Influence of Fluoride on Remineralization Via the Polymer-Induced Liquid-Precursor Process on Dentine Hardness

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Abstract

Polymer-induced liquid-precursor (PILP) process used polyaspartic acid as an analog non-collagenous protein to prevent spontaneous nucleation of calcium and phosphate before entering the intrafibrillar compartment. Fluoride was added for the formation of fluorapatite crystals. Objective of this study is determining the effect on dentinal hardness when fluoride is added in the PILP process.

Twenty-four dentin blocks were divided into four groups. Group I (normal dentin) was the control, Group II comprised demineralized dentin stored in PILP remineralization solution, Group III comprised demineralized dentin stored in remineralized PILP solution with 5 ppm fluoride, Group IV comprised demineralized dentin stored in remineralized PILP solution with 25 ppm fluoride. All samples were stored in an incubator with continuous shaking at 37 ℃ for 14 days. A Vickers hardness test was performed to measure dentin hardness along with an X-ray diffraction (XRD) test to determine the percentage of hydroxyapatite and fluorapatite crystals.

There are significant differences between the hardness values of dentin after storage in PILP solution, PILP solution with 5 ppm fluoride, and with 25 ppm fluoride. The conclusion is remineralization via PILP process with the addition of 5 ppm fluoride and 25 ppm fluoride yields higher dentinal hardness value than without fluoride.

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Introduction

The polymer-induced liquid-precursor (PILP) process is a remineralization process introduced by Gower et al., which involves adding a small amount of acidic polypeptides (e.g., polyaspartic acid) to the remineralization solution.[1,2,3] Polyaspartic acid (pAsp) has been used to precipitate the formation of hydroxyapatites in intrafibrillar remineralization. PAsp has been proven to induce remineralization in collagens under supersaturation of calcium and phosphate ions, with hydroxyapatite crystals forming on the intrafibrillar area.[4,5,6] Studies show that remineralization in the intrafibrillar area has a major effect on the elasticity and hardness of dentin.[7] In the PILP process, most of the crystals are formed in the intrafibrillar area, with very few crystals formed in the extrafibrillar area. Hence, a nanoindentation test on artificial dentin caries shows the mechanical properties of the dentin to be only about 50% that of normal dentin. In a study by Saxena et al., fluor was added in the PILP process to induce the formation of fluorapatite with crystals larger than the gap zone. Hydroxyapatite crystals were expected to form in the intrafibrillar area, with fluorapatite crystals forming in the interfibrillar and extrafibrillar areas.[8,9]

Research by Saxena using PILP resulted in the formation of minerals in the intrafibrillar and extrafibrillar areas of a rat tail tendon collagen. The study by Saxena used various concentrations of fluor and proved that using fluor in the PILP process at concentrations of 1 ppm, 2 ppm, and 5 ppm induced the formation of minerals in the intrafibrillar and extrafibrillar areas. Fluor with a concentration of 5 ppm

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induced the formation of the largest amount of minerals in collagen, while a 25 ppm fluor concentration yielded a small amount of minerals and no remineralization in intrafibrillar collagen.[8] The rat tail tendon had a composition of 97% type I collagen, which is the type of collagen present in dentin. The research on the rat tail tendon using fluor in the PILP process showed that the collagen nanostructure formed resembled normal dentin more closely than if the PILP process alone was performed. Furthermore, the mechanical strength of the dentin did not return to normal, probably because the rat tail tendon did not have interconnectivity between fibrils such as dentin, which needed to withstand the pressure applied during the nanoindentation test.[8] Therefore, this study was carried out on the dentin block to determine the effect of fluor addition on the mechanical properties of dentin. Burwell et al. observed that complete mineral deposition is formed after 14 days of treatment[2], therefore in this study, treatments were given for 14 days.

