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Evaluation of Enamel Remineralisation Using Different Remineralising Agents

Aishwarya Das, Iffat Nasim
Saveetha Dental College, Chennai

Abstract

Introduction-Dental caries is one of the most prevalent diseases of the world which affects almost all the age groups. Numerous research work has been carried out assessing the various agents which halt the progression of caries. Fluoride therapy is one of the most effective therapies for the same but it has some limitations. Newer preventive agents have been introduced from time to time in adjunct to fluoride therapy or independent of it.

Aim-The aim of this study was to assess the ability of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP ACP), CPP ACP with silver nanoparticles, Bioactive Glass (BAG) and Hydroxyapatite (HA) gel to remineralise artificial carious lesions in enamel in vitro using a 21 day pH cycling model through surface microhardness analysis and scanning electron microscopy(SEM).

Materials and Methods-Fifty enamel samples were divided into five groups of ten samples each. Group A was the control group consisting of intact enamel samples, Group B: CPP-ACP (Tooth Mousse Plus), Group C: CPP-ACP with silver nanoparticles, Group D: BAG (SHY-NM) and Group E: HA gel (ReminPro). All groups except the control group were subjected to demineralisation following which four groups were remineralised using the four different remineralising agents. The treated groups were subjected to pH cycling over a period of 21days which was followed by assessment of surface microhardness and SEM for evaluation of surface changes. The results were analysed by One-Way Analysis Of Variance (ANOVA). Multiple comparisons between groups were done by paired t-test and post-hoc Tukey test.

Results-Remineralisation of enamel was highest in samples of Group C (CPP ACP with silver nanoparticles) followed by Group B (CPP-ACP), Group D (BAG) and Group E (HA gel).There was a significant difference ($p<0.05$) in the remineralising ability between the CPP ACP and BAG groups and HA gel group. SEM photographs of the test groups exhibited either amorphous crystals or particles scattered on the surface or lines of remineralisation along the prismatic borders.

Conclusion-CPP ACP with silver nanoparticles demonstrated effective enamel remineralising ability as compared to the other groups.

Keywords-Demineralisation, Dental caries,pH cycling, Remineralisation, Remineralising agents

INTRODUCTION

Dental caries is a microbial disease of the calcified tissues of the teeth which is characterized by demineralisation of the inorganic portion and destruction of the organic substance of the tooth, leading to cavitation. It is a complex process where a multiple factors initiate the progression of disease [1]. Although numerous agents and therapies have been introduced to reduce or prevent the incidence of dental caries but still it remains a major dental disease [2].

Demineralisation and remineralisation cycle is a dynamic process that normally occurs in the oral cavity. Diet variations, poor oral hygiene and microbial activity can lead to demineralisation of the tooth. Whereas remineralisation is facilitated by the buffering action of the saliva, precipitating calcium and phosphate ions onto the tooth surface [3,4]. Hence, regulation of the demineralisation-remineralisation cycle is the key to prevention of dental caries. The conventional treatment for dental caries involved caries excavation and restoring with a suitable restorative material [5]. With the advent of "minimally invasive" approach emphasis on prevention is given rather than an invasive approach.

Fluoride has considerable cariostatic potential but it does not help to eliminate caries and increased fluoride concentration can produce detrimental effects on the tooth [6,7]. Hence many preventive agents have been introduced which function as an adjunct or independent to fluoride [8,9].

The anticariogenic potential and remineralising properties of CPP-ACP derived from milk protein casein is well known [10]. It acts as reservoir of bio-available calcium and phosphate, thus facilitating remineralisation [11]. GC Tooth Mousse plus contains 0.09% fluoride and is available as CPP–ACP paste (GC Tooth Mousse; GC Corporation, Tokyo, Japan) [12-16].

The antimicrobial mechanism of silver nanoparticles is because of their ability to penetrate the bacterial cell wall, impairing lipid peroxidation thus interrupting DNA replication and cellular respiration.

NovaMin (SHY-NM) a BAG acts as a biomimetic mineralizer . Ample of research has been carried out to support NovaMin as a successful desensitizing agent [17-21].

Remin Pro (VOCO,Germany) is another remineralising paste which in contrast to CPP-ACP products contains calcium and phosphate in the hydroxyapatite form. Also, fluoride and xylitol have been included in this product .
This in vitro study was designed to evaluate the remineralising capacity of the above agents on artificial enamel lesions, through Surface Microhardness (SMH) analysis and SEM examination.

