Remineralizing Potential of Milk and GC Tooth Mousse on Demineralized Human Enamel: An In Vitro Comparative Evaluation

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J Health Allied Sci[NU] 2023;13:107–113.

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Abstract

Introduction Caries initiation is associated with demineralization of the subsurface tooth enamel. Today, bioactive agents based on milk products have been developed to enhance remineralization under cariogenic conditions. However, there is limited information on the remineralization potential of milk.

Materials and Methods Fifty enamel specimens were prepared from sound human premolars. All enamel specimens were then placed in demineralizing solution for 4 days at 37°C to produce artificial carious lesion. Baseline surface microhardness (SMH) was evaluated using Vickers indenter. Enamel specimens were then randomly divided into three groups. Group 1 specimens were kept in artificial saliva, whereas group 2 and 3 enamel specimens were treated with milk and GC tooth mousse, respectively, for 5 minutes twice daily for 21 days. Post-treatment SMH measurements of all specimens were evaluated on the 7th, 14th, and 21st day. Data was statistically analyzed using one-way analysis of variance test and Tukey honest significant difference post-hoc test.

Results There were no significant differences in the SMH values in the control group at any time interval. There were statistically significant increases in the post-SMH values in milk and GC tooth mousse ($p < 0.001$) at the end of 21st day of remineralization.

Conclusion Milk showed remineralization potential comparable to that of GC tooth mousse.

Keywords
- milk
- GC tooth mousse
- enamel
- remineralization

Introduction

In the oral environment, the tooth structure undergoes continuous demineralization and remineralization.[1,2] If this natural balance is disturbed and demineralization predominates, it could lead to weakening of the remaining tooth structure. Caries initiation is associated with demineralization of the subsurface tooth enamel. Subsurface lesions are caused due to loss of calcium and phosphate ions.[3]

In the initial stage, the caries lesion is reversible through a remineralization process that involves the calcium and phosphate ions diffusion into the subsurface lesion and hence restoring the lost tooth structure.[3] However, children who have an increased and/or repeated episodes of

article published online
May 4, 2022

DOI https://doi.org/10.1055/s-0042-1744441.
ISSN 2582-4287.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

demineralization with a high caries risk require added strategies to enhance remineralization.

It is challenging to exactly define the effectiveness of various remineralization methods, though remineralization has been a major area of research. Until today, fluoride therapy has remained the most popular of all caries prevention and remineralization methods. Fluoride has been considered to have a profound effect on the extent of caries progression; it is, however, far from being a complete remedy. Using fluoride at any concentration that will eliminate caries totally is doubtful.[4] High fluoride stratagems cannot be followed in most instances to minimize the potential for adverse effects due to exposure to fluoride.

The demineralization and remineralization process is a delicate balance to which dental hard tissues are subjected to, and focuses on the salivary calcium, phosphate, and salivary alkaline phosphatase levels.[5] To enhance remineralization of the enamel and dentine under cariogenic conditions, bioactive agents based on milk products have been developed recently to release such elements.[6]

Casein phosphopeptides–amorphous calcium phosphate (CCP-ACP) is used in several tooth products such as toothpastes, chewing gums, mouthwash, and in dental filling materials as well. CPP are a group of eight peptides that have been shown to stabilize calcium and phosphate, preserving them in an amorphous or soluble form termed as ACP.[7] The essential components of enamel and dentin that form highly insoluble complexes are the calcium and phosphate ions. But in the presence of CPP, they remain soluble and biologically available. Dental products with CPP–ACP are used in several tooth products such as toothpastes, chewing gums, mouthwash, and in dental filling materials as well. One such product containing CPP–ACP complex is commercially available as GC tooth mousse that adheres to the dental biofilm and increases calcium phosphate, which serves as a reservoir for free calcium and phosphate ions. We have chosen GC tooth mousse for our study since it was the most easily available commercial dental product containing CPP–ACP at the time, and whose efficacy in remineralizing early carious lesions was well established.[8,9]

Milk and milk products, such as cheese, have also been shown to exhibit anticariogenic properties in humans and animal models.[10] Milk comprises of elements that have anticariogenic properties such as calcium, phosphate, casein, and lipids. The direct chemical effect from phosphoprotein casein and calcium phosphate components of cheese has been suggested to be the mechanism of anticariogenic action.[11,12] Milk and dairy products are the foods most regularly consumed by humans and are a source of good-quality protein, carbohydrate, fats, vitamins, and minerals including calcium, phosphate, iodine, magnesium, and potassium.[13,14] Milk and milk products can be perceived as a natural product, due to the presence of protective agents and hence it is also recommended on account of its nutritional properties chiefly as a source of calcium and phosphate.

