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Laboratory Research

Comparison of the Efficacy of Different Fluoride Varnishes on Dentin Remineralization During a Critical pH Exposure Using Quantitative X-Ray Microtomography

Efficacy of Fluoride Varnishes on Dentin Remineralization

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Clinical Relevance

The application of dental varnish containing fluoride on demineralized dentin could be recommended to remineralize and protect dentin lesions, and there might be no additional benefit

to incorporating calcium and phosphate in enhancing the remineralization on dentin caries in a clinical situation.

SUMMARY

Objectives:

The objective of this *in vitro* study was to quantify the amount of mineral change in demineralized dentin at pH 5.5 after the application of dental varnishes containing fluoride with casein phosphopeptide–amorphous calcium phosphate, fluoride and bioglass, or fluoride alone.

Methods and Materials:

A total of 12 extracted human sound mandibular premolar root samples were coated with an acid-resistant varnish, leaving a 2 × 3 mm window at the outer root surface. These root specimens were then randomly divided into four groups and separately subjected to the demineralizing cycle at a pH of 4.8 for five days to create artificial caries-like lesions in dentin. Subsequently, each sample was imaged using quantitative x-ray microtomography (XMT) at a 15-µm voxel size. Each test group then received one of the following treatments: dental varnish containing casein phosphopeptide–amorphous calcium phosphate and fluoride (CPP-ACP, MI varnish, GC Europe), bioglass and fluoride (BGA, Experimental, Dentsply Sirona), or fluoride alone (NUPRO, Dentsply Sirona), as well as a control group, which received no treatment. These samples were kept in deionized water for 12 hours. The thin layer of varnish was then removed. All samples including the nonvarnish group were subjected to the second demineralizing cycle at pH 5.5 for five days. The final XMT imaging was then carried out following the second demineralizing cycle. XMT scan was also carried out to varnish samples at 25 µm voxel size. The change in

mineral concentration in the demineralized teeth was assessed using both qualitative and quantitative image analysis.

Results:

There was an increase in radiopacity in the subtracted images of all varnish groups; a significant increase in mineral content, 12% for the CPP-ACP and fluoride ($p \leq 0.05$ and $p \leq 0.001$), 25% BGA ($p \leq 0.001$), and 104% fluoride alone varnish ($p \leq 0.001$). There was an increase in the size of radiolucency in the lesion area with a significant decrease in mineral content in the nonvarnish group, 10% ($p \leq 0.05$ and $p \leq 0.001$).

Conclusions:

There was encouraging evidence of a remineralization effect following the application of dental varnish on dentin and also an observed resistance to demineralization during the acidic challenge in all cases. However, a dental varnish containing fluoride alone appeared to have a much greater effect on dentin remineralization when compared with CPP-ACP with fluoride and bioglass with fluoride.

INTRODUCTION

Remineralization is a continuous process of mineral exchange within the demineralized tooth structure. The newly formed crystals ($Ca_{10}(PO^4)_6F^2$) are comparatively more resistant to dissolution.[1] This could be seen in both enamel and dentin and achieved either as part of routine daily plaque control or through the application of various remineralization agents.[2] In this respect, there is considerable evidence supporting the use of fluoride-based treatments to remineralize tooth structure.[3,4] However, the effectiveness of fluoride alone (F) with an inadequate source of calcium (Ca^{+2}) and phosphorous (PO_4^{3-}) could be limited.[5-7]

It has been reported that providing both Ca^{+2} and PO_4^{-3} together with fluoride had a superior impact in preventing dentin caries when compared with F in populations with high caries risk.[8] In this respect, different formulations of calcium phosphate have been incorporated into fluoride products, including casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) and bioglass (calcium sodium phosphosilicate).[5,9] These formulations were recently introduced as part of fluoride varnish where there is a chance to act as slow-release fluoride reservoirs due to adherence to the tooth surface for longer periods of time.[10] A recent *in vitro* study reported a significant remineralization effect using fluoride with CPP-ACP on caries-like lesions in bovine dentin that received no toothbrushing in comparison with a dental varnish containing F.[11]

