Comparison Between The Effects Of Two Recaldent® Products And Artificial Saliva On The Hardness Of Enamel – An In Vitro Study

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Abstract

This in vitro experimental study on 24 extracted sound human maxillary central incisors was conducted to evaluate the effects of two Recaldent® products and artificial saliva on the hardness of enamel. All hardness measurements were taken with Vickers hardness tester.

A two stage blinding procedure was performed. Data was entered into MS Excel spreadsheet and processed using SPSS software version 15.0. One way analysis of variance (ANOVA), Student t test and Bonferroni test were performed.

The difference in enamel microhardness between baseline and demineralization in the groups A_1, A_2, A_3 were 1.1, 0.4, 1.1 respectively. The mean difference in enamel microhardness measured afterremineralisationin the groups A_1, A_2, A_3 were 3.8, 2.9, 0respectively.

CPP-ACP and CPP-ACFP increase the microhardness of enamel. Artificial saliva increases the hardness of enamel, but to a lesser extent than CPP-ACP and CPP-ACFP. There is no difference between the remineralising capacity of CPP-ACP and CPP-ACFP.

INTRODUCTION:

Milk and milk products, such as cheese, have been shown to exhibit remineralising properties in human and animal models[1]. Casein is the predominant phosphoprotein in bovine milk and accounts for almost 80% of its total protein. Casein Phosphopeptide – Amorphous Calcium Phosphate (CPP-ACP) shows an anti caries effect by suppressing demineralization, enhancing remineralisation, or possibly a combination of both[2]. Reynolds and colleagues proposed that under acidic conditions as occurring during a cariogenic challenge, localized calcium and phosphate ions substantially increase the amount of calcium phosphate in plaque. Thus a state ofsupersaturation is maintained, inhibiting enamel demineralization and enhancing remineralisation[3]. In 2006, Sukasaem.H et al concluded that CPP-ACP can increase hardness of enamel eroded by cola and that the remineralising effect of CPP-ACP is significantly higher than that of artificial saliva in vitro[4].

These studies have primarily focused on the remineralizing capabilities of CCP-ACP on demineralized enamel. In accordance with the principle of minimum intervention dentistry, optimal noninvasive strategies are preferred not only for the management of oral lesions after their occurrence, but also to enhance the existing state of health.

Hence, the present study aims to compare between the effects of two Recaldent® products (Tooth Mousse, Tooth Mousse Plus) and artificial salivaon the hardness of enamel not exposed to and exposed to a carbonated beverage.

MATERIALS AND METHODS:

Study design:

An in vitro experimental study on extracted sound human maxillary central incisors.

Sample size calculation:

Sample size was calculated based on the difference between two group means derived from a similar study conducted by MurathaPanich et al(2009)[3]. The required sample size is **24 teeth**. This should include 8 teeth in each of the six sub groups.

Inclusion criteria:

Extracted sound human maxillary central incisors.

Exclusion criteria:

1. Endodontically treated teeth.
2. Teeth affected by Fluorosis,wasting diseases,Fractured teeth

MATERIAL SELECTION:

Casein phosphopeptides contain the cluster sequence of –Ser(P) –Ser(P) –Ser(P)-Glu-Glu from casein. Through these multiple phosphoseryl residues, CPP can remarkably stabilize calcium phosphate (which is highly insoluable) in a state that facilitates formation of CPP-ACP complexes. This complex is a nano cluster of ACP with four multiphosphorylated peptides that prevent its growth to the critical size required for nucleation, phase transformation and precipitation.

When placed on the surface of teeth, the stable CPP portion in Recaldent® attaches to enamel, plaque, bacteria, hydroxyapatite and soft tissues and delivers bioavailable amorphous calcium phosphate (ACP). The free calcium and phosphate ions dissolve out of the complex and interact with hydrogen ions forming the species calcium hydrogen phosphate. This, under a diffusion gradient can enter into the tooth, react with and consume water to produce enamel mineral, thereby removing sub-surface mineral defects[5].

Furthermore, under acidic conditions, ACP substantially increases the level of calcium and phosphate ions in the vicinity of the tooth. Thus a state of super saturation is maintained that inhibits enamel demineralization and enhances remineralisation[6]. A quick return of the oral environment to resting calcium concentration also occurs which further limits the degree of demineralization[2].

Study methodology:

Group allocation:

Stage of treatment	A group		
	A_1	A_2	A_3
Baseline	No treatment	No treatment	No treatment
Demineralisation	Artificial saliva	Artificial saliva	Artificial saliva
Remineralisation	Test material I and Artificial	Test material II and Artificial	Artificial saliva (inbuilt control)

	saliva	saliva	

Baseline measurements:
Baseline micro hardness measurements were taken on the labial surface by means of a Vickers indenter with 100 grams of force for 15 seconds. Five indentations were made on the left hand side of the middle one third of the labial surface. The indentations were 120 micro meters apart from each other. The hardness value of each sample at baseline was calculated by averaging the value of all five indentations.

