REMINERALIZATION OF ENAMEL SURFACE IN SITU BY NANO-CHITOSAN HYDROXYAPATITE

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ABSTRACT

The process of caries begins with hard tissue demineralization by organic acids produced from fermented carbohydrates by cariogenic bacteria in dental plaque. Remineralization is a slow process compared to demineralization so that the provision of remineralization material is needed to reduce the demineralization process. This research aims to compare the effect of hydroxyapatite and nano-chitosan hydroxyapatite gel in reducing enamel demineralization by using SEM-EDX to find the amount of content (weight %) of calcium phosphorus and morphological changes of enamel surface of enamel.

Twenty four recently extracted embedded molar teeth enamel were used and divided into four groups. Group I was only immersed in clarified saliva. Group II immersed in ademineralization solution for four days. Group III immersed in demineralization solution for four days and then are applied with hydroxyapatite gel. Group IV immersed in demineralization solution for four days then given a combination of nano-chitosan hydroxyapatite gel.

Statistical tests showed no significant difference in the amount of calcium and phosphorus between the email that was applied with hydroxyapatite gel and nano-chitosan hydroxyapatite gel (p-value > 0.05). Qualitative analysis using SEM shows that there is a change in the surface morphology of tooth enamel which shows the decrease in demineralization after application of hydroxyapatite gel and nano-chitosan hydroxyapatite gel. In conclusion, hydroxyapatite gel and nano-chitosan hydroxyapatite gel have the same ability to reduce the demineralization process of tooth enamel.

KEYWORDS: Email Demineralization, Hydroxyapatite, Nano-Chitosan Hydroxyapatite

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INTRODUCTION

Dental caries is a pathological process of localized tooth tissue damage. This disease begins with the demineralization of hard tooth tissue by organic acids produced from carbohydrates that can be fermented by cariogenic bacteria in dental plaque. Frequent exposure to foods containing fermentable carbohydrates, especially foods that contain sugar will cause an increase in cariogenic bacterial populations such as *Streptococcus mutans*, *Lactobacillus* spp., and other species in the biofilm layer. These bacteria are acidogenic and aciduric which produce organic acids, especially lactic acid and actively produce these acids at pH values around 5.¹

The pH value is a critical pH value for enamel apatite which will cause damage. The critical pH value is considered as the highest pH value where tooth enamel will experience mineral loss. The true value depends on the activity of Ca^{2+} and PO_4^{3-} ions on the hydration layer around the enamel crystals and this is usually balanced by the

same concentration of ions in the salivary fluid and plaque. Laboratory research results state that the critical pH of tooth enamel is around 5.5. This is based on the average Ca^{2+} and PO_4^{3-} concentrations in salivary fluid and plaque.¹

During the caries process, most organic acids produced by plaque bacteria are not in a separate form. The organic acid will diffuse into the tooth enamel through the water-filled interprismatic space so that the pH in the hydration layer around the enamel crystal becomes lower. If the pH is below 5.5, the crystalline enamel will experience mineral loss in a process called.¹

The pathophysiology of caries is highly dependent on the components of dental minerals, especially calcium and phosphate, which are demineralized and released by organic acids which are the product of bacterial production, which is in biofilms, attached to the surface. Calcium and phosphate ions are released from the enamel subsurface, causing initial lesions to form in the enamel.² These initial lesions are reversible because with the remineralization process, calcium and phosphate ions are supplied from external sources to the teeth to stimulate ion deposits into the crystal cavities which has been formed at the time of demineralization to produce minerals again. The ability of remineralization in teeth that have undergone demineralization is very dependent on the condition of the oral cavity, including saliva, individual diets, and habits of patients, especially in maintaining the cleanliness and health of their teeth and mouth.³

In laboratory studies, experimental animals and in situ caries models in humans it appears that Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) has anticariogenic activity. CPP can stabilize calcium phosphate in solution with the residual phosphoserine so that the formation of CPP-ACP bonds.¹ CPP-ACP as remineralization material still has weaknesses. CPP-ACP has low solubility in acidic conditions. This will reduce the ability of CPP-ACP to hold calcium and phosphate ions in an acidic environment.