Despite the promising results from several studies, there was a low level of evidence to suggest either CPP-ACP or bioglass plus fluoride formulation to enhance the remineralizaton on dentin lesions.[12-15] In addition, none of the conducted studies compared these formulations in more challenging conditions, such as a constant demineralizing cycle at pH 5.5. This could be valid for individuals who have an impairment in the salivary function, where saliva is unable to maintain the natural pH during an acidic challenge, or for those with a resting pH of 5.5 combined with a high-caries risk.[16] In addition, the significant drop in salivary pH is more likely to be seen in individuals with excessive sugar and fermentable carbohydrate intake who are metabolized by micro-organisms to produce acids.[17] Therefore, the aim of this study was to compare the mineral deposition of different dental varnishes containing CPP-ACP with fluoride, bioglass with fluoride, and F on dentin-like lesions at a critical pH using quantitative x-ray microtomography (XMT).

METHODS AND MATERIALS

Twelve human sound mandibular premolars that were extracted for orthodontic purposes were collected, cleaned, and polished using nonfluoridated pumice and a slow handpiece. The teeth were then stored in 1% thymol prior to sample preparation.

Preparation of Demineralized Solution

The demineralization buffer solution was prepared using 0.2205 g/L $CaCl_2.2H_2O$, 0.1225 g/L KH_2PO_4, and 50 mmol/L acetic acid. The pH was adjusted to 4.8 using 0.05 g/L KOH for the first demineralization cycle, to create artificial-like caries in dentin.[18] An Oakton pH meter (Oakton Instruments, Nijkerk, the Netherlands) was used to measure the pH of the demineralized solution. All chemical reagents were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). The same buffer solution with pH 5.5 was prepared to immerse the specimens following dental varnish application.

Preparation of Artificial Caries-like Lesions

The crowns of collected teeth were removed 1 mm below the cemento-enamel junction using a 0.3-mm-thick diamond disc under running water at 3000 rpm speed (Struers,Copenhagen, Denmark). Adhesive tapes of 2 × 3 mm were placed coronally 2 mm beyond the cutting side of the root samples, and then an acid-resistant varnish (Revlon, New York, NY, USA) was applied. A dentin window of 2 × 3 mm was then exposed by removing the piece of tape on each sample. These allocated areas represented the lesion sites. The root specimens were separately immersed in 10 mL of demineralizing solution at pH 4.8 and then kept in a shaker incubator for five days at 37°C to simulate body temperature.[18,19] The solution was replaced every 24 hours to keep the demineralization process under fresh solution.[20]

XMT Baseline Scan

Following the first demineralization cycle, each root specimen was washed thoroughly with deionized water and placed vertically inside a clear plastic tube (Sterilin, Newport, UK) and held with soft wax (6969 from Poth Hille & Co Ltd, Rainham, Essex, UK) to prevent any possible movement. The tube was then filled with deionized water to keep the samples hydrated during the scanning procedure. Subsequently, each prepared sample was placed on a movable kinematic stage, ensuring the long axis of the tooth was parallel to the XMT rotational axis. The XMT scanner was set to produce a 15 µm voxel size reconstruction, and the x-ray generator was set to 90 kV and 180 µA. Each XMT scan took around seven hours to complete.[20] A calibration carousel was repeatedly scanned at the end of each tomography scan.[21] The baseline XMT scan was subsequently carried out following the first demineralizing cycle at pH 4.8 and before the application of dental varnish.

Dental Varnish Application

Following the baseline scan, the root specimens were randomly divided into four groups (three samples each) to receive one of the allocated treatments (Table 1). The specimens were dried using a three-in-one dental syringe. A thin and uniform layer of dental varnish was applied to each specimen covering the 2 × 3 mm exposed window. The specimens were then kept separately in deionized water (10 mL) and placed in a shaker incubator at 37°C for 12 hours. The thin layer of varnish was then carefully removed using a scalpel blade (Swann-Morton, Sheffield, UK),[23] and each area was visually inspected using a 4× magnification lens to confirm the removal of all varnish remnants.

