THE EFFECT OF NaF 5% AND NANONaF TO THE PERMANENT TOOTH ENDURANCE TOWARD DENTAL CARIES

Fidya[1], Ranny Rachmawati[2], M. Chair Effendi[3], Ni Ketut Ayu Ferianti Dewi[4]*

1. Departement of Oral Biology School of Dental Medicine Faculty of Medicine Brawijaya University, Malang, Indonesia.
2. Departement of Periodontics School of Dental Medicine, Faculty of Medicine Brawijaya University, Malang, Indonesia.
3. Departement of Pediatric Dentistry, School of Dental Medicine, Faculty of Medicine Brawijaya University, Malang, Indonesia.
4. Graduate Student of School of Dental Medicine, Faculty of Medicine Brawijaya University, Malang, Indonesia.

Abstract

NaF 5% is a fluoride that commonly used as a dental topical application treatment. As the technology develops nowadays, nanoparticle is possibly used as dental material because it has greater penetration to enamel, dentin, and dentin-enamel junction. To compare the effect of NaF 5% and Nanofluid to the enamel endurance of permanent teeth toward dental caries. Twenty-seven teeth were divided into three groups; a control group, NaF 5% group, and NanoNaF group. NanoNaF was produced using HEM. NaF 5% and NanoNaF were application for 12 hours and then through demineralization and remineralization process for 14 days. Some test was used such as Microhardness Vickers, SEM, EDX and XRD tests. Miling process using HEM resulting NanoNaF particle size of 295 nm. SEM from NanoNaF group showed smoother enamel surface than from control and NaF 5% group. Microhardness Vickers test result showed 81.18 HV (control), 100.57 HV (NaF 5%), and 78.72 HV (NanoluorNaF) with p= 0.76 (p>0.05). Fluor content in control was 0.07%, NaF 5% was 0.26%, and in NanoNaF was 0.04% with p= 0.08 (p>0.05). XRD test showed that fluorapatite was detected in control (11.2%), NaF 5% (9.75%), and NanoNaF (30.11%). Kruskal Wallis test resulted p= 0.60 (p>0.05). NanoNaF penetration also maintained hydroxyapatite for 63.37%. NanoNaF application can increase the amount of fluoride, fluorapatite, and can maintain hydroxyapatite in enamel better than the NaF 5%. NanoNaF application did not affect the hardness of the enamel surface compare to NaF 5% application.

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Introduction

The idea of using tooth has already appeared in USA since 1930 when the effect of this material from drinking water was found as away to prevent teeth from dental caries.[1] Fluoride is one of the minerals that is commonly used in dental material. Fluoride can be easily given in daily drinking water, toothpaste, tablet dan drops, gel, and mouth rinses.[1] Fluoride is an anti-caries material that was found to be effective and has already been implicated as one of the so many ways to prevent caries.[2] The topical fluoride application in dental treatment is often using Sodium fluoride (NaF). It can be applied from a wide range of age (children to adult). The purpose of this material application is to strengthen teeth enamel.

***Corresponding author:**
Fidya
Departement of Oral Biology School of Dental Medicine,
Faculty of Medicine Brawijaya University, Malang, Indonesia.
E-mail: fidya.fk@ub.ac.id

Enamel contains an organic material, organic material, and water. Tooth enamel structure is a complex chemical structure with 97% mineral protein which contains hydroxyapatite in majority.[3] Hydroxyapatite is composed by "difficult to soluble" protein. There is substance between the prism that have smaller crystal size that called interprism. This enamel structure make ions in oral fluid can penetrate into a deeper portion of enamel. The enamel structure very likely to let the ion transportation from a deeper portion of enamel. Porous structure of enamel enables ions to diffuse into the deeper part. These ions are sodium, Mg, and carbonat.[4]

Nowadays, nanotechnology in medical has shown rapid development. Nanoparticle can enhance the hardness level. This particle can gather into one long macrocrystalline and prevent structure detached.[5] Nanoparticle also can be used as a strong magnet. This invention of nanotechnology enables the possibility of opening an innovation gate in the dentistry material. This material is hoped to have greater penetration to enamel, dentin, and DEJ structure[2]. Therefore, research to compare NaF and NanoNaF application in dentistry to measure the enamel endurance toward caries is necessarily needed to be done.

