Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate and Three Calcium Phosphate on Enamel Microhardness

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ABSTRACT
Aim: This study aims to investigate the effect of casein phosphopeptide-amorphous calcium phosphate and three calcium phosphate (CPP-ACP and TCP) on increasing the microhardness of human enamel after induction of erosion.

Materials and methods: A total of 26 healthy human-impacted third molar teeth were chosen, and their hardness measured using a microhardness testing machine. The samples were immersed in Coca Cola (pH = 4.7) for 8 minutes. Then, microhardness was measured again, and these samples were randomly divided into four groups (two control groups and two experimental groups). (1) Negative control group: Artificial saliva was used for 10 minutes, (2) positive control group: Fluoride gel was used for 10 minutes, (3) β-TCP group: TCP was used for 10 minutes, (4) CCP-ACP group: CCP-ACP was used for 10 minutes. The final microhardness of those samples was measured, and the changes in microhardness of teeth within group and between groups were analyzed using the paired and analysis of variance tests respectively. Results were considered statistically significant at a level of p < 0.05.

Results: No significant difference was observed in microhardness between CPP-ACP group and TCP group (p = 0.368) during the time microhardness significantly dropped after soaking in soda.

Conclusion: Casein phosphopeptide-amorphous calcium phosphate and TCP increased the microhardness of teeth.

INTRODUCTION
Erosion is irreversible destruction of tooth structure by chemical process without the activity of bacteria on the teeth. Clinical problems and complications of erosion include the loss of the glossy surface of teeth and the smooth convex surface of teeth.

Fluoride is one of the most effective remineralization agents in the treatment of demineralized lesions. However, chronic exposure to low levels of fluoride can cause problems in normal body systems (digestion, genitourinary, and respiratory). On the contrary, the prevalence of dental fluorosis has increased, especially in nonfluoridated areas. Furthermore, fluoride ion alone cannot completely remineralize dental demineralized lesions. In addition to fluoride, calcium and phosphate ions are needed to form per unit of fluorapatite. Therefore, it seems necessary to replace fluoride with materials that can remineralize the demineralized lesions.
In the last decade, there has been a tendency to use different combinations containing calcium and phosphate, which can affect dental remineralization. Different groups of foods contain these mineral compounds, some of which include β-three calcium phosphate (TCP) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).

The results of studies on the chemical composition and materials that contain calcium and phosphate, such as CPP-ACP and β-TCP, show the mineralizing action of these materials on the surface of teeth.

In the most recent studies, these materials have not been comprehensively investigated; in many, the materials were used with toothpaste, chewing gum, or mouthwashes that contain fluoride. Furthermore, the remineralizing effect of these two materials was not compared.

The purpose of this study was to investigate and compare the effect of CPP-ACP and TCP on increasing the microhardness of human enamel after erosion.

MATERIALS AND METHODS

In this research, 26 impacted third molar teeth were used. They did not have cavities, hyperplasia, enamel crack, and erosion. The teeth were placed in glass containers containing water of Tehran, after extraction by surgery. The water of these glasses was changed every 2 days to prevent changes in the surface of water and water pollution. The samples were mounted in a special frame with clear polyester after brushing teeth and cleaning up any debris. The samples were polished in the presence of water with polishers and a polish device to achieve a flat and suitable surface for measuring microhardness. Then, the surface of samples was dried, and the initial microhardness was measured using the Vickers method.

Consistent with previous articles, 50g of force was chosen for this research.

The samples were immersed in delestre for 8 minutes. The beverage was changed every 2 minutes to make sure that the buffering effect of dissolved ions from the enamel surface was not reduced and that the beverage gas remained. Then, the microhardness was measured for the second time. Thereafter, teeth were randomly divided into two groups of 10 and two groups of 3. Groups of 10 were placed into β-TCP and CPP-ACP (tooth mousse) and groups of three (positive and negative control group) were placed into fluoride gel and artificial saliva respectively, for 10 minutes.

Then, the microhardness of teeth was measured for the third time. The data were analyzed using Statistical Package for the Social Sciences version 22 software. Descriptive features, including mean and standard deviation, and inferential statistics, including analysis of variance, were performed. Comparisons between groups were made using the Duncan multiple range test, and paired sample t-test was used to compare groups before and after the experiment.

RESULTS

No significant difference was observed between two treatments groups in microhardness (p = 0.368). During the time, microhardness significantly dropped after soaking in soda (p = 0.000) (Table 1). Microhardness was significantly increased in both groups after the treatment (p = 0.000) (Table 2).

