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Effect of calcium phosphate addition to fluoride containing dental varnishes on enamel demineralization

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Effect of calcium phosphate addition to fluoride containing dental varnishes on enamel demineralization

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ABSTRACT

Background: To evaluate the ability of calcium phosphate and fluoride containing varnishes to inhibit enamel demineralization.

Methods: Six varnishes were selected for analysis: 1) Enamel Pro containing amorphous calcium phosphate; 2) [3M-ESPEClinpro](#) White containing functionalized tricalcium phosphate; 3) MI Varnish containing casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP); 4) Duraphat (first no added calcium control); 5) Profluorid (second no added calcium control) and 6) Placebo (no added calcium or fluoride control). Human enamel slabs (36) were each cut into half-slabs and covered with one of the six dental varnishes to create a window. The half-slabs were then individually immersed in a polyacrylate demineralization buffer pH 4.8 for four days at 37°C with a change of solution each day. Mineral content was determined using transverse microradiography.

Results: All fluoride-containing varnishes significantly inhibited enamel demineralization when compared with the placebo varnish. However, out of the calcium phosphate and fluoride containing varnishes only MI varnish, containing fluoride and CPP-ACP was superior to the fluoride-alone varnishes. MI varnish also released the highest levels of calcium, phosphate and fluoride ions.

Conclusion: MI varnish containing fluoride and CPP-ACP was superior to the other varnishes in protecting against enamel demineralization.

Key words: Fluoride varnish; calcium phosphate; CPP-ACP; fTCP; enamel demineralization

INTRODUCTION

Topically applied fluoride varnishes have been used extensively as an in surgery caries-preventive intervention ^{1,2}. Their ease of use, acceptability and efficacy make them an important tool in the prevention of dental caries in high-risk groups. The American Dental Association Council on Scientific Affairs recommends fluoride varnish as the preferred professionally applied fluoride for moderate to high risk patients of all age groups ³.

Fluoride varnish was developed to prolong the contact time between fluoride and the tooth surface, so that the tooth becomes more resistant to dental caries ⁴. The proposed mechanism of action is the formation of intra-oral fluoride reservoirs due to the formation of calcium fluoride ion pairs (e.g. CaF^+) which are retained on enamel and in dental plaque and slowly released to help inhibit mineral loss during demineralization ⁵⁻⁷. The formation of intra-oral CaF^+ reservoirs is limited by calcium and fluoride ion availability ⁸. This has been further demonstrated by the reported increase in anticariogenic efficacy and enhanced remineralization of early caries lesions when bioavailable calcium and phosphate ions are applied with, or in addition to, the fluoride ion in randomized, controlled clinical trials ⁹⁻¹². Hence a number of manufacturers have modified fluoride varnishes to include calcium and inorganic phosphate ions in an attempt to further improve efficacy. Fluoride containing varnishes are now available containing tricalcium phosphate modified by fumaric acid (fTCP) (~~3M-ESPE~~[Clinpro](#) White Varnish), amorphous calcium phosphate (ACP) (Enamel Pro Varnish) and casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) ¹³ (MI Varnish). However, even though the desired effect of these additions is to increase the levels of bioavailable calcium and fluoride ions intra-orally, adding calcium and phosphate salts together with fluoride ions in dental materials can lead to the opposite

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4 outcome by the formation of poorly soluble calcium fluoride phosphate phases in the
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6 material decreasing ion bioavailability^{14, 15}. It is essential therefore to ensure these
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8 innovative materials release bioavailable calcium, phosphate and fluoride ions and protect
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10 enamel against acid demineralization at least as well as, and hopefully significantly better,
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12 than the normal fluoride-alone dental varnishes. The aim of this study was to evaluate the
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14 ability of these novel calcium phosphate and fluoride containing varnishes to release
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16 calcium, phosphate and fluoride ions and to inhibit enamel demineralization.
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MATERIALS AND METHODS

Dental varnishes

Six dental varnishes were selected for analysis (Table 1): 1) Enamel Pro varnish containing amorphous calcium phosphate (ACP) with 2.26% (w/w) fluoride; 2) ~~3M-ESPE~~Clinpro White varnish containing functionalized tricalcium phosphate (fTCP) with 2.26% (w/w) fluoride; 3) MI varnish containing casein phosphopeptide amorphous calcium phosphate (CPP-ACP) with 2.26% (w/w) fluoride; 4) Duraphat varnish containing 2.26% (w/w) fluoride (no added calcium, positive control); 5) Profluorid containing 2.26 % (w/w) fluoride (second no added calcium, positive control) and 6) Placebo (no added calcium or fluoride).