Purpose

The purpose of this research is to compare the effect of NaF 5% and NanoNaF to enamel endurance of permanent tooth toward dental caries. The enamel endurance included teeth hardness, the total amount of the fluoride element, hydroxyapatite, and fluorapatite.

Materials and Methods

This research was done using experimental laboratory study. The design used was The Post-Test Only Control Group Design. The samples were 27 permanent maxillary and mandibular first premolar teeth which had just recently being extracted from orthodontics treatment, especially which were free from caries. The samples are stored in aqua distillate right after extraction. Samples were randomly divided into three groups randomly; group of NaF, group of NanoNaF, and group of control.

The places of this research were in Balai Keramik Bandung, Laboratory of Dentistry Program and laboratory of Pharmaceutic of Brawijaya University, Laboratory of Physic Malang University, and laboratory of energy of Insitute Technology Surabaya. This research was approved by Ethical committee in Medical Faculty of Brawijaya University.

Two hundred and fifty milligrams of sodium fluoride were milling using High Energy Milling (HEM) for 24 hours and 48 hours. Afterwards, the result was measured using Particle Size Analyse (PSA).

Scanning Electron Microscopy (SEM) was used to analyze the surface, form, and texture of enamel. SEM was combined with EDX (Energy Dispersive Xrays Analyse) and used to analyse the composition of the material surface quantitatively and qualitatively. XRD (Xray Diffraction) was used to find out the elements in NanoNaF. In addition, NanoNaF particle was done using PSA measurement. Later, this powder was further processed into varnish NanoNaF.

Demineralization liquid composed by from $CaCl_2$ (2.2 mM/L), KH_2PO_4 (2.2 mM/L), acetate acid (50mM/L) and added with KOH. The suitable pH was achieved (4.06). Remineralization liquid composed by $CaCl_2$ (1.5mM/L), KH_2PO_4 0.9mM/L, and KCl (130mM/L) until the suitable pH reached (6.93).

The Production of varnish in this research doing by MDS (Material Data Sheet) from varnish that has been publicly in the market. The process was mixing three ingredients, 70% rosin, 25% ethanol and 5% NanoNaF without any addition of the stabilizer, preservative, and flavoring material. To prevent damage, the result of the mixing process was directly application to teeth.

Enamel was prepared by dividing the crown part and the root part using diamond bur high speed handpicked. Tooth was divided so that the buccal side and lingual side were separated. The buccal side was cut into 4x4x3 mm, then planted in acrylic. The enamel surface was polished with brush and pumice. NaF and Nanofluid were applications using microbrews, in the control group without any material application. In order to get better penetration toward enamel surface, teeth with NaF 5% and NanoNaF were left for 12 hours. Furthermore, they were rinsed and kept in aqua distillate. In order to test the endurance of enamel, demineralization and remineralization process necessarily need to be done. Demineralization using 30 ml demineralization liquid (pH 4.06) for 5 minutes and rinsed with aqua distillate then dried. Remineralization using 30 ml remineralization liquid was processed for 30 minutes. Demineralization and remineralization liquid must be changed every cycle. This cycle was repeated until six times in a day for 14 days. All teeth were kept in aqua distillate for during and after cycle. All demineralization and remineralization activity were done in room temperature. When all cycles finished, teeth were rinsed with aqua distillate and dried it. Moreover, teeth must be kept in aqua distillate until the next cycle on the next day, was replaced once in two days.

Enamel endurance measurement was through three different kinds of measurement which are enamel hardness with Vickers microhardness test, the total amount of fluoride with SEM and EDX, and the total amount of fluorapatite with XRD.

In order to know the difference of enamel endurance by topical fluoride application between 3 groups, One-Way Anova was used in this research. If there is the difference among each group, the calculation needs to be continued by using Tukey HSD. Error degree used is 0.05.[6] Kolmogorov-Smirnov was used to test the normality of the data. If the data were not distributed normally, it needs to be continued with Kruskal-Wallis test.