Second demineralizing Cycle and the Final XMT Scan

All root specimens, including the non-varnish group, were subjected to the second demineralizing cycle at pH 5.5 for five days. The specimens were then placed in a shaker

incubator in 10 mL of demineralizing solution at 37°C. This solution was changed every 24 hours to keep the lesion area in contact with the fresh demineralizing solution.[20] All root specimens, including the nonvarnish group, were then prepared for the final XMT scan using the same settings as in the baseline scans.

Image Analysis

The obtained 2D images (>1000 projections) from the XMT scan were corrected for beam hardening,[4] and reconstructed to create the three-dimensional (3D) image. The 3D image from the baseline scan of each sample was aligned with the 3D image from the final scan using the in-house–developed alignment software running under International Date Line (IDL, Exelis Visual Information Solutions, Boulder, Colorado, USA) The initial 3D image was then subtracted from the final 3D image using in-house–developed IDL software. The volumetric subtraction was carried out to detect the mineral gain/loss as visually detected as an increase in the radiopacity/radiolucency in the demineralized area. To quantify mineral change in the demineralized area, the average linear attenuation coefficient (LAC) from a total of 15 points from the baseline scans that were randomly selected from the demineralized area was compared with the corresponding average of 15 points from the final scan. These points were chosen in five slices, with three points in each, and subsequent analyses were carried out separately for each sample.

The obtained LAC value of each point was converted to mineral concentration (g cm^{-3}) using the following equation[15]:

$$c = \frac{\mu - \mu_o}{\mu_m - \mu_o} \rho_m$$

where μ is the measured LAC, μ_o is the LAC of the deionized water and plastic that assumed soft tissue (0.268 cm^{-1}), μ_m is the pure sample material LAC that presumed a pure hydroxyapatite (3.12 cm^{-1}), and P_m is the concentration of the pure hydroxyapatite (3.16 cm^{-3}).

XMT Scan for the Test Varnishes

The contents of each varnish were mixed and placed into small centrifuge tubes and positioned on a movable kinematic stage with the long axis of the tube parallel to the XMT rotational axis. The x-ray generator was set at 90 kV and 180 μA, and to produce 25-μm voxel resolution using the high-definition XMT scanner, each scan took about 60 min.

Power Calculation

Sample size calculation was based on the data from a previous study, where the mean difference between baseline and final scans was 0.32 and the standard deviation of the differences was 0.02.[15] The statistical power was set to 80% at a level of significance of 0.05 (two sided).

Statistical Analysis

One-way analysis of variance (ANOVA) was performed to find the differences in mineral change between the groups. Paired *t*-test was used to analyze the mineral change following the application of varnish for each sample by comparing the 15 points between baseline and final scans. The mean percentages in mineral change of each group were then calculated. Data analysis was performed using IBM SPSS Statistics 24.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Qualitative Assessment of Mineral Change

The subtracted images of the root specimens that received dental varnishes showed an increase in the radiopacity in the demineralized area, whereas there was an increase in the radiolucency for the negative group (Figure 1).

Quantitative Mineral Concentration Change

One-way ANOVA showed that there was a significant difference between the test groups ($p<0.001$). Paired *t*-test revealed a significant increase in mineral content in all samples for the varnish groups. The mean percentage of mineral gain was higher in samples treated with the dental varnish containing F 104% ($p\leq0.001$) then bioglass and fluoride **(**BGA) with fluoride 25% ($p\leq0.001$) and finally CPP-ACP with fluoride 12% ($p\leq0.05$ and $p\leq0.001$). There was a significant decrease of 10% ($p\leq0.05$ and $p\leq0.001$) in mineral content for the nonvarnish samples (Table 2).

