journal of dentistry 42s1 (2014) s30–s38

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Mode of action studies on the formation of enamel minerals from a novel toothpaste containing calcium silicate and sodium phosphate salts

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KEYWORDS

Calcium silicate
Enamel
Erosion
Hydroxyapatite
Mineralisation
Toothpaste

ABSTRACT

Objectives: To investigate *in vitro* and *in situ* the deposition and formation of hydroxyapatite (HAP) on enamel surfaces following brushing with a novel toothpaste containing calcium silicate (CaSi), sodium phosphate salts and fluoride.
Methods: Polished enamel blocks were brushed *in vitro* with a slurry of the CaSi toothpaste. After one brush and four weeks simulated brushing the enamel surfaces were analysed. In an *in situ* protocol, enamel blocks were attached to first or second molar teeth of healthy subjects, exposed to 4 weeks twice per day brushing with the CaSi toothpaste and then analysed. The surface deposits were analysed using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM) and selected area electron diffraction (SAED). In addition, the CaSi toothpaste was slurried in simulated oral fluid (SOF) over a 3 hour period and the solids were isolated and analysed by Fourier transform infrared spectroscopy (FTIR).
Results: The FTIR study demonstrated that calcium phosphate phases had formed and these became increasingly crystalline over 3 hours. CaSi was deposited onto enamel surfaces following one brushing with the toothpaste *in vitro*. The deposited particles showed evidence of HAP crystalline phases associated with the CaSi. Following 4 weeks brushing *in vitro*, the deposition increased and analyses showed that the deposited material was HAP. These results were confirmed by the *in situ* study.
Conclusions: Calcium silicate can be deposited onto enamel surfaces from a novel toothpaste formulation where it can form the enamel mineral HAP.
Clinical Significance: A novel toothpaste formulation containing CaSi can form HAP on enamel surfaces. The potential of this technology is for a novel approach to the repair of demineralised enamel and the protection of enamel during acid exposure.

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1. Introduction

Tooth enamel is composed of approximately 96% by weight of hydroxyapatite (HAP), which is a mineral rich in calcium and phosphate.[1] If exposed to challenges from plaque acids or the dietary intake of acidic soft drinks or foods, calcium and phosphate can be lost from the enamel, leading to a compromise in the tooth surface integrity and strength.[2]

A natural process of tooth repair is possible from saliva, which contains calcium and phosphate ions that can re-integrate into the acid damaged enamel surface, restoring the lost enamel minerals. The enamel repair and protection process can be enhanced by the use of fluoride containing products.[3-5] A range of other actives, typically based on calcium containing materials has been described; these can promote enamel repair either on their own or in combination with fluoride.[6] Bioactive materials which can stimulate a beneficial response from the body have been described, with particular reference to their ability to bond to host bone tissue and to the formation of calcium phosphate layers on a material surface.[7] As the tooth is also a hard tissue with a similar mineral content to bone, bioactive materials have been considered for dental applications and their ability to promote enamel and dentine remineralisation has been studied.[8-11] These bioactive materials include bioglass, bioactive glass ceramics and calcium silicates.[6,7]

A novel technology has been developed based on the combination of calcium silicate, sodium phosphate salts and fluoride. The technology is proposed to augment the natural mineralisation processes of human saliva by providing additional calcium and phosphate, nucleating HAP formation and leading to overall remineralisation of tooth enamel minerals. The proposed mechanism can help to repair acid-softened enamel and to protect sound enamel from acid challenges, giving overall enamel health benefits. The technology has been formulated into a novel toothpaste containing calcium silicate, sodium phosphate salts (monosodium phosphate and trisodium phosphate) and sodium monofluorophosphate (1450ppm as F).

The aim of the current work is to investigate the mode of action of the calcium silicate and sodium phosphate salts, both as active agents and when formulated into a toothpaste, relevant to their enamel remineralisation and protection potential. This will include characterisation of the calcium silicate following incubation in simulated oral fluid, deposition studies of calcium silicate onto enamel and transformation of calcium silicate into HAP on enamel surfaces, using *in vitro* and *in situ* methods and a range of state-of-the-art measurement and analytical techniques. The null hypotheses to be tested are (a) calcium silicate will not deposit onto the enamel surface and (b) calcium silicate will not transform into HAP.

2. Materials and Methods

2.1 Deposition of calcium silicate onto enamel in vitro and analysis of deposits

Extracted human teeth were cleaned firstly by 75% ethanol with a scraper until there was no obvious calculus, dirt or stain left on the surface.

Calcium silicate (CaSi) material was dispersed in phosphate solution at pH 7. The enamel blocks were manually brushed (n=2) with the CaSi-phosphate mixture for 3 minutes and then thoroughly rinsed with water. A second set of blocks was brushed with a CaSi-water mixture as a control group. A third set of blocks (CaSi-toothpaste) was brushed with a toothpaste containing the CaSi, sodium phosphate salts (monosodium phosphate and trisodium phosphate) and sodium monofluorophosphate (SMFP, 1450ppm F). The toothpaste was slurried in water (toothpaste:water, 1:1 by weight). Following the brushing treatment, the enamel blocks were rinsed with water.

The enamel surfaces were studied using a scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX) in order to characterize the morphology and chemical composition of any deposits. A field-emission cold gun SEM (Hitachi S-4800, Japan) equipped with EDX spectroscope (X-Max 80, Oxford Instruments, UK) was used. A subset of the toothpaste-treated enamel blocks was immersed in ethanol in an ultrasonic bath to allow removal and collection of the deposited particles. These particles were imaged by a transmission electron microscope (TEM) (JEOL 2010F, Japan). Selected area electron diffraction (SAED) was used to analyze and determine the crystallography of areas of interest.

2.2 Calcium ion release from calcium silicate.

The release of Ca^{2+} by CaSi was studied in unbuffered solutions using a Ca^{2+} ion-selective electrode (Sentek Ca-ISE, Sentek, Ltd, UK). The Ca^{2+} ion concentration, following the introduction of 1 mg/ml of CaSi, was monitored at one minute intervals from one minute up to 10 minutes (n=5). pH was monitored simultaneously using a separate pH probe (Mettler InLab 413, UK).

2.3 FTIR evaluation

The formation of hydroxyapatite was investigated using Fourier Transform Infra Red spectroscopy (FTIR). In this study the CaSi/phosphate toothpaste was mixed with simulated oral fluid (SOF) in the ratio of 1:2. The composition of SOF is shown in Table 1. The resulting slurry was incubated in plastic vessels at 37°C in a thermostatic shaker bath. At given time points, the slurry was removed from the water bath, and the solid content in the slurry was separated by centrifugation at 8000 rpm for 3 minutes. The solid was washed three times with anhydrous ethanol and dried in a vacuum oven at 37°C. The FTIR spectra were recorded between 4000 and 500 cm^{-1} in transmission/absorbance mode with 4 cm^{-1} resolution (FTIR - Nicolet iN10, Thermo Fisher Scientific, USA).

2.4 Formation of HAP in vitro

Intact bovine enamel teeth were cut into blocks of approximately 3mm x 3mm and with a thickness of 0.7-1.0mm. The enamel surface was polished (Wetordry™ 401Q, 3M, US) with a final finish of 1500 grit for at least 3 minutes, and throughly rinsed with water. These blocks were placed into whole human saliva at 37°C overnight to generate a pellicle. These enamel blocks (n=5) were brushed with a 1:2 slurry of the CaSi/phosphate toothpaste in water using a mechanical brushing machine (M235 Martindale, SDL ATLAS, Switzerland) fitted with flat trim soft toothbrush heads