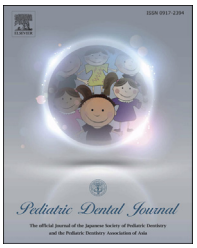
PEDIATRIC DENTAL JOURNAL XXX (2016) 1–8

Available online at www.sciencedirect.com

Pediatric Dental Journal

journal homepage: www.elsevier.com/locate/pdj



Original Article

# Evaluation of remineralizing potential of Calcium Sucrose Phosphate and CPP-ACP: An in vitro study

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ARTICLE INFO

*Article history:*
Received 13 February 2016
Received in revised form
2 June 2016
Accepted 11 July 2016
Available online xxx

*Keywords:*
Calcium Sucrose Phosphate
CPP-ACP
Scanning Electron Microscope
Demineralization
Remineralization
Vickers microhardness

ABSTRACT

*Background:* In depth understanding of dental caries progression and improved diagnostic methodologies to assess early demineralization has enabled development of novel remineralizing therapeutics. Hence, an emerging goal of modern dentistry is to manage non-cavitated carious lesions non-invasively through remineralization.

*Objective:* To evaluate the remineralizing potential of Calcium Sucrose Phosphate (CaSP) and Caesine PhosphoPeptide-Amorphous Calcium Phosphate (CPP-ACP) on early enamel lesions.

*Method:* Thirty enamel specimens were prepared from freshly extracted noncarious molars and artificial enamel subsurface lesions were induced. The sample was randomly divided into 3 groups based on the surface treatment i.e. Group A – Control (no surface treatment) Group B – CPP-ACP cream, Group C – CaSP paste. A caries progression test (pH cycling) was carried out for 12 days. All enamel specimens were subjected to quantitative and qualitative analyses at baseline, after demineralization and after remineralisation using Vickers microhardness test and Scanning Electron Microscopy respectively.

*Results:* Statistical analysis using one-way ANOVA followed by multiple comparisons test was applied to detect significant differences at $P \leq 0.05$ levels. The mean surface microhardness recovery with CaSP was significantly higher than other groups.

*Conclusion:* The CaSP paste was effective in remineralizing early enamel lesions than CPP-ACP.



## 1. Introduction

Despite advances in scientific knowledge, improved oral hygiene regime and the ever increasing availability of new commercial preventive formulations, dental caries continues to be an acknowledged global health problem. It is a dynamic process with strewed periods of demineralization and remineralization causing initiation, progression, and reversal of caries. At critical pH, the conditions favouring dissolution

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http://dx.doi.org/10.1016/j.pdj.2016.07.002
0917-2394/

Please cite this article in press as: Thabitha Rani S, et al., Evaluation of remineralizing potential of Calcium Sucrose Phosphate and CPP-ACP: An in vitro study, Pediatric Dental Journal (2016), http://dx.doi.org/10.1016/j.pdj.2016.07.002

outweigh those favouring redeposition, resulting in the initiation of enamel lesions. Prevention of initiation and interruption in progression of early lesions are the desirable modes of caries management.

There has been an explosion of interest on minimal invasive treatment of incipient carious lesions with the availability of novel preventive formulations, like fluoride [1], casein phosphopeptide (CPP-ACP; Recaldent) [2], unstabilized ACP (ACP, Enamelon), CPP stabilized amorphous calcium phosphate with fluoride (CPP-ACP F; Recaldent) [3] and Calcium Sucrose Phosphate (CaSP) [4].

Casein phosphopeptides (CPP) is a bioactive agent, produced from a tryptic digest of milk protein "casein", comprises multiphosphoseryl sequence (Ser(P)-ser(P)-Glu-Glu) which stabilizes amorphous calcium phosphate into nanocomplexes in solutions like Amorphous Calcium Phosphate [5]. Thus provide a reservoir of nonstructurally bound calcium and phosphate ions which favour remineralisation during a cariogenic attack. It dramatically decreases lesion depth and increases the mineral content of enamel [6].

