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Rapid biomimetic remineralization of the demineralized enamel surface using nano-particles of amorphous calcium phosphate guided by chimaeric peptides

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ABSTRACT

Objective. The objective of this study was to develop a rapid and effective method to remineralize human carious-like enamel using chimaeric peptide-mediated nanocomplexes of carboxymethyl chitosan/amorphous calcium phosphate (CMC/ACP), mimicking the mineralizing pattern of the oriented assembly of ACP guided by amelogenin in the biomineralization of enamel.

Methods. CMC/ACP nanocomplex solution was first synthesized through the successive addition of carboxymethyl chitosan, calcium chloride, and dipotassium phosphate into distilled water. ACP nanoparticles were degraded by 1% NaClO from CMC/ACP nanocomplexes. The morphology of the particles at different periods was tested by transmission electron microscopy (TEM). The chimaeric peptides were added to guide the arrangement of ACP nanoparticles and to bind ACP nanoparticles to the demineralized enamel surface specifically. X-ray diffraction (XRD)/scanning electron microscope (SEM)/confocal laser scanning microscopy (CLSM)/nano-indentation tests were applied to check the remineralization effects.

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Results. CMC/ACP nanocomplexes were obtained and could be kept without precipitation for a long time. After the degradation of NaClO and guidance of chimaeric peptides, ACP nanoparticles were arranged into oriented arrays before transforming into crystals, and the enamel-like crystals were tightly bound onto the demineralized surface. The newly formed enamel-like crystals were nearly well-organized and equipped with strong mechanical properties.

Significance The chimaeric peptide, which possesses guiding and binding motifs, was proven to be effective in demineralized enamel remineralization. This study provides a novel method of enamel remineralization, which is of great value in the prevention and therapy of enamel decay and tooth erosion, such as decay due to chalk caries after orthodontic therapy.

1. Introduction

Remineralization of dental hard tissue is an important curative technique in minimally invasive dentistry (MID) [1]. Biomimetic remineralization plays an important role in the prevention and early treatment of enamel caries, dentin caries, and dentin hypersensitivity [2]. It is well known that dental caries and tooth erosion begin at the outermost layer of teeth (enamel) with the damage of dental hard tissues. This process is called demineralization [3], which is attributed to the loss of mineral ions from the lattice of hydroxyapatite (HAP) through the action of organic acids produced by bacteria on the surface of enamel. Increasing numbers of researchers believe that dental caries formation is a dynamic disease process that is caused by the disturbance between the demineralization and remineralization processes. And tooth erosion is a disease that dental hard tissues were progressively etched by endogenous and exogenous acid [4]. Therefore, the prophylaxis of preliminary enamel caries and enamel erosion can eliminate the progression of dental caries and further erosion, resulting in better preservation of natural dental tissue. One approach to preventing lesion progression is to treat the lesion with a remineralizing agent to tip the balance back toward remineralization, thus reversing the pathological process of caries and erosion formation [5].

Enamel formation is a typical biomineralization process that requires the syngeneic actions of both organic and inorganic components. With the assistance of complex development and degradation of organic components in the extracellular matrix, the inorganic elements are regulated and then begin to nucleate, grow, and assemble in an orderly manner in a certain time and space [6,7]. Robinson et al. illustrated that spherical mineral particles with a diameter of 50 nm organize into chains in enamel formation [8]. It was suggested that these nanoparticles are composed of amorphous calcium phosphate (ACP) and proteins associated with biomineralization and that the ACP can transform into HAP crystals. The small particles arrange in lines and then, with the help of functional proteins, the particles disappear and form crystals parallel to the c-axis of enamel. Among the natural proteins, amelogenin is now the most acknowledged, consisting of 173 amino acids with an N-terminus and C-terminus on each side. Some *in vitro* studies have shown that amelogenin can stabilize ACP to form Amel/ACP particles and guide the particles to arrange, fuse, and transform into HAP crystals [9–11]. In addition, from the perspective of knowledge of material nanoparticles of ACP are much more active for enamel remineralization due to their small size and high surface area [12,13]. Therefore, it is possible to utilize the nanoparticles of ACP stabilized by matrix to remineralize demineralized enamel.

Inspired by the stabilization ability of amelogenin, it is speculated that the existence of macromolecules can serve as a stabilizer to directly sequester supersaturated calcium and phosphate ions in solution from precipitates. Thus, some organic macromolecules, such as polyaspartic acid (PASP), polyacrylate acid (PAA), and casein phosphopeptide amorphous (CPP), have been applied to stabilize ACP nanoparticles in solution because of their chelating function resulting from rich carboxyl groups [14–18]. However, the disadvantages of the aforementioned materials are also apparent, such as the low ability to stabilize high-ion supersaturation and the allergenic property of the materials to people who are sensitive to milk. Here, we used carboxymethyl chitosan (CMC), a derivative product of chitosan with biodegradable, biocompatible, nontoxic, and antibacterial properties [19], that is rich in carboxyl groups and is a good stabilizer of ACP nanoparticles as nanocomplexes of CMC/ACP to provide a remineralizing effect on dental tissues [20].

Regarding the remineralization materials, the economic operative time and effective binding to the enamel surface are basic demands of the dental clinical population. However, macromolecules such as PASP, PASA, CPP, and CMC, applied to stabilize amorphous calcium phosphate, cannot make the particles undergo crystal transformation quickly enough for them to have an enamel binding effect in clinical practice. *In vivo*, MMP-20 and KLK4 are the two key enamel proteases that can process amelogenin to generate the major cleavage products that accumulate during the secretory stage of amelogenesis [21]. Thus, surfactant should be used *in vitro* to mimic the natural degradation protein-like proteases to digest amelogenin and release active ACP in a timely manner. NaClO is responsible for the oxidative degradation of carbohydrate polymers [22], which develop strong abilities to decompose CMC and release active ACP.

Regarding the binding effect and crystal promotion, we created a chimaeric peptide to arrange the amorphous calcium phosphate particles in order. The novelty of the peptides utilized in this study is derived from the HA promotion part