A previous study compared salivary calcium, phosphate, and alkaline phosphatase levels in children with early childhood caries and found that calcium and phosphate levels increased after administration of milk, cheese, and GC tooth mousse.[15] However, the study did not evaluate the remineralization potential of milk. There is limited information in literature pertaining to the remineralizing potential of milk and its products.[16] Hence, the present study sought to determine, in vitro, the remineralization potential of milk on demineralized human tooth enamel.

Materials and Methods

The study was conducted in the Department of Pediatric and Preventive Dentistry, AB Shetty Memorial Institute of Dental Sciences, Mangalore, in alliance with the Department of Mechanical Engineering, Manipal Institute of Technology, Manipal, and was approved by the Ethical Committee of Nitte University.

Preparation of the Specimens

Sixty freshly extracted human premolars from patients ranging in the age group of 14 to 20 years, for orthodontic purpose, were collected, cleaned, and stored in physiological saline for 2 weeks according to the Occupational Safety and Health Administration (OSHA) guidelines.[17] Included teeth showed no evidence of caries on visual inspection, no white spot lesion, and no enamel cracks. Using a low-speed diamond disc, the coronal part of the tooth was longitudinally sectioned from the buccal surfaces and a rectangular form was given to each slab that measured 4mm*4mm*1mm.[18] Fifty enamel sections were thus prepared.

Enamel specimens were then embedded in on top of partially set self-cure acrylic resin poured into custom-made plastic cylindrical molds and were then allowed to set. Flat and smooth surfaces were obtained through grit silicon carbide bur. Finishing and polishing of the enamel surfaces were done using polishing disc (Soflex ,3M India Limited, Bangalore, India).[18] The polishing procedure removed ~200 µm of tooth surface.

Baseline surface microhardness (SMH) of sound human enamel was measured using a Vickers microhardness indenter (Matzusawa micro Vickers Hardness Tester Model- MMT*7A) with 100 g of force for 15 seconds.[19] The Vickers hardness numbers (VHN) of four indentations at spacing of 120 µm were recorded and the average value was taken as the mean baseline SMH. Enamel specimens were then placed in demineralizing solution for 4 days at 37°C to produce artificial carious lesion (20 mL of acid buffer with 2mmol/L Ca^{2+}, 2 mmol/L Po_4^{3-} and 0.075 mol/L acetate at pH 4.3).[20] Subsequently, the Vickers indentor test was performed similarly as at baseline. Specimens were then divided into three groups: one control and two test groups. Group 1 contained 10 specimens, while group 2 and 3 contained 20 specimens each.

No surface treatment was performed for group 1 (control group) specimens and was stored in artificial saliva at 37°C. Artificial saliva contained Na_3Po_4—3.90mM, $NaCl_2$—4.29mM, KCl—17.98mM, $CaCl_2$—1.10mM, $MgCl_2$—0.08mM, H_2SO_4—0.50mM, $NaHCO_3$—3.27 mM, distilled water, and the pH was set at 7.2.[21]

Group 2 specimens were immersed for 5 minutes twice daily in a kidney tray filled with milk (Amul tetra pack toned whole milk—25 mL for each use). These specimens were then cleaned in deionized water, dried and later stored in artificial saliva at 37°C.

In group 3 specimens, an applicator brush was used and a thin layer of CPP–ACP cream (GC tooth mousse) was applied and left for 5 minutes twice daily. These specimens were then cleaned in deionized water, dried and later stored in artificial saliva at 37°C. The above surface treatments with milk and GC tooth mousse were repeated every day, twice daily for the next 21 days. Artificial saliva was changed every 24 hours. Surface microhardness (VHN) of the specimens were checked periodically at 7, 14, and 21 days.

Statistical Method for Analysis

Descriptive (mean and standard deviation) SMH values of enamel specimens were calculated for all the groups at baseline and various time intervals.

Paired *t*-test was used to calculate statistical differences in the SMH values in each group (intragroup) between demineralization and remineralization periods and the intergroup differences of SMH values were analyzed by using one-way repeated measure analysis of variance test followed by Tukey honest significant difference post-hoc test. The significance was set at 5% probability level.

