RESEARCH ARTICLE

Investigation of enamel surface hardness using remineralization of white spot lesions with CPP-ACP, fluoride and CPP-ACPF

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ABSTRACT

The enamel surface hardness determination plays a key role in surface abrasion resin composite bond strength. The aim of this study was to investigate the enamel surface hardness after remineralization of white spot lesions using casein phosphopeptide amorphous calcium phosphate (CPP-ACP), fluoride and casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF). 36 bovine anterior teeth were chosen and after decoronization, the teeth were mounted in acrylic resin and their baseline surface hardness was measured. Samples were then demineralized and their surface hardness was measured. The teeth were then divided into three groups and subjected to surface treatment with CPP-ACP, CPP-ACPF and fluoride mouthwash. The surface hardness was measured and the data were analyzed using SPSS software. Paired t-test showed that surface hardness significantly changed after demineralization ($P<0.001$) and after treatment with CPP-ACPF ($P<0.001$) and fluoride ($P=0.005$), however: the change was not significant after treatment with CPP-ACP ($P=0.155$). The obtained results indicated that treatment with fluoride and CPP-ACPF may increase the surface hardness of demineralized enamel.

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INTRODUCTION

Demineralization and remineralization occur several times a day for each tooth and it is regulated by many factors such as the number and type of microbial flora present in the biofilm, diet, oral hygiene, genetics, dental anatomy, enamel and dentin composition [1-6]. In the initial phases of caries, demineralization is visible in the form of white opaque spots. Normally, the hydroxyapatite (HA) nanocrystals of the enamel are in balance with the saliva and are rich in phosphate and calcium [1-5]. In presence of calcium and phosphate ions in a neutral pH, demineralization is reversible and the HA nanocrystalline architecture may form again [6-18]. This process is referred to as remineralization and to obtain a suitable result, demineralization should eventually end and followed by remineralization [19-25]; however, this is not always the case [4-6]. Many advances in composition of dental materials and restorative techniques of dental caries have enhanced management of tooth caries and oral health maintenance [5]. Anti-caries features of milk have been previously proven and it is directly related to Ca and PO_4 elements and casein phosphopeptide (CPP) in the composition of milk [7-11]. The bond of CPP to amorphous calcium phosphate (ACP) hinders the solubility of Ca and PO_4 ions. Moreover, CPP-ACP serves as a rich source of phosphate and calcium and keeps the solution saturated. Fluoride is the most commonly used

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substance to develop remineralization.

An increase in pH results in the formation of large fluorohydroxyapatite (FHA) nanocrystals which are resistant to demineralization. Fast deposition of fluorapatite (FA) makes a hard superficial layer which is more resistant to demineralization. On the other hand, this layer inhibits deeper penetration of fluoride ions into the carious lesion [26-37]. Therefore, it should be noticed that remineralization of enamel surface with the support of fluoride is self-limited. Therefore, several studies have shown the ability of CPP-ACP for enamel remineralization and calcium and phosphate-based remineralizing agents such as CPP-ACP are increasingly applied in the clinical applications due to CPP-ACP properties which can impede demineralization and enhance remineralization [11-27]. Oshiro et al. showed that the CPP-ACP paste can stop enamel demineralization [28]. These results were confirmed by Pogio et al. [29]. Considering the contradictory results of previous studies [28-38], the influence of surface hardness on abrasion and composite bond present significant changes. In this study, we aimed to assess the effect of fluoride, CCP-ACP paste and CPP-ACPF on enamel surface hardness.

MATERIALS AND METHODS

In this *in vitro* experimental study, 36 extracted bovine anterior teeth were selected. The teeth belonged to cows older than 30 months and teeth without caries, cracks, enamel defects and discoloration were selected, also the materials used in this work is presented in Fig. 1. The teeth were stored in %0.2 thymol solution at room temperature for disinfection. Scaling and root planning were performed and the teeth were sectioned at the cementoenamel junction using a high-speed disc under water coolant. Pulp tissue was removed and the teeth were mounted in auto-polymerizing acrylic resin such that their buccal surface was exposed. Surface hardness of the teeth was measured using automated Knoop hardness tester with 25 g force applied for 10 seconds. The hardness of each surface was measured at five points, the center and four other sites with 300 µm distance from each other.