XMT Scans for Dental Varnishes

The XMT scans of the dental varnish containing CPP-ACP and fluoride showed a homogenous mixture of fine particles with a density of approximately 0.2 to 0.3 LAC. However, the BGA varnish had a non-homogenous mixture and more defined particles that settled on the base of the tube, with a higher density (0.4-0.8 LAC) when compared with the former varnish. The images of the varnish containing F were less homogenous with more defined particles when compared with images of the CPP-ACP varnish. However, the density of the F varnish was higher than the CPP-ACP formulation and lower than the BGA varnish (0.3-0.5 LAC). Most of the defined particles of the dental varnish in the F group accumulated in the apical half of the tube.

DISCUSSION

The results of this laboratory-based study showed that dental varnish containing F had a superior remineralization effect on dentin when compared with other varnishes formulated with either CPP-ACP and fluoride or BGA. A constant demineralizing model at pH 5.5 was carried out instead of demineralization/remineralization cycling. The reason for this was to simulate an extreme clinical condition, such as those with a critical saliva pH and exposed dentin surfaces, where there is a risk of mineral loss,[16,17] and thus a periodic application of fluoride varnish is recommended.[2]

In this study, the varnishes were removed after 12 hours to emphasize the chemical effect rather than the mechanical effect and also to simulate the clinical situation in which dental varnish could be retained on the tooth surface prior to removal from regular toothbrushing and mastication.[25-27] In previous studies, it is noted that dental varnishes containing fluoride were removed from the test specimens within a period ranging from 6 to 24 hours following the application.[13,28-30]

The superior remineralization effect of the varnish containing F in comparison to calcium phosphate varnishes could be related to the ability of the latter varnishes to release inorganic phosphorous in addition to calcium and fluoride, thereby decreasing the bioavailability of fluoride ions through the formation of a lower-soluble fluorapatite rather than loosely bonded CaF^{+} and CaF_2 in the immersion solution.[13,31,32] In addition, it can be speculated that both dental varnishes (CPP-ACP and BGA) with calcium and phosphate had the ability to form hydroxyapatite crystals on the lesion surface that could restrict infiltration and deposition of fluoride ions in the demineralized region in comparison with the varnish containing F.

The lowest mineral deposition for the varnishes containing CPP-ACP and fluoride could be related to the higher ability of the CPP-ACP compound to dissolve in the aqueous solution.[33] This might assist in releasing calcium, phosphate, and fluoride ions into the aqueous solution rather than retaining them in the demineralized area. This explanation was supported by Sleibi and others, who confirmed that the highest fluoride, calcium, and phosphate ion release was seen in the varnish containing CPP-ACP formulation with fluoride compared with the varnish with BGA.[34] In addition, the varnish containing F showed the lowest fluoride ion release.[34] These results were also consistent with the findings of previous studies that the dental varnish with fluoride and CPP-ACP had the highest calcium and fluoride ion release in comparison with the varnishes with different calcium phosphate formulations.[32,35]

Similarly, bioglass facilitates rapid ionic exchange of sodium ions with hydrogen cations in the aqueous solution, allowing discharge of calcium and phosphate ions from the glass to the immersion solution.[36] Although bioglass has the ability to retract free ions, the time required for completion might be insufficient for this process, since the immersion solution was constantly changed. Therefore, this process possibly then led to disposal of these free ions. In this respect, it can be speculated that the experimental BGA varnish released more ions and had a minimum capability to retain them in the lesion area in comparison to the varnish with F. This explanation, however, should be interpreted with caution, as the samples were immersed in deionized water and then subjected to a constant demineralizing solution that would not simulate natural saliva with regard to compositions and physical properties.

