Enamel Remineralization Effect using Dewaxed Shellac Varnishes with Added Carbonate Apatite and Tricalcium Phosphate

Arief Cahyanto[1,2,*], Dena Fadhilah Marwa[3], Kesya Saragih[3], Veni Takarini[1,2], Zulia Hasratiningsih[1,2]

1. Department of Dental Materials Science and Technology, Faculty of Dentistry, Universitas Padjadjaran, Indonesia.
2. Oral Biomaterials Study Centre, Faculty of Dentistry, Universitas Padjadjaran, Indonesia.
3. Graduate dental student of Faculty of Dentistry, Universitas Padjadjaran, Indonesia.

Abstract

Dental varnish is proven to prevent tooth demineralization, while remineralization effect on previous studies has not been tested. Remineralization may occur naturally in the oral cavity and the process will be optimal if remineralization agents such as carbonate apatite (CO_3Ap) and α-tricalcium phosphate (α-TCP) were added.

The aim of this present study is to investigate the dewaxed shellac varnishes with added CO_3Ap and α-TCP can remineralize enamel. All teeth samples were divided into two groups. The first group (group A) was dewaxed shellac varnishes without added CO_3Ap and α-TCP, and the second group (group B) was dewaxed shellac varnishes with added CO_3Ap and α-TCP. The SEM results of group A samples showed smooth and homogeneous surface. Whereas the results of group B samples showed rough surface. The EDS results of all groups showed high element of carbon (C), however, group B there were calcium (Ca) and phosphate (P) elements. Varnish has good penetration ability so that the microporosity of all samples can be covered. The only difference is the surface of group B where there were granules from CO_3Ap and α-TCP. The high carbon element (C) was due to shellac's composition. While the appearance of calcium (Ca) and phosphate (P) in sample B is increased because of the CO_3Ap and α-TCP.

This study concludes that varnish from dewaxed shellac raw material with added CO_3Ap and α-TCP could be synthesized and potentially remineralized enamel.

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Introduction

Demineralization is the process of dissolving important elements of the teeth that occur due to acid exposure.[1] Demineralization can be prevented and regenerated by administering remineralization-trigger agents. Remineralization does always occur naturally, yet the activity level varies according to each oral cavity condition. So that the remineralization process runs optimally, a remineralization agent is needed.[2,3] The ideal remineralization agent must have the requirement that it can deliver calcium and phosphate on the surface of the teeth, of course, to replace dissolved calcium and phosphate.[4-6]

Corresponding author:
Arief Cahyanto, Ph.D.
Department of Dental Materials Science and Technology, Faculty of Dentistry, Universitas Padjadjaran, Jalan Sekeloa Selatan 1 Bandung, 40134, West Java, Indonesia
E-mail: arief.cahyanto@fkg.unpad.ac.id

One of the materials that can form hydroxyapatite layer that resembles bone mineral phase and has a good adaptation between apatite and bone tissue is carbonate apatite (CO_3Ap).[7-16] Lee et al. (2008) stated that toothpaste that contains 20% nano CO_3Ap shows dentinal tubules closure in the average of 79.5% resulting in reduction of dentin hypersensitivity.[17] Another material that can form hydroxyapatite structure is α-Tricalcium Phosphate (α-TCP).[4] α-TCP is a biocompatible material that can form bonds to the bone surface without fibrous tissue intervention layer and has the ability to support bone growth.[18] Study-related to TCP as one of the remineralization agents had been done by Patil et al., in 2013 that the TCP has better remineralization effect than Casein Phosphopeptide-Amorphous Calcium Phosphate

Fluoride (CPP-ACPF) and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).[19]

Demineralization is proven to be prevented by using varnish. Studies on fluoride varnish with shellac as raw material had been done before. In both studies, the varnish effect of fluoride in reducing demineralization was tested, while the remineralization effect has not been tested.[18,19] Varnish has an advantage compared to toothpaste which is insoluble in water so that the content contained in it can last longer attached to the teeth surface.[20] Innovation in this study is to add CO_3Ap and α-TCP as active materials to the varnish formula and to test its remineralization effect.