Result

Milling process of sodium fluoride using HEM resulted from whole NaF with particle size of 295 nm, so that it is called NanoNaF. The first milling process in 24 hours resulted in NaF with particle size of approximately 160 nm.

After the second milling process for 48 hours, it was obtained NaF with particle size of 295 nm. Milling process could not be continued because it can cause NaF particle size enlarged. In the other group, particle size of NaF varnish was about 400 nm.

Therefore, there was the difference for about 105 nm between NaF group and nanoNaF group. The smaller the particle size, the better the penetration likely to happen.

The result from SEM analysis toward control group was presented in Figure 1 with 100x magnification (Fig. 1A & 1B) enamel was mostly exfoliate.

The exfoliation from the outside part of teeth caused dental caries. Acid simulated exfoliation of the mineral of tooth.

By 500x magnification (Fig. 1C), there was no line in the peripheral side of the enamel, damage of enamel prism form and very porous area.

By 50000x magnification, enamel prism was not well-organized form.

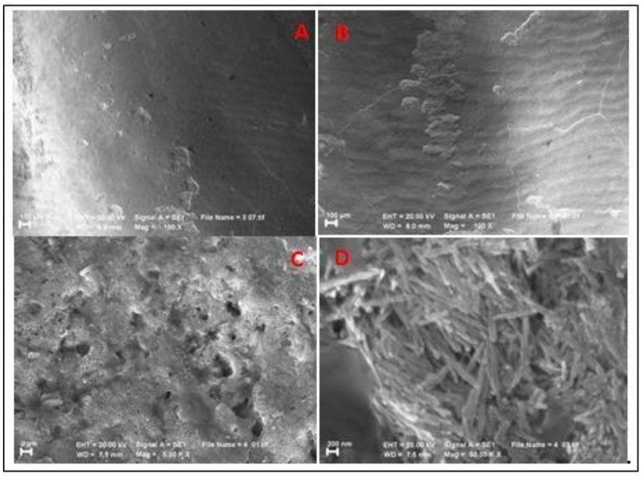

Figure 1. SEM result of control group
Description: (A and B = magnification 100x, C= magnification 5000x, D= magnification 50000x)

Figure 2 shows SEM from the enamel surface toward NaF group. In 3000x magnification (Fig. 2B), it is seen that the head and tail of enamel prism slowly disappear. In 5000x magnification (Fig. 2C), the head of fish shape as an outline form, the peripheral side slowly disappeared and separated. Some damage in enamel rod membrane and porous area can be seen. In 10000x magnification (Fig. 2D), enamel prism remains well-organized. This condition showed that damage has started to occur on the enamel surface, but not as much as in the control group.

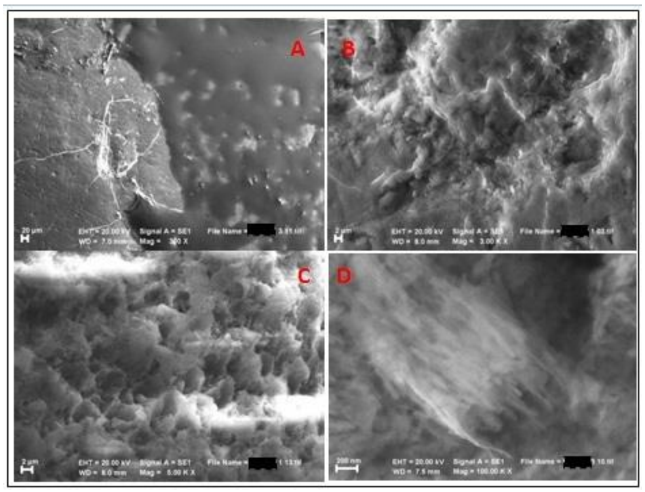

Figure 2. SEM result of NaF group
Description: (A= magnification 300x, B= magnification 3000x, C= magnification 5000x, D= magnification 100000x)

SEM result from NanoNaF group with 100x magnification in Fig. 3, showed a more smooth enamel surface than from control and NaF group. However, only a little NanoNaF layer attach to teeth. By 7500x magnification (Fig. 3C), there was a key-hole shape of enamel prism with a clear borderline, no gap between the enamel rod membrane, and no porosity occur. Enamel was fully covered from damage caused by acid. This result showed that NanoNaF varnish penetrated better to enamel than NaF varnish. By 50000x magnification, enamel prism was completely well-organized.