Results

The mean baseline SMH value of sound enamel specimens was 314.4 ± 15.3 VHN that reduced to 127.9 ± 16.8 VHN after demineralization. This difference was statistically highly significant ($p < 0.001$) (►**Table 1).**

The mean SMH value of enamel specimens of group 1 after demineralization was 130.92 ± 13.5 VHN that remained constant after 7 days of exposure to artificial saliva. However, at 14th and 21st day mean SMH values of enamel specimens were increased to 131.47 ± 13.5 VHN and 132.8 ± 12.8 VHN, respectively, which was statistically not significant ($p < 0.05$) (►**Table 2**).

The mean SMH values of enamel specimens of group 2 after demineralization were 123.92 ± 18.4 VHN that increased to 164.06 ± 17.63 VHN after 7 days of exposure to milk. At 14th day and 21st day, mean SMH values of enamel specimens increased to 185.54 ± 9.2 VHN, and 211.2 ± 16.9 VHN, respectively. Statistically highly significant differences were seen after exposure to milk at all-time intervals ($p < 0.001$) (►**Table 2**).

The mean SMH values of enamel specimens of group 3 after demineralization were 123.7 ± 19.3 VHN that increased to 173.04 ± 11.7 VHN after 7 days of exposure to GC tooth mousse. At 14th day and 21st day, mean SMH values of enamel specimens increased to 196.7 ± 7.7 VHN and 228.4 ± 12.9 VHN, respectively. We observed statistically highly significant differences after exposure to GC tooth mousse at all-time intervals. ($p < 0.001$) (►**Table 2**).

Statistically highly significant differences were found when SMH values of group 2 and group 3 were compared with corresponding SMH values of the control group at all-time intervals ($p < 0.001$) (►**Table 3**). No significant differences were found between SMH values of group 2 and 3 at any time interval (►**Table 4**)].

Discussion

Milk provides essential amino acids and organic nitrogen for humans and animals of all ages and is hence considered as an excellent protein food. It also comprises protective properties such as calcium, phosphate, casein, and lipids.[22]

A considerable quantity of research has been conducted on the dental properties of milk and its products. Studies on caries control and the benefits of milk can be traced back more than 50 years. Benefits of milk and its related products can be attributed to several principal factors: remineralization of the tooth, prevention of bacterial attachment to the tooth, and inhibition of bacterial biofilm formation ability according to researches conducted. Milk has also been shown to contain multiple proteins that serve a variety of functions important for health in addition to the most evident beneficial factor, calcium.[23]

There are other applications of remineralization potential of milk on various remineralizing agents containing CPP–ACP. These remineralization technologies show assurance as an adjunctive treatment to fluoride therapy in the noninvasive management of early carious lesions by CPP–ACP complex.[12] As fluoride did in the past for caries prevention and reduction, CPP–ACP complex has also emerged significant in remineralization of early carious lesions.[23]

However, in our study we focused on Amul toned whole milk as a natural remineralizing agent that is consumed by children on a daily regular basis and could thus provide constant beneficial effects on the oral health of children. Hence, in the present study, we evaluated and compared the

Table 1 Comparison of microhardness values of intact tooth enamel and enamel specimens after demineralization

Paired samples statistics						Paired differences				
Group			Mean	*n*	SD	Mean	SD	*t*	df	*p*-value
	Pair 1	Baseline	314.4906	50	15.36092	186.58579	25.13821	62.1	69	<**0.001**
		Demineralization	127.9048	50	16.88413					

Abbreviation: SD, standard deviation.

Table 2 Comparison of SMH after demineralization and exposure in group 1 (artificial saliva), group 2 (milk), group 3 (GC tooth mousse)

Paired samples statistics						Paired differences				
Group			**Mean**	***n***	**SD**	**Mean**	**SD**	***t***	**df**	***p*-Value**
Control	Pair 1	Demineralization	130.92	10	13.53274	Cannot be calculated.				
		Remineralization after 7 days	130.92	10	13.53274					
	Pair 2	Demineralization	130.92	10	13.53274	−0.55	0.764853	−2.274	9	0.049
		Remineralization after 14 days	131.47	10	13.49124					
	Pair 3	Demineralization	130.92	10	13.53274	−1.88	1.141928	−5.206	9	0.001
		Remineralization after 21 days	132.8	10	12.89961					
Milk	Pair 1	Demineralization	123.2775	20	18.439	−40.784	17.47255	−10.439	19	<0.001
		Remineralization after 7 days	164.0615	20	17.630					
	Pair 2	Demineralization	123.2775	20	18.439	−62.2675	18.00401	−15.467	19	<0.001
		Remineralization after 14 days	185.545	20	9.204					
	Pair 3	Demineralization	123.2775	20	18.43	−87.9775	22.91814	−17.168	19	<0.001
		Remineralization after 21 days	211.255	20	16.96766					
Tooth Mousse	Pair 1	Demineralization	123.7518	20	19.33986	−49.2933	18.54323	−11.888	19	<0.001
		Remineralization after 7 days	173.045	20	11.77435					
	Pair 2	Demineralization	123.7518	20	19.33986	−72.9633	20.10603	−16.229	19	<0.001
		Remineralization after 14 days	196.715	20	7.745102					
	Pair 3	Demineralization	123.7518	20	19.33986	−104.743	27.01476	−17.34	19	<0.001
		Remineralization after 21 days	228.495	20	12.93329					