Materials and methods

This study was conducted by synthesizing varnish made from dewaxed shellac with added CO_3Ap and α-TCP (Taihei Chemical Industrial Co. Ltd., Tokyo, Japan). The CO_3Ap powder was fabricated by the previous study.[8,16] The CO_3Ap powder then ball mill to get smaller particle size, however, the α-TCP was employed without further treatment. The Average of the CO_3Ap particle size after ball mill is 1.154 μm and the α-TCP particle size was 1.007 μm (Horiba Scientific SZ-100 Nanopartica, Tokyo, Japan). The dewaxed shellac was dissolved in ethanol 97% and mixed with 5% CO_3Ap and 5% α-TCP, then stirred until homogeneous. Extracted human maxillary premolar teeth samples were collected from the Orthodontics and Oral Surgery departments at Faculty of Dentistry Dental Hospital, Universitas Padjadjaran, according to protocols approved by the Research Ethics Committee of Universitas Padjadjaran, number 1040/UN6.KEP/EC/2019.

All teeth samples (n = 5 of each group) were cleaned and disinfected before cut through the cementoenamel junction. Then, the etching agent of H_3PO_4 37% (3M Espe, Neuss, Germany) was applied on the enamel for 30 seconds and rinsed off using water until clean. Samples were divided into 2 groups, group A and B. In group A, varnish formula without added of CO_3Ap and α-TCP was applied for 4 and 8 smears, respectively. While in group B, varnish formula with added CO_3Ap and α-TCP was applied for 4 and 8 smears, respectively. The samples were then immersed in artificial saliva (30 mL per sample) for 24 hours. Samples then tested using Scanning Electron Microscope (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS) (JEOL JSM-IT3000, Tokyo, Japan).

Results

The results of particle size analysis characterization showed that the CO_3Ap particles after milled had an average diameter size of 1.154 μm and the α-TCP of 1.007 μm as shown in Figure 1 and 2.

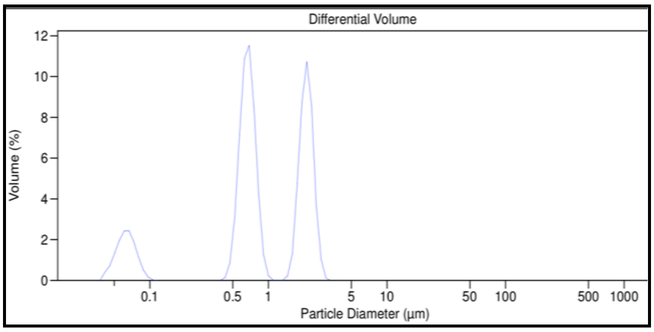


Figure 1. The average particle size of CO_3Ap.

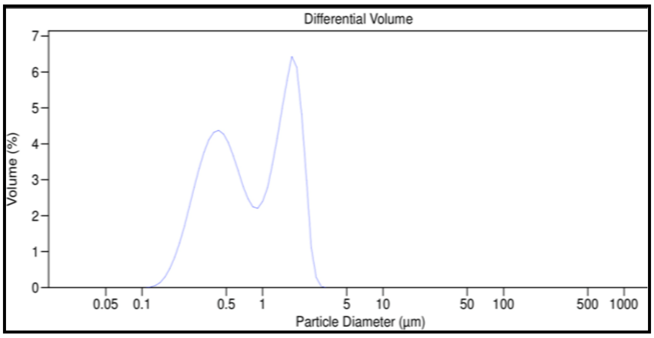


Figure 2. The average particle size of α-TCP.

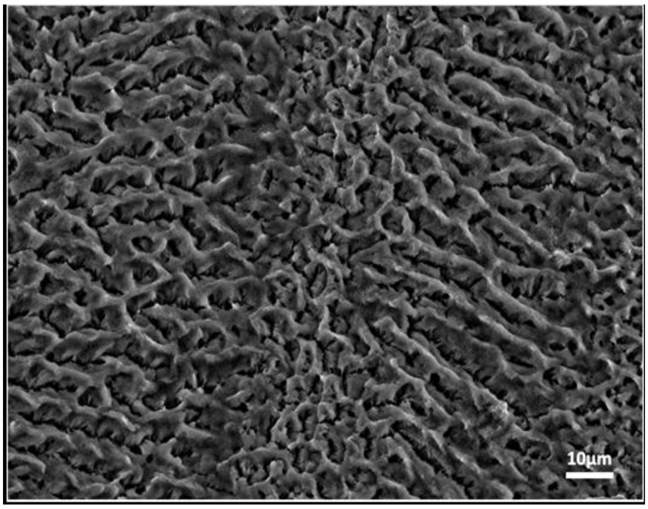


Figure 3. SEM result of etched tooth.

