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Abstract: ABSTRACT
Objectives: To compare the efficacy of fluoride varnishes either casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or bioglass particles on the severity index of root caries.
Methods: Visual-tactile assessments including lesion hardness was carried out to categorise the severity index of 80 extracted teeth with primary root caries. These teeth were randomly divided into four groups; CPP-ACP and fluoride, bioglass and fluoride, fluoride only, and no treatment. Standardised toothbrushing using a dentifrice containing 1,450 ppm fluoride was carried out twice a day for all groups. All samples were stored in remineralising solution at 37°C for 45 days. Visual-tactile assessments were carried out at baseline, and after 45 days. Surface roughness measurements (Ra) were performed at baseline and after 7, 14, 30 and 45 days. X-ray Microtomography was performed at the baseline and after 45 days for three samples from each group to quantify the change in mineral contents in the lesion area.
Results: The Visual-tactile assessment results showed a reduction in the severity index of root caries, being 20% in CPP-ACP and fluoride, 100% in bioglass and fluoride, 80% in fluoride only, and 60% in non-varnish (toothbrushing only). Non-significant change in surface roughness was observed in all groups. X-ray Microtomography assessment showed a highly significant increase in the mineral deposition in all cases ($p>0.001$).
Conclusion: The combination of bioglass with fluoride has a potentially superior effect than either CPP-ACP with fluoride or fluoride only to reverse and arrest the root caries in a laboratory setting.
Clinical significance: The combination of bioglass particles and fluoride formulation is likely to have a significant impact in reversing and arresting root caries in a minimally invasive approach. However, randomised controlled double-blinded clinical trials are required to translate these results into clinical practice.

Cover Letter

18th of December 2017

Dr. Christopher D. Lynch
Editor in Chief
Journal of Dentistry

Manuscript title '**Comparison of efficacy of dental varnish containing fluoride either with CPP-ACP or bioglass on root caries: *Ex vivo* Study**'.

Dear Dr Lynch,

This is the first laboratory-based study that assessed the effectiveness of a novel dental varnish containing bioglass particles and fluoride in comparison to dental varnish with fluoride in addition to calcium and phosphate ions on natural root caries.

To the best of our knowledge, there is no similar study which has been conducted previously, and the results highlight the necessity of using the bioglass as an active ingredient in addition to fluoride for the minimally invasive approach to manage root caries. This would have a significant influence on designing a randomised controlled clinical trial especially in compromised situations such as patients with dry mouth.

We look forward to hearing from you all.

Kind regards,
Dr Ahmed Sleibi DDS, MSc

***Title Page with Author Details**

Title page

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Short title:

Efficacy of fluoride varnishes on root caries: Ex vivo Study

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Manuscript

Comparison of efficacy of dental varnish containing fluoride either with CPP-ACP or bioglass on root caries: *Ex vivo* study

ABSTRACT

Objectives: To compare the efficacy of fluoride varnishes either casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or bioglass particles on the severity index of root caries.

Methods: Visual-tactile assessments including lesion hardness was carried out to categorise the severity index of 80 extracted teeth with primary root caries. These teeth were randomly divided into four groups; CPP-ACP and fluoride, bioglass and fluoride, fluoride only, and no treatment. Standardised toothbrushing using a dentifrice containing 1,450 ppm fluoride was carried out twice a day for all groups. All samples were stored in remineralising solution at 37^{o}C for 45 days. Visual-tactile assessments were carried out at baseline, and after 45 days. Surface roughness measurements (Ra) were performed at baseline and after 7, 14, 30 and 45 days. X-ray Microtomography was performed at the baseline and after 45 days for three samples from each group to quantify the change in mineral contents in the lesion area.

Results: The Visual-tactile assessment results showed a reduction in the severity index of root caries, being 20% in CPP-ACP and fluoride, 100% in bioglass and fluoride, 80% in fluoride only, and 60% in non-varnish (toothbrushing only). Non-significant change in surface roughness was observed in all groups. X-ray Microtomography assessment showed a highly significant increase in the mineral deposition in all cases ($p>0.001$).

Conclusion: The combination of bioglass with fluoride has a potentially superior effect than either CPP-ACP with fluoride or fluoride only to reverse and arrest the root caries in a laboratory setting.

Clinical significance: The combination of bioglass particles and fluoride formulation is likely to have a significant impact in reversing and arresting root caries in a minimally invasive approach. However, randomised controlled double-blinded clinical trials are required to translate these results into clinical practice.

