Biomimicry of the Growth of Dental Enamel from Amelogenin and MMP-20 Sols using a Continuous Titration Approach

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Statement of Purpose: Dental enamel is the hardest tissue in the mammalian body and is composed of bundles of 40 – 60 nm wide apatite crystals with the aspect ratio of up to 1:10,000. In this study, a biomimetic experimental setting is used to epitaxially grow apatite crystals with resemblance to natural enamel apatite morphologies, and at the same time to gain fundamental knowledge of the mechanism by which the morphogenesis of the enamel proceeds *in vivo*. Besides the potential clinical significance of the study, understanding the biological formation of the tooth enamel is important as it may lead to fundamental insights into protein-mineral interactions that govern biomineralization processes in general. As the formation of dental enamel proceeds via a coordinated assembly and degradation of the protein matrix, in our study we use both amelogenin, the main protein of the enamel matrix, and MMP-20, the main selective protease known to digest amelogenin [1].

Methods: Recombinant human amelogenin and MMP-20 were synthesized via their expression in BL21(DE3) plysS *Escherichia Coli* [2,3]. The programmed titration was performed using a Titrino 751 GDP device connected to a Dosimat 755 (*Brinkmann-Metrohm*). The initial reaction suspension comprised 5 ml of buffered 0.2 – 1.6 mg/ml rH174 and different concentrations of KH_2PO_4 and $CaCl_2$. The two buffered titrant solutions comprising the separate precursor ions ($CaCl_2$ and KH_2PO_4) and the electrolyte (KCl) up to the level of the physiological ionic strength were then being introduced into the reaction vessel at a controlled rate of 1.2 ml/day, cumulatively, throughout a 7-day period of time. Fluoroapatite glass-ceramics were used to facilitate epitaxial crystal growth [4, 5].

Results: Amelogenin was shown to decrease the nucleation lag time with respect to apatite in proportion to its concentration. Higher concentrations of amelogenin were thus shown to lead to earlier onset of the precipitation and to a more pronounced crystal growth over the course of the 7-day reaction time. MMP-20-induced proteolysis has the same effect on nucleation lag time as increasing rH174 concentration does. Increasing MMP-20 weight ratio with respect to rH174 induces more intensive crystal growth. Deposited apatite structures with the highest aspect ratio were obtained at pH 6.5 (Figure 1) where apatite and amelogenin carry opposite zeta-potentials and surface charges [6]. As adsorption of amelogenin nanospheres is shown to present the first step prior to nucleation of apatite, this process is markedly pronounced under these conditions as compared to pH 7.4 when both amelogenin nanospheres and the apatite substrate surface are negatively charged. Dynamic light scattering analyses showed that calcium ions tend to bond or adsorb onto rH174 nanospheres more efficiently than phosphate species, implying that the process of amelogenin-controlled nucleation is also calcium-controlled. Consequently, at similar saturation levels, nucleation is favored at low calcium concentrations and high phosphate concentrations, which is in agreement with the calcium and phosphate content of the enamel matrix at the secretory stage.

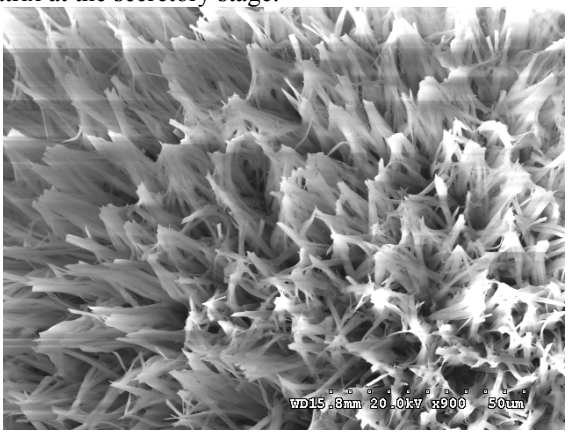

Figure 1. SEM image showing the epitaxially grown apatite with a morphological resemblance to that of apatite constituting natural enamel

Conclusions: Despite their mainly hydrophobic nature, amelogenin nanospheres were shown to act as a nucleation-promoting agent during the crystallization of apatite. The rate of proteolysis coupled to the crystal growth was also observed as directly proportional to the crystal growth rate, confirming a crucial effect of the proteolysis on the proper formation of enamel. The biomimetic experimental setting applied in this study proves as convenient for replicating the enamel-like apatite morphologies and gaining insight into the fundamental nature of the process of amelogenesis. The project is supported by the NIH/NIDCR grants R01-DE017529 and R01-DE015821.

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