The use of natural products in the field of dentistry is currently growing rapidly. This research will use high molecular chitosan and hydroxyapatite from marketed pearl shells.

Chitosan is one of the biomaterials that lately continues to be developed because it has various medical benefits and is proven safe for humans. Chitosan has special properties, including good biocompatibility, biodegradable, non-toxic, does not cause immunological reactions, does not cause cancer. With the special nature of chitosan, chitosan and modifications with other materials can be used for clinical applications as biomaterials.

Arnaud et al. (2010) investigated the effects of chitosan on the demineralization and remineralization processes of tooth enamel associated with the presence of phosphorus. The results of the study concluded that chitosan plays a role in the remineralization process by inhibiting the release of phosphorus from tooth enamel.⁴

Chitosan can also interact with metal ions. The interaction of chitosan with metal ions occurs because of the complexing process where ion exchange, absorption, and chelating occur.⁵

Calcium and phosphorus are the main minerals that makeup bones and teeth. Among calcium phosphate salts, hydroxyapatite is the most similar one to the mineral parts in bones and teeth. Hydroxyapatite is a form that describes a bond containing calcium ions which can be combined with orthophosphate, phyrofosfat, hydrogen or hydroxide which is the main ingredient in the formation of bone and tooth enamel, called bio-material.⁶ This substance has high biocompatible properties and has been used in medical treatment widely. The purpose of this study is to determine the effect of nano-chitosan when added to hydroxyapatite, can it reduce the surface demineralization process of tooth enamel.

MATERIALS AND METHODS

Preparation of 2% Hydroxyapatite Gel

Hydroxyapatite gel is made by dissolving 2 grams of hydroxyapatite obtained from commercial hydroxyapatite, added with 100 ml of aquabidest then the solution is sterilized with Jar Test at a speed of 200 rpm for ± 30 minutes so that a homogeneous gel is obtained.

Preparation of Hydroxyapatite Nano-Chitosan Gel

Chitosan gel was prepared by dissolving 1 gram of chitosan in 50 ml of a weak acid solution (1% acetic acid) and then sterilized with a Jar Test at 200 rpm so that the gel was obtained for ± 30 minutes. Then the chitosan solution was dropped with a solution of 20 drops of sodium tripolyphosphate (NaTPP 0.5%) as much as 20 drops while slowly sterilizing. Mix the chitosan solution with sodium tripolyphosphate again with jar test for ± 30 minutes. The addition of 0.5% NaTPP was done so that the surface of the solution was smooth. The solution that has formed the gel is inserted into the ultrasonic bath to break the chitosan particles into nano. Nano chitosan is filtered and the residue is washed with aquadest. The resulting residue in the form of nano chitosan gel will be added to the hydroxyapatite.

As much as 25 mL of nano chitosan solution was added to 25 mL of 2% hydroxyapatite solution slowly while stirring until homogeneous. To achieve a neutral solution, 0.05M NaOH is added until the pH reaches 7, which is seen with Universal pH.

Preparation of Research Saliva (Lemos et al., 2010)

Preparation of the Adsorption Buffer Solution

The adsorption buffer solution was prepared with a mixture of 50 mM KCl; 1 mM potassium phosphate (0.35 mM K_2HPO_4 plus 0.65 mM KH_2PO_4); 1 mM $CaCl_2$; 0.1 mM $MgCl_2$. The pH value is equal to 6.5. The solution is stored at room temperature.

Saliva Preparation (Clarified Saliva)

50 mL of saliva was collected from one donor. Saliva is mixed with an adsorption buffer solution (ratio 1: 1, v / v) and added with 50 μ L 0.1 M phenylmethyl-sulfonyl fluoride (PMSF). The mixture of the solution was centrifuged at 5,500g; 4°C for 10 minutes. Supernatant was taken and filtered with a 0.22 μ m PES low protein binding-filter.

