The Influence of Pandalus Borealis Shell Nano Chitosan on Permanent Teeth Enamel Integrity against Caries

Fidya[1*], Mohammad Chair Effendi[2], Mutiara Fauzia Nurmawlidina[3]

1. Department of Oral Biology, Faculty of Dentistry, Brawijaya University.
2. Department of Pediatric Dentistry, Faculty of Dental Medicine, Brawijaya University.
3. Undergraduate Program in Dentistry, Faculty of Dental Medicine, Brawijaya University.

Abstract

Chitosan is a polysaccharide that is formed from deacetylation of chitin. It is believed that chitosan can inhibit demineralization process. Nanoparticles are more effective conductors of small organic molecules. They can penetrate barriers that cannot be passed by larger molecules. The objective of this study is to identify the effect of shrimp nanochitosan paste on the microhardness integrity of tooth enamel surface. Twenty-seven maxillary first premolars are divided into three groups; they are the control group, microchitosan group, and nanochitosan group. All groups go through demineralization process for one hour three times a day for five days. For the treatment group, after the chitosan paste is applied to the sample for 5 minutes, the sample is brought into demineralization cycle. Vickers Hardness Tester is used to calculate the microhardness of the enamel surface. The mean result of the difference of enamel surface microhardness before and after the treatment for control group is 123,34 HV, for microchitosan group is 88,04 HV, and for nanochitosan group is 66,28 HV. The results of One Way Anova test show significant differences among the three groups. The conclusion is that nanochitosan is better in maintaining enamel integrity than microchitosan.

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Introduction

One of the efforts to prevent further caries is using chemical elements that can prevent the release of minerals of teeth; one of which is chitosan. Chitosan is the deacetylated form of chitin.[1] Chitosan is believed to inhibit the process of tooth demineralization. Chitosan as a natural biopolymer material which is non-chemical and natural and has long been used in traditional medicine.[2] Chitosan has biocompatible, biodegradable and tends to adhere to biological surfaces.[3] One of the chemical elements in chitosan is amino group (-NH2), which has high reactivity to the cariogenic acid, so it can maintain the pH stability in oral cavity by maintaining the pH of plaque above the critical pH. The amino group contained in chitosan can prevent acid dissolution on hydroxyapatite through rapid absorption processes. Chitosan can also in repel free radicals that can damage enamel structure, forming a weak area that supports demineralization and causes dental caries.[4]

The nanometer size of nanoparticles allows the particle to be used in a subcellular scale to achieve cellular targets with high accuracy, so it can achieve maximum therapeutic effect without any adverse effects.[5] Chitosan in nanometer scale can increase its surface area up to hundreds of times of micrometer-scale particles. This can improve chitosan's ability in binding other chemical groups. Nano-sized chitosan can also improve the efficiency of physicochemical processes on its surface because it allows interaction on a larger surface.[6] The objective of this study is to identify the role of nanochitosan in preventing demineralization, which causes dental caries.

Corresponding author:
Fidya
Undergraduate Program in Dentistry,
Faculty of Dentistry,
Brawijaya University.
E-mail: fidya.fk@ub.ac.id

Method

This true experimental research uses pretest posttest with control group design. The sample of this study is twenty-seven post-extraction caries-free maxillary first premolars of orthodontic patients. The sample is divided into three groups; they are the control group, the microchitosan treatment group, and the nanochitosan treatment group. Each group consists of nine teeth. This research was conducted in Pharmacy Laboratory of Faculty of Medicine of Brawijaya University in Malang, Laboratory of PT. Nanotech Herbal Indonesia Puspitek in Serpong, Materials Testing Laboratory of Mechanical Engineering Faculty of State University of Malang, and Laboratory of Mechanical Engineering of Brawijaya University in Malang. This research has received ethical approval form the Ethics Commission of Faculty of Medicine of Brawijaya University in Malang.