Demineralization and remineralization analysis of teeth have been carried out in various ways, one of which is by knowing the microhardness value of the teeth.[10] Microhardness is an important physical property that reflects the ability of a material to withstand plastic deformation and persistent indentation. Featherstone et al. stated that there is a linear correlation between microhardness and mineral content.[11] The hardness value is also related to various other mechanical properties, such as the Young's modulus and yield stress.[12] Resistance to fracture and the binding strength are also associated with microhardness values. Therefore, determining microhardness is the first step in predicting dentin properties and the restoration interface.[13] Another technique for analyzing remineralization utilizes X-ray diffraction (XRD).[14] Because XRD can determine the type of crystals and the percentage composition, it can detect whether hydroxyapatite crystals and fluorapatite crystals were formed after adding fluorine at concentrations of 5 ppm and 25 ppm for 14 days. The aim of this study is to measure the hardness value of demineralized dentin after the addition of fluorine at concentrations of 5 ppm and 25 ppm to a remineralization solution for 14 days. The study specimens were observed to see whether the hardness value would return to its pre-demineralization value.

Materials and methods

This in vitro study was approved by the Dental Research Ethics Committee, with ethical clearance no: 130/Ethical Approval/FKGUI/XII/2019. A total of 24 extracted human maxillary premolars without caries and restoration were selected as research samples. The teeth were cleaned to remove soft tissue remnants and debris and were then placed in deionized water and stored in a refrigerator at 4 ℃ for a maximum of 14 days before experimental treatment.

The experimental model described by Saxena was used in this study. Dentin blocks cut from the mid-coronal region of the teeth perpendicular to the direction of the tubules were shaped in each tooth sample with a length and width of 4.5 mm and a thickness of 2 mm thick. The surface of each sample was roughened with SiC abrasive paper to a 320–1200 grit and then polished with aqueous diamond suspension (particle size: 6.0, 3.0, 1.0, 0.25 micron). The entire surface of the sample was coated with varnish nail polish to prevent demineralization, except for an exposed area of 2.5 x 2.5 mm. After artificial caries lesions were formed, the samples were rinsed with aquabidest and immersed in a remineralization solution at a temperature of 37 ℃, except for the control group.

PILP remineralization solution was prepared using the following steps: 50 mM tris-buffered solution was added, with 0.9% NaCl and 0.02% NaN_3. The solution was divided into two. To the first solution, 9 mM $CaCl_2$ was added, and 4.2 mM K_2HPO_4 and NaF were added to the second solution. The solutions were filtered with 0.22 µm filter paper. Polyapartic acid was added to the $CaCl_2$ solution and stirred for five minutes. The two solutions were then mixed and stirred for five minutes before use. Subsequently, the solution was divided into three portions. Then, 5 ppm fluorine was added to the first solution and 25 ppm fluorine to the second solution, while no fluorine was added to the last solution. The sample was then divided into four groups: Group I comprised normal dentin as a control, Group II underwent remineralization with PILP solution for 14 days, Group III underwent remineralization with PILP solution plus 5 ppm fluorine for 14 days, and Group IV underwent remineralization with PILP solution plus 25 ppm fluorine for 14 days, all in an incubator with continuous shaking at 37 ℃.

Statistical tests were performed using SPSS 23 (SPSS inc) software. The data was analyzed using the normality distribution test in each group using Shapiro–Wilk statistics because the sample size was less than 50. A one-way ANOVA test was used—if the data distribution was normal—to analyze the differences between the four groups. Statistical analysis using the SPSS 23 software employed a significance level of <0.05.

Results

Data obtained from the test results in this study were analyzed statistically using SPSS 23 Statistics software. Based on the normality test, the data obtained is normal and the data distribution is homogeneous. Thus, a one-way ANOVA statistical data test was performed with a 95% confidence interval, along with a post hoc Bonferroni test, for a comparative analysis of the results for each treatment group.

