

## The effect of various amounts of nanohydroxyapatite on the mechanical properties and remineralization of a fissure sealant.

<sup>1</sup>Roza Haghgoo <sup>2</sup>Mohammad Ataie <sup>3</sup>Sara Tavassoli Hojjati <sup>\*4</sup>Somayeh Kameli <sup>5</sup>Sara Rahimian Imam

<sup>1</sup>Associate Professor, Dept. of Pediatric Dentistry, Dental School, Shahed University, Tehran- Iran.

<sup>2</sup>Associate Professor, Dept. of Polymer, Petrochemical and Polymer Research Center of Iran, Tehran- Iran.

<sup>3</sup>Assistant Professor, Dept. of Pediatric Dentistry, Dental School, Shahed University, Tehran- Iran.

<sup>\*4</sup>**Corresponding Author:** Postgraduate Student, Dept. of Pediatric Dentistry, Dental School, Shahed University, Tehran- Iran. **E-mail:** [so.kameli@shahed.ac.ir](mailto:so.kameli@shahed.ac.ir)

<sup>5</sup>Postgraduate Student, Dept. of Pediatric Dentistry, Dental School, Shahed University, Tehran- Iran.

### Abstract

**Objective:** The main reason for failure in application of fissure sealant is its loss of bond to tooth and development of secondary decays. Nanoparticles of hydroxyapatite have convenient biologic properties and we can try to benefit from these characteristics by adding them to repair materials. The present study was performed with the aim to assess the effect of addition of various amount nanoparticles of hydroxyapatite on mechanical properties such as microshear bond strength and physical properties like curing depth and degree of conversion as well as evaluation of teeth enamel remineralization.

**Methods:** In the present laboratory experimental study different weight percentages comprising 0% (control), 1%, 3%, 5%, 10% and 15% of nanoparticles of hydroxyapatite with 50 nm dimensions were separately added to fissure sealant. Then degree of conversion test by Fourier Transform Infrared Spectroscopy (FTIR) and curing depth according to ISO 4049 standard were performed on prepared samples. For the microshear bond strength test 35 premolar teeth without caries were used. The substance properties measurement device (Zwick) was used to do the test. Remineralization of permanent teeth enamel was assessed by Scanning Electron Microscopy (SEM). Raw data obtained were statistically analyzed by normal distribution test (Kolmogorov-Smirnov), one way ANOVA and Tukey Post Hoc.

**Results:** Results showed that microshear bond strength and degree of conversion had no significant difference in various concentrations of fissure sealant containing hydroxyapatite nanoparticles ( $P > 0/05$ ). Between microshear bond strength and degree of conversion means of 3M commercial fissure sealant and usual fissure sealant there was no significant difference ( $P > 0/05$ ). Curing depth in concentrations of 10% ( $P = 0$ ) and 15% ( $P = 0$ ) statistically significantly decreased compared to previous groups ( $P < 0/05$ ). Curing depth of 3M commercial fissure sealant group was lower compared to conventional fissure sealant group ( $P = 0$ ) and the difference was statistically significant ( $P < 0/05$ ). A remineralized region on the surface between the fissure sealant and tooth enamel was observed by SEM. This region was more remarkable in higher concentrations.

**Conclusion:** Fissure sealant containing hydroxyapatite nanoparticles with effect on remineralization on the surface of tooth enamel can lead to reduction of micro-leakages and prevention of development of secondary caries while mechanical properties do not decline.