KEY WORDS: Root caries; dental varnish; CPP-ACP; bioglass; surface roughness; XMT

1. Introduction

The world population aged 60 and above is increasing and is set to rise from 11% in 2000 to 20% in year 2050 [1]. A recent data revealed an increased risk of developing root caries in older population due to exposed root surfaces following gingival recession. This could be related to the aging process or due to a history of periodontal disease [2,3,4,5]. Nowadays, it is clearly stated that older people retain most of their natural teeth [6]. This brings a constant need for preventive and restorative dental services amongst older adults, especially for underserved populations such as those institutionalised.

Exposed root surface becomes susceptible to developing carious lesion as the demineralisation of dentine can occur at pH values of 6.0 [7]. To manage these lesions using a minimally invasive approach, regular toothbrushing with a dentifrice having different fluoride concentration, ranging from 1100 to 5000 ppm, has been employed with a variation in the success rate [8,9,10]. Following the introduction of dental varnishes, there is a potential benefit from increasing fluoride release through maximising contact time on the tooth surface [11]. This offers an intraoral reservoir of fluoride ions to challenge the cariogenic process over a longer period of time which would contribute to the enhancement of the remineralisation process [12,13]. The use of dental varnishes is considered safe, particularly for patients who are unable to use other fluoride delivery systems e.g. mouthwash [14]. Ekstrand [15] reported a significant improvement in the management of root

caries following the application of a dental varnish containing fluoride (22.6 ppm) when compared with a dentifrice containing 1,450 ppm fluoride. Furthermore, the use of dental varnish was found to perform only slightly better in comparison to a dentifrice containing 5,000 ppm fluoride [15].

Several studies have been conducted to assess the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) for the management of enamel caries. CPP-ACP has the ability to stabilise calcium phosphate, and further to maintain its potential availability during an acid challenge [16,17]. Reynolds [18] reported a considerable reduction in caries progression with the application of CPP-ACP with additional fluoride when compared with the use of either fluoride, or CPP-ACP alone.

Recently, calcium sodium phosphosilicate bioglass has been shown to play a key role in the hard tissue remineralisation of both enamel and dentine [19,20]. Bioglass has the ability to release calcium, sodium, and phosphate ions into aqueous solution. This allows for an ionic exchange of sodium with H^+ or H_3O^+ at the glass-liquid interface, permitting the formation of supersaturated ionic reservoir. The remaining silanols that result following bioglass dissolution act as a nucleation site on the tooth surface to form an apatite structure by attracting the released calcium and phosphate ions, forming a calcium phosphate rich layer [21,22]. A recent systematic review by Fernando [23] demonstrated that bioglass has the ability to boost remineralisation in dentine. However, this was not fully tested, and further studies are required.

Therefore, both CPP-ACP and bioglass formulations, combined with fluoride, have an effect on the remineralisation process within root carious lesions. This could be particularly useful for older populations for whom routine oral hygiene activities become challenging due to the deterioration in mobility, resulting in compromised oral health as a result of insufficient salivary function and impaired plaque control. Thus, the aim of this *ex vi*vo study was to compare the efficacy of a fluoride varnish with CPP-ACP, with a novel varnish containing fluoride and bioglass and with

regular toothbrushing (control group) for a period of 45 days on root caries, using visual-tactile assessments for lesion hardness, non-contact optical profilometry to assess the changes in surface roughness, and finally using X-ray microtomography to investigate the changes in mineral concentration.

As both CPP-ACP and bioglass could be a source of calcium and phosphorous to which enhances the remineralisation process within the root carious lesion. The hypothesis of this study was to confirm that there are no differences in effectiveness of CPP-ACP with fluoride, and bioglass with fluoride on root caries.

2. Materials and methods

2.1. Root caries sample collection

80 extracted teeth with primary active root caries were collected from the Dental Emergency Clinics at the Institute of Dentistry, Bart's and the London, Queen Mary University of London. Ethical approval was obtained from the Office for Research Ethics Committees Northern Ireland (ORECNI, 16/NI/0101) prior to the study. The teeth were stored in 1% thymol prior to sample preparation. They were then cleansed and polished using a slow speed handpiece and polishing cup with a non-fluoridated prophylaxis paste (NUPRO DENTSPLY, USA). The study flow chart is illustrated in figure 1.

