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A comparative evaluation of remineralizing capacity of primary versus permanent teeth using Enafix and GC tooth mousse - An in vitro study

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Abstract

Aim: To evaluate the remineralizing capacity of Enafix versus GC Tooth Mousse on primary and permanent teeth and to compare the remineralizing capacity of primary versus permanent teeth using Enafix and GC Tooth Mousse.

Materials and Method: The teeth were sectioned longitudinally to get two halves (buccal and lingual) using a diamond disc mounted on a straight handpiece. The primary and permanent enamel sections were randomly divided into 6 groups. All the test specimens were subjected to demineralizing and remineralizing cycle respectively. Artificial saliva was renewed every 24 hours. Each section was analyzed for depth of the lesion under a polarized microscope.

Results: The mean lesion depth values for the primary and permanent teeth were 77.27 ± 21.06 µm and 51.78 ±16.05 µm for GC Tooth Mousse and 89.12 ± 21.06 µm and 74.45 ± 13.30 µm for Enafix. Both the experimental groups showed a significant amount of remineralization for both primary and permanent teeth.

Clinical Significance: GC Tooth Mousse demonstrated higher remineralizing capacity than Enafix. Permanent

teeth showed a superior remineralizing capacity than primary teeth.

Keywords: Demineralization, Enafix, GC Tooth Mousse, Polarized microscope, Remineralization.

Introduction

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic portion of the tooth, which often leads to cavitation.[1] Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain.[2]

GC tooth mousse is a commercially available formulation containing Casein phosphopeptide - amorphous calcium phosphate (CCP-ACP).[3] The proposed mechanism for anticariogenicity of CPP–ACP is the localization of ACP in dental plaque thus buffering free calcium and phosphate ion activities. This provides a state of supersaturation of these ions with respect to tooth enamel, inhibiting demineralization and enhancing remineralization.[4] The multiple Serine (P) residues bind to forming nanoclusters of ACP in supersaturated solutions preventing their growth to the critical size for phase transformations.[5]CPP-ACP may act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth mineral, depressing enamel demineralization, and enhancing remineralization.[6]

Enafix (Commercial toothpaste containing Calcium sucrose phosphate (CaSP))[7], is a combination of calcium salts of sucrose phosphate esters, mixed with inorganic calcium. It is composed of 10%–12% calcium (wt %) and 8%–10% phosphorous (wt %). It acts as an ideal carrier for calcium and phosphate in water. Enafix acts by adsorption of sucrose phosphate ion rapidly on the enamel surface, thereby reducing the rate of acid dissolution of hydroxyapatite and quick remineralization by calcium and phosphate ion by common ion effect.[8] It prevents acid dissolution of enamel by depositing a layer of sucrose phosphate ion layer over the exposed hydroxyapatite of tooth.[9]This study assessed and compared the remineralizing capacity of Enafix and GC Tooth Mousse on primary and permanent teeth.

The Null Hypothesis: There is no statistically significant difference in the remineralizing capacity using Enafix and GC Tooth Mousse.

Materials And Methods

Nine, non-carious therapeutically extracted primary molars and permanent third molars each, were collected from the Department of Pediatric and Preventive Dentistry and the Department of Oral and Maxillofacial Surgery, Vydehi Institute of Dental Sciences and Research Center respectively for the purpose of the study. After extraction, the teeth were stored in accordance with ISO specification / TS 11 405:2015.[10]

Study Type: Experimental In-Vitro Study, Double blind study.

Method: The teeth were sectioned longitudinally to get two halves (buccal and lingual) using a diamond disc mounted on a straight handpiece. In all the sections a 3x3mm window of enamel was exposed on the middle third of the buccal/lingual surfaces by coating the remaining enamel with acid resistant nail varnish.

These test specimens were stored in deionized distilled water until further use.

Group Division: The primary and permanent enamel test specimens were randomly divided into 6 groups.