Calcium Sucrose Phosphate (CaSP) is a mixture of calcium sucrose mono and diphosphate, disucrose monophosphate and inorganic calcium phosphate that contains 11% calcium, 9.5% organic phosphate and 2.5% inorganic phosphate [7]. It reduces the rate of dissolution of hydroxyapatite in acid buffers and decreases enamel demineralization. It creates an environment conducive for remineralization of tooth surface and inhibits the formation of plaque [8,9].

Till date there is only limited research available over the remineralizing efficacy of CaSP. Hence, the present study was designed to comparatively evaluate the remineralization potential of CaSP containing toothpaste (Enafix™) and CPP-ACP containing tooth crème (GC Tooth Mousse™) on artificially demineralised human enamel through Surface Microhardness (SMH) and Scanning Electron Microscope (SEM) examination.

## 2. Materials and methods

### 2.1. Sample selection

A total of 30 human permanent mandibular molars free from caries, restorations, cracks, and enamel defects, extracted due to compromised periodontal conditions were selected. The teeth were cleaned of calculus, soft tissue debris and stored in deionised water containing 0.1% Thymol until use. The institutional ethical committee clearance was obtained before commencing the study.

### 2.2. Enamel specimen preparation and grouping

The teeth were decoronated at CEJ and sectioned mesiodistally using a diamond disc bur at slow speed with water as coolant. Custom made plastic cylindrical moulds were prepared and self-cure acrylic resin was sprinkled into them. The buccal fragment of each enamel specimen was then embedded onto the top of partially set acrylic resin and was allowed to set completely. The buccal surfaces were then ground flat and hand polished progressively using 400- and 600-grit silicon carbide paper (Allied High Tech Products Inc.), followed by 0.004- and 0.0600-grit silicon carbide paper (Allied High Tech Products Inc.) and followed by 1.0 μm and 0.05 μm alumina suspensions. All enamel specimens were subjected to quantitative and qualitative analysis using Vickers microhardness test (VHN) and SEM respectively and the data was recorded at Baseline.

### 2.3. Solution preparation

Demineralising and remineralising solutions were prepared according to the recommendations of Tencate and Duijsters [10].

Demineralizing solution: Acetic acid (50 mM) solution containing 2.2 mM $Ca(NO_3)_2$, 2.2 mM $KH_2PO_4$ and 0.1 ppm NaF adjusted to pH 4.5.

Remineralizing solution: 20 mmol. HEPES, 1.5 mM $CaCl_2$, 0.9 mM $KH_2PO_4$, 130 mM KCl and 1 mM $NaN_3$ the pH was adjusted to 7.0 with KOH.

### 2.4. Artificial enamel lesion formation

Early artificial caries lesions representing preliminary stage of subsurface enamel demineralisation were produced in the enamel according to Tencate and Duijsters [10]. Each specimen was immersed into a glass tube containing 8 ml of demineralising solution for 72 h at 37 °C [10]. After artificial caries lesion formation, Surface microhardness (SMH) and SEM analysis was carried out.

### 2.5. Grouping of samples

The resultant 30 enamel specimens were then randomly allocated into three groups with 10 in each based on the remineralizing agent used.

Group A – Deionised water (Control)

Group B – CPP-ACP (GC Tooth Mousse™) (GC CORPORATION),

Group C – CaSP (Enafix™)

Sources of supply of commercial products should be given with the address (town, state and country)

Group B – CPP-ACP (GC Tooth Mousse™) (GC CORPORATION, 76/1 HASUNUMA – CHO, ITABASHI – KU, TOKYO, JAPAN)

Group C – CaSP (Enafix™) (GROUP PHARMACEUTICALS LIMITED, PLOT NO. 41, Nasigere Village, Kasaba Hobli, KIADB indi. Area, Malur-563130)

Enamel specimens of Group B and C were surface treated with the respective remineralizing agents following manufacturer's recommendations using applicator brush and left undisturbed for 3 min.

### 2.6. pH cycling model

The pH cycling schedule was followed to simulate pH dynamics of the oral environment as proposed by White, was carried out for 12 days [11] (Table 1). The de- and remineralizing solutions were freshly made every third day. Each cycle involved 2 h of demineralization in order to simulate the daily acid challenges occurring in the oral cavity.

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