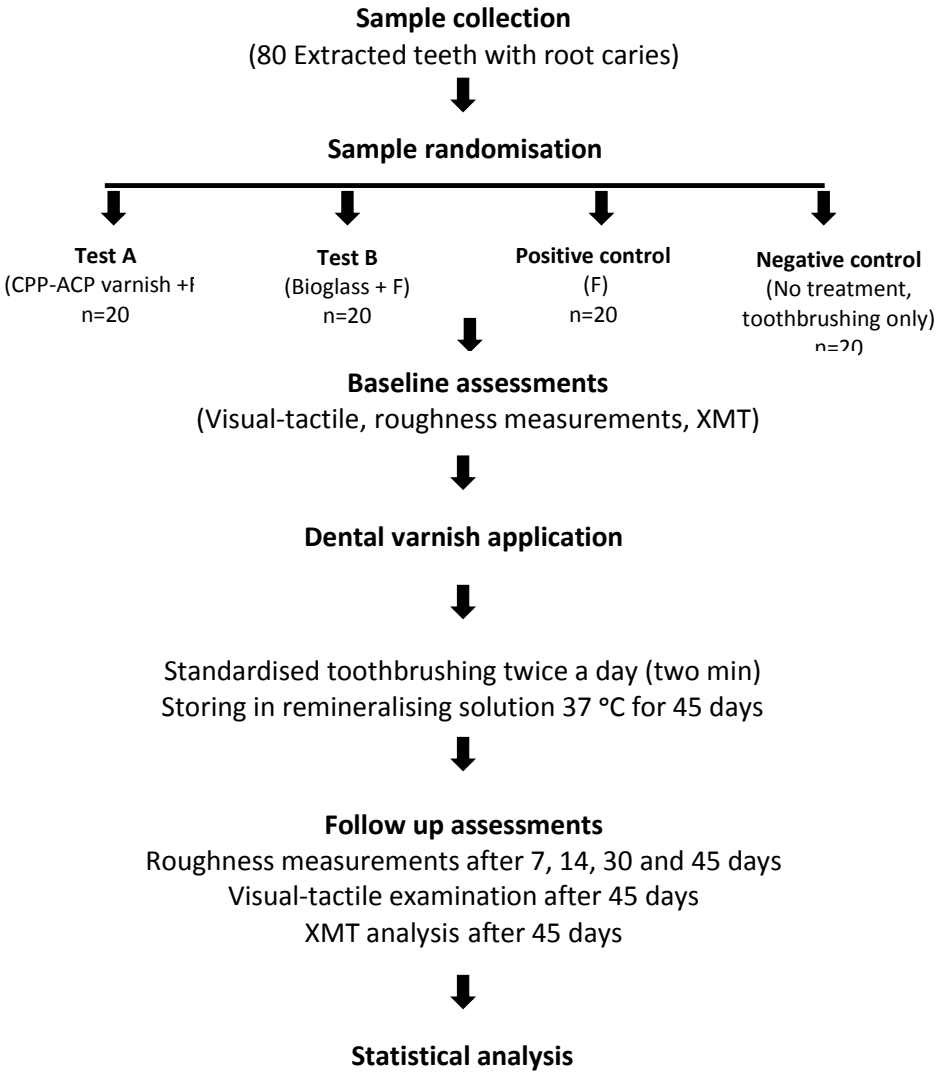


Figure 1. Study flow chart. SEM assessment was carried out to three samples from each group.

2.2. Treatment

Four different treatment strategies were tested with a total of 80 samples. Each group (n=20) received one of the allocated treatments as described in Table 1. The samples were dried using a three-in-one syringe, and a thin and uniform layer of dental varnish was applied to each root caries according to manufacturer's instructions. The area was then gently wetted with deionised water to accelerate varnish set.

Table 1. The allocated test groups: MI varnish (5%NaF+ CPP-ACP), NUPRO Experimental varnish (5%NaF & bioglass), NUPRO varnish (5%NaF), and non-varnish group. All groups received standardised toothbrushing with 1,450 ppm F dentifrice.