Sample Preparation

This study used six molar teeth. The root of the tooth is cut using a disc bur with water spray. Each tooth will be cut into four parts in the mesiodistal and buccolingual / buccopalatal direction so that 24 sample pieces will be obtained which will be divided into four groups. The buccal / lingual part is planted in a 3cc syringe which is cut to a height of 1 cm using acrylic resin and an open area with a diameter of about 2x2 mm is obtained.

Figure 1: Sample Preparation (A) Preparation of Sample procedures; (B). Sketch of the Direction of Cutting the Sample and the Sample to be Treated and Tested.

Sample Treatment and Testing

The samples are numbered 1 to 24 and divided randomly into four groups so that each group consists of six samples. The sample was conditioned as if in the oral cavity by soaking the clarified saliva for 24 hours. The treatment for each group modified from the research conducted by Hedge and Moany (2012) is as follows:

- Group I was only soaked in clarified saliva and incubated in an incubator shaker at 37°C for seven days.
- Group II soaked in the demineralization solution for four days. Then the sample was immersed with clarified saliva and incubated in an incubator shaker at 37°C for seven days.
- Group III soaked in the demineralization solution for four days and then given a hydroxyapatite gel once a day for five minutes. Then the sample was immersed in clarified saliva and incubated in an incubator shaker at 37°C for seven days.
- Group IV soaked in the demineralization solution for four days then given a nano-chitosan hydroxyapatite combination gel once a day for five minutes. Then the sample was immersed in clarified saliva and incubated in an incubator shaker at 37°C for seven days.

All samples were given thermocycling treatment on the seventh day. Thermocycling was carried out as many as 500 cycles using 5°C and 55°C temperatures. Sample testing with SEM-EDX was carried out on the eighth day. Testing the sample with EDX aims to find out the ratio of% by weight of the elements Ca and P in each group. While the SEM examination aims to obtain a microstructure description of the sample.

DATA ANALYSIS

The data obtained was performed one-way analysis of variance statistical tests (ANOVA) with a significance level ($\alpha = 0.05$) to determine differences in remineralization of email in groups I, II, III, and IV. Then a Post Hoc Least Significant Difference (LSD) test was performed to determine the difference in the reduction in email demineralization between the treatment groups.

RESULTS

Chemical Elements on the Email Surface of Each Group

The content of elements on the email surface of each group is known by observation through EDX. Email each treatment group contains elements of carbon (C), nitrogen (N), oxygen (O), phosphorus (P), and calcium (Ca). Descriptive data of the elements contained in the emails of each treatment group can be seen in Table 1.

Table 1: Descriptive Data Showing Mean Values and Standard Deviations of Chemical Elements Contained in the Emails of each Treatment Group

Group	C (% Weight)	N (% Weight)	O (% Weight)	P (% Weight)	CA (% Weight)
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
I	6,36 $\pm$ 2,15	17,23 $\pm$ 3,33	20,29 $\pm$ 4,54	19,20 $\pm$ 0,65	36,15 $\pm$ 1,73
II	9,70 $\pm$ 5,08	17,92 $\pm$ 3,23	21,28 $\pm$ 4,45	17,63 $\pm$ 0,64	33,37 $\pm$ 2,27
III	9,32 $\pm$ 5,77	17,54 $\pm$ 4,48	19,23 $\pm$ 3,74	18,59 $\pm$ 0,86	35,33 $\pm$ 2,01
IV	6,58 $\pm$ 1,00	19,05 $\pm$ 2,72	19,25 $\pm$ 3,65	19,14 $\pm$ 0,72	35,98 $\pm$ 1,91

I: groups that are only soaked in clarified saliva.

II: groups immersed in the demineralization solution.

III: immersed in the demineralized solution and then given a hydroxyapatite gel.

IV: immersed in the demineralization solution then given nano-chitosan hydroxyapatite combination gel.

Content of Calcium (Ca) and Phosphorus (P) Element on the Email Surface of Each Group

The results of observations with EDX obtained data content of elements of Ca and P in% by weight. To find out the data obtained were normally distributed then the data normality test was first performed with the Shapiro-Wilk Test.

The test results showed all data content of Ca, P and Ca / P ratios in each group were normally distributed with $p > 0.05$. Homogeneity test results for all data variants showed a value of $p > 0.05$ which means that the data variants were homogeneous.