Demineralization assay

Thirty six extracted human third molars were obtained from private dental practices after obtaining informed patient consent. Any extracted soft tissues were removed and the teeth washed thoroughly in distilled de-ionized water (DDW) and stored in DDW before being sterilized by exposure to 10% neutral buffered formalin solution for 2 weeks. Sound relatively planar buccal and lingual surfaces free of cracking, staining and fluorosis (as viewed under a dissecting microscope) were selected. The outer enamel surfaces were polished wet to a mirror finish using Soflex™ discs on a slow speed contra-angle dental handpiece. Each polished surface was cut from the tooth as an approximately 8×4 mm slab, using a water-cooled diamond blade saw. Each slab was cut into two halves. For one half-slab (used as the control sample), a window (approximately 1×7 mm) was created in

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4 the middle by applying acid-resistant nail polish on both sides of the window (as shown in
5 Fig. 1). For the other half-slab (used as the experimental sample), a window (approximately
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7 1×7 mm) was created in the middle by applying one of the six dental varnishes (randomly
8
9 selected) on both sides of the window (Fig. 1). Each half-slab was coded and then
10
11
12 suspended in 10 mL of unagitated demineralization buffer, consisting of 80 mL/L Noverite
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14 K-702 polyacrylate solution (Lubrizol Corporation, Wickliffe, OH), and 0.1 mol/L lactic
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16 acid (Ajax Chemicals, Auburn NSW) pH 4.8, for 4 days at 37°C¹⁶ A change of solution
17
18 was made every 24 hours at which time the slabs were removed from the solution, rinsed
19
20 thoroughly with deionised water, blotted dry and placed into fresh demineralization buffer.
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23 The slabs were similarly rinsed and dried after four days of demineralization.
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28 After demineralization the varnish was carefully removed with a scalpel from each
29
30 demineralized control enamel block and test block treated with the dental varnish. The
31
32 blocks were placed into freshly poured transparent cold curing methacrylate resin with the
33
34 lesion windows parallel. The resin vial was marked at the top corner to identify the coded
35
36 test and control blocks and the resin was allowed to set at room temperature overnight.
37
38 Sections approximately 300 µm thick were cut from the embedded blocks perpendicular to
39
40 the lesion surface using an internal annulus saw microtome. The sections were lapped down
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42 to 100 ± 5 µm using a RotoPol/RotoForce lapping instrument (Struers, Denmark) with
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44 1200 grit lapping paper. The lapped sections were removed from the lapping instrument
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46 with absolute ethanol and rinsed in deionised water, blotted dry and stored on soft tissue
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48 between glass slides. Each section, which contained the lesion treated with the coded dental
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50 varnish and the demineralized control lesion from the same enamel slab, was radiographed
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4 along with an aluminum stepwedge with 7×37.5 μm thick increments using Microchrome
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6 High Resolution glass plates (3 x 3 x 0.06 in., Microchrome, USA) and copper Kα radiation
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8 at 20 kV, 30 mA for eight minutes. Each glass plate was developed in Microchrome
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10 Developer D5 for five min, placed into glacial acetic acid stop bath for 30 seconds and then
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12 fixed in Microchrome Fixer F4 for five min.
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16 Radiographic images of the lesions were viewed via transmitted light through a Leica
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18 DM 5500B microscope (Leica, Germany). The images were acquired by a ProgRes® MF
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20 scan digital camera (Jenopik, Jena, Germany) under the control of Image-Pro Plus version
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22 7.0 imaging software on a Sci-Tech Imaging Workstation (SciTech, Preston, VIC,
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24 Australia). Images of the lesions and the neighbouring areas of sound enamel were scanned
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26 by an operator blinded to the varnish code using the program's line luminance function that
27
28 gives readings in grey values. An area free of artifacts or cracks was selected for analysis.
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30 Each scan comprised 200 readings taken from the tooth surface to sound enamel.
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35 The stepwedge image on each slide was scanned and the averaged step grey value
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37 readings were plotted against aluminum thickness. The readings for the tooth section
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39 images were within the linear portion of the stepwedge curve and linear regression was
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41 used to convert the grey value data into values of equivalent thicknesses of aluminium. The
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43 section thicknesses was measured and the %-percent mineral data computed using the
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45 equation of Angmar *et al.*¹⁷ and the linear absorption coefficients of aluminium, organic
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47 matter plus water and apatite mineral (131.5, 11.3, and 260.5 respectively). The image of
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49 the sound enamel was scanned six times and averaged to give a control sound-enamel
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51 densitometric profile. The lesion images of the dental varnish treated windows and
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4 demineralization control windows were similarly scanned and the vol% mineral content
5 profiles determined. The start and end of the lesion were defined as the points where the
6 mineral density was 20% and 95% respectively that of the sound enamel. These
7 measurements were then used to determine lesion depth. The depth of the lesion on the
8 half-slab not exposed to the dental varnish was designated LDc for lesion depth control.
9 That of the lesion on the half-slabs treated with dental varnish was designated LDv for
10 lesion depth varnish.

