

Comparison of Various Concentrations of Tricalcium Phosphate Nanoparticles on Mechanical Properties and Remineralization of Fissure Sealants

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Abstract

Objective: The aim of this study was to investigate the mechanical properties (flexural strength, micro-shear bond strength) and remineralizing potential of fissure sealants by adding various concentrations of β -tricalcium phosphate nanoparticles.

Materials and Methods: This in-vitro study consisted of five experimental groups containing prepared nano-fissure sealants (1-5 wt.% β -TCP nanoparticles) and two control groups containing a prepared and a commercial fissure sealant.

Flexural/micro-shear bond strength values were measured using Zwick test machine. Cavities on sixty healthy premolar teeth were filled with the fissure sealants containing 0-5 wt.% of nano β -TCP. The samples were assessed for remineralization under scanning electron microscopy (SEM) and EDAX. Kolmogorov-Smirnov test, One-way ANOVA and Tukey's Post Hoc analysis/HSD were used to analyze the data.

Results: There was no significant difference between the flexural strengths/elastic modulus of the 0-5 wt.% nano β -TCP groups ($p>0.05$). The average flexural strength/elastic modulus of the prepared fissure sealant group (0%) was significantly higher than the commercial fissure sealant group (Clinpro) ($p<0.05$). There was no significant difference between micro-shear bond strengths of the experimental groups (1-5 wt.%), and between the commercial and the prepared (0%) fissure sealant groups ($p>0.05$). Examining the samples under SEM showed a significant increase in thickness of the intermediate layer with increasing concentrations of β -TCP nanoparticles ($p<0.05$).

Conclusion: Addition of 1-5 wt.% β -TCP nanoparticles to the fissure sealants significantly increased the remineralization potential without affecting the mechanical properties.

Key Words: Nanoparticles; Fissure Sealants; Tooth Remineralization

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INTRODUCTION

One of the main concerns regarding fissure sealants is weakening of the bond of this material that may lead to secondary caries. As fissure sealants consist of a resin matrix, po-

lymerization shrinkage may lead to weakening of the bond, resulting in microleakage that increases with time and leads to secondary bacterial attack/caries [1]. By adding remineralizing agents to fissure sealants, the enamel be-

comes more resistant to carious attack, which in turn leads to a reduction in secondary caries [2].

On the other hand, it is a requirement of fissure sealants as preventive agents to remain on the surface of fissures for many years. Therefore, consideration of the mechanical properties of fissure sealants is particularly important for their longevity [1, 3]. Tricalcium phosphate [TCP, chemical formula: $\text{Ca}_3(\text{PO}_4)_2$] is a biomaterial with high potential for biological application [4]. This material is available in a calcium: phosphate ratio of 1:5 and is available in two forms of α and β (β particles are less soluble than α particles). TCP has been known to increase salivary calcium levels, and is one of the materials that can improve the process of tooth remineralization due to its calcium and phosphate content [5].

It was shown in a study that adding β -TCP to toothpaste containing 5000 ppm fluoride led to a 30% increase in subsurface carious remineralization and enamel microhardness [6]. Besides, addition of β -TCP has been shown to result in improved mechanical properties of polymeric materials. In a study that investigated the addition of β -TCP particles to hydroxyapatite crystals, the resulting composite material displayed double shear bond strength values compared to using hydroxyapatite alone; there was also an increase in the compressive bond strength of the composite [7].

In another study, β -TCP nano-particles were added to a glass ionomer cement that led to an increase in the compressive bond strength of glass ionomer [8]. The field of nanotechnology has recently been applied to many scientific areas including dentistry. Nano-sized particles have desirable characteristics and numerous applications due to their higher surface to volume ratio.

In addition, it is possible to control certain characteristics of the powder, such as size, shape, particle dispersion and agglomeration in order to improve mechanical/biologic properties. These materials have superior biologi-

cal properties compared to materials with a larger particle size [9].

Using nanotechnology, it is possible to prepare β -TCP nanoparticles with improved cariostatic and remineralizing properties on the enamel surface [7]. The purpose of this experimental study was to investigate the effect of varying concentrations (1, 2, 3, 4 and 5 wt.%) of β -TCP nanoparticles on the mechanical properties (flexural strength, micro-shear bond strength) and remineralizing potential of fissure sealants.

