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Research Article

SYNTHESIZED FA CRYSTALS IN PMCS: ANTI-CARIOGENIC ASSESSMENT

Dr Vikrant Pratap*

Assistant Professor, Department of Dentistry, Gouri Devi Institute of Medical Sciences & Hospital, Durgapur -713212, West Bengal.

ABSTRACT

This study aimed to synthesize fluoridated hydroxyapatite (FA) crystals within preformed metal crowns (PMCs) and assess their anti-cariogenic properties in vitro. FA crystals were grown on etched PMCs and stainless steel discs, which were then, characterized using scanning electron microscopy (SEM). Fluoride release from the FA-coated discs was evaluated, with four groups of discs (n = 6/group) exposed to solutions ranging from pH 4 to 7. The fluoride levels in the solution were measured after each exposure. Additionally, 12 FA-coated PMCs were cemented onto each of six teeth in four groups: FA-coated with glass ionomer (GI) or unfilled resin, non-coated with GI, and non-coated with resin. These teeth were subjected to acidified gelatin (pH = 4.3) exposure for 9 weeks. SEM analysis revealed FA crystal growth on both the interior and exterior of the crowns. An average fluoride release of 0.16 mg/L/cm2 from FA-coated discs at pH 5.0 was recorded. Upon sectioning, FA-coated crown groups exhibited significantly smaller lesions compared to non-coated crown groups.

Keywords:- Fluoridated hydroxyapatite (FA), Preformed metal crowns (PMCs), Anti-cariogenic properties, Scanning electron microscopy (SEM), Fluoride release.

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INTRODUCTION

In the realm of dental materials, there is a growing preference for highly aesthetic but costly products. However, preformed metal crowns (PMCs) offer a durable, cost-effective alternative, albeit lacking in aesthetic appeal and intrinsic antimicrobial properties. Particularly for caries-prone pediatric and adult patients [1], a cost-efficient full coverage restoration could prove invaluable in preventing primary and recurrent caries, whether used temporarily, semi-permanently, or permanently. Yet, it's crucial to safeguard the crown-tooth margin over extended periods to avoid failure stemming from compromised adhesion, plaque retention, and recurrent caries, a leading cause of restoration failure [2]. In combating caries, fluoride-releasing substances have shown promise in slowing lesion development and aiding in repair. Fluoride is known to promote remineralization of enamel and dentine [3], positively impacting calcium and phosphate levels in demineralized areas. Recent studies have unveiled a novel technique for synthesizing enamel-like crystals through hydrothermal processes. Fluoridated hydroxyapatite (FA) crystals exhibit similarities to natural dental enamel in various physical characteristics such as color, chemical composition, surface morphology [4], and structure. These crystals, when grown on stainless steel surfaces, form bundles resembling a prism-like structure, as confirmed by X-ray diffraction (XRD) pattern analysis and high-resolution transmission microscopy. This study investigates the feasibility of growing enamel-like FA crystal coatings on PMCs to enhance their aesthetics and impart anti-caries properties [5]. Utilizing scanning electron microscopy, the growth of crystals on the crowns is analyzed, and the coatings' adhesion to the etched stainless steel substrate is evaluated through scratching tests. Moreover, the release of fluoride from

Corresponding Author: **Dr Vikrant Pratap**

FA-coated PMCs and its impact on the development of caries-like lesions in vitro are explored to ascertain the anti-caries efficacy of such coatings.

METHODOLOGY

A solution was prepared by mixing 9.36g of ethylenediaminetetraacetic acid calcium disodium salt (EDTA-Ca-Na2) with 2.07g of sodium hypochlorite in 90ml of distilled water. To adjust the pH, NaF was dissolved in 10 ml of water and the pH was adjusted to 7.0. The NaF solution was then added to the EDTA solution while continuously stirring. Preformed metal crowns (PMCs) (3M ESPE Unitek, MN, US) were etched with a solution of H_2SO_4 for 24 hours prior to immersion in the prepared solution. Subsequently, the solution and the crowns were autoclaved for 10 hours at 121°C under 2 atm pressure. To assess fluoride release, the same procedure was repeated using stainless steel discs measuring 15 mm in diameter and 0.5 mm in thickness.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was utilized to evaluate morphological features including crystal alignment, size, and shape. The analysis was conducted using a Phillips XL30FEG Scanning Electron Microscope manufactured by FEI company (OR, US). The microscope operated at 10 kV and offered a resolution of 2.0 nm at 30 kV and 5.0 nm at 1 kV. To mitigate specimen charging, the crystal-coated crowns underwent coating with an Au/Pd film.