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21 The %-percent mineral profile of each enamel block's demineralized control and
22 dental varnish-treated lesion was compared with the sound enamel %-percent mineral
23 profile of the same section. The difference between the area under the densitometric profile
24 of the control treated lesion and the sound enamel, calculated by trapezoidal integration,
25 was represented by ΔZc . The difference between the area under the densitometric profile
26 of the dental varnish-treated and the sound enamel, calculated by trapezoidal integration,
27 was represented by ΔZv . These parameters were then used to calculate integrated mineral
28 loss between the control and varnish-treated enamel $\Delta Zc - \Delta Zv$ and % percent Inhibition of
29 demineralization:
$$\frac{\Delta Zc - \Delta Zv}{\Delta Zc} \times 100$$

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46 **Varnish ion and CPP release**

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49 Seven polyvinyl chloride plastic strips (Cumberland, NSW, Australia), 25 mm long by 16
50 mm wide, were completely coated with a single layer of one of the varnishes. The amount
51 of varnish painted on each strip was determined by weighing the strip before and after
52 applying the varnish. The painted strips were placed into 10 mL of demineralization buffer
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4 solution, coded and then placed in an incubator at 37 degrees celsius. At 24 hours each
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6 strip was removed from the demineralization solution and placed in fresh demineralization
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8 solution for another 24 h period (48 h exposure). The coded demineralization solutions
9
10 were then analysed using ion chromatography to determine the release of calcium,
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12 inorganic phosphate and fluoride ions.
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16 The ion chromatography system was equipped with cation (IonPac CS12; Dionex,
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18 CA, USA) and anion (IonPac AS18; Dionex, CA, USA) columns and two separated
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20 conductivity detectors (ICS-3000; Dionex, CA, USA). A combined seven anion standard
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22 (#56933; Dionex, CA, USA) and a combined six cation standard (#046070; Dionex, CA,
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24 USA) were diluted with distilled deionized water to several concentrations to calibrate and
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26 quantify the conductivity readings. The cumulative release of calcium, inorganic phosphate
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28 and fluoride ions was expressed as μM and μmol per gram of varnish applied to the strips.
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33 Samples were also analysed by mass spectrometry (MS) using an Ultraflex III
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35 MALDI-TOF/TOF instrument (Bruker Daltonics, Bremen, Germany) utilising a 2,5-
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37 dihydroxybenzoic acid matrix (50 mg/mL in 40% acetonitrile/60% milliQ water plus 0.1%
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39 TFA) spotted onto a ground steel plate (MTP384). MS/MS was carried out using LIFT and
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41 FlexAnalysis software (Bruker Daltonics) used to analyse the spectra and label fragment
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43 peaks. To identify casein peptides the labelled MS/MS spectra were imported into the
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45 Bruker BioTools software and Mascot (Matrix Science, London UK) used to search a
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47 database of bovine casein sequences from SwissProt.
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52 The ionic strength, calcium, phosphate, fluoride and hydrogen ion concentrations of
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54 the demineralization solutions were used in an iterative computational procedure with the
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56 expanded Debye – Hückel equation to calculate ion activity fugacities¹⁸. The ion activities
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4 were then used to determine the apparent ion activity products (IAP) for hydroxyapatite
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6 (HA) and fluorapatite (FA). The solubility products (Ksp) for HA¹⁹ and FA²⁰ were then
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8 used together with the respective IAPs to determine the apparent degree of saturation (DS)
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10 of the solutions with respect to HA and FA using the following equation: $DS =$
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12 $(IAP/Ksp)^{1/n}$ where IAP is the ion activity product, Ksp the solubility product of calcium
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14 phosphate phase and n equals the number of ions in a unit cell¹⁸.
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20 21 **Statistical analyses**

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23 The data were statistically analyzed using a linear mixed model with SPSS Version 22
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25 statistical software (SPSS Inc, Chicago, IL, USA). For all statistical tests, the significance
26
27 level was set at $\alpha = 0.05$. Comparisons between the varnish-treated lesion depth, integrated
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29 mineral loss and percent% inhibition of demineralization were determined using pairwise
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31 comparisons with a Sidak adjustment for multiple comparisons. Once all the densitometric
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33 and ion release data were entered, locked and statistical analysis completed the code for the
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35 different varnishes was released.
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RESULTS

Inhibition of demineralization

There was no significant difference in lesion depth and mineral loss for the control enamel half-slabs which were used to pair with their matching half-slab treated with one of the dental varnishes (Fig. 1). The mean depth of the lesion in the control enamel half-slabs (LDc) was $111.05 \pm 9.82 \mu\text{m}$ and mean integrated mineral loss was $3,789.45 \pm 516.91 \text{ vol\%min.}\mu\text{m}$ (Table 2). All fluoride-containing varnishes tested significantly inhibited enamel demineralisation when compared with the placebo varnish (Table 2). There was no significant difference in inhibition between the calcium phosphate-containing fluoride varnishes Enamel Pro and [3M-ESPE Clinpro White](#) and the fluoride-alone control varnishes Duraphat and Profluorid (Table 2). The only calcium phosphate-containing varnish that inhibited demineralization significantly ($p < 0.001$) better than the fluoride-alone varnishes was the MI varnish containing CPP-ACP, which provided 130% greater inhibition when compared with the Duraphat fluoride-alone control (Table 2). The superior inhibition of demineralization by MI varnish was shown both in reduction of mineral loss and reduction of lesion depth (Table 2).