**Key words:** Fissure sealant, Hydroxyapatite nanoparticle, Microshear bond strength, Degree of Conversion, Curing depth, Remineralization

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### Introduction:

One of the concerns existing regarding fissure sealant is its loss of bond to tooth which leads to development of microleakages and subsequent

secondary caries. This is explained by the fact that fissure sealant has a resin matrix and contraction during polymerization causes loss of bond and formation of microleakages between fissure sealant and enamel (1, 2). Therefore different materials are added to fissure sealant in

order to improve its mechanical properties, clinical durability and to prevent secondary caries (3). In PH under crisis limit, mouth liquid have an unsaturated state in relation to hydroxyapatite, so hydroxyapatite is dissolved. At that moment, presence of small amounts of fluoride in solution will cause a state of unsaturation in relation to Fluorapatite and particularly to Fluorhydroxyapatite present in enamel. Because of better access of superficial layers of enamel to environment ions and surface being covered by a film of saliva which acts as a barrier limiting diffusion of ions out of the enamel, the amount of calcium, phosphate and fluoride saturation in tooth surface is higher, and probability of remineralization in this region is high and likelihood of demineralization is low. Thus as time goes, a high amount of Fluorhydroxyapatite resistant to acid is accumulated in the superficial layer. Minerals are mainly lost in the subsurface, which leads to primary enamel caries lesions with a distinct appearance of completely mineralized 20-50 microns in thickness superficial layer and subsurface body. Formation of the superficial layer has a protective effect against continuation of dissolution of underneath minerals. In addition, exit of calcium and phosphate ions from tooth and diffusion of hydrogen ions, confines acid to regions under the surface, which delays spread of the lesion and cavity formation in the tooth. It is proven that if the aforementioned condition of saturation in relation to calcium and phosphate in the enamel environment establishes, primary caries lesions are reversible before cavity formation, and it is possible that these ions become crystallized again on the surface of crystal residues of demineralization. This process is called remineralization and the ions necessary for it are mainly provided by the saliva (4).

In 1972, Silverstone stated that remineralization by saliva's calcium and phosphorus ions is a slow process and that because of the low concentration gradient from saliva to the inside of the lesion, accumulation of minerals occurs mainly in the superficial layer. He also claimed that to perform effective repairs of lesion, other systems beside saliva are needed to supply calcium and phosphate ions (5). For this reason today, with a change of approach in treatment of

caries, and the importance of conservative dentistry, a considerable part of researches are focused on evaluation of substances for remineralization of dental caries in their initial stages. And since fissure sealant have to stay on dental grooves for many years as a preventive substance, their bond strength as well as mechanical properties are of the most importance (6).

Hydroxyapatite (HAP) is a calcium and phosphate crystalline which is found in enamel, dentin, cementum and bone. Hydroxyapatite, because of its characteristics such as similarity to the principal mineral part of human body hard tissues, biological compatibility and low solubility in humid environments, has been widely used in biology, medicine and dentistry (7). Another important feature of HAP is its antibacterial properties; studies performed on it showed that this substance limited growth of *Streptococcus Mutans* and other oral pathogens (8).

Park et al in their research demonstrated that fissure sealant containing nanohydroxyapatite (NHAP) can lead to remineralization of enamel in the region beneath the fissure sealant (9). In another study, by adding hydroxyapatite to glass ionomer, its mechanical properties such as flexural strength and tensile strength have been improved (10).

One of the most important subjects of recent studies is the use of nanoparticles in order to improve the properties of a material. By producing particles in nanometer size, their surface to volume ratio increases considerably which makes them effective in very low concentrations. Since till now no study has been performed in this field, the purpose of this study is to assess physical properties such as conversion degree and curing depth, mechanical properties like shear strengths well as remineralization of permanent teeth enamel following application of fissure sealant with addition of nanohydroxyapatite in various amounts (1, 3, 5, 10 and 15%).

## Methods:

In the present laboratory experimental study, first, fissure sealant with the following formula

was made by the Petrochemical and Polymer Research Center of Iran, 70% BIS-GMA, 30% TEGDMA, 0.5% Comphorquinone, 0.5% DMAEMA, 7% Silanised Silica (Rohmchem, Darmstadt, Germany).