MATERIALS AND METHODS

This in-vitro study consisted of seven groups as follows: five experimental groups of fissure sealants containing β -TCP-NPs in different concentrations of 1, 2, 3, 4, and 5 wt.%, and two control groups containing fissure sealants with no additives. The first control group consisted of Concise (3M ESPE, USA) as commercial pit and fissure sealant and the second control group consisted of a (70% BIS-GMA, 30% TEGDMA, 0.5% DMAEMA, 0.5% Camphorquinone and 7% Silanized Silica) manufactured by the Iranian Polymer and Petrochemical Institute.

Preparation of Fissure sealant containing β -TCP nanoparticles: In this experiment; all groups, five measurements (0.1g, 0.2g, 0.3g, 0.4g and 0.5g) of β -TCP nanoparticles (plate-like structure with a thickness of 100-200 nm, Ca/P= 1.4) were weighed using a digital scale up to four decimal points), added to 10g of fissure sealant and mixed homogeneously in a dark room for 20min with a glass spatula. The resulting mixtures were stored in completely opaque containers until each test was performed.

Measurement of Micro-shear Bond Strength: In order to measure the micro-shear bond strength of 25 permanent maxillary/ mandibular premolar human teeth (extracted in the previous six months for orthodontic reasons),

the samples were observed under $\times 2$ magnification using a stereomicroscope (Carton optical Industries, Thailand). Teeth that did not contain abrasive wear facets, caries/decalcification were selected and placed in 12% formaldehyde solution for one week. The teeth were then transferred to 0.9% sodium chloride solution until the time of the experiment [10].

The crown of each tooth was removed using a paper disk and air turbine (W8H, Austria) under water irrigation. Each tooth was then sectioned into two buccal and lingual segments, resulting in 50 tooth sections for the experiment. The enamel surfaces were polished using silicon carbide abrasive disks (280 grit, Matador, Germany) under water irrigation in order to remove the non-prismatic enamel. The samples were then randomly divided into seven groups of seven samples each. The polished enamel surfaces were etched using 37% phosphoric acid gel (Total Etch 37%, Ivoclar Vivadent, USA) for 15 seconds, rinsed for 20 seconds and air dried for 5 seconds.

The prepared enamel surfaces were covered by bonding agent (Exite, Ivoclar Vivadent, USA) according to the manufacturer's instructions. The bonding layer was spread out by gentle air blow, and a cylindrical tube (internal diameter: 0.75mm, height: 1.0mm) was placed on the enamel surface prior to curing for 20 seconds. The cylindrical tubes were gently filled with various fissure sealants, and a celluloid strip was placed on the molds in order to achieve a smooth surface. The prepared samples were cured from above for forty seconds, removed from the tubes using surgical blade #11 and cured from each side for another forty seconds. The samples were stored in a steam incubator at 37°C and 100% moisture for 24h. After removal of the samples from the incubator, each sample was attached to the metal surface of the testing machine using cyanoacrylate adhesive.

A thin wire (0.2mm diameter) was looped around each resin cylinder, so that the wire

was in contact with the lower half-circle of the cylinder and the tooth surface. Pulling up the wire, a shear force was applied to each specimen at a cross-head speed of 1mm/min using the Zwick Roell universal testing machine (Zo20, Germany) until failure occurred [11]. The shear bond strength was calculated using the following formula:

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

whereby τ = shear bond strength, F =maximum force at point of fracture and d =diameter of the sample. The mode of fracture and morphology of all the samples were initially observed under a stereomicroscope (Carton Optical Industries, Thailand) and then further examined under a scanning electron microscope (SEM, Microscopy VEGA // TESAN, Czech Republic). The samples were mounted on the aluminum stub using carbon-coated double-sided adhesive tape and then coated with gold using sputter coater. The mode of fracture was either adhesive, cohesive or a combination of both.