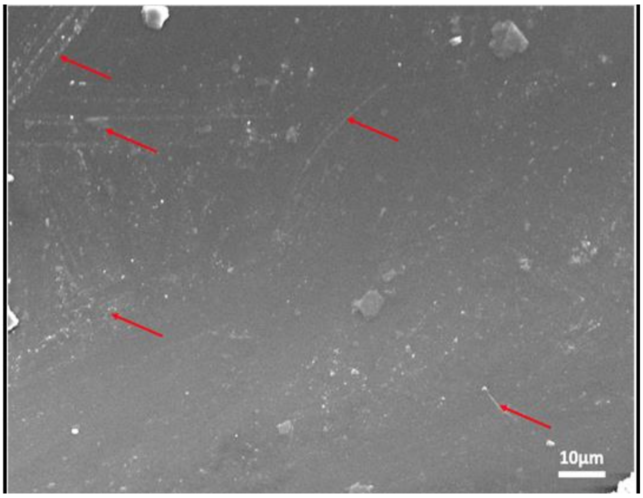


Figure 4. SEM result sample varnish without CO_3Ap and α-TCP (4 smears).

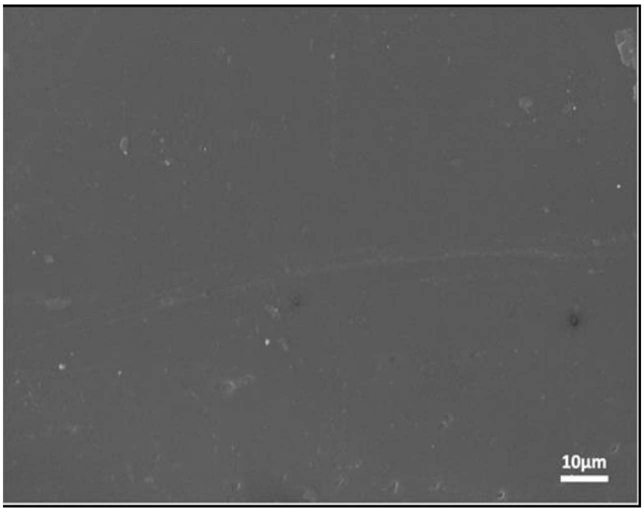


Figure 5. SEM result sample varnish without CO_3Ap and α-TCP (8 smears).

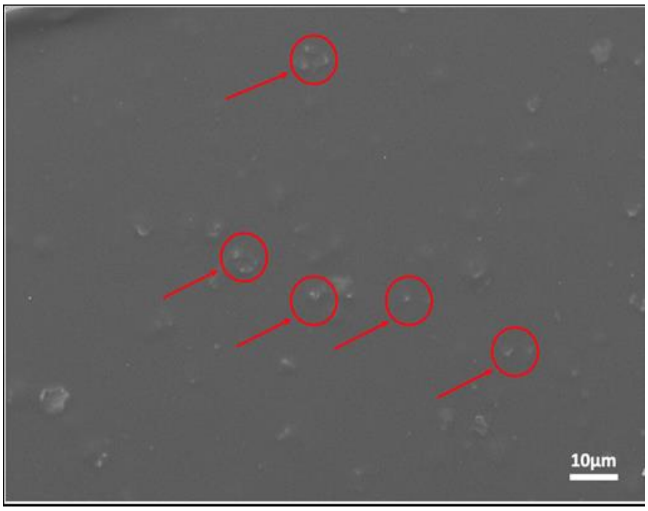


Figure 6. SEM result sample varnish with CO_3Ap and α-TCP (4 smears).

The SEM result of etched tooth showed enamel demineralization area with honeycomb structure and uneven surface that was shown in Figure 3. Figure 4 shows the SEM result of group A tooth sample with 4 varnish smears showed microporosity closure on the teeth surface although it was still imperfect because there were white lines and small dots scattered (pointed by the red arrows) almost all over the tooth surface. Figure 5 that was group A with 8 smears, the microporosity closure of the sample was way better and the surface produced was smooth and even.

