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Comparative Remineralization Efficacy on Artificial Lesions by Vickers Microhardness Evaluation

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ABSTRACT : The aim of this study is to evaluate potential remineralization capacity of calciumglycerophospahte and tricalciumphospahte pastes on artificial lesions on dentine using Vickers microhardness analysis. Thirty dentine surfaces of human molar teeth were randomly divided into four groups after creating artificially demineralized lesions (G1: calciumglycerophosphate, G2: tricalciumphospahte, G3: control group (distilled water); 10 per group) and then treated with the respective remineralization agents. The remineralization cycle repeated twice daily for 10 days. The groups were evaluated with Vickers microhardness before and after remineralization. Statistically significant difference of microhardness was observed between demineralized dentine and remineralized dentine with group 1 and group 2 ($p<0,005$). No statistically significant difference of microhardness was observed between two different pastes (p >0,005). Within the limitation of this *in vitro* study, calciumphospahte based agents enhance the remineralization process as an alternative to fluoride.

KEY WORDS: Tricalciumphosphate, calciumglycerophosphate, microhardness

I. INTRODUCTİON

Artificial caries lesions can be clinically defined as structures of the surfaces similar to white spot lesions that are softer than sound enamel and become more opaque when dried.[1] White spot lesions, defined as formations similar to artificial caries lesions, can be a problem especially for pediatric patients with low cooperation. Treatment of white spot lesions with appropriate remineralization agents can prevent formation of cavity formation.[2]While fluoride accelerates the growth of fluoroapatite, it also neutralizes the acidity created by harmful bacterias.[3] It is very important to arrange the appropriate doses in fluoride treatment. High fluoride concentrations are toxic. Recently, researchers have been trying to find an alternative material that could provide beneficial remineralization effects without the potential side effects of fluoride.[5]

Calcium phosphate based materials have been developed as an effective material in bone regeneration. It is a material with high biocompatibility. Recently, Novamin, a calciumphosphate-based agent, has also been introduced in the industry as an effective alternative remineralization agent to fluoride.[6-7]Vickers microhardness test is used to determine the degree of hardness in dental tissues and to investigate the effects of surface changes and application materials on hard tissues. There are two techniques for using a surface microhardness device. In both cases, the diamond tip is applied to the surface for a fixed time and force.[8] It gives Knoop hardness value (KHN) and Vickers hardness values .[9] First, a constant force is applied to the load. After the applied force is removed from the load, it creates a permanent mark on the material. The scar area is calculated by measuring from its corners. The found number is placed in the formula. The difference in Knoop and Vickers hardness value calculation is due to the difference in the tips used in measurement.[10] This study aims to compare the remineralization capacities of tricalciumphosphate and calciumglycerophosphate application on dentin tissue using Vickers surface microhardness analysis. The null hypothesis is that there is no significant difference between the two different remineralization agents.

II. MATERİALS AND METHODS

This was in vitro experimental study that was performed in the Marmara University, Faculty of Dentistry.

Specimens' Preparation : The teeth were cleaned from residual soft tissue and organic debris and then stored in 0.5% thymol solution less than 1 week for disinfection. The teeth were sectioned mesiodistally with a diamond disk. A 4 × 4 mm square was created in the middle third of the labial and lingual surfaces using nail varnish to certain the dentin surfaces to examine and then fixed firmly into an acrylic mold for secure handling. The demineralized Vickers surface microhardness was measured for all specimens after numbering them from 1 to 30.

Vickers Surface Microhardness Testing : SMH measurements were made with the Zwick/Roell ZHV10 Vickers Microhardness Tester (Germany) device, with the power of 50 g force for 10 seconds, with programming made with the relevant formula from 3 different points for each sample. SMH measurements were made after the demineralization protocol. After applying the remineralization protocols, SMH measurements were performed again to observe the degree of remineralization. Measurements were repeated in both stages on 30 dentin samples.

Demineralization Cycle : Teeth were immersed containing 20 mL of demineralization solution (2 mM $CaCl_2$, 2 mM NaH_2Po_4, 50 mM CH_3COOH, with the addition of 0.1M NaOH to pH 4.55). Specimens were then rinsed with 10 mL deionized water and immersed for 22 hours in 20 mL of remineralization solution (2 mM $CaCl_2$, 2 mM NaH_2Po_4, with the addition of 0.1M NaOH to pH 6.8) at room temperature.

Remineralization Cycle : The remineralization cycle was repeated twice daily for 10 days.9 All teeth were then soaked in deionized water until SMH was measured to determine the acquired microhardness.

Statistycal Analysis : Analysis of variance test (ANOVA) was used to identify statistically significant differences in micro-hardness evaluation between demineralized specimens and remineralized samples treated with two different pastes. Data were analyzed using SPSS version 22 (IBM Corp.; Armonk, New York, United States), where the *p*-value was set at 0.05, and the level of confidence was set at 95%.
The distribution of the mean, standard deviations and p values of the SMH values of the dentin surfaces after demineralization and remineralization according to groups are shown in Table 1.

Surface Microhardness Values			
	After demineralization cycle	After remineralization cycle	
	Mean±Standard Deviation	Mean±Standard Deviation	*p-Value*
Group 1 (calciumglycerophosphate based toothpaste)	55 ± 21,12	72,4 ± 20,72	0,025
Group 2 (tricalciumphosphate based toothpaste)	52,09± 31,72	80,04 ± 30,70	0,023
Group 3 (control group)	42,17± 13,77	44,98 ± 12,22	0,37

Table 1: The results of analysis of variance test regarding the microhardness between demineralized specimens and remineralizing agent pastes applied samples.

III. DISCUSSION

Remineralization and demineralization processes are dynamic events that occur in the oral cavity over time.[11] When the balance is disrupted and shifts towards demineralization, white spot lesions occur on tooth surfaces. Resaturation of these WSLs with calcium and phosphate ions will help convert the cavity formation.[11-12] Therefore, this study aimed to determine the importance of calciumphosphate based pastes in the remineralization of the tooth structure. Vickers surface microhardness test appeared for detection of remineralization grade. This test gives reliable results. It is a fast and economical test method.[13-14] The results of this research showed that the SMH values after demineralization were lower than the SMH values after remineralization and there was a significant difference between them. This difference between the control group and the groups containing toothpastes applied samples indicates that each performs that tricalciumphosphate and calciumglycerophosphate trigger the remineralization process, as shown in previous testing of these agents.[15-16-17]

When calciumphosphate-based pastes are activated in the oral cavity, they increase the pH, releasing sodium, calcium, phosphate and silica. Thus, remineralization processes begin by this way.[18-19] Pairwise comparisons shown in this figure explain that there is no significant difference in SMH values between two different calciumphosphate based pastes.It would be not true to say that calcium phosphate-based remineralization agents have similar remineralization effect as fluoride.[20-21] However, an alternative to fluoride can be found by performing more researches about this subject. Elimination of potential fluoride toxicity and dental fluorosis

resulting from excessive use of fluoride containing toothpastes during daily tooth brushing routine may be one of the benefits of preferring pastes containing calcium-based compounds instead of fluoride based pastes.[22-23]

IV. CONCLUSION

The use of calcium phosphate-containing toothpastes to perform remineralization mechanism of non-cavity lesions is a promising treatment due to its safety, but more researches are needed to prove their effectiveness completely.

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