DISCUSSION

Erosion is the destruction of tooth structure without interference or the activity of microorganisms. Erosion plays an important role in the destruction of dental tissues.

| Table 1: Primary and secondary microhardness (before and after soaking in soda) statistical indicator |
|-----------|-----------------|-----------------|-----------------|          |
| Group     | Sample size     | Time of effect  | Mean of primary | Mean of secondary |
|           |                 | (in minute)     | microhardness   | microhardness    | p-value |
| S         | 3               | 8               | 459             | 261              |         |
| F         | 3               | 8               | 359             | 268              | 0.000   |
| TM        | 10              | 8               | 490             | 275              |         |
| TCP       | 10              | 8               | 472             | 259              |         |

S: Saliva, F: Fluoride, TM: CPP-ACP (tooth mousse)

| Table 2: Primary microhardness, after demineralization and after intervention |
|-----------------|-----------------|-----------------|-----------------|          |
| Experimental groups | Number | Primary evaluation | After demineralization | After intervention |
|                    |         |                   |                  |          |
| S                  | 3       | 88/459            | 450              | 13/892       | 261       | 38       | 326     |
| F                  | 3       | 55/561            | 359              | 16/462       | 268       | 54/781   | 358     |
| TM                 | 10      | 56/272            | 490/10           | 54/319       | 275/10    | 41/884   | 378/60  |
| TCP                | 10      | 53/442            | 472/70           | 28/386       | 259       | 30/840   | 366     |
| Total              | 26      | 68/485            | 463/65           | 39/229       | 269/69    | 39/903   | 365/31  |

S: Saliva, F: Fluoride, TM: CPP-ACP (tooth mousse)
Therefore, application of repairing material of erosive lesions is necessary.

In this research, the effect of β-TCP and CPP-ACP on remineralization of erosive lesions created by exposure to delestre was investigated.

According to the results, the microhardness of tooth enamel decreased significantly by 41% after exposure to carbonated beverage.

The results of Ghajari and Razavi’s research, which investigated the effect of Iranian and foreign beverage pH on the amount of dental erosion by analysis of calcium method, showed that calcium was taken from the surface of tooth enamel after exposure to the beverage, consistent with our findings.

The results of the study by Haghgoo et al., which investigated the effect of nano-hydroxyapatite solution on mineralization of permanent teeth exposed to delestre, showed that delestre significantly decreases the microhardness of tooth enamel, consistent with our research.

According to the results of this research, the increase of microhardness in the artificial saliva group was 24%. Saliva is capable of keeping the mouth environment over-saturated with calcium and phosphate ions. This leads to the protection of hydroxyapatite crystals and increases the power of repairing the tooth enamel structure. However, oral conditions can proceed to demineralization because of various causes and, in some conditions, the use of remineralization agents is necessary to promote conditions that favor of remineralization.

According to the results of this research, microhardness of tooth enamel increased by 37% after exposure to ACP-CPP. This increase could be due to the presence of amorphous and settle ability of calcium and phosphorus ions in this material. On the contrary, saturation with calcium and phosphorus ions changes acidity into alkalinity. Furthermore, ACP-CPP can act as a calcium reservoir along with remineralization. This material is designed so as to stabilize calcium and phosphorus ions on the surface of the tooth.

The results of this research showed that microhardness of tooth enamel increased by 41% after exposure to β-TCP. The base of β-TCP is calcium and phosphate, and its chemical formula is Ca₃(PO₄)₂. This calcium and phosphate base confers on this material the ability to increase microhardness.

The results reported by Robert and Rezvani indicate that the impact of β-TCP and ACP-CPP on improving lesions is consistent with the results of our research.

In the present research, 10 minutes was allocated for exposure to remineralization agents, because longer times are difficult for patients and would create an experimental scenario that diverges from clinical criteria. In previous research, the time of exposure to remineralization materials was too long to be clinically difficult.

In this research, we investigate the impact of two materials β-TCP and ACP-CPP on the microhardness of tooth enamel after erosion in vitro.

It is suggested that this research is done in situ because of the simulation of the mouth and considering the impact of diet.

**CONCLUSION**

According to the results of the present study, TCP and CPP-ACP can affect remineralization of erosive lesions.

**CLINICAL SIGNIFICANCE**

Three calcium phosphate and CPP-ACP can affect the remineralization of erosive lesions.

**REFERENCES**