The mineral gain was higher with the BGA varnish when compared with the CPP-ACP technology. This might again be due to the presence of silanols in the BGA structure that remain attached to the tooth structure following release of ions from the BGA. Subsequently, this

process would act as a nucleation site on the tooth surface to retract calcium and phosphorous, forming a calcium-phosphate–rich layer that can resist an acidic environment.[37,38] In a previous study, BGA had more favorable remineralization potential than CPP-ACP on the enamel structure. Mehta and others suggested that CPP-ACP failed to be retained for a longer period on the tooth structure, due to its amorphous nature, such that it was unlikely to adhere to the tooth surface when compared with the firmly attached bioglass particles.[39] The superior mineral gain for the BGA formula over CPP-ACP was consistent with the findings of a recent study in which natural dentin caries specimens were assessed under a constant remineralizing condition.[15] Those authors proposed that BGA plus F had a higher remineralization efficacy than the CPP-ACP with F formula not only in the net-demineralizing but also in the net-remineralizing condition.

Regarding the CPP-ACP plus F, the findings of the present study were consistent with the results of Mohd Said and others, who reported that the F had a significant remineralization effect on CPP-ACP with fluoride varnishes on enamel caries.[13] Thereby, within the limitations of this study, it could be assumed that CPP-ACP failed to enhance the remineralization effect of fluoride. However, these results were in contrast to the findings of Shen and others,[35] who reported that CPP-ACP varnish had a superior effect on the F varnish in protecting against enamel demineralization. This could well be due to the fact that the dental varnish in the later study was applied to the area adjacent to the demineralized area, whereas in this study, it was applied directly onto the lesion. It can be speculated that it is better to manage the already formed demineralized lesions with the application of a dental varnish containing F, rather than calcium phosphate–containing fluoride varnishes, considering that the application of the F varnish is in contact with the lesion surface. The superior remineralization effect of the F compared with the CPP-ACP formula was also inconsistent with the findings of Wierichs and others and Pithon and

others.[11,40] There was a significant mineral gain observed for the CPP-ACP plus fluoride varnish compared with F on artificial-like enamel and dentin caries.[11,40] However, in these previous studies, the varnishes were not removed from the samples, which might have led to an additional remineralization effect as well as a mechanical effect from the extended retention period.

The decrease in mineral concentration in the lesion area of the nonvarnish samples (negative control) following the second demineralization cycle that was also identified as an increase in the radiolucency in the subtracted image (Figure 1) confirmed the considerable effect of the fluoride varnish application, not only to remineralize dentin but also to prevent these dentin lesions from further demineralization. These results were in agreement with the finding of an *in situ* study by Zaura-Arite and ten Cate, in which the application of a placebo varnish did not inhibit mineral loss in dentin samples compared with fluoride varnish.[41]

Nevertheless, the variances in the physical properties and chemical composition, including the presence of artificial resin in the composition of both BGA and F varnishes, could be the cause of these differences in mineral precipitation.[42-44] In this study, the XMT image of varnish liquid with CPP-ACP showed the most homogenous and small-size particles that could easily be detached from the immersion solution, whereas the large particles of both BGA and F varnishes might assist the ions to be settled (attached) on the tooth structure following the application. This probably led to a decrease in ion detachments of both BGA and F varnishes. The above explanation can be clearly seen in Figure 2, where large defined particles were mostly accumulated on the base and middle half of the bottle in both BGA and F varnishes, whereas there was a homogenous mixture for the CPP-ACP varnish. This explanation could also be applicable in a worse scenario in which the large particles represented the inactive ingredients

only. This was due to the possible entrapment of small active particles (ions) underneath the large particles, restricting their release in the aqueous solution.