Group A: Primary teeth

Group A1: Demineralization only**. (n=6)**

Group A2: Demineralization followed by remineralization using Enafix. **(n=6)**

Group A3: Demineralization followed by remineralization using GC tooth mousse. **(n=6)**

Group B: Permanent teeth

Group B1: Demineralization only. **(n=6)**

Group B2: Demineralization followed by remineralization using Enafix. **(n=6)**

Group B3: Demineralization followed by remineralization using GC tooth mousse. **(n=6)**

All the samples of Group A and Group B were immersed into a glass container containing 50 ml of Demineralizing solution (2.2mM calcium chloride, 2.2mM monosodium phosphate, 0.05M lactic acid, The final pH was adjusted to 4.5 with 50% sodium hydroxide).[11] for a period of 48 hours at 37^0 C in a universal incubator. After 48 hours of incubation the samples were washed with deionized distilled water, dried with the help of an air syringe and placed in respective glass container until further evaluation. The samples of Group A2, A3 and B2, B3 were treated with respective remineralizing agents at every 24 hours for 10 days. The remineralizing agent was applied to the test specimens with the help of cotton applicator tip for 4 mins.Following which the specimens were washed with deionized distilled water and placed in artificial saliva, in a universal incubator at 37°C, between each remineralizing cycle. Artificial saliva was renewed every 24 hours. Each enamel specimen was sectioned using hard tissue microtome to 100-150µm thickness and analyzed for depth of the lesion under polarized microscope.

Results

Statistical Analysis

The collected data were analysed with IBM.SPSS statistics software 23.0 Version. To describe about the data descriptive statistics Mean & Standard deviation were used. To find the significant difference between the bivariate samples in Independent groups (Group A & B) the Mann- Whitney U test was used. For the multivariate analysis the Kruskal Walli's test was used. In both the above statistical tools the probability value 0.05 is considered as significant level.

Group A3 (GC tooth mousse) demonstrated least Mean score 77.27±24.31µm followed by Group A2 (Enafix) 89.12±21.06 µm and Group A1 (Demineralization) 151.49±54.99 µm.**Table-1**

Group B3 (GC tooth mousse) demonstrated least mean score 51.78±16.05µm followed by Group B2 (Enafix) 74.45±13.30µm and Group B1 (Demineralization) 89.59±34.31µm.**Table-2**

P- Value for Primary teeth (Group A) is 0.054 and for Permanent teeth (Group B) is 0.057. This denotes that p-value is not significant at $0.01 < P \leq 0.050$.**Table-3**

Table 1: Intragroup comparison of lesion depth before and after remineralization with Enafix (Group A2) and GC Tooth Mousse (Group A3) of demineralization of primary teeth (Group A1).

Groups = Primary teeth	N	Mean	Std. Deviation
Group A1	6	151.49	± 54.99
Group A2	6	89.12	± 21.06
Group A3	6	77.27	± 24.31
TOTAL	18	105.96	± 48.12

Table 2: Intragroup comparison of lesion depth before and after remineralization with Enafix (Group B2) and GC Tooth Mousse (Group B3) of demineralization of permanent teeth (Group B1).

Groups = Permanent teeth	N	Mean	Std. Deviation
Group B1	6	89.59	± 34.31

Group B2	6	74.45	± 13.30
Group B3	6	51.78	± 16.05
TOTAL	18	71.94	± 27.01

Table 3: Shows demineralized and remineralized mean depth of penetration & significant differences of primary (Group A) versus permanent teeth (Group B).

Groups= Primary teeth (Group A)		Values
	Chi-square	5.87
	Df	2
	Asymp. Sig.	0.054
Groups= Permanent teeth (Group B)		Values
	Chi-square	5.71
	Df	2
	Asymp. Sig.	0.057

(*P- value is Significant at $0.01 < P \leq .050$)

Discussion

Remineralization of white-spot lesions may be possible with a variety of currently available agents, such as fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and bioavailable calcium phosphate. This concept bridges the traditional gap between prevention and surgical procedures.[12] CPP-ACP could be incorporated into the pellicle in exchange for albumin and it inhibits the adherence of S. mutans and S. sobrinus.[13] CPP-ACP crème is effective in remineralizing early enamel lesions of the primary teeth, a little more effectively than 500ppm NaF and can be used for the prevention of ECC.[14] Casein phosphopeptide based technology has been established as a strong non-fluoridated remineralizing agent fulfilling all the criteria of an ideal remineralizing material.[2] CPP–ACP inhibits demineralization, enhance remineralization or possibly both.[15]

The efficacy and ease of Polarized light microscope in caries research studies has been established previously. High degree of differentiation between demineralized area and normal area of tooth sample can been achieved in this analysis, which has been shown to be better than with microradiography.[16]As lesion depth measurement was a parameter under consideration in the present study, PLM analysis was chosen for quantification of demineralization and remineralization of hard tissues.