In Table 1, the highest mean value of dentin hardness was 71.9, recorded for Group IV (PILP + 25 ppm fluorine), followed by 64.8 for Group III (PILP + 5 ppm fluorine), 63.3 for Group I (control), and 51.0 for Group II (PILP). Based on the results of the one-way ANOVA statistical test, there are significant differences between the treatment groups. To analyze for the statistical differences between the groups, a Bonferroni post hoc test was performed.

Group	Mean (SD)	P value
(I) Control	63.3 (23.0)	0.000*
(II) PILP	51.0 (9.1)	
(III) PILP + 5 ppm fluorine	64.8 (7.9)	
(IV) PILP + 25 ppm fluorine	71.9 (9.4)	

Table 1. Comparison of the dentin hardness of Group I (control), Group II (PILP), Group III (PILP + 5 ppm fluorine), and Group IV (PILP + 25 ppm fluorine).

Subsequently, an XRD test was conducted to confirm the percentage of hydroxyapatite crystals and fluorapatite crystals formed in the three treatment groups. An XRD PANalytical X'pert with a Cu radiation source with a wavelength of 1.54 Å, a voltage of 40 KV, and a current of 30 mA. The data obtained was analyzed using HighScore (plus) software, which is compatible with crystallographic databases. The results of the examination of the percentage crystallinity of the hydroxyapatites and fluorapatites with XRD were confirmed, with the peak intensity identified for each group.

Figure 1 shows the difference in peak intensity of the treatment groups, and it appears that Group III and Group IV have two peaks, while there is only one peak in Group II. Figure 2(a) shows the percentage of hydroxyapatite crystals (97.8%) and fluorapatite crystals (2.2%). Figure 2(b) shows the percentage of hydroxyapatite crystals (0.5%) and fluorapatite crystals (99.5%). Figure 2(c) shows the percentage of hydroxyapatite crystals (0.4%) and fluorapatite crystals (99.6%).

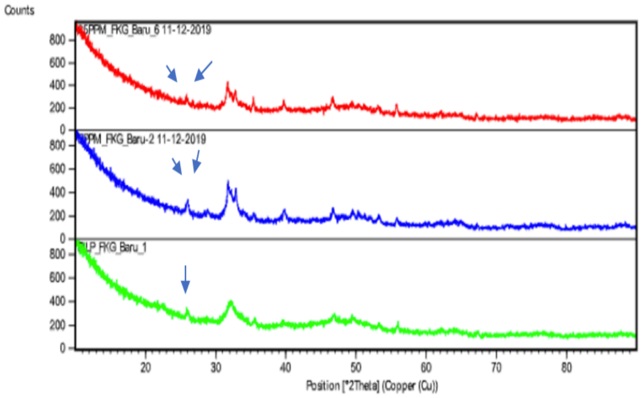

Figure 1. Comparison of the peak intensity of Group II (PILP), Group III (PILP + 5 ppm fluorine), and Group IV (PILP + 25 ppm fluorine).

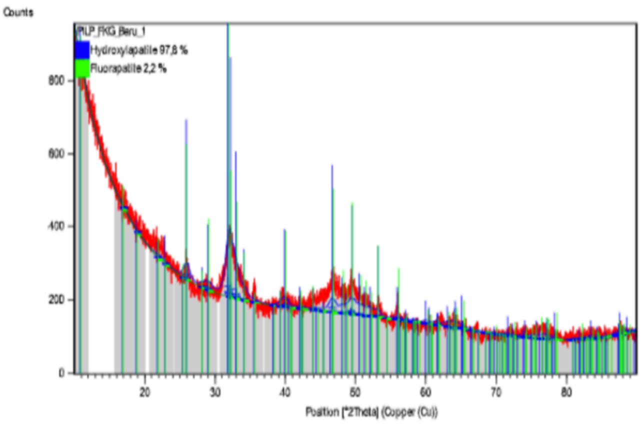

Figure 2a. Shows the percentage of hydroxyapatite crystals (97.8%) and fluorapatite crystals (2.2%).