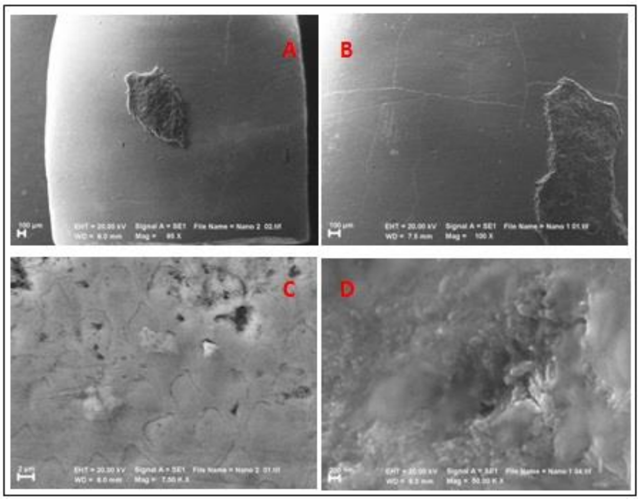

Figure 3. SEM result of Nanofluor group
Description: (A= magnification 65x, B= magnification 100x, C= magnification 7500x, D= magnification 50000x)

Results obtained from the microhardness calculation from three groups were 81.18 HV for a control group, 100.57 HV for NaF group, and 78.72 HV for NanoNaF group. Normality test from Kolmogorov-Smirnov shows $p>0.05$, which means the data was distributed normally and can be continued using One-Way Anova test. From this test, shows $p= 0.76$ ($p>0.05$). It is can be concluded that there is a mean difference between the three group hardest test.

From EDX result, it was obtained average total amount of the fluoride element from this research; the control group has 0.07%; NaF group has 0.26%, and NanoNaF group has 0.34%. The largest total amount average was in NanoNaF group, and the smallest total amount average is in the control group. Normality test using Kolmogorov-Smirnov showed $p>0.05$, which means that data was distributed normally and can be continued using One-Way Anova. From this test, show $p=0.08$ ($p>0.05$). It can be concluded that there was no mean difference between the total amount of the fluoride element from each group. Other elements such as Calcium (Ca), and Phosphor (P) also play an important role in forming crystal hydroxyapatite. The total amount average of Ca and P in the control group are 21.55% and 10.26%. In NaF group, the total amount average of Ca and P are 21.92% and 10.28%. In NanoNaF group, the total amount average of Ca and P are 30.48% and 13.49%. This result shows that NanoNaF can prevent mineral release better than NaF.

Result from XRD test showed that a hydroxyapatite element from the control group was 19.22%, whereas in NaF group was 45.65% and in NanoNaF was 63.33%. The normality test are p= 0.200, 0.198, 0.001 which means that the data was not distributed normally. Therefore, it was the need to be continued using Kruskal-Wallis test. Through this test shows $p= 0.06$ which mean there was no mean difference between the control group and NaF, control group and NanoNaF; and also NaF group and NanoNaF group.

In XRD test analysis, showed that only a little of fluorapatite detected from some of the teeth which were 11.22% (Control), 9.75% (NaF), and 30.11% (NanoNaF). This result happens probably because only a little fluoride element that can penetrate to enamel the surface that was similar to EDX test result. In EDX test result, it showed that Fluor element for the control group was 0.0256% and in NanoNaFgroup was 0.385%. NanoNaF varnish penetration can stabilize the total amount of hydroxyapatite (63.33%) and increased the total amount of fluorapatite (30.11%).