To simulate the oral environment, the surface of each tooth was coated with one layer of thick artificial saliva with the composition mentioned in Table 1.

For the process of demineralization, the teeth were subjected to pH cycling using Tencate method, which included 30 min of immersion in the demineralizing solution and 150 min of immersion in the remineralizing solution 6 times a day followed by immersion in remineralizing agent for 6 hours for a total of 3 days. After formation of white spot lesions, the surface hardness was measured again as explained earlier as shown in Fig. 2.

The teeth were then divided into 3 subgroups for treatment with CPP-ACP paste, CPP-ACPF and fluoride mouthwash. The teeth were subjected to therapeutic pH cycling over 30 days as shown in Fig. 3.

For remineralization of the white spot lesions in groups CCP-ACP and CPP-ACPF, the pastes were applied on the buccal enamel surface by a micro-brush (0.5mm thickness) for 3 minutes according

Fig. 1: Materials used in this study for hardness analysis.

Table 1. Chemical composition of artificial saliva, remineralizing and demineralizing solution.

Artificial saliva pH=6.75	Remineralizing solution pH=7.0	Demineralizing solution pH=5.0
Methyl-p-hydroxybenzoate, 2g	1.5 mM Ca	1.4 mM Ca
Sodium carboxymethyl cellulose,10g	0.9 mM P	0.9 mM P
KCl 0.625 g	0.1 Tris buffer	0.05 M Acetate buffer 0.03 ppm F
$MgCl_2.6H_2O$ 0.059 g	0.05 ppm F	
$CaCl_2$ -H_2O. 0.166 g		
K_2HPO_4, 0.326 g		
KOH		