Ion release

The release of calcium, phosphate and fluoride ions from the varnishes into the lactic acid demineralization buffer over 24 h and 48 h is shown in Table 3a and 3b respectively. None of the varnishes significantly altered the pH of the demineralization buffer hence the inhibition of demineralization was attributed to ion release. It was interesting to note that the two varnishes without added calcium (Duraphat and Profluorid) did release calcium

ions, as did the placebo varnish, at 5-6 μmol of Ca per g of varnish. This amount of calcium was fully released in the first 24 h of exposure to the demineralization buffer (Table 3a and 3b). The 3M-ESPEClinpro White varnish with added tricalcium phosphate did not release any more calcium in the first 24 h than the control varnishes without added calcium (Table 3a). Only two varnishes significantly released more calcium than the control varnishes in 24 h (Table 3a). MI varnish significantly released more calcium than all the other varnishes in 24 h including those with added calcium phosphate (3M-ESPEClinpro White and Enamel Pro) (Table 3a). After 48 h the release of calcium from 3M-ESPEClinpro White and Enamel Pro with added calcium phosphate had increased relative to the control varnishes without added calcium (Table 3b). However, the varnish with the greatest release of Ca at 48 h was still MI varnish at 53.52 μmol Ca/g compared with 3M-ESPEClinpro White at 19.56 $\mu\text{mol/g}$ and Enamel Pro at 19.39 $\mu\text{mol/g}$ (Table 3b).

Only two varnishes released inorganic phosphate at levels significantly higher than the control and placebo varnishes in 24 and 48 h of exposure to the demineralization buffer and these were Enamel Pro and MI varnish (Tables 3a and 3b). Surprisingly Enamel Pro released a large amount of inorganic phosphate, more than ten times the level of calcium released from the same varnish (Tables 3a and 3b). The level of phosphate release from MI varnish was stoichiometrically similar to that of the calcium ion released from the same varnish (Tables 3a and 3b).

For fluoride ion MI varnish exhibited the highest release in 24 h (1,149 $\mu\text{mol/g}$) which represented close to full release (96%) of the added fluoride. Enamel Pro released approximately 41% (506.72 $\mu\text{mol/g}$) of its added fluoride in the first 24 h. 3M-ESPEClinpro White and Profluorid released approximately 16% (175.87 $\mu\text{mol/g}$) and

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4 17% (201.34 $\mu\text{mol/g}$) respectively of their added fluoride in 24 h. The order of fluoride
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7 release for the different varnishes after 48 h was the same as at 24 h with MI varnish
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9 showing the highest level (1,183.27 $\mu\text{mol/g}$) of fluoride ion release at 48 h.
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11 ~~In conclusion~~Overall, MI varnish released the highest levels of calcium, phosphate
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13 and fluoride ions and also produced the highest degree of saturation with respect to
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15 fluorapatite. MI varnish also released intact CPP into the demineralization buffer (Fig. 2).
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DISCUSSION

The results of this study showed that all of the commercial dental varnishes containing 5% sodium fluoride significantly protected enamel from demineralization by 22-58% when compared with the placebo varnish not containing fluoride. All the varnishes were found to release calcium ions as well as fluoride ions to some degree however only one varnish showed high calcium, phosphate and fluoride release and was significantly superior in the inhibition of enamel demineralization and that was MI varnish containing CPP-ACP and fluoride.

Fluoride varnish has a short life span in the oral environment as it is removed by the action of the cheeks and tongue, salivary flow, mastication and oral hygiene procedures. Therefore, varnishes should release their ions in a relatively short time period before the varnish is lost. It has been estimated that varnishes only remain *in situ* for up to 24 hours²¹. Therefore, in this current study fluoride, calcium and phosphate release were examined in the first 24 hours and 48 hours. Differences were found in the ion release of calcium, inorganic phosphate and fluoride ions from the different varnishes.

Duraphat was chosen as the positive control in this study as it is a commonly used fluoride varnish that has been tested by other authors in similar studies and is well defined in terms of fluoride release and caries prevention^{2, 22, 23}. Considering the importance of fluoride ion release from the varnishes, it is imperative that the addition of calcium and phosphate ions do not reduce the availability of the fluoride ions. All calcium-containing varnishes in the current study were found to have a cumulative release of fluoride ions at 24 hours and 48 hours that was significantly greater than that of the positive control Duraphat.