Table 2 is a descriptive ANOVA test for the average content of Ca, P and Ca / P ratios with the calculation of the significance level $\alpha = 0.05$. The results of the Ca element content test showed a p value = 0.048 (p - value < 0.05) statistically meaning there were significant differences in the amount of Ca element content among all treatment groups. The P element shows the value of $p = 0.004$ (p - value < 0.05) which statistically also means that there are significant differences in the amount of P element content among all treatment groups. Ca / P ratio shows the value of $p = 0.979$ (p - value > 0.05) statistically means there is no significant difference in Ca / P ratio between all treatment groups.

Table 2: Descriptive Data Showing Mean Values and Standard Deviations from the ANOVA Test in Testing the Amount of Calcium (Ca), Phosphorus (P), and CA / P Ratio on Enamel Surface of Each Group

Group	CA Element (% Weight)		P Element (% Weight)		CA/Prasio	
	$\bar{X} \pm SD$	p value	$\bar{X} \pm SD$	p value	$\bar{X} \pm SD$	p value
I	36,15 $\pm$ 1,73	0,048*	19,20 $\pm$ 0,65	0,004*	1,88 $\pm$ 0,09	0,979
II	33,37 $\pm$ 2,27		17,63 $\pm$ 0,64		1,89 $\pm$ 0,09	
III	35,33 $\pm$ 2,01		18,59 $\pm$ 0,86		1,90 $\pm$ 0,95	
IV	35,98 $\pm$ 1,91		19,14 $\pm$ 0,72		1,88 $\pm$ 0,89	

I: groups that are only soaked in clarified saliva.

II: groups immersed in the demineralization solution.

III: immersed in the demineralized solution and then given a hydroxyapatite gel.

IV: immersed in the demineralization solution then given nano-chitosan hydroxyapatite combination gel.

* p value <0.05 significant

Figure 2 shows descriptively the average amount of Ca, P, and Ca / P ratio. The highest Ca element was seen in the sample group which was only immersed in clarified saliva and incubated in a shaker incubator at 37°C for 7 days (group I), while the mean value of the lowest amount of Ca element was seen in the group immersed in demineralization solution for four days, then immersed in clarified saliva, and incubated in an incubator shaker at 37°C for seven days (group II).

The highest average value of P content was seen in the sample group that was only immersed in clarified saliva and incubated in a shaker incubator at 37°C for 7 days (group I). The lowest mean value of P content was seen in groups immersed in demineralization solution for four days, soaked with clarified saliva, and incubated in an incubator shaker at 37°C for seven days (group II).

The highest average Ca / P ratio was seen in the sample group immersed in demineralization solution for four days and then given a hydroxyapatite gel once a day for five minutes, immersed in clarified saliva and incubated in an incubator shaker at 37°C for seven days (group III). The lowest average Ca / P ratio was seen in the group immersed in demineralization solution for four days and then given a nano-chitosan hydroxyapatite combination gel once a day for five minutes, immersed in clarified saliva, and incubated in an incubator shaker at 37°C for seven days (group IV).

Figure 2: Graph of the Average Value of the Amount of Ca, P, and Ca / P Ratio after Treatment.

Effect of Given Hydroxyapatite Gel on the Content of Calcium and Phosphorus Email Surface

To find out for certain groups that have different Ca, P, and Ca / P ratios in the presence of hydroxyapatite gel, post hoc LSD testing was performed. The results of the post hoc LSD test in Table 3 show that the Ca element between groups I and III had a significant difference in content with a value of $p = 0.025$ (p - value <0.05). Element P between groups II and III has a significant difference in content with a value of $p = 0.032$ (p - value <0.05). The Ca / P ratio did not show significant differences between any groups.