Groups	**Varnish active ingredients**	**Other treatment**
Test A (MI varnish, GC, Japan)	CPP-ACP and 5%NaF	Toothbrushing 1450 ppm F
Test B (NUPRO Experimental , DENTSPLY, USA)	Bioglass and 5%NaF	Toothbrushing 1450 ppm F
Positive control (NUPRO, DENTSPLY, USA)	5%NaF	Toothbrushing 1450 ppm F
Negative control (Non-varnish)	-	Toothbrushing 1450 ppm F

2.3. Remineralisation buffer solution

A remineralisation solution was prepared as previously described by Ten Cate [24] using 1.5 mmol/L $CaCl_2$, 0.9 mmol/L KH_2PO_4, 20 mmol/L HEPES and 130 mmol/L KCl. 1.5 mmol/L NaN_3 was added to the solution to prevent microbial growth. The pH was adjusted at 7 using 0.5 M KOH [25]. All chemical reagents were obtained from Sigma–Aldrich, UK.

The prepared specimens of each group were immersed in 300 mL of remineralising solution, and the solution was kept in an incubator at 37^{o} C for 45 days. The remineralising solution was replaced every two days.

2.4. Toothbrushing procedure

The mechanical brushing procedure was set to simulate the clinical setting were the recommended brushing time was two minutes for the entire mouth [26]. The first brushing process started after 12 hours following the application of the dental varnish. Toothbrushing was simulated

with a medium bristle toothbrush (Oral-B) with 1,450 ppm fluoride dentifrice (Aquafresh GSK, UK) twice a day for 45 days. The procedure was performed using an electrically powered toothbrushing machine (Weybridge Equipment, UK) designed to produce a constant reciprocal movement in a linear pattern. For each specimen the force applied to the brush bristle was 150 gm [27], the brushing process was performed using 1 mL of a 1:3 slurry of dentifrice and deionized water, for 10 seconds [28]. The specimens were then left for two minutes before washing with deionised water.

2.5. Visual-tactile assessment for root caries

Visual-tactile examinations were carried out to categorise the severity index of root caries with regards to lesion hardness. This index is based on a score system from 0 to 4 as illustrated in Table 2 [29,30,31,32].

Table 2. Categories of the severity index of root caries

Severity index Score	**Lesion surface hardness**
0	Hard
1	Leathery to hard
2	Leathery
3	Leathery with local softening
4	Soft

The hardness of each lesion was assessed using an Ash No.6 blunt probe with a pressure of around 100 g. Soft lesions were easily penetrated by the probe whilst leathery lesions had some resistance to withdrawal and hard lesions failed to have any penetration [29]. The selected lesions in this study at baseline presented leathery types in hardness with severity indices between 1, 2 and 3. Lesions that scored 4 were excluded as they require a restorative approach.

The intra examiner reliability to assess severity index and hardness of root caries was performed twice within a one week interval for examiner 1 (AS). The assessments for root caries then were carried out independently by two examiners (AB&AS) at baseline before varnish application and after 45 days.

2.6. Surface roughness measurement (Ra)

Non-Contact Optical Profilometry (NCOP) has been used as a method to quantify the changes in surface roughness (Ra) of enamel following demineralisation [33,34]. NCOP is a non-invasive and precise technology utilising the different refractive indices of the components of white light to measure sample topography.

The crowns and the apical parts of the teeth were removed using a 0.3 mm thickness diamond disc under running water at 3000 rpm speed (Struers, Germany) keeping the root carious lesions. The 20 samples for each group were fixed onto a customised aluminium tray using cold-cured acrylic resin (Pegasus Plus, England), keeping the lesion area and 2 mm of the surrounding sound dentine exposed. The kinematic tray was designed to relocate the samples during multiple repeated NCOP scans throughout the study.

NCOP was carried out using a Proscan 2000 (Scantron, UK) with a S13/1.2 sensor to measure the surface roughness (Ra) of each sample at various time points throughout the study. The specimens were dried for a period of 5 min at 37^{o}C prior to each NCOP scan. An initial area scan of 1.5×1.5 mm was carried out to ensure suitability of the selected area. Following this, three NCOP line scans were selected within this scanning area, for measurement of Ra of each sample. NCOP line scans with operational parameters (step size: 0.001 mm, number of steps: 1500) were carried out. A sampling rate of 30 Hz was used [35]. Surface roughness measurements (Ra) of all samples were carried out at baseline before varnish application, and then after 7, 14, 30 and 45

days. A correlation test was performed to analyse the relation between the change in Ra measurement and severity index for each group.

