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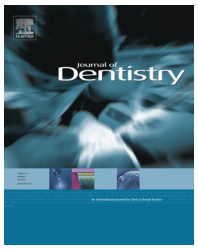

Enamel prism-like tissue regeneration using enamel matrix derivative

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ABSTRACT

Backgrounds: Enamel matrix derivative (EMD) has been shown to promote periodontal regeneration, but its effect on biomimetic mineralisation of enamel is not reported.
Objectives: This *in vitro* study aimed to investigate the effect of commercially available EMD on promoting biomimetic mineralisation in demineralised enamel using an agarose hydrogel model.
Methods: Human enamel slices were demineralised with 37% phosphoric acid for 1 min. They were covered with a 2-mm-thick EMD-calcium chloride ($CaCl_2$) agarose hydrogel. Another 2-mm-thick ion-free agarose hydrogel was added on top of the EMD-$CaCl_2$ hydrogel. They were incubated in a phosphate solution containing fluoride at 37 °C for 96 h. Scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) were used to evaluate the crystals formed on the demineralised enamel surface. A nano-indenter was used to evaluate the elastic modulus and nanohardness on the surface of the enamel slices.
Results: SEM observed enamel prism-like crystals formed on the enamel. They had typical apatite hexagonal structures, which corroborated the enamel's microstructure. EDX revealed that the elements were predominantly calcium, phosphorus, and fluorine. XRD confirmed that they were fluorinated hydroxyapatite. The mean elastic modulus before and after remineralisation was 59.1 GPa and 78.5 GPa ($p < 0.001$), respectively; the mean nanohardness was 1.1 GPa and 2.2 GPa, respectively ($p < 0.001$).
Conclusions: EMD promoted *in vitro* biomimetic mineralisation and facilitated enamel prism-like tissue formation on demineralised human enamel.
Clinical significance: This study is the first to report on using EMD in biomimetic mineralisation, which may serve as a biomaterial for enamel repair.

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1. Introduction

Enamel remineralisation is a well-accepted concept for repairing enamel defects in dental erosion. Many clinical products have been developed to improve enamel remineralisation, including fluoride varnish,[1] fluoride dentifrice,[2] and casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) paste.[3] Although CPP–ACP can promote remineralisation of subsurface enamel lesions, the crystals formed were loosely structured and morphologically irregular after treatment with these agents.[4] Therefore, studies were performed to explore enamel remineralisation through biomimetics.[5] Moreover, researchers are interested in how these enamel crystals form during tooth development.

Enamel has a unique morphological structure and distinctive mechanical properties, making it different from other mineralised tissues such as the bone and dentin in the human body. It is composed of more than 95% highly organised hexagonal carbonated hydroxyapatite crystals by weight. These crystals are roughly parallel to form highly organised architectural units known as enamel prisms. The unique shapes and organisations of enamel crystals determine the excellent mechanical properties of tooth enamel with increased hardness and resistance to fracture and acid erosion. Ameloblast activity and the protein-mediated process of mineralisation are crucial to achieving such precisely organised structures. Regeneration of enamel is not feasible after trauma or decay due to the acellular and protein-free composition of mature enamel.

Several *in vitro* methods have been reported for regenerating enamel-like tissue comprising hydroxyapatite crystals. By applying certain chemical synthetic techniques under extreme conditions, such as a high temperature hydrothermal method[6,7] or using extremely low acidity,[8,9] the synthesis of enamel hydroxyapatite nanorods is possible. However, many of these methods are expensive or are performed under conditions of high temperature, high pressure, or extremely low acidity. Moreover, preparation of a protein-containing nanocomposite is not feasible under such non-physiological conditions. It is therefore important to develop biomimetic methods that do not require extreme conditions.

Comparing to these non-physiological conditions, a biomimetic mineralisation method for enamel tissue regeneration is a desirable approach and under certain conditions is relevant to the clinical setting. A glycerin-enriched gelatin system has been used to form dense fluorapatite layers on human enamel.[10,11] Formation of enamel-like structures using different organic additives has been performed *in vitro*.[12,13] Surfactants have also been used as reverse micro-emulsions to modify the apatite nanorods into a prism-like structure.[14] Recently a carboxyl-terminated poly (amido amine) induced *in situ* remineralisation of nanorod-like hydroxyapatite on enamel,[15] and an electrospun hydrogel mat of ACP/poly (vinylpyrrolidone) nanofibres was developed to guide and promote *in vitro* remineralisation of enamel.[16] Although the results are promising in the study of enamel-like tissue regeneration, there are still challenges in the application of the biomimetic strategies in dentistry.

In enamel mineralisation, enamel develops through complex interactions among organic and inorganic components and gradually transforms from a proteinaceous substance into a hard and durable highly mineralised tissue.[17] Ameloblasts secrete an enamel extracellular matrix, which occupies the extracellular space between ameloblasts and dentine controlling the initiation, habit, orientation, and organisation of enamel crystals. The extracellular matrix is composed mainly of enamel matrix proteins (EMPs): amelogenin, enamelin, and ameloblastin.[18] Studies have been performed to prepare enamel-like materials using amelogenin to control the crystallisation of calcium and phosphate.[19,20] Some researchers suggested that interactions between these proteins may be crucial for normal enamel formation.[21–23] They also believed that the interactions between crystal and proteins dynamically and delicately control not only the growth rate of the crystal but also its growth direction and morphology.

Enamel matrix derivative (EMD) is a product of EMPs. EMD is extracted from the porcine foetal tooth. Emdogain (Straumann, Basel, Switzerland) is a commercially available EMD and has been used in dentistry—mainly in periodontal therapy. A systematic review found that it has been extensively used in periodontics to support periodontal tissue regeneration.[24] Recent studies demonstrated that EMD might have other applications, such as in dental implants[25] and dental pulp capping.[26] However, whether EMD can be used to promote the remineralisation of enamel has not been reported. We have developed an agarose hydrogel biomimetic mineralisation model that mimics the gel-like environment for regeneration enamel prism-like tissue.[27] It is noteworthy that the initial formation of enamel apatite in nature occurs when a unique gel-like organic matrix protein interacts with mineral ions. Based on our previous studies on enamel regeneration in agarose hydrogel model,[27] we envisaged that EMD might be used for protein-guided mineralisation on apatite templates. The present study is a follow-up to investigate the effect of EMD on the formation of enamel prism-like tissue.

2. Materials and methods

2.1. *Enamel slices preparation*

This study was approved by The University of Hong Kong/Hospital Authority Hong Kong West Cluster Institutional Review Board (IRB UW10-210). Extracted human third molars with no detectable caries or restorations were collected with patients' consent. The teeth were treated with 3% sodium hypochlorite to remove bacteria and rinsed with phosphate-buffered saline. Tooth slices of 2 mm thickness were cut perpendicular to the longitudinal axis of each tooth by a water-cooled diamond saw (IsoMet low-speed saw, Buehler, Lake Bluff, IL, USA). Ten enamel slices without cracks were selected for use in this study. The slices were polished with 600-, 1200-, 2400-, and 4000-grit silicon carbide papers and then ultrasonically cleaned with acetone, ethanol, and deionised water. They were stored at 4 °C before treatment.