Preparation of fissure sealant containing nanoparticles of hydroxyapatite:

To eliminate air humidity from particles' surface, nanoparticles of hydroxyapatite were placed for 1 hour in an oven at 100° C. Nanohydroxyapatite with 50nm dimensions and determined weigh percentages (1, 3, 5, 10 and 15%) were measured to 4 decimals by a digital balance.

Preparation of test groups was performed in a dark space under dark room special red light. This study comprised two control groups and 5 experimental groups. The first control group comprised commercial fissure sealant (3M concise, USA) and the second group was composed of fissure sealant produced by the Petrochemical and Polymer Research Center of Iran. In each experimental group after determination of 10 grams of fissure sealant, this digit was multiplied by 1%, 3%, 5%, 10% and 15%. In the experimental groups respectively 0/1, 0/3, 0/5, 1 and 1/5 grams of nanohydroxyapatite were measured by a digital balance to 4 decimals and added to the fissure sealant made by the Petrochemical and Polymer Research Center. Then for mixing and appropriate distribution, glass and plastic spatulas were separately used for 10mn for each weigh group in order to achieve mixing of nanoparticles with fissure sealant and obtain a uniform distribution of particles.

To assess remineralization, 70 healthy pre molar teeth without cracks, fractures or repairs were collected. Teeth were conserved in a 12% formaldehyde solution for 1 week. Then they were transferred to a sterile saline solution. Teeth surfaces were polished at 280 grits and under cooler with silicon carbide abrasive papers with commercial name of Matador and made in Germany. After what, cubic cavities with 1x4x5 dimensions were prepared on the buccal surface. Teeth were divided in 7 groups of 10 and fissure sealant with various percentages was applied on them. Then samples were placed in 36/5° C and artificial saliva for 4 weeks to simulate the oral environment. Prepared samples were then cut.

Samples were evaluated for remineralization regions by T Scan Electronic Microscopy (VegaII model of Czech Republic). Thickness in microns of the interstitial layer of each group was measured. Also, in order to determine the dispersion and distribution of calcium and phosphor elements, prepared samples for SEM evaluation were assessed in 3 different locations by EDAX device INCA model (Oxford Instrument, England); Concentrations of calcium and phosphor elements in layers of fissure sealants, interstitial and underneath were measured.

To perform microshear bond strength test, 35 premolar teeth without caries and after disinfection were conserved for 1 week in saline solution. After polish, enamel pieces were randomly divided in 7 groups of 5. Then enamel surface was etched by an acidic gel for 20 sec, after what they were rinsed with water and dried. Following what, they were recovered by Excite bonding, and before curing, a silicon band in cylindrical shape with 1mm height and 0/8mm internal diameter was placed on enamel surface to limit the bonding surface. Then they were filled by each percentage of fissure sealant containing nanohydroxyapatite and cured for 40 sec. After one month of conservation of the samples in artificial saliva and at 37°C, Universal testing Machine (ZwickRoell model, Germany) was used to measure microshear bond strength. An orthodontic wire with 0/2mm diameter was circled around resin cylinder in such a way to be in contact with the inferior half of the cylinder and the teeth surface. To precisely measure forces, load cell with maximum measurement of 5KN was used in the device. Force with a speed of 1mm/mn was applied for breaking to occur. The way of breaking and their morphology was assessed with electronic microscope. Microshear bond strength was calculated using the following formula:

$$\tau = \frac{4F}{\pi d^2}$$

$\tau$  = Shear strength, F= Maximum force in breaking point, D = Sample diameter

To assess the curing depth, fissure sealant was

measured in conformity with ISO (4049). Fissure sealant without (0%) and containing various percentages of nanohydroxyapatite and 3M fissure sealant were separately placed in two steel cylindrical frames with 4mm diameter and 10mm height between two plates. Then the samples were cured for 40 sec from above. After irradiation, non cured material on the other side of the sample was scratched by a plastic spatula and thrown away. Following what, the height of the remaining part was divided by 2. The average of 3 repetitions for each percentage was considered the curing depth.