Abbreviations: SD, standard deviation; SMH, surface microhardness.

Table 3 Intergroup comparison of post-SMH values between the groups at all-time intervals (one-way ANOVA)

		n	**Mean**	**SD**	**Mean square**	***f***	**Sig.**
7-d	Control	10	0	0.00e + 00	6.77e + 03	30.132	
	Milk	20	40.784	17.47255			<0.001
	Tooth mousse	20	49.29325	18.54323			
	Total	50	31.6585	22.55981			
14-d	Control	10	0.55	0.764853	13506.87	57.075	
	Milk	20	62.2675	18.00401			<0.001
	Tooth mousse	20	72.96325	20.10603			
	Total	50	49.79807	28.52401			
21-d	Control	10	1.88	1.141928	25755.68	63.56	
	Milk	20	87.9775	22.91814			<0.001
	Tooth mousse	20	104.7433	27.01476			
	Total	50	73.29379	38.82539			

Abbreviations: ANOVA, analysis of variance; SD, standard deviation; SMH, surface microhardness.

Table 4 Post-hoc test for comparison of SMH values between the groups at all-time intervals (Tukey HSD)

Dependent variable	(i) group	(j) group	Mean difference (i-j)	SE	*p*-Value	Inference
7-d	Control	Milk	−40.784	5.803539	<0.001	
		Tooth mousse	−49.2933	5.803539	<0.001	
	Milk	Tooth mousse	−8.50925	4.73857	0.285	No difference between milk and tooth mousse
14-d	Control	Milk	−61.7175	5.958015	<0.001	
		Tooth mousse	−72.4133	5.958015	<0.001	
	Milk	Tooth mousse	−10.6958	4.864699	0.134	No difference between milk and tooth mousse
21-d	Control	Milk	−86.0975	7.796315	<0.001	
		Tooth mousse	−102.863	7.796315	<0.001	
	Milk	Tooth mousse	−16.7658	6.365664	0.05	No difference between milk and tooth mousse

Abbreviations: HSD, honest significant difference; SE, standard error; SMH, surface microhardness.

remineralizing potential of milk and GC tooth mousse using SMH measurements.

GC tooth mousse is a water-based, lactose-free crème containing 10**%** w/w Recaldent CPP–ACP. When CPP–ACP is applied in the oral environment, it will bind to biofilms, plaque, bacteria, hydroxyapatite, and soft tissue, localizing bioavailable calcium and phosphate.[24] It is proven as an adjunctive treatment to fluoride in the noninvasive management of early carious lesion, root dentinal caries, dental erosion, and dentine hypersensitivity.[25,26]

Contemplating the significance of the surface layer in caries progression, the assessment of changes in this region was relevant. SMH measurement was an appropriate technique for this purpose. For a material having a fine microstructure, nonhomogeneous, or prone to cracking like enamel, the microhardness measurement is appropriate. SMH indentation delivers a comparatively simple, nondestructive and rapid method in demineralization and remineralization studies.[27] Hence, in this study, microhardness measurements at baseline, after demineralization, and following remineralization were measured using SMH.

A flat and polished surface is needed to assess microhardness to facilitate accurate measurements; therefore, the area exposed to demineralization was not the ideal surface enamel. Scratch lines were developed during polishing in few samples and we faced difficulty in attaining smooth enamel surface. Thus, to overcome this problem, indentations at least 120 µm apart were taken and the average of four indentation was used as the microhardness value.