The information on the packaging indicated that MI Varnish, [3M-ESPE Clinpro White](#),

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4 Enamel Pro, Profluorid and Duraphat all contained 2.26 wt% fluoride. ~~Therefore, a~~At 24
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6 hours the percentage fluoride release from MI Varnish, Enamel Pro, ~~3M-ESPE~~Clinpro
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8 White, Profluorid and Duraphat was 96%, 41%, 16%, 17%, and 5% respectively.
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11 Therefore, MI Varnish released nearly all of its fluoride in 24 hours. Overall, the MI
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13 varnish performed best in terms of fluoride ion release at 24 hours and 48 hours.
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17 Calcium ions were released from all varnishes tested. The greatest cumulative
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19 calcium release at 24 hours and 48 hours was from MI Varnish followed by Enamel Pro
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21 and ~~3M-ESPE~~Clinpro White. ~~3M-ESPE~~Clinpro White had comparable calcium ion release
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23 to Profluorid and Duraphat at 24 hours but its calcium ion release at 48 hours was greater
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25 than the control varnishes.
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29 The release of inorganic phosphate ion from Enamel Pro was the highest followed by
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31 MI Varnish. The other varnishes showed very low phosphate release. High inorganic
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33 phosphate levels may be problematic for the retention of bioavailable fluoride reservoirs as
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35 relatively high phosphate levels can inhibit the formation of CaF^+ and CaF_2 and favour the
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37 formation of poorly soluble phases²⁴. The ectopic formation of poorly soluble fluoride
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39 phases may decrease the bioavailability of the fluoride ion and may promote calculus
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41 formation. Therefore, the relatively high inorganic phosphate release from Enamel Pro
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43 varnish may be counter-productive in terms of localizing bioavailable fluoride ions in the
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45 oral environment.
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50 Recently a study by Schermerhorn *et al.*²¹ examined the fluoride uptake into and
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52 onto sound and demineralized bovine enamel that had been treated with an ACP (Enamel
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54 Pro) and fTCP-containing dental varnish (Omni Vanish White Varnish). The varnishes
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56 were applied to the sound enamel core adjacent to a demineralized core and then both were
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4 exposed to artificial saliva for 24 hours. It was found that the ACP varnish promoted
5 significantly more fluoride deposition onto sound and demineralized enamel than the fTCP
6 containing varnish. The authors postulated that this was due to the ACP varnish containing
7 a higher level of available calcium and phosphate ions ²¹. The 24 hour ion release data from
8 the current study is consistent with this explanation as the ACP containing varnish had
9 significantly higher 24 hour release of calcium, inorganic phosphate and fluoride ions than
10 the fTCP-containing varnish. However, it is not known whether the ACP containing
11 varnish deposited this fluoride as a precipitate or was incorporated into the enamel crystal
12 structure. As discussed earlier *in vitro* precipitated fluoride as a surface solid may be of
13 limited value in influencing the dynamics of the dental caries process *in vivo*. The authors
14 of that study did not distinguish between the precipitated fluoride and the subsurface
15 fluoride or fluorapatite formed through remineralization nor did they measure subsurface
16 mineral content ²¹. The low release of inorganic phosphate ions from [3M-ESPEClinpro](#)
17 White may be explained by the low amount of fTCP added to the varnish ²⁵ or by the low
18 solubility of tricalcium phosphate. The results of our current study did not show a
19 significantly superior protection of subsurface enamel demineralization by the Enamel Pro
20 varnish (ACP varnish) when compared with the [3M-ESPEClinpro White](#) varnish even
21 though it did release more calcium and fluoride ions than the latter varnish at 24 h (Tables 1
22 and 2).

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49 Overall MI Varnish released very high amounts of calcium and inorganic phosphate
50 ions which is consistent with the bioavailable nature of calcium and phosphate ions in CPP-
51 ACP contained within the varnish ²⁶. Enamel Pro released a high amount of inorganic
52 phosphate ions but a relatively lower level of calcium ions, which is consistent with the
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4 order of ingredients listed on the material safety data sheet (Table 1). 3M-ESPEClinpro
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6 White released a small amount of calcium and low levels of inorganic phosphate per g of
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8 varnish. The 3M-ESPEClinpro White varnish was quite viscous and required much greater
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10 application of the varnish to cover the same surface area, e.g. 106% more than MI varnish
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12 and 39% more than Profluorid. Hence, ion release levels per g of varnish were not high for
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14 this varnish. All varnishes released measurable fluoride and calcium, however MI Varnish
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16 containing CPP-ACP had the highest release of calcium, phosphate and fluoride ions and it
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18 was the only varnish significantly superior to the other varnishes in preventing subsurface
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20 enamel demineralization. The results of the current study are consistent with previous
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22 studies suggesting superiority of the MI varnish in terms of very high ion release and
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24 protection of enamel demineralization as measured using optical coherence tomography ²⁷,
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33 The high release of calcium, inorganic phosphate and fluoride ions from MI varnish
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35 as well as the release of CPP is consistent with the presence of bioavailable CPP-ACFP
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37 nanocomplexes ²⁹⁻³¹. These nanocomplexes have a hydrodynamic radius of 2.12 ± 0.26 nm
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39 ²⁹ and are electroneutral ion clusters allowing rapid diffusion out of the varnish and into the
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41 enamel subsurface through intraprismatic spaces ³². The presence of the CPP-ACFP
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43 nanocomplexes would explain the superior ability of MI varnish to inhibit demineralization
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45 in this study as the combination of CPP-ACP and F to form CPP-ACFP nanocomplexes has
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47 been shown to be superior to F alone in inhibiting enamel demineralization and promoting
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49 remineralisation in a number of *in situ* and *in vivo* randomized controlled clinical trials ^{11, 12,}
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CONCLUSION

Dental varnishes containing calcium and fluoride significantly inhibited enamel subsurface demineralization. MI Varnish containing CPP-ACP and fluoride exhibited the highest fluoride and calcium ion release and produced the greatest inhibition of demineralization. These novel varnishes may have potential as part of a preventive regimen to lower caries risk.