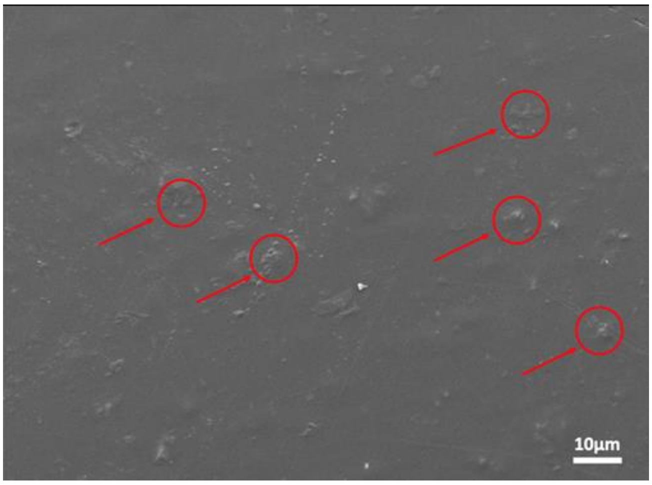
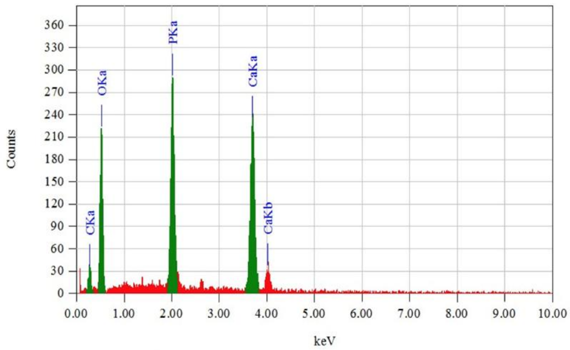


Figure 7. SEM result sample varnish with CO_3Ap and α-TCP (8 smears).



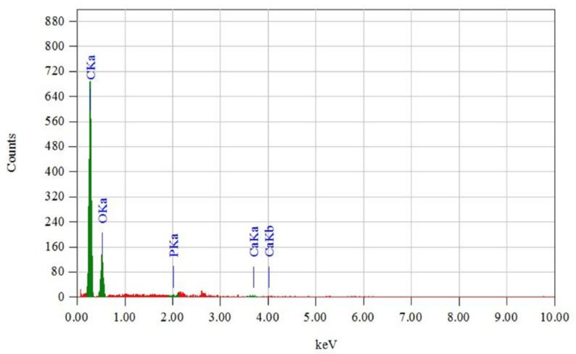
ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.1198

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	K
C K	0.277	8.65	0.36	14.64				2.9424
O K	0.525	47.87	1.22	60.79				35.0467
P K	2.013	16.95	0.41	11.12				22.8893
Ca K	3.690	26.53	0.65	13.45				39.1215
Total		100.00		100.00				

Figure 8. EDS results of etched tooth.

Figure 6 shows the group B sample with 4 smears. The result indicated very good microporosity closure, there were granules (pointed by the red arrows and circles) on the surface which considered as CO_3Ap and α-TCP so the sample surface became uneven. Group B sample with 8 smears as shown in Figure 7 had a similar picture with the previous sample but with more CO_3Ap and α-TCP granules due to more smears applied.

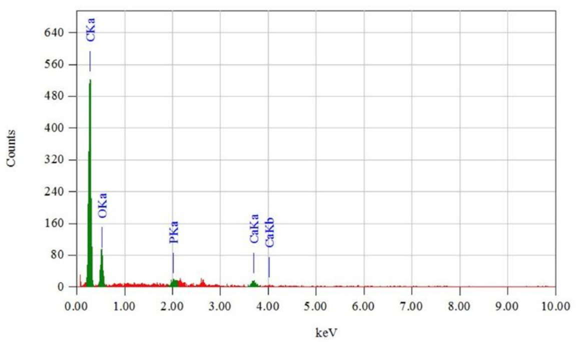
EDS testing was performed to determine the elements found on the teeth surface which had been applied varnish. Some elements that will be seen on EDS testing are Carbon (C), Oxygen (O), Phosphate (P), and Calcium (Ca) as seen in Figures 8, 9, and 10.



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.2114

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	K
C K	0.277	69.90	0.60	75.65				78.0853
O K	0.525	29.89	1.05	24.28				21.5816
P K								
Ca K	3.690	0.21	0.08	0.07				0.3331
Total		100.00		100.00				

Figure 9. EDS results of varnish without CO_3Ap and α-TCP.



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.2331

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	K
C K	0.277	71.03	0.68	77.50				77.6644
O K	0.525	26.34	1.12	21.58				18.1484
P K	2.013	0.69	0.12	0.29				1.0349
Ca K	3.690	1.95	0.21	0.64				3.1523
Total		100.00		100.00				

Figure 10. EDS results of varnish with CO_3Ap and α-TCP.