To measure flexural bond strength according to ISO 4049 standard, stainless-steel molds (2 \times 2 \times 25 mm in size) were selected. The steel molds were then placed on a glass slab completely covered by lubricant in order to allow complete removal of the materials after hardening. The resins were poured into the molds using a plastic spatula by avoiding formation of air bubbles. Then, the mold was covered with another glass slide and specimens were polymerized using LED light cure machine (Demetron, Kerr, USA). The length of the mold was polymerized five times (40 seconds each) and the bottom surface was polymerized once. Samples containing air bubbles/cracks were excluded. After removal of the specimens from the molds, they were immediately placed in saline and stored in an incubator (100% moisture, 37°C temperature) for 24 hours in order to avoid desiccation.

In order to measure flexural bond strength, the specimens were removed from the incubator and polished using 600 grit silicon carbide paper in order to remove any excess material and achieve a polished surface. The specimens were placed in the universal testing machine (Z20, ZwickRoell, Germany) at a cross-head speed of 0.5 mm/min. Flexural bond strength was measured by calculating the length/width of the samples and the maximum flexural force using the following formula:

$$\sigma = \frac{3PL}{2bd^2}$$

Whereby σ = flexural strength (MPa), p=maximum flexural force (N), L= distance of two pivots from each other (20mm), b=sample width (mm), d=sample height (mm) [3].

In order to investigate the process of remineralization, thirty-six tooth specimens were prepared by sectioning the crown of each tooth into two segments and excluding samples that appeared to be cracked under the stereomicroscope.

A cavity 3×3 mm in size and 0.5mm depth was prepared in the middle third of the buccal/lingual enamel surface.

The enamel surfaces were etched using 37% phosphoric acid gel for 15 seconds, rinsed for 20 seconds and air dried for 5 seconds. The prepared enamel surfaces were covered by bonding agent according to the manufacturer's instructions. The bonding layer was spread out by gentle air blow and cured for 20 seconds. Fissure sealant was then placed into the cavity and cured for 40s. The cavity edges were polished using a cylindrical bur in slow speed handpiece.

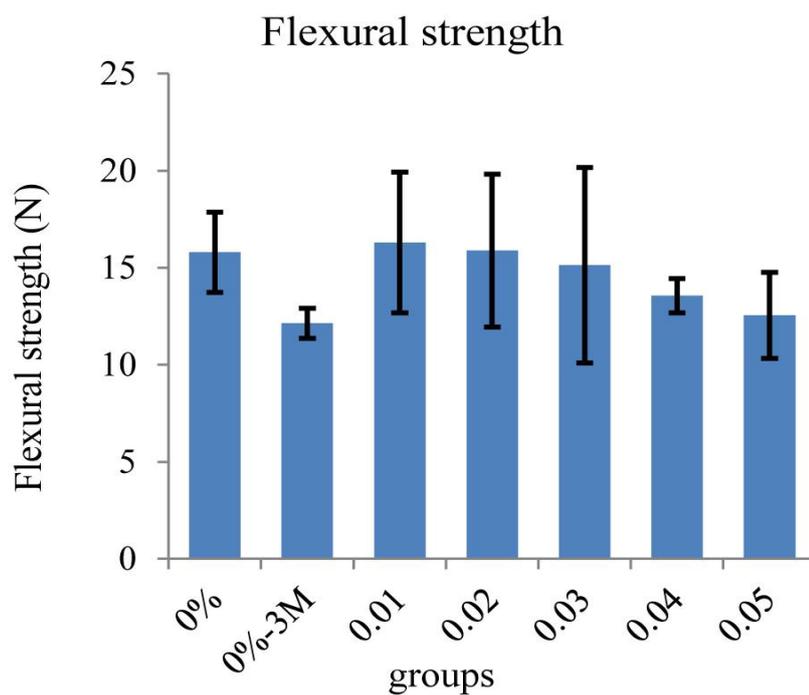
The crown of each tooth and 1mm of the restoration periphery were then covered by nail polish in order to only allow a 5×5mm window exposure to the environment and prevent liquid absorption at the other surfaces.