To evaluate the degree of conversion (Dc %), the control fissure sealant (0%) was placed between two bands of polyethylene. Fissure sealant was placed in the Fourier transformation infrared spectroscopy (Burker model, Germany) before curing, and spectroscopy performed. In the next step DC % was calculated according to the formula. In different groups, DC % was also

calculated in the same way. The average of 3 repetitions for each percentage was considered the amount of DC %.

Raw data obtained were analyzed by SPSS. Normal distribution test (Kolmogorov-Smirnov) was used in order to determine the normality of data distribution. And one way ANOVA was used to test the difference of variables means.

## Results:

Results of the study using one way ANOVA test showed that microshear bond strength in different concentrations of fissure sealant containing nanoparticles of hydroxyapatite didn't have a significant difference ( $P>0/05$ ,  $P=0/143$ ). Also between the mean of microshear strength of 3M commercial fissure sealant and the usual fissure sealant no significant difference was observed ( $P>0/05$ ,  $P=0/105$ ). (Table 1)

**Table 1- Microshear Bond Strength in different groups**

<b>3M-0%</b>	<b>0%</b>	<b>1%</b>	<b>3%</b>	<b>5%</b>	<b>10%</b>	<b>15%</b>
<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>	<b>Microshear Bond Strength (Mpa)</b>
7.67	8.07	8.9	8.89	16.78	9.6	11.68
7.76	8.32	13.94	10.84	10.97	12.06	8.81
9.03	18.56	15.98	14.58	13.17	7.62	7.69
9.13	15.07	18.78	15.21	9.96	10.25	13.22
12.26	7.2	15.17	15.04	17.37	9.91	9.08

Also, using the one way ANOVA test, it became clear that the degree of conversion in different concentrations of the new fissure sealant compound in fine layers didn't have a significant difference ( $P>0/05$ ). And between the mean of degree of conversion of the 3M commercial group with the usual fissure sealant there was no significant difference ( $P>0/05$ ). (Table 2)

Fissure sealant curing depth in concentrations 10 and 15% decreased, and by using the one way ANOVA test and Tukey Post Hoc Test it became clear that it was statistically significant ( $P<0/05$ ). But in concentrations 0%, 3%, and 5% it was not statistically significant ( $P>0/05$ ). In concentrations 10 and 15%, viscosity of the

substance increases. The reason for that is the accumulation of nanoopaque particles in the high thickness of the substance. Also the mean of curing depth of the 3M commercial fissure sealant group was lower compared to the usual fissure sealant and had a statistically significant difference ( $P<0/05$ ). (Table 3)

According to observations by SEM, a remineralized region in the surface between fissure sealant and teeth enamel was seen, which was more remarkable in higher percentages. And thickness of the interstitial layer remarkably increased, which was measured in microns in each experimental group. But this region was not observed in the control group (0%). This region

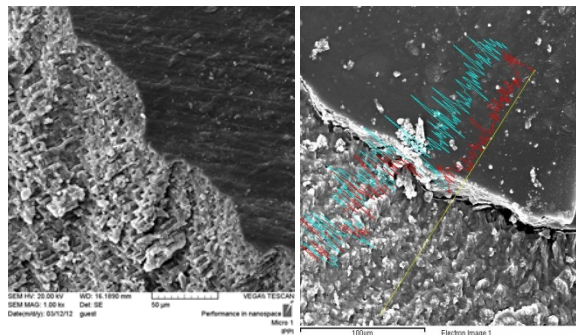
appeared as an amorphous and white region in the surface between the tooth enamel and fissure sealant (Figure 1).