The chitosan paste used in this study is 0.2% chitosan nanoparticle paste, which is applied on nanochitosan group, and microparticle chitosan paste, which is applied on microchitosan group. This paste is produced by PT. Nanotech Herbal Indonesia. The particle measurements are performed using Particle Size Analyzer. The demineralization solutions which are used in this study are 2.2 mM/L of $CaCl_2$, 2.2 mM/L of KH_2PO_4, and 50 mM of acetate buffer. The acidity is regulated using KOH until it reaches the pH of 4.06.[7]

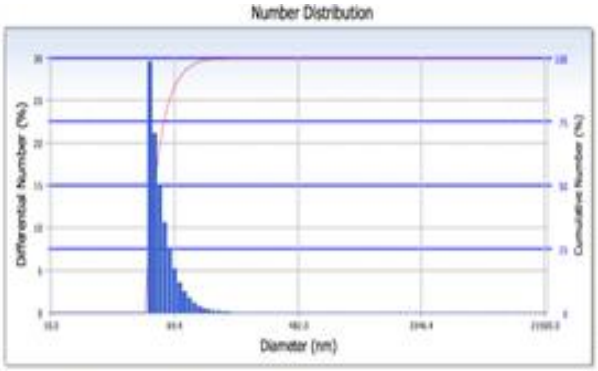
All samples are prepared until the buccal surface of the teeth was obtained, which was then planted in acrylic resin. The root of the teeth is cut using a low-speed micromotor with Carborundum Disc until the crown remains. Each premolar is split into two parts in the direction of buccal-palatal. The buccal part is planted into a 1- cm-diameter PVC pipe using acrylic resin. The sample surface is cleaned with a brush for 3 minutes until a debris-free surface is obtained.

Prior to treatment, the enamel surface's microhardness of each sample is measured using Vickers Hardness Tester. The criterion of the mean initial enamel hardness in this study is 227-424 HV. The tip of the diamond indenter is pressed on the surface of the sample for ten seconds with the load of 300 grams. The hardness of each sample is measured at three different points; they are the upper, middle, and lower (near-cervical) regions. Then, the mean is calculated. For the treatment group, microchitosan and nanochitosan pastes are applied topically on the surface using a tip applicator for five minutes. Then, the sample is washed using a syringe. After six hours of chitosan paste application, each sample is immersed in 20 ml of demineralization solution (pH 4.06) for one hour. The immersion is also performed on the control group, which excludes chitosan paste application process. The procedure above is repeated for three times a day for five days.[4]

After the treatment has competed, final enamel surface hardness testing is performed on each sample at similar points where the initial enamel microhardness test was performed. Then, mean microhardness difference before and after treatment is calculated. The value of mean difference in hardness indicates the level of mineral release on the enamel. Therefore, the value can be used to measure the enamel integrity of each sample. Analysis on the morphological features of enamel surface is conducted to evaluate the mineral release on enamel surface. The analysis is performed on two samples from each group. The morphological depiction of enamel is obtained using Scanning Electron Microscope with 5000x magnification.

Results

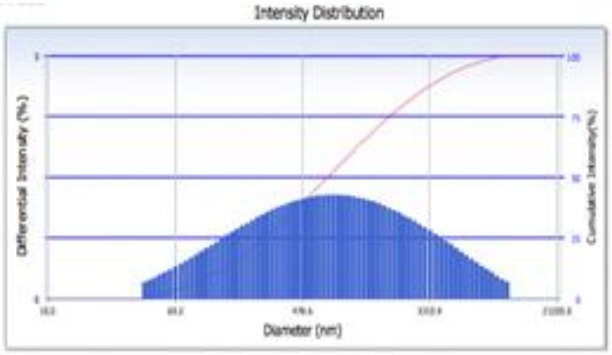
Average size of chitosan nanoparticles produced by PT. Nanotech Herbal Indonesia through ionic gelation method was 57,6 nm (Figure 1). The size qualifies the size of nanoparticle is 1-100 nm.[5] The average size of micro chitosan was 1.4815 micrometer. The chart and distribution table of average diameter of micro chitosan was shown in Figure 1. The average values of difference of enamel surface's microhardness before and after the treatments were 123,34 HV for control group is, is 88,04 HV for micro chitosan group, and 66,28 HV for nano chitosan group. The chart of mean microhardness Vickers values before and after the treatment, as well as its difference, are shown in Table 1.



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	57.6	15.0
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	57.6	15.0

(A)



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1,481.5	1,888.5
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	1,481.5	1,888.5

(B)

Figure 1. Chart and distribution table of average diameter of chitosan particles**.** Note: A= nanoparticle; B= microparticle.

The results of Shapiro-Wilk normality test and Levene homogeneity test showed p value of > 0.01, which meant that the data of the three groups were normally distributed and homogeneous. The results of One Way Anova test showed significantly differences among the control group, micro chitosan and nano chitosan treatment group (p <0.01).