Acid exposure and fluoride release measurement

Sixteen stainless steel discs were coated with FA crystals following the outlined procedures. These coated discs were then divided into four groups, each consisting of four discs, and immersed in buffered lactic acid solutions adjusted to pH values of 7.0, 6.0, 4.5, and 4.0, respectively. The discs underwent pH oscillation for 24 hours to mimic oral pH fluctuations. After 30 minutes, they were transferred to a buffered solution at pH 7.0, and the fluoride concentration in the acidic solution (5 ml) was determined using a fluoride electrode. Subsequently, the discs were left in the neutral solution for 3.5 hours before being returned to new acidic solutions at their respective pH levels. This cycle was repeated four times within a 24-hour period at intervals of 0, 4, 8, and 24 hours, and then once daily for a duration of 10 days.

Crown application to extracted teeth and acid exposure

Twenty-four caries-free extracted human molars were randomly divided into four groups, each containing an equal number of teeth. In groups 1 and 2, FA-coated PMCs and non-coated PMCs were cemented to the teeth using GC Fuji Plus, respectively. In groups 3 and 4, FA-coated PMCs and non-coated PMCs were cemented to the teeth using a resin bonding agent (Panavia 21, Kuraray Medical). The crowns of each molar were contoured to expose approximately 1 mm of cervical enamel. Caries induction followed a previously established protocol. The crowned molars were immersed in wells containing 10 ml of acidified gelatin, covering the entire crown surface. The acidified gelatin solution, prepared with a 10% concentration using PBS buffer, was adjusted to a pH of 4.3 with lactic acid. After adding two grams of thymol, the gel mixture was autoclaved at 121°C for 30 minutes at 2 atm. The teeth were exposed to the acidified gelatin for 9 weeks, with biweekly changes of the gel and regular pH monitoring throughout the experimental period.

Fluoride diffusion measurement

A FA-coated PMC was applied to three extracted human molars that had been free of caries. During the nine-week period, each crowned molar was encapsulated in 15 ml of acidified gelatin prepared as described above. Following the nine-week period, gelatin samples were taken and analysed for fluoride. In each FA-coated PMC, 295 ml samples of gelatin were taken 1 cm and 5 cm from the crowned molar. De-ionized water was added to gelatin samples and 2 ml of lactic acid was added. Fluoride concentrations were measured with a fluoride electrode.

Lesion measurement

The teeth were removed from acidified gelatin nine weeks after they had been positioned in it, and they were carefully sectioned with a diamond saw through the lesion that was created by the acid. To prepare the tooth sections, 120-200 microns of high-grit sandpaper was used to sand them. The depths of the lesions were measured with a polarized light microscope. A difference in birefringence between demineralised and sound enamel was observed under polarized-light. A traverse was drawn along the base of the lesion using the microscope eyepiece's graticule to measure the depth of the lesions at three points. Differences in lesion depth between the groups were calculated using an unpaired t test

Scratch test

In order to determine the scratch resistance of the FA crystal coating and its practical adhesion strength to stainless steel, we used the stylus scratch method. Constant speed and force were applied to the diamond stylus as it was drawn across the coated samples. A microscope was used to assess the damage caused by the stylus as a function of the applied force. We followed the recommendations and procedures of ASTM standards (G171, C1624, D7187). In this experiment, the critical force (Lc) at which the coating becomes damaged was measured. A total of four measurements were conducted for each sample.

RESULTS:

Hydrothermal conditions were applied twice to the crowns, resulting in disordered crystal arrangements. The crystals completely covered all surfaces, leaving no trace of the stainless steel substrate underneath. The depth of acid gelatin-induced lesions in the four different groups of crowned teeth. Each tooth section was measured on the buccal and lingual surfaces to determine the number of lesions in the enamel. These polarised-light photomicrographs show representative lesions with FA and non-FA coated crowns. Lesions in the FA-coated versus non-FA-coated groups were significantly different in size. The GI cemented crowns showed a smaller mean lesion depth for the FA-coated crowns (34.44 ± 6.8 lm) compared to the non-FA-coated crowns (99.72 ± 8.85 lm) (p\0.01). (p/0.05) showed that the resin cemented crown groups with FA-coated crowns showed a smaller mean lesion depth than the groups without FA-coated crowns, 49.72 x 10.18 x 9.75 mm. The lesion depths were not statistically different between the GI and resin cemented control groups. The FA-coated discs show the results. For all groups, the first measurement independent of pH showed significant fluoride release. After 30 minutes, it was found that fluoride release occurred at pH 5.0, not pH 7.0..