Remineralisationprocess :
A thin layer of test material I (CPP-ACFP) was applied on the enamel surfaces of the samples in group A1 for four minutes, as recommended by the manufacturer and then stored in artificial saliva for 6 hours. In group A2 , a thin layer of test material II (CPP-ACP) was applied on the enamel surface for four minutes and stored in artificial saliva for 6 hours. Group A3 were immersed in artificial saliva for 6 hours and served as controls.
After the remineralisation process was completed, the samples were washed in distilled water and blotted dry. The hardness of the enamel surfaces were measured using Vickers indenter with 100grams of force for 15 seconds. Five indentations were made, 120 micrometer apart from each other in the right hand side of the middle one third of the labial surface. The hardness value of each sample, after the remineralisation process was obtained by averaging the values of all five indentations.

Blinding procedures :
A two stage blinding procedure was performed. The tubes of the two commercially available Recaldent® products were covered with opaque paper and labeled as test I and test II. Also, the investigator performing the Vickers Hardness Test was blinded as to which sub group was under investigation.
After completion of the experiment, the opaque wraps were removed and test material I was found to be Tooth Mousse Plus and test material II was found to be Tooth Mousse.

Data analysis:
Data was entered into MS Excel spreadsheet and processed using SPSS software version 15.0

RESULTS

Table 6: Mean Vickers Hardness Measurement at baseline, after demineralization and after remineralisation.

Stage	**Groups (Mean ± SD)**		
	Artificial Saliva and CPP-ACFP (A_1)	**Artificial Saliva and CPP-ACP (A_2)**	**Artificial Saliva and Artificial Saliva (A_3)**
Baseline	336.06±6.27	328.49±7.49	333.98±11.14

Remineralisation	341.00±5.07	331.78±7.22	334.99 ± 9.91

Table 6 depicts the mean Vickers Hardness Measurement at baseline and after remineralisation. Bonferroni test performed on group A_1 showed the difference in enamel microhardness after demineralization and after remineralisation was statistically significant ($p = 0.01$). In group A_2, the difference in enamel microhardness at baseline and after remineralisation ($p = 0.000$) and after demineralization and remineralisation ($p = 0.028$) were found to be statistically highly significant and significant respectively. In group A_3, the difference in enamel microhardness was not statistically significant at any stage.

DISCUSSION:

Mineral gain or loss in enamel can be measured as hardness change. Return of the enamel's original stability against physical forces is indicative of mineral gain. The baseline enamel microhardness values in our study ranged from 318.2 to 344.9 VHN and the mean enamel microhardness was 333.6VHN. these values are similar to the values reported by MurathaPanich et al, Gedalia et al and Maupome et al but are higher than the values reported by Wongkhatee et al (260 to 279 VHN) and Sukasame et al (244.8 VHN). This difference may be explained by the fact that both the above mentioned studies used premolar teeth. Also, Cuy and colleagues found that enamel hardness varies depending on the degree of mineralization of the enamel, local variations resulting from enamel rods and tufts and increased porosity near the dentino-enamel junction.

It was found that after immersion in artificial saliva, microhardness of the teeth increased. This is in accordance with several studies that have shown that artificial saliva can reharden acid softened enamel. For the remineralistion procedure in this study, casein phosphate and artificial saliva were used in combination as it more representative of the oral environment and studies have shown that this combination is more effective in remineralising teeth.

However, the amount of remineralisation obtained in terms of Vickers Hardness Number is lower than in the study by MurathaPanich et al (37.8 VHN). This may be due to difference in the composition of artificial saliva used in these studies. This can be substantiated by the fact that the increase in hardness by artificial saliva alone in the study by MurathaPanich et al and in the present study were 7.3 and nil respectively.

In this study the difference in remineralising capacity of CPP-ACP and CPP-ACFP did not show statistically significant difference. This is in agreement with a study by S.K Rao et al to evaluate the efficacy of CPP dentrifrices and fluoride tooth paste containing 1,190mg/kg of sodium monofluoro phosphate among school children in Bangalore. The authors concluded that 2% CPP paste was as effective as the fluoride paste.

The difference in quality of remineralisation between CPP-ACP and CPP-ACFP is microvolumetric. Those studies that indicate the synergistic effect of casein phosphate and fluoride have used techniques such as laser fluorescence and electron probe microanalyser to detect this micro volumetric difference. The present study protocol using Vicker's Hardness Tester is not ideally suited for this purpose.

Although this study was designed to simulate the natural conditions as closely as possible, the complexities of the oral environment such as the effect of pellicle, plaque formation and salivary flow rate could not be completely incorporated into this model. Some authors have pointed out that in vitro assessment might overestimate the amount of erosion. This has further been supported by Hooper and coworkers who conducted a study paralleling an in vitro and an in vivo enamel erosion model.

Though it can be concluded that casein phosphate may enhance remineralisation of normal and eroded enamel surfaces, further studies are needed to ascertain this effect in vivo. Furthermore, the stability of the mineral deposit may be determined by imposing an acid challenge post

remineralisation. The potential formation of calculus resulting from the supersaturated calcium phosphate state in plaque is another avenue that warrants future research.

CONCLUSION:

Through this in vitro study, it can be concluded that carbonated beverages decrease the microhardness of tooth enamel. CPP-ACP and CPP-ACFP increase the microhardness of enamel, irrespective of whether the tooth has been exposed to or unexposed to the carbonated beverage. Artificial saliva increases the hardness of enamel, but to a lesser extent than CPP-ACP and CPP-ACFP. No difference was found between the remineralising capacity of CPP-ACP and CPP-ACFP.

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