Group	Mean Microhardness Vickers (HV) values (Before Treatment)	Mean Microhardness Vickers (HV) Values (After Treatment)	The Difference Value (HV) ±SD
Control	308,61	190,33	123,34± 41.75
Micro chitosan	304,66	217,36	88,04± 33,36
Nano chitosan	297,54	231,29	66,28± 35.93

Table 1. The mean of Microhardness Vickers Values in Control, Micro Chitosan, and Nano Chitosan Group (HV).

The results of LSD post hoc test indicated significant differences between control group and nano chitosan group (p <0.01). However, the difference between control group and microchitosan group, and between treatment groups, were not significant (p> 0,01).

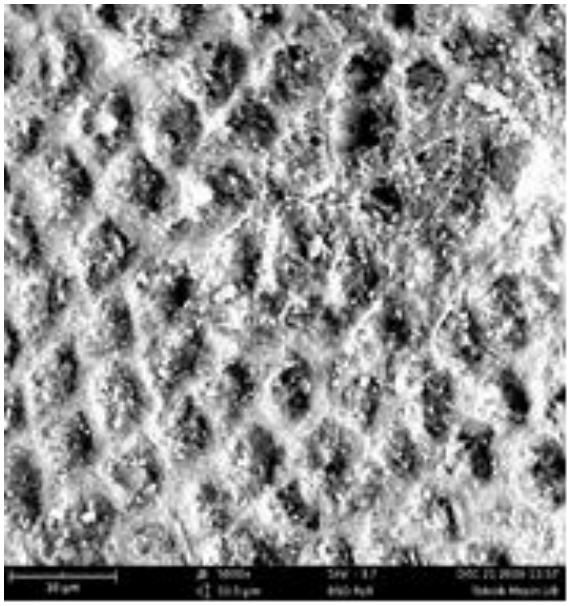


Figure 2. SEM result on control group (5000x magnification).

Observation using Scanning Electron Microscope (SEM) on control group showed that the morphological feature of enamel surface was filled with porosities due to demineralization process. Depressions of deep porosity can also be seen in figure 2. The porosity formed on the enamel surface tended to be irregular and formed distinctive features of demineralized enamel rod structures, i.e. honeycomb-like. The morphological feature of

enamel surface of micro chitosan treated group as presented in Figure 3 showed clearly several porosities in some parts of the enamel surface. The porosity was fewer than those on control group. The figure also showed that chitosan microparticles were still attached to the enamel surface.

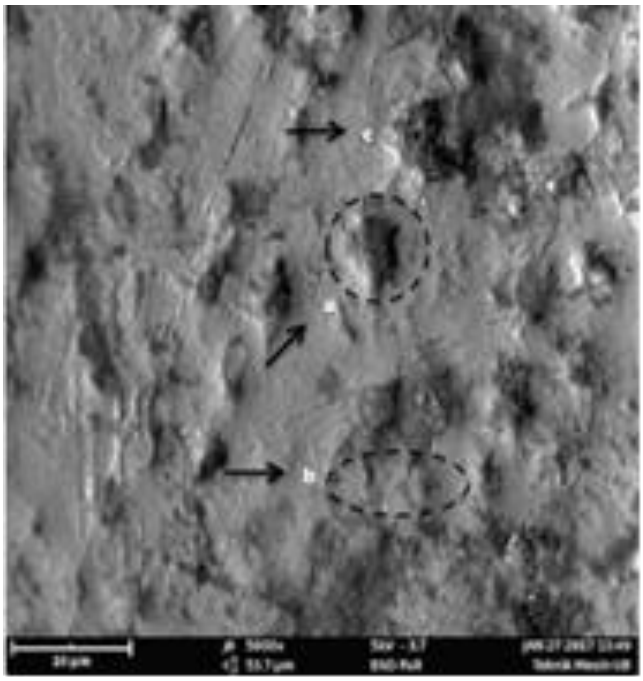


Figure 3. SEM result on microchitosan treatment group (5000x magnification). (Notes: a= deep porosity; arrow; b = shallow porosity; arrow; c = chitosan microparticle paste).

Figure 4 showed the morphological feature of enamel surface of nano chitosan treated group. The enamel surface was intact, without porosity. The porosity did not fill the entire surface. It was only formed on some enamel surfaces. The picture also showed that chitosan nanoparticles were still attached to the enamel surface.