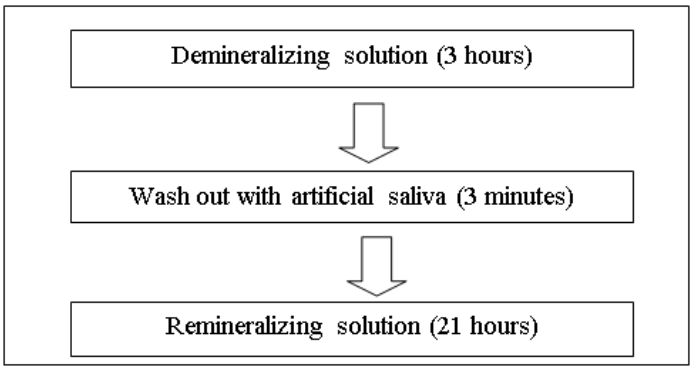

Fig. 2: Demineralization pH cycling

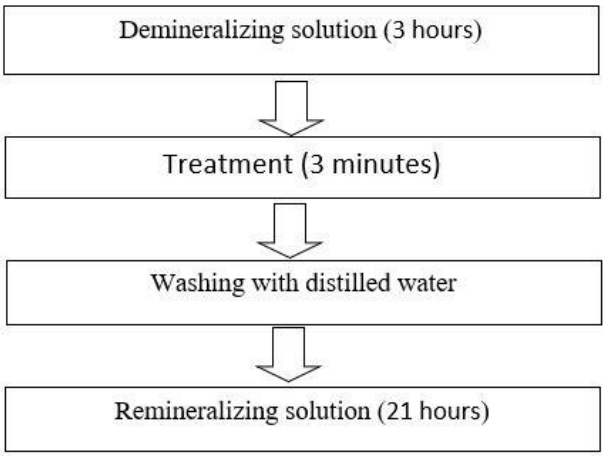

Fig. 3: Remineralization pH cycling

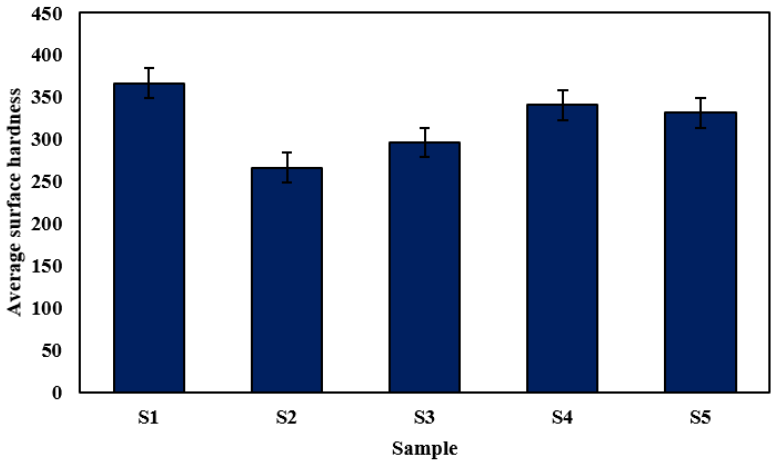

Fig. 4: The average surface hardness for Samples S1, S2, S3, S4 and S5 measured in this study

Table 2: Mean surface hardness of groups.

Base line surface hardness mean ±SD= S1		366.7±46.89
Demineralized surface hardness mean±SD =S2		266.6±49.18
Treatment group	CPP-ACP = S3	296.2±37.11
	CPP-ACPF = S4	340.5±36.87
	F = S5	331.3±51.29

to the manufacturers' instructions. In the time interval among the 2 cycles, the teeth were washed with distilled water to avoid contamination of the demineralizing solution with the remineralizing solution. Then, the teeth were dried by paper towel and exposed to fresh materials in a new cycle. The surface hardness was then measured again as explained earlier. The surface hardness values were statistically analyzed using SPSS 22 software. The values were compared using repeated measures ANOVA, paired t-test and independent t-test at P=0.05 level of significance. The null hypothesis was that CPP-ACP, fluoride and CPP-ACPF would not have a significant effect on surface hardness.

RESULTS AND DISCUSSION

Results indicated that the mean surface hardness and the repeated measures ANOVA were used to compare surface hardness at baseline, after demineralization and after treatment. The surface hardness of CCP-ACP group had significant differences with those of other two groups (P<0.001) [38-47].

However, when microorganisms were inoculated from animals with active caries, they developed tooth decay in the presence of carbohydrates. Fig. 4 shows the average surface hardness for five specimens. Fig. 4 shows that the average surface hardness for sample S1 is the highest value whereas the sample S2 has the lowest value. The following diagram indicates the average hardness for all samples before demineralization S1. The average hardness value of total samples after demineralization S2 shows the average hardness value of samples in the three treated groups S3, S4, and S5 (see Table 2). The hardness table shows 36 samples on top of each other called base line and then the hardness of the 36 samples in demineralized state. Then, these 36 samples were divided into three parts and in three treatment groups of 12, hardness were determined. The highest surface hardness belonged to teeth before demineralization (366.7 ± 46.89) and the lowest value was noted in teeth after demineralization (266.6 ± 49.18). Among the treatment groups, the maximum and minimum surface hardness values

Fig. 5: The bovine anterior teeth without any defects were tested

after treatment belonged to CCP-ACPF (340.5 ± 36.87) and CCP-ACP (331.1 ± 51.29) groups, respectively.