For Review Only

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FIGURE LEGENDS**Fig. 1. Enamel slab window preparation.**

The two half-slabs, one with dental varnish applied (blue) and the other with acid-assistant nail polish applied (red) as a control were incubated separately in the demineralization buffer and then matched together for embedding, sectioning and mineral content analysis using TMR.

Fig. 2. MALDI-TOF mass spectrum of MI varnish 48 hr sample. The peak at m/z 3122.406 is the major casein phosphopeptide of CPP-ACP, beta-casein tryptic fragment 1-25. The peaks at 3144, 3166 and 3188 are sodium ion adducts of the same peptide. The peak at m/z 3024 is a MS fragment peak indicating the loss of one phosphate further confirming the release of the intact phosphopeptide.

Table 1 - Varnishes selected for analysis

Product	Manufacturer	Batch No.	Expiry Date	Composition (w/w)	Source
Placebo	GC, Tokyo, Japan	120613	n/a	30-50% polyvinyl acetate, 10-30% hydrogenated rosin, 20-30% ethanol, 1-5% silicon dioxide	Provided
Duraphat	Colgate Oral Care, Sydney, NSW	127948	06-2015	30-60% colophonium, 10-30% ethanol, 5% sodium fluoride, other ingredients	MSDS†
Profluorid	Voco GmbH, Cuxhaven, Germany	1204258	01-2014	10-25% ethanol, 5% sodium fluoride	MSDS
Enamel Pro	Premier Dental Products, PA, USA	38002	08-2013	rosin, ethanol, 5% sodium fluoride, dibasic sodium phosphate, calcium sulfate dihydrate	MSDS
MI Varnish	GC, Tokyo, Japan	1204172	04-2015	30-50% polyvinyl acetate, 10-30% hydrogenated rosin, 20-30% ethanol, 5% sodium fluoride, 1-5% CPP-ACP, 1-5% silicon dioxide	MSDS
3M ESPE Clinp ro White	3M ESPE, MN, USA	C14726 K1L	5-2013	30-75% pentaerythritol glycerol ester of colophony resin, 10-15% n-hexane, 1-15% ethyl alcohol, 5% sodium fluoride, 1-5% flavour enhancer, 1-5% thickener, 1-5% food grade flavour, <5% modified tricalcium phosphate	MSDS

†MSDS = material safety data sheet

Table 2 - Lesion depth change, difference in integrated mineral loss and percentage inhibition of demineralization produced by the various dental varnishes.

Product	LDC-LDv† (µm)	ΔZc-ΔZv‡ (vol%min.µm)	%Inhibition of Demineralization
Placebo	1.79 ± 2.73*	1.68 ± 133.67*	0.16 ± 3.42*
Duraphat	27.52 ± 8.92	825.89 ± 402.04	22.25 ± 9.34
Profluorid	28.71 ± 12.03	1006.76 ± 218.12	30.54 ± 9.30
Enamel Pro	29.22 ± 8.13	1219.73 ± 83.44	32.17 ± 3.01
3M-ESPE wClinpro White	33.64 ± 3.46	1253.03 ± 217.76	33.55 ± 2.40
MI Varnish	45.19 ± 4.39*	2210.09 ± 431.27*	58.28 ± 8.61*

† LDC = lesion depth of control half-slab without exposure to varnish (control). There was no significant difference in LDC values for the different varnish-treatment groups. The mean LDC was 111.05 ± 9.82 µm. LDv = lesion depth of varnish treated lesion.

‡ ΔZc= integrated mineral loss of control half-slab without exposure to varnish (control). There was no significant difference in ΔZc values for the different varnish-treated groups. The mean ΔZc was 3,789.45 ± 516.91 vol%min.µm. ΔZv = integrated mineral loss of varnish-treated lesion.

* Values so marked are significantly different to all other values in the column (p < 0.001). Values joined by a vertical line are not significantly different.

Table 3a. Calcium, phosphate and fluoride ion release from the varnishes into the demineralization solution after a 24 hour exposure.