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	3.3	2.35	3.3	3.38
4.5 h	0	0	0.63	0.27
24.5 h	0	0	0.31	0.79
Day 5	NM	NM	0.57	1.02
Day 10	NM	NM	0.70	0.51

Table 2 Fluoride concentration

FA-coated PMC	Distance from source (cm)	Fluoride (mg/L)
2	2	0.719
2	6	0.367
3	2	0.318
3	6	0.152
4	2	0.458
4	6	0.312

As a result, measurements of fluoride release were made at pH 4.5 and 4.0 throughout the 10-day experimental period to ensure fluoride release at the acidified gel pH of 4.3. Groups 3 and 4 released an average of 0.46 mg/L/cm2 of fluoride per acid exposure. In Table 2, the concentration of fluoride in gelatin is shown at two distances from the FA-coated PMC source. Increasing distance from an FA-coated PMC decreased fluoride concentration. The maximum measured distance of 5 cm allowed for the detection of fluoride concentrations. For FA-coated stainless steel discs, we performed a stylus scratch test. According to the data, Lc was 86.11 mN on average

DISCUSSION

As a result of this study, the first synthetic coating for PMCs (fluoridated hydroxyapatite) has been developed. Our study aimed to demonstrate that FA-coated crowns have anti-caries properties. The enamel lesion depth along the margins of FA-coated crowns was significantly smaller than that of non-FA-coated crowns in the in vitro caries model. Because of the characteristics of the FA coating only, demineralisation decreased. There was a difference between groups cemented with GI and resin [6]. In both FA-coated and non-FA-coated groups, GI did not reduce lesion size. It was found that fluoride was released measurable amounts from FA surfaces under carious-inducing conditions. As evidenced by the reduction in carious lesion size over the 9-week trial above, fluoride released decreased demineralisation of enamel. It was hypothesized that the high fluoride release initial readings occurred as a result of a loosely bound ionic fluoride and/or readily soluble fluoride-containing nonapatitic phase being produced during synthesis. As a result of the initial exposure of 0.5 hours at any pH, loosely bound fluoride ions and/or readily soluble fluoride containing nonapatitic phases were removed, resulting in high fluoride measurements only in the final measurement in all groups [7]. After this initial wash, the fluoride release from FA crystals was only below the critical pH of 5.5. It was advantageous to use FA-coated crowns because the crystals were near the tooth/crown margin, as they were present on the inner and outer surfaces. FA crystals were exposed to the oral environment and fluoride ions were released at the tooth margin under acidic conditions. The release of fluoride in this study reduced lesion progression effectively. As pH rose, the crystals' essential ions were preserved, which increased their longevity and effectiveness. Fluoride-released lesion size was only examined at the crown margin due to the limited extent of the study. Under acidic conditions, fluoride release could also benefit calcified tissues adjacent to FA surfaces, for example, interproximally, which is another highly susceptible site to caries development [8]. The fluoride diffusion profile in the gelatin also suggested this, with F detected 5 cm from the FA-coated PMCs. Fluoride might be introduced more generally to the oral environment through FA-coated PMCs since they are not only designed to affect the adjacent interproximal area, but also neighboring enamel/dentine. According to scratch test results, the crystals bonded strongly to etched stainless steel surfaces [9]. By doing so, it would be possible to minimise delamination and deformation of the coating as a result of mastication. FA-coated crowns could be fitted and seated without significant loss of FA crystals without substantial manipulation during this procedure. In the event that the FA coatings delaminated, it would not be possible to recoat the PMCs [10]. We still need to investigate the limits of manipulation that FA-coated crowns can withstand. FA coating adds a negligible amount of thickness to the crown. SEM measurements showed coating thicknesses ranging from 50 to 100 microns. With this thickness of coating, the tooth preparation remains conservative, as it does not need to accommodate the coating as it would with a veneered PMC. The acid gelatin had previously been used to produce caries-like lesions in vitro at pH = 4.3. Consequently, we exposed the FA crystal-coated stainless steel discs to pH values of 4.0 and 4.5 to determine whether they would release fluoride. According to the results, this was the case. In order to maximize the effect of the fluoride release on demineralisation around the crown margins, the acid gelatin pH was set at 4.3. It was below the 5.5 critical pH reported for enamel. It is intended to use this model over longer time periods at different pH values ranging from 4.5 to 5.5 to investigate the effect of FA coatings on enamel and dentine demineralization in the future [11]. Providing FA-coated crowns in a clinical setting would make them affordable. Restorative materials such as PMC are among the least expensive options, and the crystal coating is inexpensive and takes only a few seconds to apply. In addition, FA coatings provide a more natural tooth appearance, thus providing a more aesthetic option for restorative dentistry

CONCLUSION

An in vitro study demonstrated that synthetic fluoridated hydroxyapatite (FA) crystals coated on stainless steel crowns exhibited anti-cariogenic properties. Additionally, the FA crystal coverage provided a durable enamel-like appearance, significantly enhancing aesthetics compared to standard preformed metal crowns (PMCs). These restorative options, characterized by their anti-cariogenic properties and improved aesthetics, could be considered for temporary, semi-permanent, or permanent use, offering comparable costs to partial mouth reconstruction procedures.

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