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Casein phosphopeptide–amorphous calcium phosphate remineralization of primary teeth early enamel lesions

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ABSTRACT

Early childhood caries (ECC) is a serious problem that progresses rapidly and often goes untreated. Current traumatic treatments may be replaced by safe and effective remineralization at very early stages.

Objective: The aim of this *in vitro* study was to evaluate the remineralization effects of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) paste on enamel lesions by assessing ultrastructure, nanomechanical properties, and compound and elemental analysis.

Methods: Enamel specimens from 6-year-old children were divided into groups: (1) native enamel; (2) water as negative control; (3) 500 ppm NaF as positive control; and (4–7) CPP–ACP paste for 4, 8, 12, and 24 h, as test groups. Ultrastructure and roughness were observed by atomic force microscopy (AFM); nanohardness and elastic modulus were measured by nanoindentation; compound and crystal size of enamel surface patterns were investigated by X-ray diffractometer (XRD). An electron microprobe (EPMA) was used for element analysis. Data were analyzed using one-way ANOVA.

Results: The CPP–ACP paste repaired the microstructure of enamel, including prism and interprism, through significantly increased hydroxyapatite crystal size (12.06 ± 0.21 nm) and Ca/P molar ratios (1.637 ± 0.096) as compared with NaF (8.56 ± 0.13 nm crystal size and 1.397 ± 0.086 Ca/P, $p < 0.01$). Both CPP–ACP and NaF decrease roughness, and increase the nanohardness and elastic modulus, with no significant differences between the materials.

Conclusions: The CPP–ACP paste is more suitable for children than NaF, due to advantages for remineralization. The AFM, nanoindentation, EPMA, and XRD are very helpful methods for further understanding of microscale and nanoscale remineralization mechanisms.

1. Introduction

Early childhood caries (ECC) is a term used to describe any form of caries in infants and preschool children.[1,2] It is a major public health problem worldwide. ECC begins with white-spot lesions, and caries can progress continuously, leading to complete destruction of the crown.[3] Calcium and phosphate ions are more readily lost for children because they eat mainly soft and sweet foods, and due to lower

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mineralization of deciduous enamel. The disease usually develops very quickly and causes many childhood health problems, such as caries-related toothache and infection. Also affected may be chewing, nutrient absorption, and maxillofacial growth and development, which can negatively influence children's psychological and emotional conditions and be very difficult and costly to treat. Any treatment agent must be convenient and safe at the early caries stage for cure and prevention. Demineralization and remineralization are dynamic processes in caries initiation, progression, and reversal. Therefore, regulation of the demineralization–remineralization balance is a key to caries prevention and treatment.[4,5] The ideal method of increasing remineralization is reconstructing the depleted tissues with hydroxyapatite, which is the same inorganic component as the enamel.[6] Fluoride has a profound effect on the level of caries prevalence, but it is far from a complete cure.[7] Furthermore, research has indicated that only localized high concentrations of fluorine can significantly enhance anticaries efficacy and promote remineralization. But fluoride can also cause fluorosis through overexposure, especially in young children.[8] Therefore, it is necessary to seek an alternative, effective nonfluoride agents for ECC.

Milk products have been long-found to have an anti-caries role, and in recent years, studies reported this effect with respect to casein phosphopeptide (casein phosphopeptides, CPPs) in cheese. The CPP, a biologically active peptide rich in phosphoserine, is produced from a tryptic digest of the milk protein casein by aggregation with calcium phosphate and purification by ultrafiltration.[9] As an inorganic ion carrier, CPP can promote intestinal absorption and utilization of iron, zinc, selenium, and especially calcium,[10,11] with the advantage of being noncytotoxic, safe, and reliable, and causing little sensitization.[12] Casein phosphopeptides contain a cluster of phosphoseryl residues in the motif -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- which markedly increase the apparent solubility of calcium phosphate by stabilizing amorphous calcium phosphate (ACP) under neutral and alkaline conditions, forming metastable solutions that are supersaturated with respect to calcium phosphates. The CPP- and ACP-formed nanoclusters buffer the activities of free calcium and phosphate ions in the plaque fluid, helping to maintain supersaturation with respect to enamel mineral, and acting as a calcium-phosphate reservoir, thereby depressing enamel demineralization and enhancing remineralization.[13,14] By competition of calcium binding sites, CPP–ACP can also inhibit adhesion between streptococcus and streptococcus sobrinus, as well as their adhesion to plaque.[15] The high concentrations of calcium have been shown to invade bacteria and destroy normal their metabolism. CPP can also promote infant absorption of calcium and phosphorus to form dental hard tissue and improve acid resistance of enamel, and this effect is not affected vitamin D deficiency.[16] Currently, many applications of CPP–ACP are found in other areas, such preventing decalcified enamel lesions during fixed orthodontic therapy,[17] without affecting the shear bond strength of bonded brackets.[18] It is also used for treating dentinal hypersensitivity.[19] Current assessment is limited to traditional detection methods, study of remineralization mostly at microscale, with less known about changes at the smaller nanoscale. Atomic force microscopy (AFM) provides an opportunity to observe the shape of softened enamel surfaces at the nanometer scale. Nanoindentation, for measurement of nanomechanical properties, such as surface nanohardness and modulus for indentation depths of less than 100 nm,[20] can accurately measure mechanical properties of very small (submicrometer) volumes, with fine spatial resolution.

This *in vitro* study was designed to evaluate the remineralization potentials of CPP–ACP paste, which was used on artificial early enamel lesions of children's primary teeth, through observing the surface microstructures and nanomechanical properties by AFM and nanoindentation, and the chemical composition/element and crystal structure by X-ray diffractometer (XRD) and electron microprobe (EPMA). With these materials-engineering microanalysis strategies, we can research the activity patterns of calcium and phosphorus ions at the nanoscale in the process of *in vitro* enamel remineralization, and furthermore, to provide theoretical references for CPP–ACP remineralization mechanism explorations. We conducted these experimental studies to test the validity of the null hypothesis: the remineralization effects of CPP–ACP and NaF are significant different; CPP–ACP cannot achieve the same remineralization effect as NaF.

2. Materials and methods

2.1. Sample

The CPP–ACP cream was a tooth mousse, a water-based, sugar-free topical cream containing RECALDENT™. The topical cream contained 10% (w/w) CPP–ACP nanocomplexes with bioavailable calcium and phosphate (GC Corp., Tokyo, Japan).

2.2. Specimen preparation

The Institutional Ethical Committee of Beijing Stomatology Hospital affiliated to the Capital University of Medical Science, approved this study. A total of 70 lower retained deciduous incisors from 58 children at about 6 years old (28 female, 30 male) were obtained from the clinic of Pediatric Dentistry, School of Stomatology, Capital Medical University, Beijing City, China, and the patients were informed about and consented to the use of their teeth. Immediately after the extraction of the teeth, the roots were removed. The teeth were rinsed with the tap water, and the specimens were cleaned of debris and stored in distilled water with 0.5% thymol until use. Under the stereomicroscope (ACT-1, Nikon, Japan), any teeth with defects, erosions, or microcracks on their enamel surfaces, or visible stains on the facial surfaces were excluded. Each whole tooth was embedded in long-cure epoxy resin (Leco, St. Joseph, MI, USA), the surfaces were polished sequentially using 800-grit and 1200-grit silicon carbide sand papers, followed by 3, 2, 1, and 0.5 μm Al_2O_3. All polished samples were individually sonicated in distilled water for 10 s to remove the residual abrasives while not disrupting structure and properties.