Effects of Nano-Hydroxyapatite and Casein Phosphopeptide-Amorphous Calcium Phosphate in preventing loss of minerals from teeth after exposure to an acidic beverage

(An in vitro study).

A Thesis

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Abstract

Background: Hydroxyapatite (HA) is one of the most biocompatible and bioactive materials and is widely applied to coat artificial joints and tooth roots. Nanosized particles have similarity to the apatite crystal of tooth enamel in morphology, crystal structure and crystallinity. In recent years, an increasing number of reports have shown that Nano-Hydroxyapatite (NHA) has the potential to remineralize artificial erosive lesions following addition to tooth pastes, mouth washes, etc. Casein phosphopeptides (CPP) are peptides derived from the milk protein casein that are complexed with calcium and phosphate. In this complex, the CPP maintains the calcium and phosphate in an amorphous form. Its technical name is Casein PhosphoPeptides-Amorphous Calcium Phosphate (CPP-ACP). Calcium phosphate is normally insoluble (crystalline structure) at neutral PH. However, the CPP keeps the calcium and phosphate in an amorphous, non-crystalline state. In this amorphous state, calcium and phosphate ions can enter the tooth enamel and enhance remineralization.

Aims of the study: To evaluate the effect of antierosive agents (10% Nano-Hydroxyapatite (NHA), 10% Casein Phophopeptide-Amorphous Calcium Phosphate (CPP-ACP), and combination of 10% NHA and 10% CPP-ACP) on loss of minerals from enamel surface of permanent teeth treated with antierosive agents when exposed to an acidic beverage and investigate the morphological changes of enamel surface after demineralization with cola based beverage under Scanning Electron Microscope (SEM).

Materials and Methods: The teeth sample consisted of 60 maxillary first premolars, teeth were randomly divided into four groups, 15 teeth for each group. Group I treated with 10% NHA, Group II treated with 10% CPP-ACP, Group III treated with 10% NHA and 10% CPP-ACP, and Group IV did not treated with any remineralizing agents. A circular window of 6 mm in diameter

was prepared on the buccal surface of the tooth. It painted with an acid resistant nail varnish except for the circular window. The teeth were immersed in the remineralizing solutions (10% NHA, 10% CPP-ACP, and combination of 10% NHA and 10% CPP-ACP) for 4 minutes twice daily, once in the morning and once at night for 28 days. Each tooth was rinsed with de-ionized water and dried with a piece of cotton before and after each immersion for 2 minutes and then stored in the artificial saliva between exposures to remineralizing solutions and for the remaining 12 hours overnight. Incubator was used to maintain the temperature at 37ºC.

After the samples were remineralized for 28 days, they were immersed for 40 minutes in 20 ml Pepsi cola (PH=2.5). Atomic Absorption Spectrophotometer (AAS) was used to record the calcium and phosphorus concentrations in Pepsi cola before and after demineralization with cola based beverage. SEM also used to examine the morphological changes occurs in enamel surface of each group after demineralization with cola based beverage.

Results: Statistically, there is a highly significant increase in calcium concentration in Pepsi cola (mg/dl) after demineralization with cola based beverage. Group I showed the lowest changes in calcium concentration values among the three studied groups (1.72 ± 0.08). Group II was the next, which also showed lower changes in calcium concentration values (1.96 ± 0.12), then group III (2.31 ± 0.08) while the highest changes were recorded in group IV (3.72 ± 0.12). There is a highly significant reduction in phosphorus concentration in Pepsi cola (mg/dl) after demineralization with cola based beverage. Group I showed the lowest changes in phosphorus concentration values among the three studied groups (9.56 ± 0.24). Group II was the next, which also showed lower changes in phosphorus concentration values (8.71 ±

0.18), then group III (7.68 ± 0.19) while the highest changes were recorded in group IV (5.59 ± 0.19).

Statistically, a highly significant difference was showed in calcium and phosphorus concentrations between the four studied groups after demineralization with cola based beverage. Group IV has a highly significant difference in comparison to group I, group II, and group III.

Microscopical examination of enamel surface under SEM revealed that new protective layers were formed on enamel surface after treatment with remineralizing agents and this new protective layers were resist demineralization with different degrees of resistance. NHA formed better new protective layer as compared with CPP-ACP and combination of NHA and CPP-ACP.

Conclusion: Both the remineralizing agents (NHA and CPP-ACP) were found to be effective in inhibiting the demineralization caused by cola based beverage. And among the remineralizing agents used in this study, NHA was found to be more effective than CPP-ACP and combination of NHA and CPP-ACP. The combination of NHA and CPP-ACP had no synergistic effect on remineralization.