**Table 2-Comparison of the amount of Degree of Conversion in different groups**

Sample name	Mean (DC%)	SD of 3 repeats
3M	43.34129	5.783241
0%	35.39665	2.374652
1%	39.09441	1.178566
3%	38.56956	10.95008
5%	38.24114	7.073335
10%	25.78348	14.95229
15%	37.15019	4.098489

**Table 3- Comparison of the amount of Curing Depth in different groups**

Sample name	Mean (DC%)	SD of 3 repeats
0%	2.983333	0.002886
1%	2.981666	0.002886
3%	2.988333	0.005773
5%	2.926666	0.007637
10%	2.803333	0.012583
15%	2.513333	0.028431
3M	1.431616	0.015275



**Figure 1- Interface between tooth and fissure sealant by SEM (x1000) (a)- Control Group (b)- Fissure sealant 15% weight NHAP**

Under SEM and investigating with EDAX, crystals of NHAP have been observed on the surface of fissure sealant and enamel that were uniformly dispersed. The image was compared to the control group (0%).

**Discussion:**

Hydroxyapatite is known as a remineralizing substance when applied on the surface of tooth

enamel (9). Nanohydroxyapatite is the most similar compound to the dental tissue. Characteristics such as biologic compatibility, antimicrobial properties, remineralization potential and reduction of microleakages in dental cavities caused this substance to attract a lot of attention in recent researches (10).

Nanoparticles of hydroxyapatite having a higher surface to volume ratio are more effective than bigger hydroxyapatite particles. In recent years nanotechnology by reducing the size of particles and controlling their properties in terms of shape and distribution, have provided very bioactive calcium and phosphates, which probably have the potential to penetrate more in porosities beneath demineralized region as potential remineralizing substances (11, 12).

Arcis et al in 2002 in a study evaluated the effect of hydroxyapatite filler in the composition of dental composites as well as its effect on water absorption. Results showed that hydroxyapatite filler in composite provokes the reduction of composite water absorption. In that study nanoparticles and also various concentrations of hydroxyapatite weren't used. Also other mechanical properties were not assessed (13).

Tschoppe et al in 2011 assessed the effect of tooth paste containing nanohydroxyapatite on remineralization of enamel and dentin. Results demonstrated that tooth paste containing nanohydroxyapatite in comparison to tooth paste containing fluoride amine had a significant effect on remineralization of dentin and enamel (14).

Kim et al in 2007 investigated the remineralizing effects of nanohydroxyapatite in combination to sodium fluoride mouth wash on human tooth primary caries lesions and used various concentrations of nanohydroxyapatite. Results indicated that amount of remineralization of tooth enamel increased with mouth wash containing nanohydroxyapatite (15).

In several studies effects of remineralization properties of nanohydroxyapatite on dentin and enamel demineralized lesions were evaluated (16, 17). But since fissure sealant should ideally put on healthy tissues, in the present study in order to simulate oral environment, samples were conserved in artificial saliva at a temperature of 36/5°C which is a temperature similar to body temperature. Ca and P ions in

artificial saliva were profusely dissolved, thus it was possible to create conditions for mineralization stages.

Park et al in 2005 assessed the effects of various concentrations of hydroxyapatite on remineralization of fissure sealant. They added concentrations of 1, 5, 10, 15 and 20% of hydroxyapatite to fissure sealant and placed them on 80 samples of human teeth. Then samples were conserved for a month in artificial saliva. By adding hydroxyapatite to fissure sealant, bond strength between sealant and tooth increased compared to control group, and an enamel remineralized region was observed by electron microscopy beneath fissure sealant. He referred a 20% concentration as desirable. In the study mentioned nanoparticles were not used. Also to evaluate the remineralized region EDAX was not used (9).

A repair material with the same color as the tooth should ideally have convenient physicochemical characteristics, appropriate biological compatibility and be able to support dental repair and mastication (14, 15). In the present study NHAP in 0% (control), 3, 5, 10 and 15% concentrations was added to fissure sealant in order to be able to investigate the physical and mechanical characteristics of this new substance. Also curing depth, microshear bond strength and degree of conversion of this new substance were compared to control group. Remineralization effect of NHAP was assessed as well.

