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Controlled remineralization of enamel in the presence of amelogenin and fluoride

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ABSTRACT

Reconstructing enamel-like structures on teeth have been an important topic of study in the material sciences and dentistry. The important role of amelogenin in modulating the mineralization of organized calcium phosphate crystals has been previously reported. We used amelogenin and utilized a modified biomimetic deposition method to remineralize the surface of etched enamel to form mineral layers containing organized needle-like fluoridated hydroxyapatite crystals. The effect of a recombinant amelogenins (rP172) on the microstructure of the mineral in the coating was analyzed by SEM, XRD and FT-IR. At rP172 concentrations below 33 μg/mL, no significant effect was observed. In the presence of 1 mg/L F and at a concentration of 33 μg/mL rP172, formation of fused crystals growing from the enamel surface was initiated. Amelogenin promoted the oriented bundle formation of needle-like fluoridated hydroxyapatite in a dose dependent manner. Biomimetic synthesis of the amelogenin fluoridated hydroxyapatite nano-composite is one of the primary steps towards the development and design of novel biomaterial for future application in reparative and restorative dentistry.

1. Introduction

The highly organized hierarchical microstructure provides dental enamel its high strength and anti-abrasive properties [1]. Mature enamel is acellular, has more than 95% mineral content and does not remodel. The crystals found in enamel are carbonated hydroxyapatite nanocrystals, 50–70 nm in width, 20–25 nm in thickness, with length to width aspect ratio over 1000. Enamel has a high packing density of inorganic material, evident when comparing enamel density (2.85–3.00 g/cm^3) [2] to pure monolith HAP (3.08 g/cm^3). Recent approaches for *in vitro* synthesis of enamel-like hydroxyapatite (HAP) nanorods include: hydrothermal method with control release of calcium from Ca–EDTA [3], hydrothermal transformation of octacalcium phosphate (OCP) rod to HAP nanorods in the presence of gelatin [4], surfactant supported HAP self-assembly [5,6], hydrogen peroxide containing calcium phosphate paste [7] and electrolytical deposition taking place at 85 °C [8]. The majority of these synthesis methods were developed under the condition of high temperature, high pressure, extreme acidic pH or in the presence of a concentrated solution of surfactants. The investigation of *in vitro* biomimetic synthesis of enamel-like calcium phosphate structures under physiological conditions is therefore essential in dentistry as an alternative dental restorative material. However, the synthesis of rod-like apatite crystals under physiological temperature is a challenging task. *In vitro* formation of enamel-like apatite crystals under relatively mild conditions was reported for the first time by Moriwaki et al. [9] in a mineralization device using a cation-selective membrane system. In such a device the direction of calcium ion transport was controlled and the crystals formed in the membrane isolated chamber contained bundles of needle-like OCP and HAP. Using a similar system, Iijima et al. applied a dual-membrane device as a model of enamel formation to investigate the function of amelogenin proteins on calcium phosphate mineralization [10,11]. The co-existence of amelogenin and fluoride (F) was found to be crucial for the organized rod-like apatite crystal formation on the membrane [11,12].

In enamel mineralization, superamolecular assembly of the extracellular macromolecules is a crucial step [13]. The amelogenin-rich extracellular organic matrix of enamel is continuously secreted, assembled, processed, and mineralized during enamel development. Amelogenin self-assembly is believed to be a key factor in controlling the oriented and elongated growth of the carbonate-containing fluoridated hydroxyapatite crystals within enamel prisms. Numerous *in vitro* experimental approaches have

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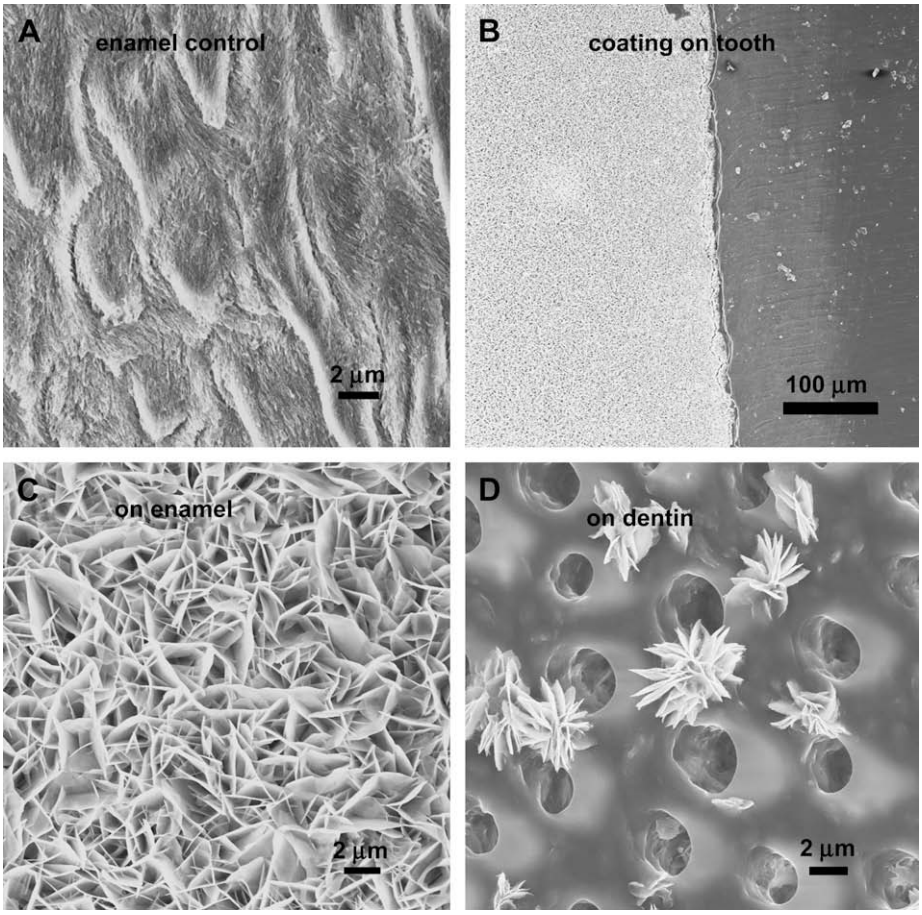