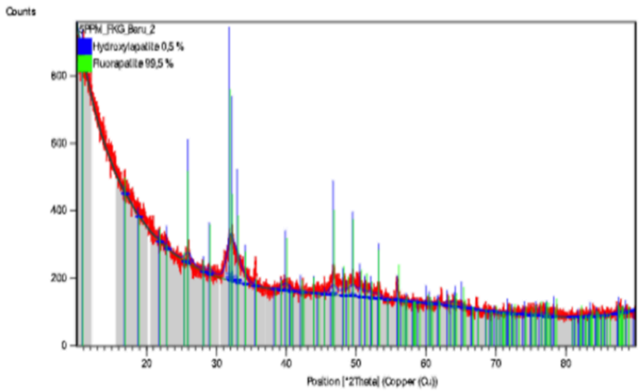

Figure 2b. Shows the percentage of hydroxyapatite crystals (0.5%) and fluorapatite crystals (99.5%).

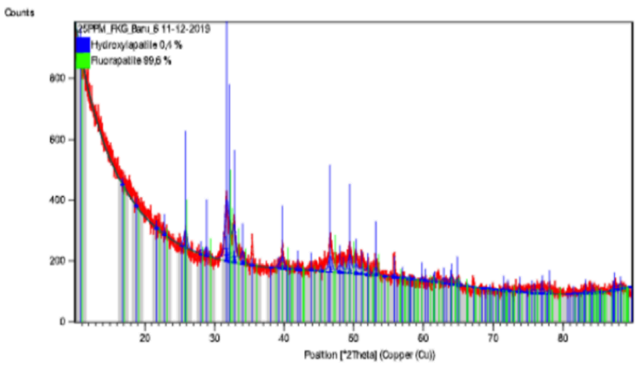

Figure 2c. Shows the percentage of hydroxyapatite crystals (0.4%) and fluorapatite crystals (99.6%).

Discussion

This study analyzes the differences in dentin hardness value after immersion in remineralization solutions using the PILP process, without the addition of fluorine and with the addition of 5 ppm fluorine and 25 ppm fluorine. The PILP process uses non-collagen protein analogs such as polyaspartic acid to prevent spontaneous nucleation of calcium and phosphate ions before entering the intrafibrillar area.[15,3] In a study using the PILP process by Burwell et al. (2012), it was observed that most of the crystals were formed at intrafibrillar areas, with very few crystals formed in the extrafibrillar areas; therefore, the nanoindentation test showed that the mechanical properties of the dentin returned only 50% comparable to normal dentin.[2] Saxena et al. added fluorine in the PILP process in rat tail tendon collagen. However, the mechanical strength of the dentin also did not return to normal, probably due to the rat tail tendon not having interconnectivity between fibrils such as dentin that was needed to withstand the pressure applied during the nanoindentation test.[8] Therefore, this study used dentin blocks, and the mechanical strength of the dentin was shown return to that of normal dentin.

The samples used were maxillary premolar teeth freshly extracted for orthodontic purposes, which were immersed in deionized water and stored at 4 ℃ until just before preparation. Preparation was performed to form a dentin block with a length and width of 4.5 mm and a thickness of 2 mm. The entire surface of the sample was then coated with nail polish varnish to prevent demineralization, except for an exposed area (2.5 mm x 2.5 mm). The sample was immersed in an acetate buffer remineralization solution for 66 hours, then rinsed with aquabidest and immersed in a remineralization solution at 37 ℃ in an incubator with continuous shaking. The entire procedure in this study is based on research conducted by Burwell et al. (2012), which examined remineralization via the PILP process, and research by Saxena et al. (2018), which investigated remineralization via the PILP process with fluoride added.[2,8]

When compared with the control group composed of healthy dentin, it appears that the value of dentin hardness for GROUP II did not return as healthy dentin. These results are consistent with the findings of the Burwell et al. study (2012), which showed that the mechanical properties of dentin do not return to that of healthy dentin after remineralization via the PILP process. The remineralization process using PILP forms hydroxyapatite crystals deposited in the intrafibrillar area, but very few crystals are formed in extrafibrillar areas, and the mechanical properties of the dentin can be restored to normal if both areas are filled with hydroxyapatite crystals.[8]