Product	Weight of varnish (mg)	pH †	Ca ⁺² released		PO ₄ ⁻³ released		F released	
			μM	μmol/g	μM	μmol/g	μM	μmol/g
Placebo	24.92 ± 6.78	4.78 ± 0.02	11.41 ± 2.90	4.94 ± 1.08	17.57 ± 7.81	6.96 ± 2.71	0.00 ± 0.00	0.00 ± 0.00
Duraphat	47.43 ± 4.38	4.78 ± 0.02	26.49 ± 17.09	5.66 ± 3.78	15.87 ± 11.29	3.32 ± 2.33	264.69 ± 17.27	56.30 ± 7.11
Profluorid	49.92 ± 5.49	4.76 ± 0.03	23.22 ± 7.67	4.80 ± 2.00	6.79 ± 2.74	1.36 ± 0.51	987.57 ± 125.68	201.34 ± 43.24
Enamel Pro	49.17 ± 12.27	4.80 ± 0.01	54.62 ± 10.55	11.49 ± 2.99*	808.95 ± 342.54	159.48 ± 49.88*	2383.72 ± 143.37	506.72 ± 114.29*
3M-ESPE Clinpro White MI Varnish	69.25 ± 8.02	4.78 ± 0.03	28.43 ± 10.67	4.16 ± 1.55	13.52 ± 6.70	2.02 ± 1.12	1,278.76 ± 74.41	175.87 ± 21.01
	33.67 ± 6.55	4.80 ± 0.01	117.48 ± 58.73	36.77 ± 21.36*	145.99 ± 55.59	44.75 ± 20.39*	3,828.47 ± 476.62	1,149.31 ± 81.10*

† pH: no significant difference between any of the varnishes.

* Values so marked are significantly different to all other values in the column (p < 0.05).

Table 3b. Calcium, phosphate and fluoride ion release from the varnishes into the demineralization solution after a 48 hour exposure.

	Weight of varnish (mg)	pH †	Ca ⁺² released		PO ₄ ⁻³ released		F ⁻ released	
			μM	μmol/g	μM	μmol/g	μM	μmol/g
Placebo	24.92 ± 6.78	4.83 ± 0.04	11.41 ± 2.90	4.94 ± 1.08	17.57 ± 7.81	6.96 ± 2.71	0.00 ± 0.00	0.00 ± 0.00
Duraphat	47.43 ± 4.38	4.83 ± 0.02	26.49 ± 17.09	5.66 ± 3.78	15.87 ± 11.29	3.32 ± 2.33	674.36 ± 69.38	143.18 ± 18.81
Profluorid	49.92 ± 5.49	4.85 ± 0.03	23.22 ± 7.67	4.80 ± 2.00	6.79 ± 2.74	1.36 ± 0.51	1,781.84 ± 207.94	362.54 ± 70.86
Enamel Pro	49.17 ± 12.27	4.81 ± 0.01	98.63 ± 48.17	19.39 ± 6.22	1,007.25 ± 462.11	196.81 ± 67.31*	2,826.12 ± 199.14	598.53 ± 124.54
3M ESPE Clinpro White MI Varnish	69.25 ± 8.02	4.82 ± 0.02	138.86 ± 45.85	19.56 ± 5.84	13.52 ± 6.70	2.02 ± 1.12	2,647.97 ± 186.63	385.71 ± 44.53
MI Varnish	33.67 ± 6.55	4.82 ± 0.02	176.23 ± 60.67	53.52 ± 20.85*	167.49 ± 58.09	51.24 ± 21.36*	3,940.09 ± 479.31	1,183.27 ± 85.84*

† pH: no significant differences.

* Values so marked are significantly different to all other values in the column (p < 0.05).

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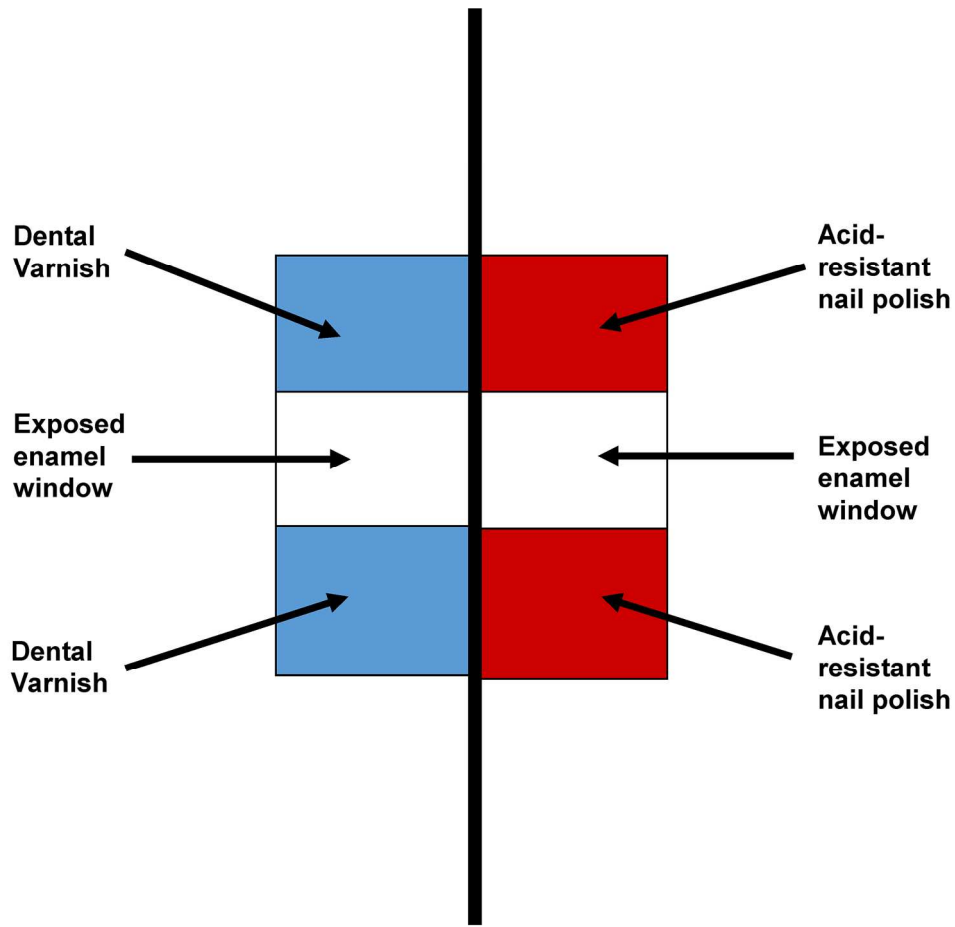