2.7. X-ray Microtomography (XMT)

The XMT assessment to measure the change in mineral concentration was carried out to three samples with score 2 severity index from each group, using the MuCAT 2 scanner designed at Queen Mary University of London [36]. Each sample was separately located inside a clear plastic tube filled with deionised water to carry out the XMT baseline scan to keep the specimen fully hydrated during the long scanning procedure (around 12 hours). The 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 at 15 µm voxel size resolution (3D). The x-ray generator was operated at 90 kV and 180 µA. After each sample scan a calibration scan was performed and the projection data was transformed to 40 keV monochromatic energy equivalent [36]. The reconstructed linear attenuation coefficient (LAC) was converted to mineral concentration using the following equation:

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

where μ is the measured linear attenuation coefficient (LAC),

μ_o is the pure organic component LAC of the tooth structure (0.268cm^{-1}),

μ_m is the pure sample material LAC which assumed a pure hydroxyapatite (3.12 cm^{-1}), and

ρ_m is the concentration of the pure hydroxyapatite (3.16 cm^{-3}).

Note that a good approximation is given where water, plastic and soft tissue are all considered as part of the organic component. Likewise, although the mineral in dental hard tissue is not pure hydroxyapatite, this is a good approximation in terms of X-ray attenuation [36].

The XMT scan was performed to the three samples at the baseline before varnish application and after 45 days. The obtained 2D images (>1000 projections) from the XMT scans were reconstructed to create the 3D image. The 3D image from the baseline scan of each sample was aligned with the reconstructed 3D image from final scan using in-house developed alignment software running under IDL (Exelis Visual Information Solutions, Inc). The difference in mineral concentration was then visually detected by subtraction of the final image from the baseline one. The mineral change in the lesion region was calculated by comparing the mineral concentration of 15 randomly selected points from the baseline image with the final image.

2.8. Statistical Analysis

The intra- and inter-examiner reproducibility for the severity index were assessed using the Intra-class correlation coefficient (ICC). The percentage change in the severity index was calculated, then followed by paired t-test to find the significant difference between the baseline and final assessments.

The data from the surface roughness (Ra) were analysed using repeated measures ANOVA and pairwise comparisons. Bivariate correlation was also used to investigate the correlation between the changes in the severity index, hardness and surface roughness (Ra).

The percentage in mineral concentration change was calculated then followed by paired t-test to compare the difference in mineral content in each sample. A significance level of 0.05 was performed using IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Severity index for root caries assessment

Excellent intra-examiner reliability for the severity index for root caries was shown for examiner 1 (AS). The intra-class correlation coefficient (ICC) value was 0.92. Good inter-examiner reliability was reported at baseline and after 45 days, the ICC value was 0.80 for severity index.

The severity index for the root caries is shown in Table 3. After 45 days, there was a significant decrease in the severity index of root caries in all groups. The maximum reduction was for bioglass and fluoride (F) (100%), and lowest was for CPP-ACP and F (20%).

Table 3. The change in the severity index (SI) of root caries for the test groups at the baseline and after 45 days in relation to each score stage, and the percentage of improvement and deterioration rates. The selected lesions at baseline presented leathery types in hardness with severity indices between 1, 2 and 3. After 45 days, there was a decrease in the SI, improving to be hard in texture (score 0), and none of the lesions had score 3.