The window area was cleaned using acetone and the surface of the fissure sealant was polished in order to remove any remnants. An acid-base demineralizing solution was prepared using 50mm acetic acid (pH5), 1.2 mm calcium chloride and 2.2 mm NaH₂PO₄. All of the thirty-six tooth samples were immersed in 30ml demineralizing solution and stored in an incubator (Fannshimi, 2.57, Iran) at 37°C and 100% moisture for four days. The acid solution was replaced every 24 hours. At the end, the buccal window was sectioned into two mesial and distal segments using a paper disk in slow speed handpiece (Austria, W8H). Specimens were placed in a dry incubator (MMM Medcentel, Incucell EinrichtungenGmb, Germany) at 37°C for two weeks in order to allow evaporation of the remaining moisture from the samples [8,12]. To measure the amount of remineralization of the fissure sealants, the samples were prepared and observed under SEM at ×5000 and ×1000 magnifications in order to measure the depth of the intermediate interface in microns. For EDXA (elemental composition analyzer) examination, one sample was selected from each group; the groups were then studied under 20 micrometer magnification and irradiated for two minutes. the extent/distribution of calcium/phosphor ions in the above samples, the concentrations of the ions were assessed at three different points (fissure sealant/intermediate/enamel layers) using EDX (EDXA, QX2, RONTEC Co, coupled with SEM) [12].

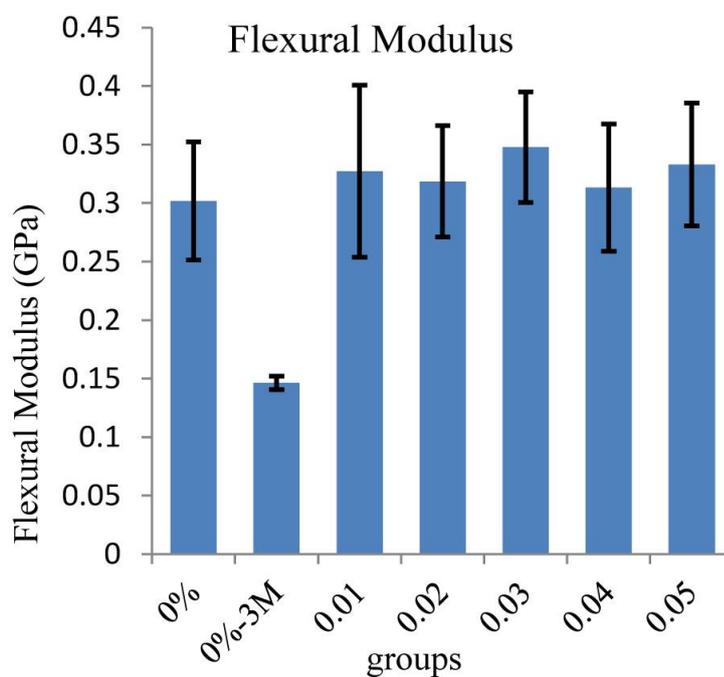
Data were assessed by a statistician using SPSS for Windows version 21 (SPSS Inc., Chicago, Illinois, USA). Kolmogorov-Smirnov test, One-way ANOVA and Tukey's Post Hoc analysis/HSD tests were used for analysis.

RESULTS

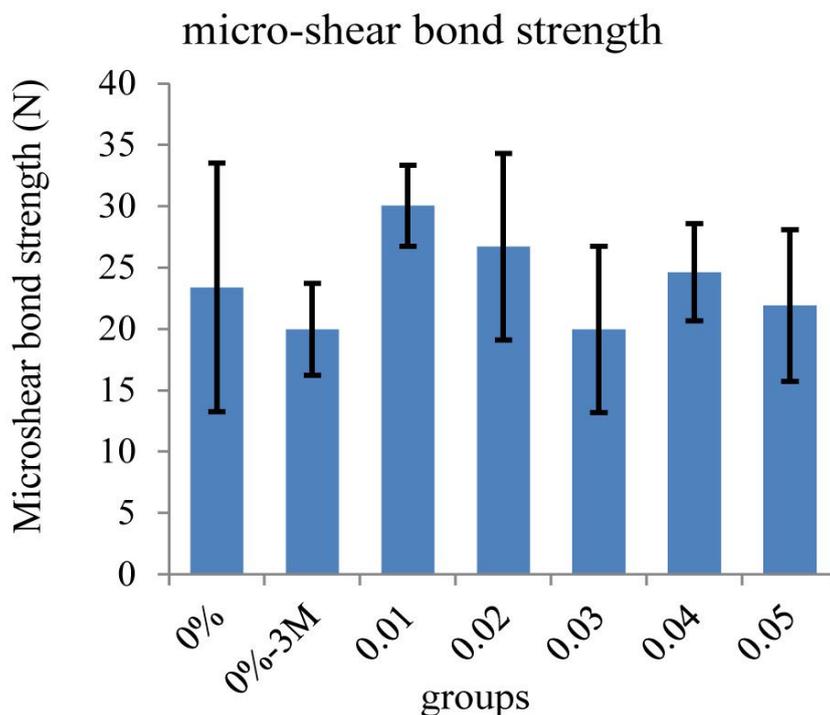
The results of ANOVA test did not show a significant difference ($p>0.05$) in the average micro-shear bond strength values between the