Fig. 1. Enamel slab window preparation.
The two half-slabs, one with dental varnish applied (blue) and the other with acid-assistant nail polish applied (red) as a control were incubated separately in the demineralization buffer and then matched together for embedding, sectioning and mineral content analysis using TMR.
180x181mm (300 x 300 DPI)

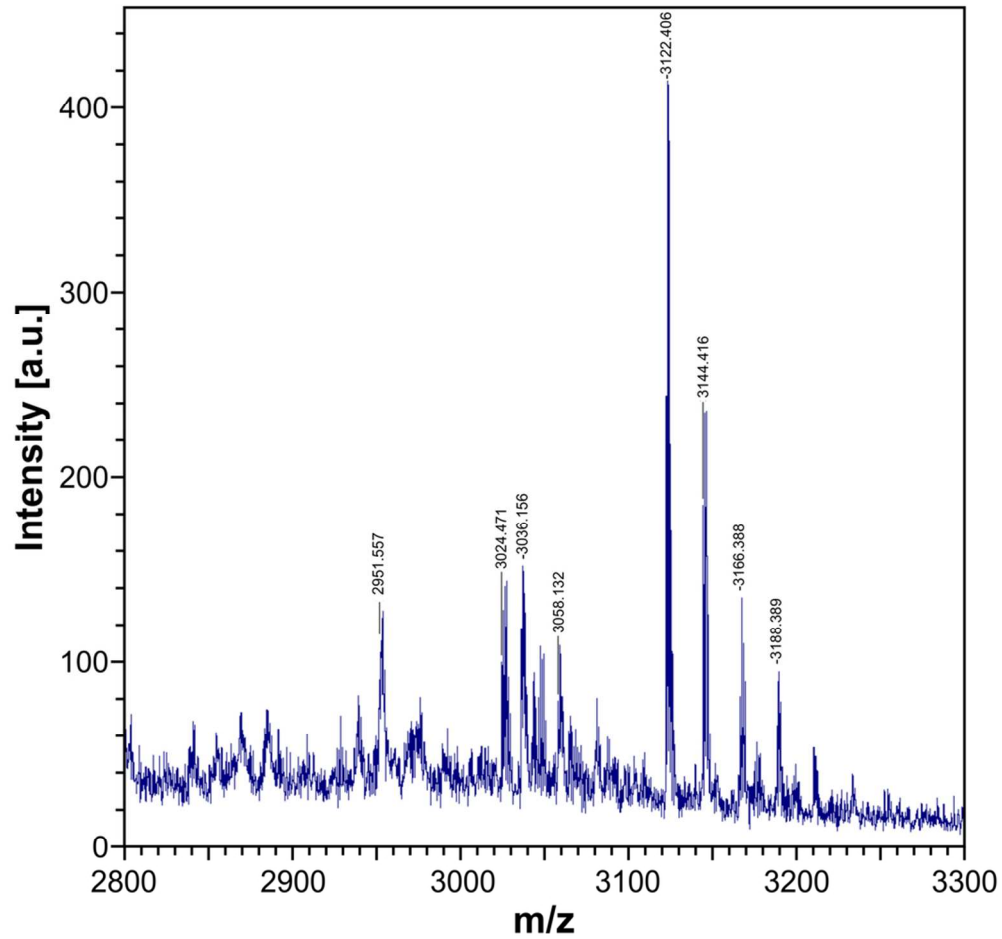


Fig. 2. MALDI-TOF mass spectrum of MI varnish 48 hr sample. The peak at m/z 3122.406 is the major casein phosphopeptide of CPP-ACP, beta-casein tryptic fragment 1-25. The peaks at 3144, 3166 and 3188 are sodium ion adducts of the same peptide. The peak at m/z 3024 is a MS fragment peak indicating the loss of one phosphate further confirming the release of the intact phosphopeptide.

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