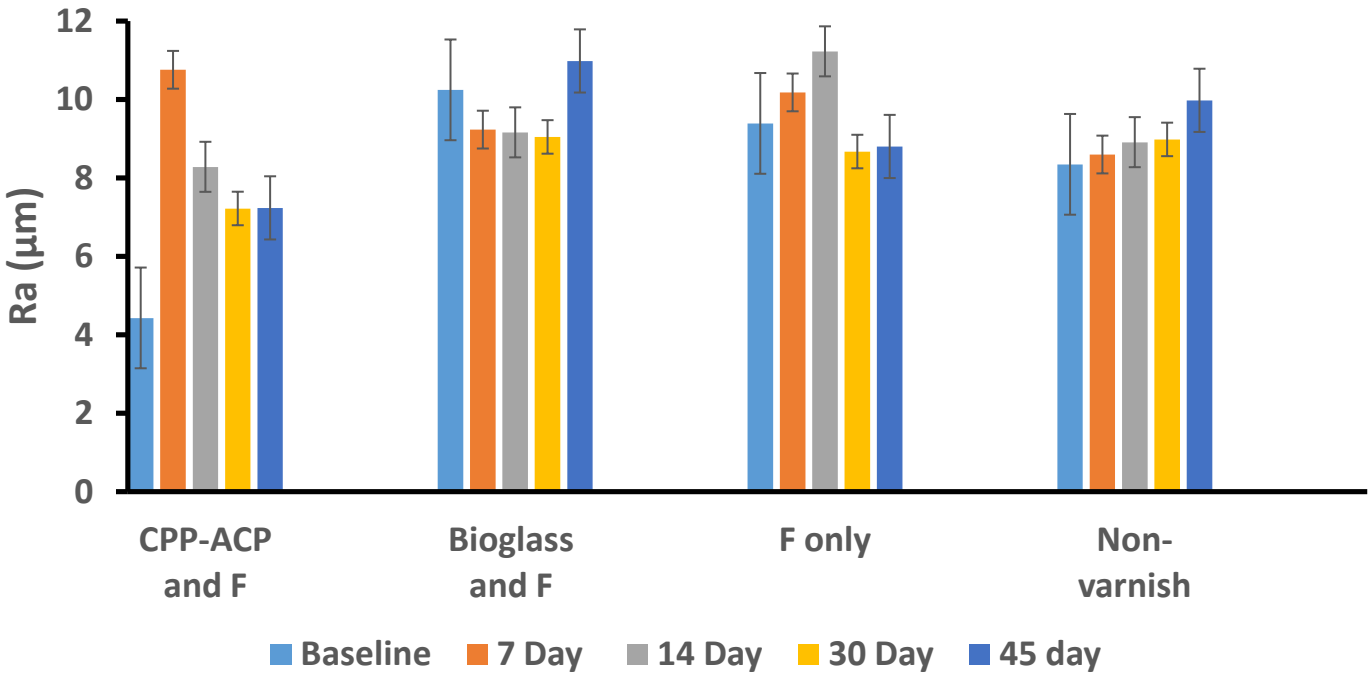
Groups	**Baseline SI scores**				**Day 45 SI scores**				**Change summary**	***p* value**
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**		
CPP-ACP and F	**-**	**14**	**4**	**2**	**1**	**19**	**-**	**-**	20% improvement 80% same SI	.042*
Bioglass and F	**-**	**1**	**14**	**5**	**3**	**16**	**1**	**-**	100 % improvement	<.001**
F only **(positive control)**	**-**	**6**	**8**	**6**	**2**	**13**	**5**	**-**	80 % improvement 10% same SI 10% worse	<.001**
Non-varnish **(Negative Control)**	**-**	**8**	**10**	**2**	**2**	**17**	**1**	**-**	60 % improvement 40% same SI	<.001**

*Significant p≤.05, ** highly significant p≤.001

3.2. Surface Roughness measurements (Ra)

The surface roughness measurements showed an increasing in Ra for CPP-ACP and F, bioglass and F, and non-varnish groups, and a decreasing trend in Ra for the F only group (Figure 2). There was a continuous increase for the non-varnish group (toothbrushing only), an irregular increase for CPP-ACP and F and bioglass and F, and an irregular decrease for the F only group. However, both test groups showed a significant change between baseline and day 45, the *p* value was 0.264 for CPP-ACP and F, and 1.00 for the other groups ($p \leq 0.05$).

Figure 2. Mean with standard error of surface roughness (Ra) for each group at each time point **(coloured)**.



3.3. Correlation between the severity index and surface roughness measurement (Ra)

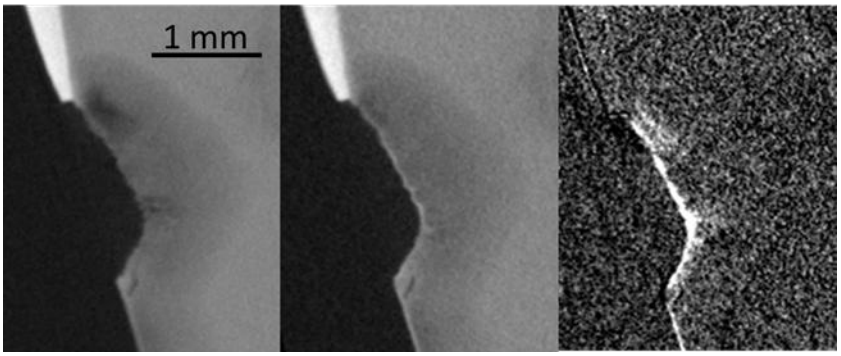
There was no correlation for the varnish groups; CPP-ACP and F (ICC=0.20), bioglass and F (0.03), and F only (0.07). A negative correlation (-0.49) was observed between the changes in the

severity index with hardness and surface roughness measurements (Ra) after 45 days in the non-varnish group only (toothbrushing only).