Graph 1. Average micro-shear bond strength values in the seven experimental groups. (Y-error bars represent standard deviation)



Graph 2. Average flexural strength values in the seven experimental groups. (Y-error bars represent standard deviation)



Graph 3. Average flexural modulus in the seven experimental groups. (Y-error bars represent standard deviation)

various experimental groups (0-5%), and between the commercial (3M ESPE) fissure sealant and the prepared fissure sealant (0%) groups. In addition, there was no significant difference between the flexural strengths of the experimental groups (0-5%) ($p > 0.05$). The average flexural strength of the prepared fissure sealant group (0%) was significantly higher than the commercial (3M ESPE) group ($p < 0.05$). Results of ANOVA revealed significantly higher flexural elastic modulus values in the prepared fissure sealant group (0%) compared to the commercial (3M ESPE) group ($p < 0.05$), although there was no significant difference ($p > 0.05$) between the flexural elastic modulus values of the various groups (0-5%).

The results are shown in graphs 1-3.

After placement of the samples in acid buffer solution for four days, one sample was selected from each group for examination under SEM (Figure 1).

Comparison of various concentrations of tricalcium phosphate showed a reduction in enamel irregularities/crack lines (resulting from demineralization) with increasing concentrations of β -TCP in groups 1-4 (Figures 1b, 1c and 1d); a homogenous layer was formed at the enamel-fissure sealant interface in groups 5 and 6 (containing 4-5 weight percent β -TCP).

In recent years, nanotechnology has enabled construction of highly bioactive calcium phosphate crystals by reducing the size and controlling the properties of this material in regards to shape/distribution of the crystals.

These particles have higher potential for diffusion into the irregularities of the demineralized layer, and are currently investigated as potential remineralizing agents [7, 16, 17]. This is considering the higher solubility of β -tricalcium phosphate compound (β - $\text{Ca}_2(\text{PO}_4)$) at the critical pH of 3.5-4, whereby it will dissolve quicker than hydroxyapatite compounds