Table 3: Differences in the Content of Ca, P and Ca / P Ratios Between Each Group and Groups that Applied Hydroxyapatite Gel

Element	Group	$\bar{X} \pm SD$	Average Difference	p value
Ca	I	36,15 $\pm$ 1,73	0,825	0,025*
	III	35,33 $\pm$ 2,01		
	II	33,37 $\pm$ 2,27	-1,959	0,103
	III	35,33 $\pm$ 2,01		
	IV	35,98 $\pm$ 1,91	0,650	0,577
	III	35,33 $\pm$ 2,01		
P	I	19,20 $\pm$ 0,65	0,611	0,158
	III	18,59 $\pm$ 0,86		
	II	17,63 $\pm$ 0,64	-0,961	0,032*
	III	18,59 $\pm$ 0,86		
	IV	19,14 $\pm$ 0,72	0,549	0,202
	III	18,59 $\pm$ 0,86		
Ca/P Element	I	1,88 $\pm$ 0,09	-0,017	0,740
	III	1,90 $\pm$ 0,95		
	II	1,89 $\pm$ 0,09	-0,009	0,859
	III	1,90 $\pm$ 0,95		
	IV	1,88 $\pm$ 0,89	-0,021	0,695
	III	1,90 $\pm$ 0,95		

I: groups that are only soaked in clarified saliva.

II: groups immersed in the demineralization solution.

III: immersed in the demineralized solution and then given a hydroxyapatite gel.

IV: immersed in the demineralization solution then given nano-chitosan hydroxyapatite combination gel.

* p value <0.05 significant

Effect on Addition of Hydroxyapatite Nano-Chitosan Gel to the Content of Calcium and Phosphorus Email Surface

To find out for certain groups that have differences in the elemental content of Ca, P, and Ca / P ratio with the addition of nano-chitosan hydroxyapatite gel, post hoc LSD testing was performed. The post hoc LSD test results in Table 4 show that the Ca elements in groups II and IV had significant differences in content with p values = 0.034 (p values <0.05). The P element also shows a significant difference in content between groups II and IV. The Ca / P ratio did not show significant differences between any groups.

Table 4: Differences In The Content Of Ca, P, And Ca / P Ratios Between Each Group And Groups That Applied Nano-Chitosan Hydroxyapatite Gel

Element	Group	$\bar{X} \pm SD$	Average Difference	P Value
Ca	I	36,15 $\pm$ 1,73	0,174	0,881
	IV	35,98 $\pm$ 1,91		
	II	33,37 $\pm$ 2,27	-2,609	0,034*
	IV	35,98 $\pm$ 1,91		
	III	35,33 $\pm$ 2,01	-0,650	0,577
	IV	35,98 $\pm$ 1,91		
P	I	19,20 $\pm$ 0,65	0,062	0,883
	IV	19,14 $\pm$ 0,72		
	II	17,63 $\pm$ 0,64	-1,509	0,002*
	IV	19,14 $\pm$ 0,72		
	III	18,59 $\pm$ 0,86	-0,549	0,202
	IV	19,14 $\pm$ 0,72		
Ca/P Element	I	1,88 $\pm$ 0,09	0,003	0,113
	IV	1,88 $\pm$ 0,89		
	II	1,89 $\pm$ 0,09	0,012	0,830
	IV	1,88 $\pm$ 0,89		
	III	1,90 $\pm$ 0,95	0,021	0,695
	IV	1,88 $\pm$ 0,89		

I: groups that are only soaked in clarified saliva.

II: groups immersed in the demineralization solution.

III: immersed in the demineralized solution and then given a hydroxyapatite gel.

IV: immersed in the demineralization solution then given nano-chitosan hydroxyapatite gel.

* p value <0.05 significant

Morphological Illustration of Each Group's Email Surface

Observation of the surface morphology description of each treatment group (figure 3) was carried out using a Scanning Electron Microscope (SEM) tool. The e-mails observed in this study were e-mail surfaces that were clinically and microscopically intact and were not polished. Morphological description of tooth enamel seen in each group can be seen in table 5.