MATERIALS AND METHODS

This in-vitro study was conducted over a period of two months in the Department of Conservative dentistry and Endodontics, Saveetha Dental College and Hospital, Chennai, India. A total of 50 human maxillary and mandibular premolars which were extracted for orthodontic purposes were selected for the study. the teeth were stored in 10% formalin solution until further use. Teeth were sectioned one mm below the cementoenamel junction, the roots were discarded and the crowns were used for the study. The specimens were stored in an antifungal solution containing 0.1% thymol solution. Custom made plastic cylindrical moulds were prepared and self-cured acrylic resin was poured in them and each tooth crown was embedded in the resin. The buccal surface was flattened and polished. A 6 mm × 6mm window of exposed enamel was created in the middle of the sample surface and a uniform coat of the nail varnish was applied to render it resistant to acid attack.

Baseline surface microhardness (B-SMH) was checked with Vicker's Microhardness Testing machine (VMT) for all the tooth samples. The indentations were made with VMT at the rate of 100 g load for 10 seconds. The average microhardness of the specimen was determined from three indentations to avoid any sort of discrepancy.

The demineralising solution contained 2.2 mM calcium chloride, 2.2 mM potassium phosphate, and 0.05 M acetic acid and the pH was adjusted with 1 M sodium hydroxide to 4.4. The remineralising solution contained 1.5 mM calcium chloride, 0.9 mM sodium phosphate, and 0.15 M potassium chloride, with a pH of 7.0. Each of the samples was immersed in the demineralising solution (20 ml) for 96 hours to produce artificial carious lesions in the enamel. After the initial demineralisation, Surface Microhardness values (D-SMH) were checked with VMT, post demineralisation.

A total of 50 samples were randomly divided into five groups of ten samples each.

Group A: Sound enamel, no treatment (Control)
Group B: CPP-ACP (Tooth Mousse Plus)
Group C: CPP-ACP with silver nanoparticles
Group D: Bioactive Glass (SHY- NM)
Group E: Hydroxyapatite gel (ReminPro)

A pH cycling model was used to replicate the changes occurring in the oral cavity. The remineralising pastes were applied with applicator tips and left for two minutes after which the samples were thoroughly washed with deionized water. The samples were again immersed in 20 ml of demineralising solution (pH 4.4) for three hours and were then washed with deionized water. This was followed up using respective remineralising agents for two minutes which was then washed off with deionized water. All the samples were then immersed in 20 ml of remineralising solution (pH 7) for 18 hours. The pH cycling was done for a period of 21 days. The remineralising and demineralising solutions were replaced every 48 hours and five days respectively. After the pH cycling process, all the enamel samples were assessed for SMH using Vickers hardness tester and the morphological variations were studied using scanning electron microscope(SEM).

Results were analysed by ANOVA and multiple comparisons between groups were performed by paired t-test and post-hoc Tukey test. $p<0.05$ was considered to be statistically significant.

RESULTS

Comparison between the baseline surface microhardness values and demineralised surface microhardness values is displayed in Table 1. Statistically significant difference was noted, suggestive of a decrease in SMH following the demineralisation cycle.

Multiple comparison between the experimental groups and control is displayed in Table 3. The highest post remineralisation SMH values were recorded in the Group C (CPP ACP with silver nanoparticles) followed by Group B (CPP-ACP), Group D (BAG) and Group E (HA gel).There was a significant difference ($p<0.05$) in the remineralising ability between the CPP ACP and BAG groups and HA gel group. SEM photographs of the test groups exhibited either amorphous crystals or particles scattered on the surface or lines of remineralisation along the prismatic borders.