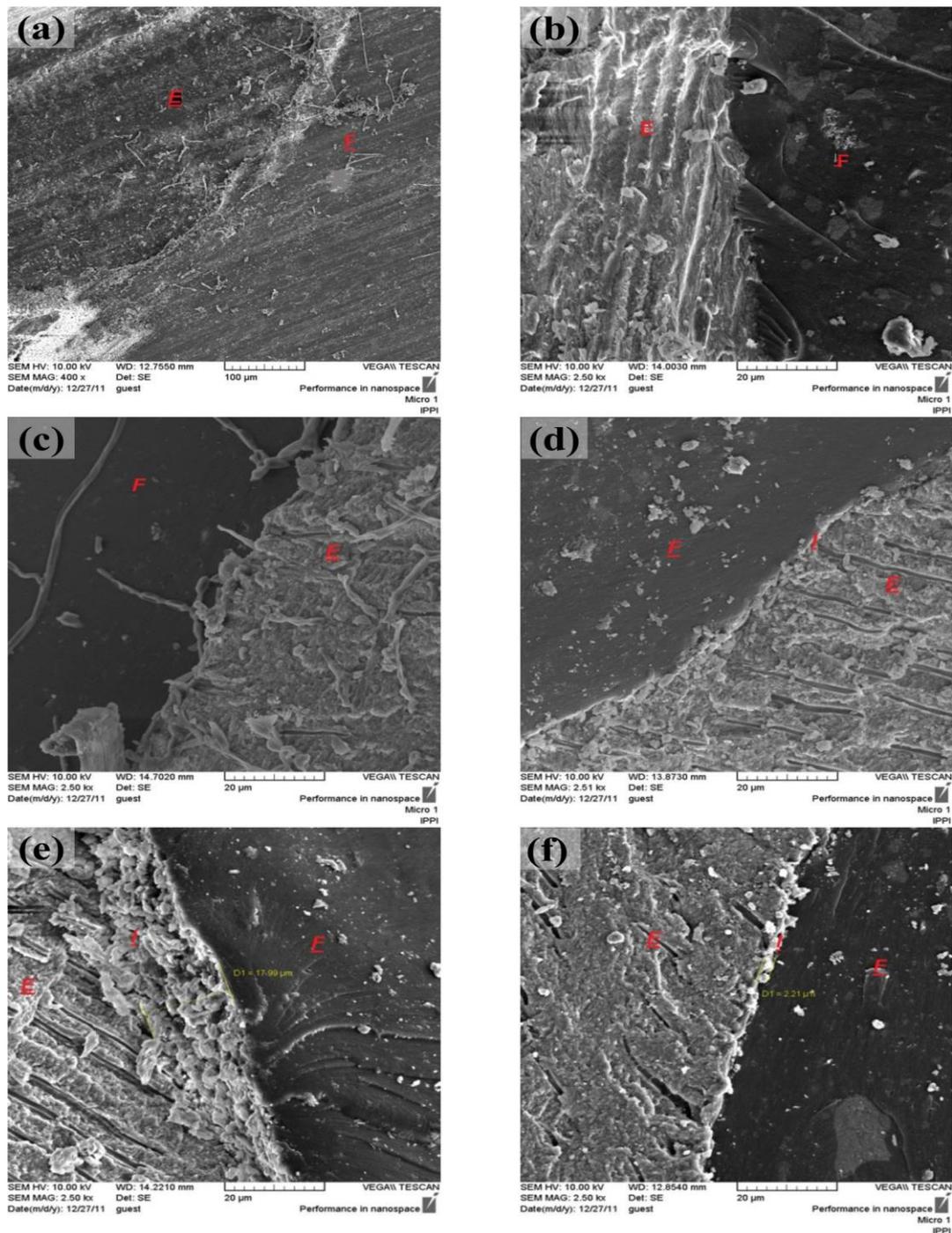


Fig 1. SEM micrographs of samples containing 0 to 5 wt% β -TCP nanoparticles under $\times 1000$ magnification have been shown respectively in a, b, c, d, e, f.

a (0%): two areas are shown: demineralized enamel surface (E) and fissure sealant (F). b (1%) and c (2%): crack lines resulting from demineralization can be observed at some areas of the tooth surface. d (3%): A residual mineral layer containing small irregularities at the enamel surface (I). There are no visible crack lines. e (4%): The intermediate homogeneous remineralized layer between the tooth and fissure sealant (I). The thickness of the intermediate layer is $D=2.21$. f (5%): An intermediate homogeneous remineralized layer with increased thickness compared to five experimental groups ($D=17.99$).

if surrounded by bodily tissues [18]. This is a superior property compared to hydroxyapatite for a remineralizing material that supplies calcium and phosphate ions in a carious lesion [19]. Considering the above properties of tricalcium phosphate, addition of these nanoparticles to resin materials can be effective in promoting remineralization and preventing demineralization of the adjacent enamel.

Previous studies have investigated the remineralizing potential of various concentrations (0.04-8 wt.%) of β -TCP in toothpaste, mouthwash and varnish using microhardness tests [5, 7, 19-21]. However, investigation of the effect of various concentrations of β -TCP in fissure sealants has not been carried out previously. Initial assessment showed that β -TCP concentrations between 6% and 10% were unsuitable as they resulted in higher viscosities of the fissure sealants. As a result, 1-5wt.% β -TCP concentrations were selected and included in this study. Creation of the subsurface lesion while maintaining the surface lesion requires the use of weak organic acids in a demineralizing solution. Therefore, in this study, acetic acid along with calcium and phosphate were added to the demineralizing solution in order to achieve gradual demineralization while maintaining the surface layer [22, 23].

