin. This was below the reported critical pH for enamel of 5.5. Future experiments using this model over longer time periods at different pH values ranging from 4.5 to 5.5 will be undertaken to ascertain the effect of these FA coatings on enamel and dentine demineralisation.

If the FA-coated crowns were made available in a clinical setting, they could be provided at a low cost. PMCs are one of the least expensive options for a restorative material and the crystal coating uses inexpensive materials and the coating takes only a short time. In addition, the FA coating masks the metallic appearance of the PMCs and provides a more natural tooth appearance, thus providing a more aesthetic restorative option.

Conclusions

The synthetic FA crystals coated on stainless steel crowns were shown to be anti-cariogenic in an *in vitro* study. In addition, the FA crystal coverage had a durable enamel-like appearance where the aesthetics were drastically improved from that of a normal PMC. The above qualities would be available as a temporary, semi-permanent or permanent restorative option at a cost comparable to PMCs.

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