Table 1 : Comparison between B-SMH and D-SMH values of test groups (Paired sample statistics)

	Mean	n	Standard deviation	Standard error mean
Baseline (B-SMH)	337.77	40	117.532	17.874
Post demineralisation (D-SMH)	233.55	40	115.318	17.563

Table 2 : ANOVA

	Sum of squares	df	Mean square	F	Sig
Between groups	56767.567	4	14191.892	13.370	<0.001
Within groups	58383.167	55	1061.512		

Table 3: Comparison of remineralisation values (Post hoc tukey test)

		Mean difference	Sig
Control	CPP-ACP	49.667*	.004
	CPP-ACP with silver nanoparticles	72.583*	<0.001
	BAG	88.250*	<0.001
	HA Gel	33.750*	.097
CPP-ACP	Control	-49.667*	.004
	CPP-ACP with silver nanoparticles	22.917	.429
	BAG	38.583*	.041
	HA Gel	-15.917	.753
CPP-ACP with silver nanoparticles	Control	-72.583*	<0.001
	CPP-ACP	-22.917	.429
	BAG	15.667	.764
	HA Gel	-38.833*	.039
BAG	Control	-88.250*	<0.001
	CPP-ACP	-38.583*	.041
	CPP-ACP with silver nanoparticles	-15.667	.764
	HA Gel	-54.500*	.001
HA Gel	Control	-33.750	.097
	CPP-ACP	15.917	.753
	CPP-ACP with silver nanoparticles	38.833*	.039
	BAG	54.500*	.001

*: Mean difference is significant at 0.05 level

Fig 1: SEM image (Sound enamel)

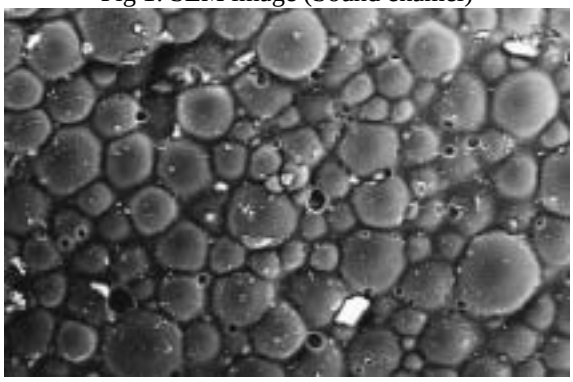


Fig 2: SEM image (demineralised enamel)

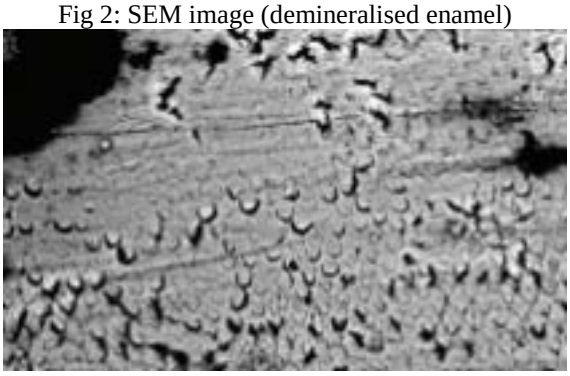


Fig.3: SEM image (CPP-ACP)

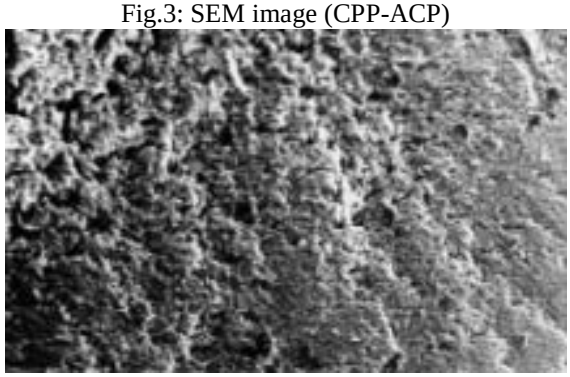


Fig.4: SEM image(CPP-ACP with silver nanaoparticles)

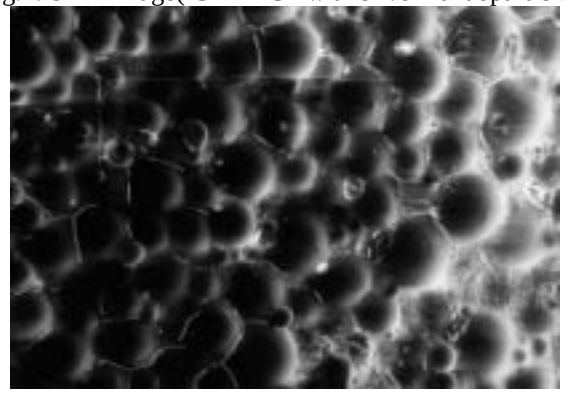


Fig 5: SEM image(BAG)