The small sample size could be a limitation of this study; however, the XMT scanner combined both improved accuracy and contrast sensitivity, and with its noninvasive nature, this scanner enables precise analysis of mineral gain/loss.[21] Each sample was also evaluated and statistically analyzed through comparing 15 different points from the baseline and from the final scans that had the same 3D positions. Each sample took about 14 hours to complete for baseline and finals scans (in total: 28 hours). In addition, the samples were assessed using both qualitative and quantitative measurements with consistent results, which supported these findings. The power calculation was carried out by considering a previous study as a reference, which employed the XMT technique.[15]

CONCLUSIONS

Within the limitation of this study, there was encouraging evidence of a remineralization effect with dental varnish containing fluoride on dentin in all cases, and the ability of this varnish to resist demineralization during the acidic conditions was evident in this laboratory setting. However, a dental varnish containing F seemed to be promising when compared with the dental varnish containing fluoride either with bioglass or CPP-ACP. Without the application of dental varnish containing fluoride, there was a risk of potential mineral loss. Further studies are required to confirm whether these results could be replicated in a clinical situation, particularly for those who are at high risk for caries initiation.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Research Ethics Committees Northern Ireland. The approval code for this study is ORECNI, 16/NI/0101.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to publication of this article.

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Figure 1. *The baseline XMT scan (left), final XMT scan (middle), and subtracted image (right) of the test groups. The contrast of the subtracted images had been increased by a factor of 8 to show subtle differences in mineral concentration. The arrow refers to the lesion area. The radiopaque area demonstrated an increase in the mineral contents of all varnish samples, whereas the radiolucency in the nonvarnish sample revealed more mineral loss following the*

second demineralization cycle (bubbles presented in only one image showed as the differences in the content between air and water).

Figure 2. *XMT images of each dental varnish.*

Table 1: *Dental Varnish Used for the Study*

Group	**Varnish Name Company**	**Active Ingredients**	**Other Ingredients**
CPP-ACP	MI varnish GC, Japan	5%NaF and CPP-ACP	Polyvinyl acetate, hydrogenated rosin, ethanol, silicon dioxide
BGA	Experimental Dentsply Sirona, USA	5% NaF and bioglass	Urethane methacrylate, hydrogenated rosin, resin, alcohol, sodium, sucralose, flavor, titanium dioxide
F	NUPRO White Dentsply Sirona, USA	5% NaF	Urethane methacrylate, hydrogenated rosin, resin, alcohol, sodium, sucralose, flavor, titanium dioxide
NC	Negative control non-varnish	—	—

Table 2: *Mean Mineral Concentrations (g cm^{-3}) at Baseline and Final XMT Scans With Standard Deviations (SD), Mean Differences in Mineral Content,* p-*Value of the Mean Differences for Each Sample, and the Overall Change in the Mean Percentage of Mineral Gain/Loss for Each Test Group*

Varnish Group	**Sample**	**Baseline Scan**	**Final Scan**	**Mean Difference**	***p*-Value**	**Overall Change in Percentage**

		Mean ±SD	**Mean ±(SD)**			
CPP-ACP	1	0.22 ± 0.08	0.24 ± 0.1	0.02	0.05*	12% increase
	2	0.22 ± 0.05	0.25 ± 0.06	0.03	<0.001**	
	3	0.23 ± 0.02	0.26 ± 0.02	0.03	<0.001**	
BGA	1	0.26 ± 0.04	0.32 ± 0.06	0.07	<0.001**	25% increase
	2	0.2 ± 0.02	0.25 ± 0.04	0.05	<0.001**	
	3	0.23 ± 0.03	0.28 ± 0.07	0.06	<0.001**	
F alone	1	0.25 ± 0.03	0.47 ± 0.05	0.22	<0.001**	104% increase
	2	0.19 ± 0.01	0.45 ± 0.03	0.26	<0.001**	
	3	0.26 ± 0.02	0.49 ± 0.03	0.23	<0.001**	
NC	1	0.36 ± 0.02	0.33 ± 0.02	−0.03	<0.001**	10% decrease

2	0.33 ± 0.07	0.3 ± 0.03	−0.03	0.03*
3	0.2 ± 0.06	0.17 ± 0.04	−0.02	0.01*

* Significant at $p \leq 0.05$; ** Highly significant at $p \leq 0.001$.