In the present study, intragroup comparison of lesion depth before and after remineralization with Enafix (Group A2) and GC Tooth Mousse (Group A3) of demineralization of primary teeth, GC tooth mousse had least lesion depth after remineralization (77.27±24.31µm) compared to the Enafix Group (89.12±21.06µm). Therefore, GC Tooth Mousse demonstrated better remineralization potential compared to Enafix group in primary teeth. A similar result was obtained in an in vitro study, comparing the remineralizing potential of GC Tooth Mousse and Fluoridated toothpaste, conducted by Agnihotri Y. et al (2012). Shah SP and Birur PN reported that GC Tooth Mousse promoted remineralization of the carious lesions by maintaining a supersaturated state of enamel mineral and had better remineralizing potential when compared with fluoridated toothpaste.[16, 17, 18]

In the present study, the intragroup comparison of lesion depth before and after remineralization with Enafix (Group B2) and GC Tooth Mousse (Group B3) of demineralized lesions in permanent teeth, showed that GC Tooth Mousse was superior in remineralization than Enafix with the measured lesion depth being (51.78 ±16.05µm) and (74.45 ±13.30 µm) for the two formulations respectively.

Sabel N et al demonstrated that the enamel responds to demineralization with different lesion depths and this correlates to the composition of the enamel. The lesion is deeper when the porosity of enamel is greater and stated

that there is a variety of chemical composition between the individual enamel analyzed.[19] Gutierrez et al. found that the micropores between the hydroxyapatite crystals appeared to be laminar in the enamel in the high-risk caries group while the micropores were considered to be cylindrical in the low-risk caries group.[20]

In the present study, intergroup comparison of lesion depth after demineralization of primary teeth versus permanent teeth assessed the percentage increase in depth of lesion of demineralized enamel after demineralization. Permanent teeth (Group B1) showed a less penetration and demineralization when compared with the primary teeth (Group A1). The mean depth of lesion was (89.59±34.31µm) and (151.49±54.99µm) for Group B1 and Group A1 respectively.

Murakami et al stated that primary and permanent enamel surfaces are morphologically different from one another and the former is more porous, less mineralized, has more carbon dioxide and carbonate. The outline and arrangement of enamel rods are similar in primary and permanent teeth but primary enamel has less organized micro crystals and a greater diffusion coefficient .[21]

Lussi et al demonstrated that the porosity of primary enamel seems to be greater than for permanent teeth, which leads to greater susceptibility to an acidic attack.[22]

In the present study, in the intergroup comparison, between primary and permanent teeth, of lesion depth after remineralization with Enafix, the permanent teeth (Group B2) showed a greater decrease in depth of lesion than the primary teeth (Group A2).

According to Wang et al, primary enamel with higher organic content dissolved considerably faster than permanent enamel. The authors reported that the demineralization of primary and permanent enamel in acidic media showed significant differences, with primary enamel having a greater susceptibility to demineralization. [23]

In the present study, the permanent teeth (Group B3) treated with GC Tooth Mousse showed a greater reduction in lesion depth than the primary teeth (Group A3).

No other publication on remineralization capacity of GC Tooth Mousse on primary versus permanent teeth could be found after a detailed search of the print and online literature at the time of writing this article.

Hence based on these results, GC tooth mousse can be considered to possess superior remineralizing capacity when compared to Enafix.

Manufacturer Name

1. GC tooth mousse :School of Dental Science at the University of Melbourne Victoria/ Australia.3
2. Enafix:Group Pharmaceuticals Pvt Ltd,Bengaluru, Karnataka, India.7

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