3.4. Mineral concentration using X-ray Microtomography

As a qualitative assessment, changes in the mineral concentration were seen as an increase in radio-opacity in the subtracted images particularly at the top surface layer of the lesion area in all groups, demonstrating an increase in the mineral concentration in the lesion area after 45 days exposure (Figure 3).

Figure 3. Part of a single slice from the reconstructed XMT image of a tooth treated by a dental varnish containing CPP-ACP and fluoride; (left) root caries before treatment, (middle) 45 days after treatment, and the difference between the two images with 8× contrast enhancement (right), shows an increase in mineral deposition as identified by an increase in radiopacity in the lesion area in the subtracted image.



The quantitative measurement showed a significant increase in mineral content in the lesion area in all groups following 45 days. The highest percentage increase was for bioglass and F samples (60%), and the lowest increase was for the non-varnish samples (22%) as illustrated in Table 4.

Table 4. The mineral concentration (g cm^{-3}) in the lesion area at baseline and 45 days (±SD), mean differences and *p* value, and the percentage of mineral change for the test groups.

Groups	Samples	Baseline	45 days	Mean difference	*p* value	overall change
CPP-ACP and F	1	1±0.14	1.4±0.15	0.4	<0.001	31% increase
	2	0.96±0.2	1.17±0.34	0.21	0.001	
	3	1.07±0.18	1.38±0.13	0.31	<0.001	
Bioglass and F	1	0.86±0.22	1.48±0.19	0.62	<0.001	60% increase
	2	0.99±0.24	1.45±0.16	0.46	<0.001	
	3	0.84±0.18	1.37±0.26	0.53	<0.001	
F only	1	0.73±0.13	0.95±0.14	0.22	<0.001	25% increase
	2	0.78±0.1	0.89±0.17	0.11	0.048	
	3	0.83±0.18	1.09±0.21	0.26	0.001	
Non-varnish	1	1.09±0.11	1.44±0.22	0.35	<0.001	22% increase
	2	1.1±0.18	1.32±0.22	0.22	0.001	
	1	1.13±0.15	1.28±0.18	0.15	0.041	

4. Discussion

This laboratory based study aimed to assess a novel experimental dental varnish containing bioglass and fluoride in comparison with a dental varnish containing CPP-ACP and fluoride on root caries. To simulate a clinical setting, specimens with natural root caries rather than artificial lesions were tested. The method of lesion assessment was carried out using validated index based on the clinical change in lesion hardness. Micro Surface Vickers Hardness Tester was not considered in this study due to the natural structure of the selected samples that could be soft with irregular surfaces. This might have disguised some parts of the Vickers micro-indenter, resulting in unreliable measurements.

The maximum reduction in the severity index was in the samples treated with bioglass and fluoride varnish (100%). This could be related to the ability of bioglass to release calcium and phosphate ions leaving silanols on the dentine surface. Bioglass has the ability to act as a nucleating agent to

re-attract calcium and phosphate ions forming a hard and acid-resistant layer in a form of hydroxyl-like crystals on the tooth surface [37,21].

The minimum reduction in the severity index was in those samples that treated with CPP-ACP and fluoride varnish (20%). This could be due to the amorphous nature of the CPP-ACP that is unlikely to adhere to the tooth structure [38]. This result confirms the observation that dental varnish with fluoride and CPP-ACP had the highest calcium and fluoride ion release in comparison to varnishes either with other calcium phosphate formulations and fluoride or with fluoride only [39, 40]. In this study, the increase in the turbidity of remineralising solution for the samples treated only with CPP-ACP and fluoride varnish that was clearly visually inspected could explain the formation of apatite crystals in the immersion solution. This suggests that fluoride varnish with CPP-ACP has the ability to release ions but is unable to retain them within the lesion area. In addition, the brushing procedure that was carried out throughout the study could challenge the retention of such a varnish has an amorphous consistency. Nevertheless, the improvement in the severity index for the CPP-ACP and fluoride group was significant ($p<0.05$). Also, none of the samples deteriorated since they either improved or remained in the same severity index category, demonstrating the effectiveness of CPP-ACP in the management of root caries. This result is consistent with the systematic review findings of Yengopal and Mickenautsch [17] who reported a significant impact of CPP-ACP in the remineralisation of both enamel, and dentine, particularly on early carious lesions.