Table 5: Morphological Depiction of Tooth Enamel seen in Each Group

Group	Surface image of an email
I	The surface of the enamel is uneven and smooth in the presence of enamel pores and uniform aprismatic surface forms
II	The surface of the enamel is rough and has porosity with a very large and irregular size
III	Rough surface of enamel with a smaller porosity size and more regular pore size of enamel from demineralized email in group II
IV	The surface of the enamel is more subtle and the size of porosity is much less than demineralized enamel in Group II

Figure 3

Figure 3 Description of the surface morphology of the email. a) Emails that have only been soaked in clarified saliva and incubated in an incubator shaker at 37°C for seven days; b) Email that is only immersed in the demineralization solution for four days, then soaked with clarified saliva, and incubated in an incubator shaker at 37°C for seven days; c) Email were applied with the hydroxyapatite gel once a day for five minutes, immersed in clarified saliva, and incubated in an incubator shaker at 37°C for seven days; d) Emails were applied with nano-chitosan hydroxyapatite gel once a day for five minutes, soaked in clarified saliva, and incubated in an incubator shaker at 37°C for seven days (magnification of 5000X).

DISCUSSIONS

The Effect on Addition of Hydroxyapatite Nano-Chitosan Gel on the Content of Calcium and Phosphorus Email Surface

Statistical analysis by post hoc LSD test showed that the calcium content of the sample group that was given demineralization treatment was then applied to nano-chitosan hydroxyapatite gel and the email sample group that was only given demineralization treatment had a significant difference with $p = 0.034$ ($p < 0.05$). Descriptively it was seen that the % calcium weight of the group given demineralization treatment was then applied to nano-chitosan hydroxyapatite gel (35.98 ± 1.91) higher than the email sample group which was only given demineralization treatment (33.37 ± 2.27).

The content of weight% phosphorus analyzed statistically by the post hoc LSD test showed a significant difference $\{p = 0.002 (p < 0.05)\}$ between the email sample groups that were given demineralization treatment then the nano-chitosan hydroxyapatite gel was applied and the group that was only given demineralization treatment. Descriptively it was seen that the weight% of the elemental phosphorus group that was given demineralization treatment was then applied to nano-chitosan hydroxyapatite gel (19.14 ± 0.72) higher than the email sample group that was only given demineralization treatment (17.63 ± 0.64).

Statistical analysis with post hoc LSD test showed that the calcium element between the email sample groups that were given demineralization treatment then applied nano-chitosan hydroxyapatite gel with the group given demineralization treatment then given the application of hydroxyapatite gel did not have a significant difference $p = 0.577 (p > 0.05)$. Phosphorus content between the two groups also did not have significant differences with $p = 0.202 (p > 0.05)$.

Descriptively it was seen that the email sample group which was given demineralization treatment then applied nano-chitosan hydroxyapatite gel containing calcium (35.98 ± 1.91) and phosphorus (19.14 ± 0.72) higher than calcium ($35, 33 \pm 2.01$) and phosphorus (18.59 ± 0.86) groups treated with demineralization were then given the application of hydroxyapatite gel.

Chitosan can absorb metals and form complex ions with metals. Chitosan as a cationic polymer can bind to metals where the amino groups contained in chitosan bind with metals and form covalent bonds. One of the forces acting on this bond is the Van der Waals force that is on the surface of the absorbent where there is a difference in energy or Van der Waals tensile strength between the absorbent and adsorbate which causes the adsorbate to bind or be attracted to the adsorbent molecule.⁵

The addition of nano-chitosan to hydroxyapatite will accelerate the absorption of calcium which will be released at the time of demineralization. The amino group contained in chitosan will bind with calcium which will be released and form covalent bonds. The Van der Waals force that occurs between the chitosan amino group with calcium will cause calcium to be bound to the amino chitosan group.⁵ Thus the release of calcium from tooth enamel can be avoided and demineralization of tooth enamel will decrease.

The chitosan ($-NH_2$) amino group in aqueous acid conditions will capture H^+ from its environment so that its ammonia group is protonated to $-NH_3^+$. The positive charge $-NH_3^+$ can be used for adsorption of negatively charged substances (anionic).⁵ On the tooth surface the positive charge $-NH_3^+$ from chitosan can be utilized for the adsorption of negative substances in the form of PO_4^- so that the phosphorus element is not separated from the enamel surface and the demineralization process can be inhibited.