In order to assess remineralization, thickness of the remineralized layer was measured under SEM. The results showed that a greater amount of tricalcium phosphate forms a uniform residual layer at the fissure sealant/enamel interface. This intermediate layer increases in thickness by the increasing weight of the nanoparticles, so that it shows the greatest thickness at 5% weight of tricalcium phosphate. In 2011, Rezvani et al. investigated the effect of various concentrations of β -TCP nanoparticles on remineralization of initial enamel carious lesions. He exposed samples of teeth to various concentrations of tricalcium phosphate nanoparticles, NaF (positive control) and deionized water (negative control) for various durations of 4, 8, 12 and 16 days.

The samples were then examined under SEM. The results of this study revealed that a homogeneous layer can be observed on the demineralized enamel surface, the thickness of which increased with increasing concentration/time in the experimental groups and was nearly constant in the negative control group [unpublished study]. EDXA is a machine that uses x-ray absorption to measure the mineral components/elements of a material [3]; it was used to assess levels of calcium/phosphate minerals in the fissure sealant, surface enamel and deep enamel layers. The results showed a significant difference in the amounts of calcium/phosphate ions at the surface enamel with increasing concentration of nanoparticles. Therefore, by increasing the percentage of nanoparticles from 0% to 5%, the amount of calcium and phosphate ions increased so that the intermediate enamel-fissure sealant interface (5 wt% β -TCP) showed the highest amount of calcium/phosphate ions. Considering the results of EDXA, it appears that entry of nanoparticles into the subsurface layers is lower than the surface enamel layers; therefore the interference effect of TCP is mainly limited to the surface layers. Therefore, fissure sealants containing β -TCP nanoparticles can be effective materials in caries prevention and can be used to increase the resistance of surface enamel to demineralization when placed on teeth with superficial enamel demineralization or microleakage. Considering the confidentiality of the composition of dental materials, any additions to the composition and assessing such effects are accompanied with certain difficulties. Addition of new components to fissure sealants may interfere with existing components thus leading to unwanted reactions. In the present study, a fissure sealant containing a basic main formula was prepared, whereby its mechanical properties (flexural strength/micro-shear bond strength) were comparable to commercial fissure sealants such as Clinpro. Our results showed that increasing the concentration of nanoparticles to

5% did not show a significant difference in bond strength compared to the control group. In addition, the results of this study showed that although the addition of tricalcium phosphate nanoparticles to the prepared fissure sealant resulted in a reduction in flexural strength, there was no significant difference in flexural strength of the resins containing various concentrations (0-5 wt.%) of tricalcium phosphate nanoparticles ($p > 0.05$). Hong and colleagues (2008) investigated the compressive strength of glass ionomer after addition of 15% β -TCP nanoparticles. They found that the compressive strength of glass ionomer with 15% β -TCP nanoparticles was slightly higher than pure glass ionomer, although this difference was not statistically significant [9]. Comparison of flexural strength of the prepared and commercial fissure sealants showed that the flexural strength of our prepared fissure sealant was significantly higher than the commercial samples.

This may be due to smaller particle size in the prepared fissure sealant (0.012 micrometers) compared to the commercial samples (0.016 micrometers). Therefore, reduction of the size of silica fillers in the prepared fissure sealant may have led to improved mechanical properties and increased flexural strength of this material.

CONCLUSION

Considering the limitations of this study, it can be concluded that by increasing the concentration of tricalcium phosphate from 1% to 5%, there was a significant increase in the remineralization potential of the fissure sealants, whilst there was no deterioration in mechanical properties such as flexural strength/microshear bond strength. We recommend to perform further laboratory and clinical studies for the investigation of other mechanical/physical properties such as marginal integrity, wear resistance, thermal expansion, longevity, and long-term efficacy, all of which are important in the prevention of dental caries.

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