Discussion

Nanomaterial is a material that is commonly imagined at least one smaller size from 100 nm.[7] However, Friends of the Earth Australia Nanotechnology Project[8] recommended that the nanoparticle is a particle that has approximately 300 nm that is used in medical necessity. Particle with 100 nm has a very reactive characteristic, good bioactivity, and bioavailability. Therefore, this particle has great surface adhesion and a strong protein-binding[9]. The particles with a size smaller than 300 nm can be captured by the cells of the body.[10] A particle with size smaller than 100 nm has a higher risk toward health issue.[11] In Milling process of the material, there might be a new physical characteristic of the particle.[12] The synthesized of nanoparticles may result in the difference structure that would effect the characteristic of the material. In processing of nanoparticle, there was difficulty to preserve the size at nanoscale. Because of their high surface area, nanomaterials showed high and strong reactivity, that made high tendency towards agglomeration.[13]

NaF has a hygroscopic characteristic.[14] It means that NaF can attach and endure the water molecule from its surrounding. This condition happens either in absorption and adsorption process which can cause physical alteration such as volume enhancement, attachment and other characteristics of NaF. This hygroscopic characteristic of NaF causes it can be agglomerated easily (union process of small particles into bigger particle). This condition cause NaF is difficult to dissolve under 100 nm. Fluoride element that becomes the major element in this material is a halogen element and the most chemically reactive compared to other elements. Fluoride is never found in free shape. It is usually found in the compound with other elements.[15]

HEM is a milling using high energy. There are some factors affecting the result of HEM including the speed rotation (rpm), milling ball and ratio of ball mass and the element mass that need to be milled. This mechanical milling may be used to make particles of a certain size and shape include nanoparticle.[16] The particle size produced depends on many parameters such as milling duration, milling speed, ratio between ball and powder weight, and rotation speed.[17] HEM is one of technique to produce NanoNaF. Compared to other techniques, HEM has some advantages. Benjamin (1990) quoted by Yadav et al[18], reported that HEM was easily application, relatively more economic, can be used in various materials, and can be produced in a large scale. However, specific material such as NaF can cause the material melt and stick again together into one particle, that cause NaF powder in this research has the larger size. Other techniques beside HEM in nano synthesis can be application as an alternative in changing the NaF particle size. Right after milling process finished, PSA need to be directly used to measure the particle size.

The hardness of enamel between three groups showed no significant difference. This result might happen because NaF has greater attachment than NanoNaF during the application process. This condition was observed through the greater amount of the detached NanoNaF particle compared to NaF particle. This result might occur because the production of NanoNaF is still simple so that the attachment difference in NaF made from industry has more complexity and more advanced production process. That process makes NaF attaches more effective to enamel than NanoNaF. Therefore, the remain attached material might increase the hardness in the enamel surface. Fluoride content of enamel in NanoNaF group was greater than the fluoride content in control and NaF group. That result happens because NanoNaF has smaller particle size so that it can penetrate easier than NaF 5%. On the other hand, enamel in a control group contained the lowest fluoride because demineralization process occurred was not replaced by fluoride ions. It is obviously occurring because there is no fluoride application in the control group. Hydroxyapatite in the control group is mostly detached from enamel. This condition happens because there is no great coverage to this particle during the demineralization process.

Only a little fluoride element detected in fluorapatite form in this research. However, fluoride is also bonded with other elements such as Chlor and Calcium Phosphate becoming Chlor apatite and Calcium phosphate apatite. Another result obtained from XRD test is Calcium Hydrogen Phosphate.

Conclusion

1. NaF has hygroscopic characteristic that cause agglomeration in nanoparticle production process using HEM.
2. NanoNaF application in enamel does not affect the hardness of the enamel surface compare to NaF application.
3. NanoNaF application can increase the amount of fluoride (0.1289%) and fluorapatite (20.35%) compare than NaF application.
4. NanoNaF can maintain hydroxyapatite in tooth until 63.33%.
5. NaF 5% and NanoNaF application can influence the enamel endurance toward the caries.

Further research about characteristic of NaF using a proper milling technic and milling process is necessarily needed.

Declaration of Interest

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