Dental caries and erosion are currently a major public health concern, and prevention is still the best approach to control them. White spot lesions are the first sign of initial caries in smooth surfaces [22, 48-58]. The most important features of initial carious lesions are presence of a sound superficial layer over underlying demineralized layers. Although, the superficial layer is intact, its mineral content is reduced. Therefore, its hardness is lower than that of sound enamel. Various hardness tests such as Vickers and Knoop have been used in previous studies [59-67]. Alves et al. [68] studied HMV-2000 with 25g force for 10 seconds was used. Several researchers performed the Knoop hardness test with 25 g force for 5 seconds and 50g force for 15 seconds. According to Tantbirojn et al. [22] the Knoop test is preferred to Vickers test because the longer diagonal is less affected by elastic recovery. Also, Knoop test was used in our study to measure the surface hardness. Use of calcium- and phosphate-based remineralizing agents such as CPP-ACP is increasing in the clinical setting since they can prevent demineralization and enhance remineralization [27, 68-70]. The mean baseline surface hardness was 366.7 in this study similar to the values reported by Rehder et al, [31] (350).

These values confirm the similarity of bovine teeth to human teeth. Rehder et al. [31] reported the mean surface hardness after demineralization to be 234.16; this value was 288.724. These values after the application of CPP-ACP were +29.4 in our study, +5.3 in the study by Rehder et al. [31]. These values after the application of CPP-ACPF were +74.1 in the present study and +0.6 in the study by Rehder et al. which may be due to use duration of CPP-ACP and CPP-ACPF in different studies. In the study by Rehder [31], this time was 90 second while in the present study remineralizing agents were used for three minutes for a total duration of one month. Thus, it may be concluded that duration of use of remineralizing agent is an important factor affecting the hardness. In this article, fluoride and CCP-ACPF were effective for enamel remineralization and increased the surface hardness to a value close to the baseline value but single application of CCP-ACP was less effective for this purpose. Therefore, it may be concluded that fluoride and CCP-ACPF can increase the surface hardness close to the baseline value but CCP-ACP is less efficient for this purpose. The results of this study showed that all remineralizing agents were effective for increasing the surface hardness and enamel remineralization.

Although remineralization and demineralization have been extensively studied in the recent decades, there are only a few studies on depth, mineral content and mechanical features of lesions. One limitation of this study was that the effect of remineralizing agents on surface hardness of sound teeth in superficial and underlying layers was not evaluated to compare the values with those of demineralized enamel. Fig. 5 demonstrated that 36 bovine anterior teeth without any defects were chosen and immersed in 0.2% thymol and after decoronization. Thus, future studies with

a sound control group are required to further elucidation of this topic which showed that both fluoride and CPP-ACP caused remineralization of demineralized enamel and their combination had a synergistic effect on intensifying remineralization [68-70]. Tantbirojn et al. [22] studied the change in hardness of enamel eroded by cola drink after treatment with fluoride and CPP-ACP and found that although fluoride and CPP-ACP effectively increased the surface hardness, the combination of the two did not have a synergistic effect.

CONCLUSION

The obtained results of this study indicated that treatment with fluoride and CPP-ACPF (in contrast to CPP-ACP) can increase the surface hardness of demineralized enamel. The advantage of this work was investigation of surface hardness of demineralized teeth after their treatment with different remineralizing agents. The highest surface hardness belonged to teeth before demineralization (366.7 ± 46.89) and the lowest value was noted in teeth after demineralization (266.6 ± 49.18). Among the treatment groups, the maximum and minimum surface hardness values after treatment belonged to CCP-ACPF (340.5 ± 36.87) and CCP-ACP (331.1 ± 51.29) groups, respectively. Considering the controversial results, further studies are required to obtain more reliable findings. One limitation of this study was that the effect of remineralizing agents on surface hardness of sound teeth in superficial and underlying layers was not evaluated to compare the values with those of demineralized enamel.

CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

AUTHORS' CONTRIBUTIONS

Farzaneh Shirani: Conceptualization, Methodology, Validation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition.

Shirin Rostami: Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration.

Parastoo Afghari: Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration.

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COMPETING INTERESTS

The authors declare that they have no conflict of interests.

ETHICS APPROVAL

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