Discussion

Nanoparticle are material with quantum size that resulting changes in physical and chemical properties. When compared with large particles, the decreased size of nanoparticle increase the surface area.[5]

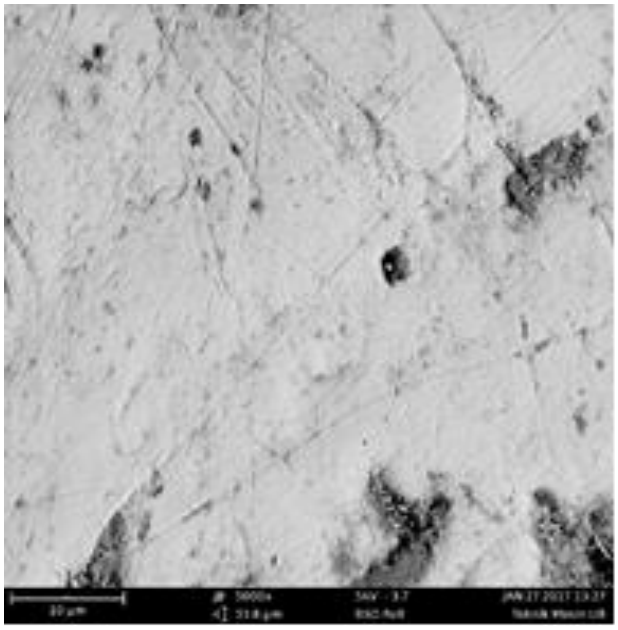


Figure 4. SEM result on nanochitosan treatment group (5000x magnification).

Nanoparticle can penetrate spaces that cannot be penetrated by particles of larger size. Nanoparticle also increase its solubility, so it make the bioavailability of the particle increase.[7]

Based on the hardness tests result in this research, nanochitosan treatment group has the lowest enamel hardness decrease of all groups. The lower the microhardness of enamel before and after the treatment, the lower the enamel hardness decrease. This shows the low level of inorganic component release from enamel. The results of the enamel hardness test show that nanochitosan is better at maintaining the microhardness of enamel surface than microchitosan. This result is supported by several theories about the superiority of nano-sized particles. The smaller the particle size, the higher its surface area. The high surface area of the particle increases its distribution efficiency because its ability to bind other chemical groups increases along with the increase of its surface area.[5] The results of this study show that nanochitosan can inhibit demineralization by acting as a barrier against acid penetration and preventing mineral release on enamel.

Another indicator for mineral release on enamel is morphological features of enamel surfaces, which are obtained from Scanning Electron Microscope (SEM). In some samples, porosity that is formed on enamel surface is found. Its formation is caused by

demineralization process, in which mineral on tooth enamel is released. The porosity is caused by dissolution process of enamel rod, so material loss in the rod core occurs, and honeycomb-like /keyhole-like structure is formed.[8,9] Porosity is mostly prevalent in the morphological feature of the enamel surface of control group. This result is supported by several theories and research results, which prove that chitosan is able to prevent demineralization of tooth enamel. With its flexibility, efficiency, and ability to act as a barrier against acid penetration, chitosan has an important role in prevention effort in the field of dentistry.[10]

The feature of the enamel surface of nanochitosan treatment group show a smaller number of porosity compared to that of microchitosan treatment group. This happens because of the superior properties of nanoparticle. The larger surface area of nanoparticles which is larger than its volume, makes them highly reactive/catalytic. They can pass through membranes more easily, and they can interact quickly with biological systems.[11] On the morphological features of enamel surface of both treatment groups, it appears that chitosan is still attached to the enamel surface. This happens because, under acidic conditions, the amino group in chitosan captures the hydrogen ion, so its charge becomes positive. The charge makes chitosan bioadhesive against surface with negative charge, such as enamel surface.[7]

Conclusions

There is a difference regarding the effect of pandalus borealis chitosan nanoparticle and the effect of pandalus borealis chitosan microparticle on the integrity of tooth enamel surface's microhardness. The mean value of microhardness difference of enamel surface in microchitosan treatment group is higher than that of the nanochitosan treatment group. This result suggests that nanochitosan is better at maintaining the microhardness of enamel surfaces than microchitosan. There is a significant difference between nanochitosan treatment group and microchitosan treatment group in samples 2 and 4.

However, there are no significant differences in other samples. Analysis on the morphological feature of enamel surfaces suggests that nanochitosan is better at maintaining the enamel surface morphological structure and preventing enamel demineralization than microchitosan.

Declaration of Interest

None declared.

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