EDAX is one of the devices able to measure minerals and elements in a material via absorption of X-rays (3). In the present study, EDAX was used to evaluate elements present in fissure sealant and enamel. EDAX assessed the amount of Ca and P elements in three layers: fissure sealant, enamel superficial layer and deep enamel layer. Results showed a specific difference in amounts of Ca and P elements in the enamel superficial layer with increase of nano percentage. In a way that amounts of Ca and P elements increased with augmentation in weight percentage of nanoparticles from 0 to 15%. The layer between enamel and fissure sealant with 15% weight of nanohydroxyapatite had the highest amount of Ca and P.

In SEM evaluation, the mineralized superficial layer was measured. A greater weight

percentage of nanohydroxyapatite uniformly precipitates between fissure sealant and enamel. This interstitial layer has a higher thickness with the increase of nanoparticles weight percentage. In such a way that at 15% weight the formed layer has the highest thickness.

In the present study microshear bond strength and degree of conversion DC% tests which are part of the most important physical and mechanical characteristics of dental materials were performed in laboratory as complement of fissure sealant remineralization properties assessment.

High amount of DC% causes better physical and mechanical properties. Insufficient polymerization of dental material leads to post treatment sensitivity, microleakages, secondary caries, increased potential for degradation, marginal pallor, color change and decline of mechanical properties. Many studies used hardness (Vickers and Knoop) tests to determine the amount of polymerization. Though hardness has a strong relation with DC%, studies based on the amount of carbon covalent bonds C=C are more reliable. It has been proven that FTIR is a sure technic for analysis of DC% of monomers of dental materials, which was used in the present study (18).

In this research, microshear test was used to assess the bond strength, which is an easy, reliable and common method for measurement of composite material bond to dental structures. Samples were prepared using silicon generators with 1mm height and 0/9mm diameter. It is claimed that because of a small bond area, probability of cracks and their spread to the inside of each sample decreases in this test. Control of location of the knife edge was difficult in this test, it is possible that some bending occur which causes changes in the results. For this reason in this study it has been tried to put the wire parallel to the tooth and fissure sealant contact surface in order for the knife edge to preserve itself (2).

In concordance with the results observed in SEM, it is possible to predict that the new compound of sealant containing NHAP particles induce formation of a remineralized layer. And this layer resistant to decay lead to decrease of bacteria invasion as well as reduction of secondary caries development. But in the

examination of physical and mechanical properties of the new sealant it has been observed that microshear bond strength and degree of conversion did not have a significant difference in various concentrations of nanohydroxyapatite. While the curing depth decreased in concentrations of 10 and 15%. This is due to collection of opaque nanoparticles in the high thickness of the substance. Therefore in high concentrations very fine layers of this substance have to be used and curing time should be at least 40sec. Also in higher concentrations (10 and 15%) the viscosity of the substance increases and to overcome this problem substance concentration of filler silica has to be lowered in order to obtain a convenient concentration.

### Conclusion:

The present study showed that adding nanoparticles of hydroxyapatite to concentrations up to 15% of weight has no effect on microshear bond strength. Also adding nanoparticles of hydroxyapatite to concentrations up to 15% of weight has no effect

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on degree of conversion in fine layers. Increase in amount of nanoparticles of hydroxyapatite to 10 or 15% leads to significant decrease in curing depth.

Adding nanoparticles of hydroxyapatite also lead to formation of a remineralized layer in a surface between fissure sealant and tooth enamel, and from 1 to 15% of weight remineralization showed remarkable increase.

Therefore addition of nanohydroxyapatite to fissure sealant can help in remineralization of enamel as well as in prevention of eventual caries. Addition of nanohydroxyapatite does not make mechanical and adhesive properties decline.

### Suggestions

Taking into account the limitations of the study, it is suggested in order to evaluate the remineralized region more precisely, to use disks of fissure sealant containing nanoparticles of hydroxyapatite in various times in artificial saliva solution and to measure their microhardness.

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