The results of this study are in line with the research of Arnaud et al. (2010) who examined the effects of chitosan on the process of demineralization and remineralization of tooth enamel associated with the presence of phosphorus elements.⁴ The research shows that chitosan plays a role in the remineralization process by inhibiting the release of phosphorus from tooth enamel.

Chitosan used in this study had a degree of deacetylation of 84.20% with a molecular weight of 893,000 Mv. The degree of deacetylation plays an important role in the absorption process. Increasing the value of the degree of deacetylation causes an increase in the number of free amine groups.⁷ The molecular weight of chitosan and the degree of

deacetylation can also facilitate solubility in acidic conditions and affect the absorption process. The increase in molecular weight and the degree of deacetylation of chitosan will affect the physical and physicochemical.

The nanometer-scale chitosan particle size in this study will improve the efficiency of the physical-chemical process of chitosan surface with hydroxyapatite derived from calcium-rich pearl shells. Nano-chitosan hydroxyapatite gel in this study more easily diffuse into the enamel prism which has a micrometer scale with a diameter of about 5-6 μm . The size of the nanometer-scale chitosan particles will increase the surface area up to hundreds of times compared to micrometer-sized particles. This will increase the efficiency of the chitosan surface physico-chemical process, because it allows interaction on a larger surface.⁸

Morphological Illustration of Each Group's Enamel Surface

The enamel used in this study is the surface of enamel that is still intact macroscopically and not polished. The results of qualitative analysis using SEM obtained morphological description of each treatment group enamel.

Enamel groups that are only incubated in artificial saliva (Figure 5.2 a) show that the surface of the enamel is not completely flat and there is an enamel tip which is a normal variation of enamel.

Figure 3 b is a picture of enamel that has only been immersed in the demineralization solution. In demineralized enamel, porosity with irregular size and a bumpy surface indicates demineralization in tooth enamel.

Figure 3 c is a picture of the surface of the enamel subjected to demineralization then the application of hydroxyapatite gel is given. In the picture we can see less porosity of enamel and smoother surface compared to enamel that only gets demineralized (Figure 5.2b). This shows that the hydroxyapatite gel from pearl shells in this study can inhibit the reduction of demineralization in tooth enamel with the penetration of calcium ions in demineralized enamel.

Figure 3 d is an image of the surface of the enamel applied to a combination of nano-chitosan hydroxyapatite gel. The surface of the enamel becomes smoother compared to enamel after the hydroxyapatite gel is applied (Figure 5.2b). This is thought to be related to the ability of chitosan in metal adsorption and the positive charge of $-\text{NH}_3^+$ from chitosan can be used for absorption of negatively charged substances.⁵ In this study, chitosan plays a role in the absorption of calcium and amino groups ($-\text{NH}_2$) chitosan which is protonated to $-\text{NH}_3^+$ under aqueous acid conditions it is thought to absorb negative ions in the form of PO_4^{4-} so that the phosphorus element cannot be separated from the tooth enamel surface. Besides, diffusion of nano-chitosan hydroxyapatite gel into enamel prisms which have a micrometer scale with a diameter of about 5-6 μm in this study will also facilitate gel diffusion so that deeper remineralization of enamel can occur.

The results of this study indicate that the hydroxyapatite gel and nano-chitosan hydroxyapatite gel can inhibit and reduce the demineralization process on the surface of tooth enamel. The reduction in enamel demineralization can be seen from the increase in calcium, phosphorus, the Ca / P ratio in tooth enamel that can be detected by EDX, and the morphological picture of enamel seen through SEM.

CONCLUSIONS

Addition of nano-chitosan hydroxyapatite gel can reduce the process of demineralization of tooth enamel. There was no significant decrease in enamel demineralization between the hydroxyapatite gel and the nano-chitosan hydroxyapatite combination gel, as well as the release of calcium and phosphorus. The morphology of enamel surface in the application of hydroxyapatite gel and hydroxyapatite gel shows a different picture, where the administration of nano-chitosan

hydroxyapatite gel shows the morphology of enamel surface morphology is much better than that applied by hydroxyapatite gel.

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