The values obtained during the initial baseline microhardness measurements in our study were in the range of VHN 253 to 363, which fulfill the VHN range of normal enamel tissue.[28] At the end of 4 days of demineralization, the SMH for the enamel specimens of all groups decreased to a range of VHN 123 to135 (►**Table 2**), which is in agreement with the study conducted by Maupome.[28]

In our study, when the demineralized enamel specimens were exposed to milk and GC tooth mousse, respectively, we observed that there was a substantial and steady increase in the post-remineralization microhardness values from the 7th day up to the 21st day, in both groups that were was found to be statistically highly significant ($p < 0.001$, [►**Table 2**]). From this, we deduce that milk and GC tooth mousse have definite remineralization potential on demineralized enamel surfaces. However, in the control group exposed to artificial saliva, we found no significant differences in the microhardness values of the demineralized enamel surfaces even after 21 days of exposure (►**Table 2**). The outcomes of our study are in agreement with the results of a previous study which found that cow milk has a better effect in raising enamel hardness compared with artificial saliva.[29]

The hydroxyapatite of the enamel is in equilibrium with saliva in a neutral environment that is saturated with calcium and phosphate ions.[30] When the pH is at or below 5.5, H^+ ions produced by the bacterial metabolites react preferentially with the phosphate group of the enamel crystals initiating the enamel dissolution process called as demineralization, which marks the beginning of early enamel caries.[31,32]

Remineralization occurs when pH is neutralized and there are sufficient calcium and phosphate ions available in the immediate environment. This enables the rebuilding of partly dissolved apatite crystals. To restore the natural equilibrium, remineralization must be boosted or demineralization must be retarded. The early enamel lesions have a potential for remineralization, with an increased resistance to further acid challenge, particularly with the use of enhanced remineralization treatments.[26]

Synthetic octapeptide calcium phosphate complex significantly reduced caries activity, confirming that the calcium

phosphate stabilizing portion of the CPP is associated with anticariogenicity as reported by Reynolds.[12]

In our study, the mean post-remineralization SMH values obtained after 21 days with milk and GC tooth mousse were 211.2 ± 16.9 and 228.4 ± 12.9 VHN, respectively, which demonstrate the significant remineralization potential observed at the end of 21 days, although mean SMH values of intact enamel were not achieved.

Further analysis revealed no significant differences when SMH values of enamel specimens treated with milk and GC tooth mousse were compared on all three post-remineralization time intervals (►**Tables 3** and **4**). From this, we infer that milk and GC tooth mousse have comparable remineralization potential. A recent study by Al-Ani and Al-Naimi concluded that CCP-ACP tooth mousse, raw fresh buffalo, and cow milk were all effective remineralizing agents, reducing the enamel surface roughness after a pH challenge.[33] They however, in contrast to our findings, stated that CCP–ACP tooth mousse showed more superior results.

Children and adolescents with low incidence of dental caries drank more milk than those with high caries incidence according to the epidemiological studies done in recent years.[34,35] The role of milk and dairy products in dental caries prevention has been described in various reviews.[36–38] El Deeb and Moneim recommend the consumption of milk following every meal containing acidic diet to reverse the demineralization process.[39]

GC tooth mousse is a very expensive product and children's compliance is questionable, on the other hand, milk is highly economical, easily available, and is necessary for a child's normal growth and development. Thus, the strength of our study was that we focused on evaluating the remineralizing potential of milk, a natural product that is an essential part of a child's daily diet.

Since our study was an in vitro study, it has some innate limitations such as the natural oral environment could not be reproduced and factors such as role of microorganisms could not therefore be taken into consideration. Further in vivo studies are required with longer periods of demineralization to determine the period of remineralization for milk that is needed in the natural oral environment.

SMH of demineralized human enamel exhibited significantly increased values after exposure to both milk and GC tooth mousse. These values were seen to be comparable, with no significant differences. We, therefore, conclude that milk had remineralizing potential comparable to GC tooth mousse and could be used as a natural, easily available alternative to synthetic products.

Nutritional counseling offered by dental hygienists usually focuses on prevention of caries that covers discussion of nutrients for enamel health and remineralization of weakened enamel. Dietary daily recommendation should be included in patient education and caries prevention plans. We recommend that children should daily swish and swallow milk to achieve maximum protective effect on their general as well as oral health. Children could thus benefit from both local remineralizing effect of milk and from the systemic effects. We further recommend that milk consumed should not contain added sugar.

Clinical Significance

Milk, being a natural product, can be recommended not only for its nutritional benefits but also for its protective effect on oral health.

Funding

Nil.

Conflict of Interest

None declared.

Acknowledgment

None.

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