Fluorine is a remineralization agent that is widely used today.[9] The addition of fluoride ions causes the formation of new apatite crystals and fluorapatite [FHA, Ca_5 (PO_4) 3F], forming fluorapatite with crystals larger than the gap zone. Hydroxyapatite crystals are expected to form in the intrafibrillar spaces, while fluorapatite forms in the interfibrillar and extrafibrillar areas. The intrafibrillar and extrafibrillar areas filled with hydroxyapatite and fluorapatite crystals are expected to restore the hardness of the dentin to normal values.[8]

In Table 1, it can be seen that the Vickers hardness value for the remineralization group via the PILP process without the addition of fluoride ions (Group II) was almost the same as for the control group and Group III (the PILP + 5 ppm fluorine group).

Based on this result, the first hypothesis is accepted. The first hypothesis states that there is a difference in dentinal hardness on the 14^{th} day of remineralization via the PILP process with the addition of fluorine at concentrations of 5 ppm and 25 ppm, and without fluoride.

An XRD test was carried out to confirm the composition of the dentin block in each treatment group. In Figure 3, we can see the peak ratio of each crystal. In Group II (the PILP group), only one peak is observed, while in Group III (PILP + 5 ppm fluorine) and Group IV (PILP + 25 ppm fluorine), there are two peaks. Figure 1(a) shows the percentage of crystals formed, with hydroxyapatite crystals dominating at 97.8%. In Figure 1(b) and Figure 1(c), the percentage of crystals was dominated by fluorapatites, at 99.5% and 99.6%, respectively. This shows that in the PILP process, with the addition of fluoride ions, the hydroxyl groups from hydroxyapatites will be replaced with fluoride and form fluorapatites. Rey et al. posited that hydroxyapatite has lower resistance to fatigue compared to apatite containing fluoride. The hardness of hydroxyapatite increases if about 80% of the OH^- group is replaced with fluoride. In apatite, the modulus elasticity also increases with an increase in fluoride content.[16] This study confirms that with more than 80% of hydroxyapatite crystals replaced with fluorapatite, dentin hardness increases. In this study, at a higher fluoride concentration of 25 ppm, the dentinal hardness value not only returned to normal but also became harder. Based on this observation, the second hypothesis is accepted. The second hypothesis states that there are differences in the percentage ratios of hydroxyapatite crystals and dentin fluorapatite crystals on the 14^{th} day of remineralization via the PILP process with the addition of fluorine at concentrations of 5 ppm and 25 ppm, and without fluoride.

The Bacino et al. research on the application of remineralization materials via the PILP process was carried out using two methods: adding polyaspartic acid (pAsp) to generic resin modified glass ionomer (RMGIC) and applying a solution with a high concentration of pAsp to hydrate the lesion before restoration with RMGIC. In the group that had pAsp mixed into RMGIC, there was a thin layer at the junction between the dentin and RMGIC, composed primarily of polymers probably formed from the reaction between the pAsp and RMGIC. In the nanoindentation test, the group rehydrated with pAsp solution showed a significant increase in modulus of elasticity and hardness compared to the group with pAsp mixed into RMGIC, but the hardness and modulus of elasticity did not return to normal dentin values.[17] Therefore, fluoride can be added to pAsp solution to evaluate the clinical hardness and modulus of elasticity values.

Conclusions

The dentin micro hardness value for the remineralization group using the PILP process with the addition of fluorine at 5 ppm and 25 ppm is greater than for the group without fluoride. The percentage ratio of fluorapatite crystals is higher than that of the hydroxyapatite crystals in the remineralization group using the PILP process with the addition of fluorine at 5 ppm and 25 ppm compared to the PILP process without fluoride.

Declaration of Interest

The authors declare that they have no conflicts of interest to disclose.

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