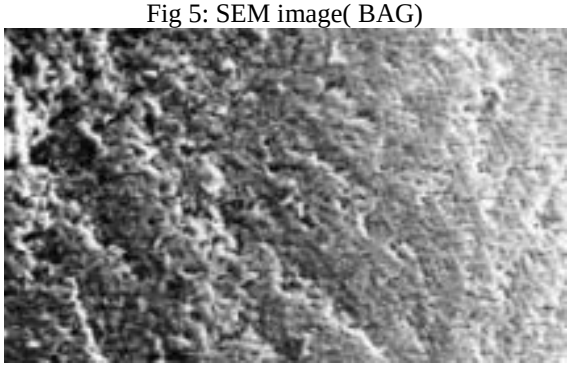
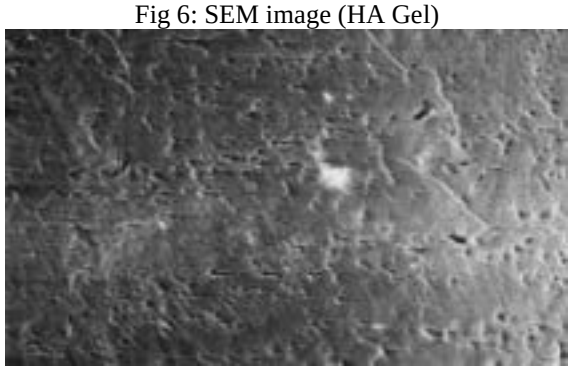


Fig 6: SEM image (HA Gel)



DISCUSSION

The pH-cycling protocol exposes enamel to a series of demineralisation and remineralisation. These studies are designed to simulate the dynamics of inorganic material loss and gain involved in caries formation . The pH cycling protocol used for this study was described by Featherstone JDB et al. In this model adopted, the dynamic cycles of demineralisation and remineralisation was replicated by sequentially immersing enamel specimens in demineralising and remineralising solutions. Surface micro hardness evaluation is a quick, non destructive method, showing mineral changes that have occurred due to the therapeutic procedures. The method also permits repeated measurements of the same specimen. Hence, surface micro hardness measurement is a suitable technique for studying de-remineralisation process and therefore employed in this study .

The use of fluoride is an effective method for promoting the remineralisation of early enamel lesions through the formation of fluorapatite. However, for every two fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite $\{Ca_{10}(PO_4)_6F_2\}$. Hence, when topically applying fluoride, an inadequate amount of available calcium and phosphate ions can limit net enamel remineralisation. CPP-ACP has demonstrated superior properties in situ in terms of anticariogenic activity, increasing levels of calcium and phosphate ions significantly in supragingival plaque, and promoting the remineralisation of enamel subsurface lesions [12]. The synergistic effect of CPP-ACP and fluoride in reducing caries may be attributable to the formation of CPP-stabilized amorphous calcium fluoride phosphate, resulting in the increased incorporation of fluoride ions into plaque, together with elevated concentrations of bioavailable calcium and phosphate ions.

BAG is an extensively studied biomaterial in the field of tissue engineering, bone regeneration and dentin remineralisation due to the remarkable capability of forming Hydroxycarbonate Apatite (HCA) [14]. Bioactive glass 45S5 (BAG) has been incorporated into dentifrices, desensitizing pastes and glass ionomer cements (experimentally). Although, it has been successfully proven that materials based on bioactive substance have the potential to promote remineralisation, only a limited number of studies have quantitatively monitored the remineralisation process.

It has been reported that, when BAG comes in contact with saliva or any aqueous media, its active ingredient, calcium sodium phosphosilicate, binds to the tooth surface in order to initiate the remineralisation process. The BAG thereby reacts with saliva inducing dissolution of calcium, phosphate and silicate ions at the glass surface and subsequent precipitation of a polycondensed silica-rich layer which serves as a template for the formation of calcium phosphate which subsequently crystallise into HCA [22].

Qualitative assessment was carried out using SEM analysis. SEM images of the sound enamel showed well organised enamel rods . The enamel crystals were homogeneously arranged with a clear outline. In contrast, the demineralised enamel was disorganized, with loss of structural characteristics . All the test groups demonstrated either amorphous crystals or particles scattered on the surface or lines of remineralisation along the prismatic borders .

CONCLUSION

Silver nano particles can be effectively used with remineralising agents for enamel remineralisation.

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