Discussion

The SEM result in Figure 3 shows the formation of microporosity on the tooth that has been etched that similar to Lopes et al. (2007) study.[21] Chemically, etching can improve enamel topography, which changes from a surface with low reactivity to a surface that is more susceptible to adhesion. Etch will react with hydroxyapatite from enamel or enamel rods, causing the solubility of certain products from teeth such as calcium and phosphate, and forming pores on its surface.[21] The diameter of pores is about 6 µm and the depth is about 5-50 µm.

SEM results in Figure 4 are dental samples given varnish application with compositions containing dewaxed shellac and ethanol. Sample was then applied with 4 times smear, because the first film layer when the application will protect 55% of the tooth surface, and the application of the second film layer will protect 85% of the tooth surface. Based on this fact, the third film layer and so on will be able to protect the tooth surface much better.[22] Varnish applied to the tooth surface will leave a thin layer of film, due to the evaporation of ethanol. The formed layer is yellowish, translucent and glossy and cannot be penetrated by the liquid in the oral cavity, so as to prevent further demineralization. The SEM results showed that the microporosity was closed but not perfect because there were still white streaks or lines scattered on the tooth surface.

The comparison of SEM test results in Figure 5 starts to show a much flatter and smoother surface when compared to Figure 4. The difference treatment is the amount of smear in sample 3 is done for 8 times. More smear will form a layer that can cover the microporosity more optimally.[22] Figure 8 shows the EDS results on a sample that was only been etched contained Phosphate (P) element with the highest relative mass percentage of 47.87% and Calcium (Ca) as the second-highest element of 26.53%. The high element of phosphate and calcium is because it is the basic composition of enamel.[23,24] EDS results of the sample that were given varnish application without added CO_3Ap and α-TCP showed a very high carbon element, this could be incorporated of varnish composition using dewaxed shellac. Shellac contains a mixture of esters and polyesters from carboxylic polyhydroxy acid. The structure of shellac consists of aleuritic acid ($C_{16}H_{32}O_5$) and shelolic acid ($C_{15}H_{20}O_6$) components, which make up 70% of the total composition of shellac.[20]

Figure 6 is given a varnish formula 4 times smear applying with a composition added with active ingredients in the form of CO_3Ap and α-TCP. The SEM result in Figure 6 shows the closure of the microporosity but there are granules or granules which are thought to be deposits of CO_3Ap and α-TCP scattered on the enamel surface so that the surface becomes uneven. Scattered deposits on the surface happened because of layered varnish applications so that the particles possibly attached to the varnish layer underneath or on the previous layer.

The SEM result in Figure 7 almost has a similar image to Figure 6. Sample in Figure 7 is 8 times smear applying. Visually, the surface of the film is not homogenous with other samples. This can occur when it is too thick to apply each layer so that the underlying layers that are not yet completely dry again are lifted when applying the next layer and cause an uneven surface of the varnish.[22] Thick varnish solutions are caused by the evaporation of the solvent contained in the varnish preparation period. This can be prevented by re-diluting the varnish formula that has been taken using the same solvent as the solvent used when preparing the formula.[22]

The EDS results of dewaxed shellac varnishes with added CO_3Ap and α-TCP showed different results because there was a small phosphate that previously not found on the EDS results of dewaxed shellac varnishes without added CO_3Ap and α-TCP and also there was a slight increase in calcium. The emergence of calcium and phosphate elements allegedly due to the addition of active materials, nevertheless the amount of calcium and phosphate appeared was too small. This appeared may be due to less homogeneous formula so that there were still particles that settled. Another possible reason for small calcium and phosphate because all active particles of CO_3Ap and α-TCP entered the microporosity. The particle size of CO_3Ap used was 1.154 µm, and α-TCP particle size was used of 1.007 µm, while the size of the microporosity itself was 6 µm. The presence of calcium and phosphate elements on the EDS result of dewaxed shellac varnishes formula added CO_3Ap and α-TCP had the potential to form hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ so that initiated the remineralization.

Conclusions

Varnish made from dewaxed shellac added CO_3Ap and α-TCP were successfully syntheses. The morphology showed the teeth microporosity closure using varnish was effective. The synthesized varnish has potentially remineralized the enamel. Further study is awaited based on these initial findings.

Acknowledgment

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Declaration of Interest

There are no conflicts of interest.

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