The decrease in the severity index in the non-varnish group that received only toothbrushing with1450 ppm fluoride (F) was consistent with the general concept of the effectiveness of fluoride in the hardening and deactivation of root caries [9,10,41]. The outcome of this study was also supported by the clinical findings that regular toothbrushing twice a day with 1,450 ppm F dentifrices can manage 50% of the root carious lesions, whilst professional application of fluoride varnish once a month in addition to same brushing regimen can manage 80% of the root carious lesions [15]. Despite the promising outcome in severity index of the non-varnish group,

enhancement of the remineralisation process through selecting other treatment options such as bioglass and F or F only varnishes would be of a great value, especially in older and compromised patients since most of this cohort of patients are unable to carry out the guidelines suggested in the UK Department of Health Tool kit i.e. standardised toothbrushing twice a day for a period of two min.

The greater improvement in the severity index in the non-varnish in comparison to CPP-ACP and fluoride group could be related to the interference of the treatment with the effectiveness of fluoride from the dentifrice, as the varnish might act as a barrier inhibiting the penetration of fluoride into the demineralised area.

All groups showed difficulty to change from leathery lesions with a severity index 1 into completely hard status score 0 according to the severity index within 45 days. This may perhaps be related to the short study period using samples with natural root carious lesions of 0.5-0.1 mm in depth, rather than artificial caries with typical lesions of only microns depth. It should be noted that the minimum time for root caries to become either arrested or reversed *in vivo* was reported as three months [9,32]. Although the methodology of this study was designed to accelerate the process by keeping the samples in a remineralising solution rather than remineralisation/demineralisation cycling, a longer period is recommended for future studies with natural root caries. The use of a constant remineralisation model was also supported by other studies, since this model would provide the regular monitoring process to assess the dental products for the management of dental caries on a short-time basis, thereby simulating a best-case scenario, even if the extent of related biological aspects was limited i.e., pH cycling and demineralisation periods [42,43]

The slight increase in Ra coupled with the decrease in the severity index for CPP-ACP and fluoride, bioglass and fluoride, and non-varnish groups was inconsistent with the general concept of caries description endorsed by the International Caries Detection and Assessment System

(ICDAS). The ICDAS system indicated that root caries clinically tends to shift from rough to smooth texture whilst changing from active to inactive [44]. This could well be due to the short study period where might have been insufficient to achieve the optimal desired smoothness.

The trend of Ra measurements was irregular in varnish groups (Figure 2). This might be related to the ability of some varnish parts to retain on the lesion surface as clinically detected despite brushing twice a day for 45 days. The finding was in accord with the ability of fluoride containing dental varnishes to be well attached to the tooth structure after application, and to be retained for a longer period [45,11]. The regular trend of increase in Ra in non-varnish group that treated with toothbrushing only might be due to the precipitation of minerals from both the dentifrice and remineralising solution. The negative correlation between Ra measurements and the severity index of the non-varnish group might again thought to be related to the short study period that was insufficient to change from rough to a smooth surfaces.

The significant increase in mineral concentration in the lesion area in all groups, demonstrates the potential of these varnishes with the brushing procedure to precipitate minerals and remineralise root carious dentine. However, the greater increase in the mineral concentration was for bioglass and fluoride (60%) is possibly corresponding with the maximum improvement in severity index (100%), proving the significant impact of bioglass and fluoride on root caries management.

The pattern of mineral deposition that was mostly detected in the top surface layer was supported by the clinical finding that the surface layer of root caries mineralised more, particularly in the presence of fluoride, whilst the subsurface layer might remain unmineralised and may have been filled with dead micro-organisms [46].

5. Conclusion

This *ex vivo* study showed that the fluoride containing dental varnish with or without CPP-ACP in addition to the regular toothbrushing is effective in the hardening and arrestment of root caries. However, combination of both bioglass and fluoride potentially can enhance its effectiveness. Therefore, randomised control clinical trials are required for a period of at least two years to investigate the effectives of these dental varnishes on root caries.

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*Permission Note

Permission note

18th of December 2017

Dear Editor,

I am writing this letter to confirm that this paper entitled "**Comparison of efficacy of dental varnish containing fluoride either with CPP-ACP or bioglass on root caries: Ex vivo study**" has been submitted solely to the Journal of Dentistry, and it is not concurrently under consideration for publication in another journal. I can confirm that the submitted work, including images, are original.

Authors	Signature
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