Fig. 1. SEM images of A) enamel surface after acidic etching, B) biomimetic mineralization coating, the left white region, formed on enamel surface in calcium phosphate containing solution after 16 h. There is no continuous coating on dentine surface (the dark area on the right). As seen under higher magnification, C) the calcium phosphate coating grew on left enamel surface part and D) isolated crystals were found on right dentin part. The round holes are dentine tubules.

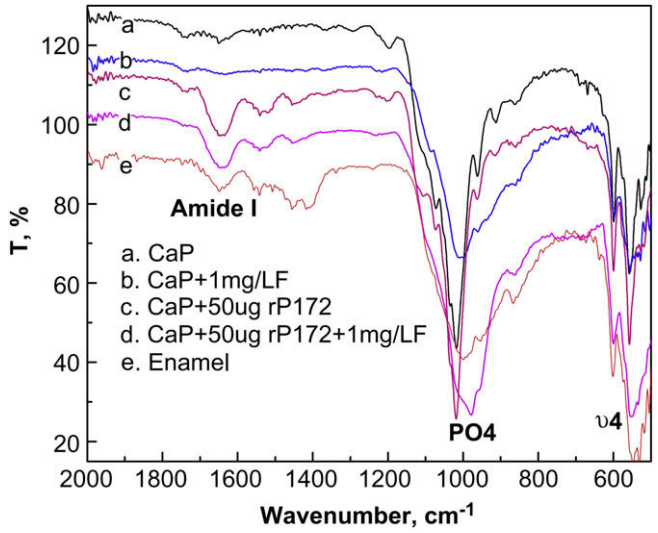

Fig. 2. ATR FT-IR spectrum of the biomimetic coating on enamel prepared after 16 h compared with enamel control. The presence of protein amide I band at 1680 cm^{-1} in the composite coating with rP172 was significant. The mineral phase of the calcium phosphate coating was mainly OCP. Spectrum of coating with fluoride and rP172 was close to native enamel, which was fluoridated HAP.

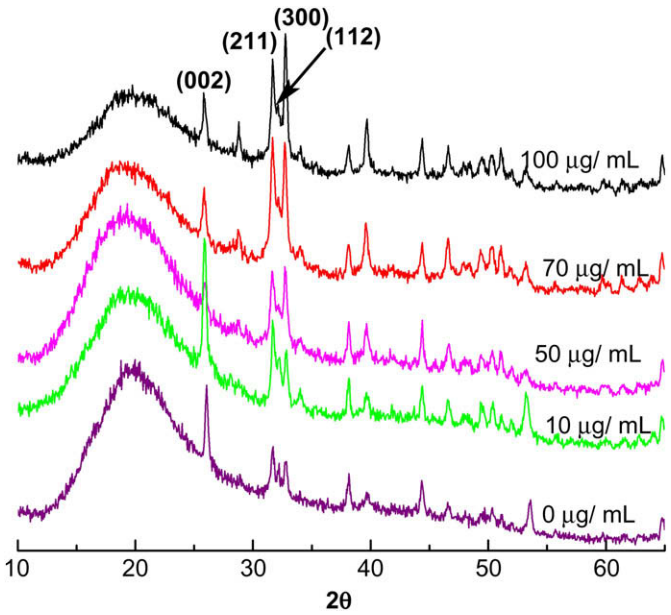

Fig. 3. The XRD spectrum of the biomimetic coating on enamel with 1 mg/L F and concentrations of rP172 amelogenin of 0, 10, 50, 70, 100 μg/mL. The presence of hydroxyapatite diffraction band (002) at $2\theta = 12.9$, (211) at $2\theta = 15.9$, (112) at $2\theta = 16.1$, and (300) at $2\theta = 16.4$ was clearly detected. The addition of rP172 didn't alter the HAP phase significantly.