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Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure

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Abstract

The application of surfactants as reverse micelles or microemulsions for the synthesis and self-assembly of nanoscale structures is one of the most widely adopted methods in nanotechnology. These synthesized nanostructure assemblies sometimes have an ordered arrangement. The aim of this research was to take advantage of these latest developments in the area of nanotechnology to mimic the natural biomineralization process to create the hardest tissue in the human body, dental enamel. This is the outermost layer of the teeth and consists of enamel prisms, highly organized micro-architectural units of nanorod-like calcium hydroxyapatite (HA) crystals arranged roughly parallel to each other. In particular, we have synthesized and modified the hydroxyapatite nanorods surface with monolayers of surfactants to create specific surface characteristics which will allow the nanorods to self-assemble into an enamel prism-like structure at a water/air interface. The size of the synthetic hydroxyapatite nanorods can be controlled and we have synthesized nanorods similar in size to both human and rat enamel. The prepared nanorod assemblies were examined using transmission electron microscopy (TEM) and atomic force microscopy (AFM). The specific Langmuir–Blodgett films were shown to be comprised of enamel prism-like nanorod assemblies with a Ca/P ratio between 1.6 and 1.7.

Keywords: Hydroxyapatite; Nanorod; Self assembly; Dental enamel

1. Introduction

Nanotechnology has been studied extensively in the past decade for the preparation of nanoscale structures with specific size, shape and physicochemical properties. It has also created many ways to direct the assembly of nanoparticles and one-dimensional nanoscale building blocks, such as nanotubes, nanowires, and nanorods, into novel functional superstructures [1–5]. Among them, the application of surfactants as reverse micelles or microemulsions for the synthesis and self-assembly of nanoscale structures is one of the most widely adopted methods reported in the literature [5–14]. So far it has been successfully used to prepare and control the size and shape of various nanostructures with different chemical compositions, such as barium chromate [6], calcium phosphates [7–10], barium sulfate [11], cadmium selenide [12], gold nanorods [13], tellurium nanorods [14], etc. The above studies have been focused on the application of surfactants for making nanostructures of uniform shape and size. These synthesized nanostructure assemblies sometimes have an ordered arrangement. The nanorods are able to self-assemble into smectic-like arrays or liquid crystalline assemblies by solvent evaporation [13,14]. Recently there have been some reports of trying to assemble the one-dimensional nanowires and nanorods directly into organized superstructures with the assistance of surfactants. Kim et al. explored the organization of barium chromate nanorods at the water–air interface using Langmuir–Blodgett (LB) technique [5]. The inorganic nanorods were successfully assembled into isotropic, nematic, and smectic phases depending on the different surface pressure. The TEM pictures

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of the nematic and smectic nanorods assemblies they produced were very similar to the prism structure of human enamel. The same group has also successfully applied the LB technique to assemble the silver nanowires into an ordered structure by rendering the nanowires hydrophobic using 1-hexadecanethiol ligands [15].

Our previous study showed that the hydroxyapatite crystals isolated from rat incisors were able to bind –COOH and $–NH_2$ terminated polyamidoamine (PAMAM) dendrimers. This binding survived rinsing with water whereas the bound acetamide ($–NHC(O)CH_3$) terminated, neutral charge dendrimers could be easily removed [16]. Chemical force microscopy (CFM) studies have revealed that the surfaces of individual biological hydroxyapatite crystals are comprised of a series of discrete and alternating charge arrays aligned along and perpendicular to the crystal *c*-axis [17]. Further atomic force microscopy (AFM) studies have showed that many proteins, serum albumin, amelogenin, dentin phosphoprotein (DPP), and dentinal sialoprotein (DSP) were able to bind to the crystal surfaces under physiological conditions [18–20]. This protein binding gave a "banded" appearance to its crystal surfaces, which seemed to be of a very similar periodicity to the charge arrays found by using CFM. All these proteins were expected to carry overall negative charge at physiological pH. These charge arrays on the crystals were assumed to play a key role in the binding to the proteins and charged dendrimers via electrostatic interactions. This indicated that the crystals would have a reactive surface to which a surfactant could bind. There are literature reports that the HA is able to adsorb ionic surfactants, sodium dodecylsulfate or long-chain alkyl ammonium [21]. It is believed that both types of surfactant are bound by electrostatic interactions and by exchange with surface-active ions, leaving externally the hydrocarbon chains, which result in the modification of the HA surface to a hydrophobic one. Then using the LB technology the nanorods would assemble into ordered structures similar to the prisms of dental enamel.

Dental enamel is the outermost layer of the teeth. The fully developed mature dental enamel is made of enamel prisms, highly organized micro-architectural units, which consist of bundles of nanorod-like calcium hydroxyapatite crystals arranged roughly parallel to each other. This structure spans the entire enamel thickness and is likely to play an important role in determining the unique physicochemical properties of the enamel [22,23].

Ninety-five percent (by volume) of human enamel comprises of nanorod-like calcium hydroxyapatite crystals which have a rough cross section ([width + thickness]/2) of 33–65 nm and a length of 100–1000 nm along the *c*-axis [23–25]. There is evidence that during enamel development these crystal rods project into the enamel matrix from the enamel/dentin junction and are separated by nanospheres of amelogenin; the major enamel protein constituting approximately 90% of all organic matrix material in developing enamel [23,26]. The amelogenin is believed to play a vital role in developing enamel by stabilizing newly formed enamel crystals and influencing their subsequent growth [26]. In amelogenin knockout mice the enamel is hypoplastic and the characteristic prism pattern is completely absent [27]. Amelogenins are synthesized and secreted by ameloblast cells. The only hydrophilic part of the nascent amelogenin molecule is comprised of a carboxy-terminated teleopeptide of 12 amino acid residues [23]. The cleavage of this C-terminated motif would decrease its apatite binding ability and lower the inhibitory potential of the molecule to apatite growth [26]. It has been proposed by Fincham et al. that the amelogenin can self-assemble into nanosphere structures approximately 20 nm in diameter with the hydrophilic C-terminals externalized and form a negatively charged surface. These nanospheres will then interact electrostatically with the enamel hydroxyapatite crystal faces parallel to the *c*-axis, and prevent crystal–crystal fusions. Enzymes (proteinase-1) eventually digest away the charged surface of the nanospheres, producing hydrophobic nanospheres that further assemble and stabilize the growing crystallites to form well organized prism patterns [26].

On the basis of the above observation, we planned to mimic the natural biomineralization process to create an ordered enamel prism-like structure. Initially in this study hydroxyapatite nanorods of similar size and composition to those isolated from enamel had to be synthesized. Hydroxyapatite powders consisting of individual irregular particles are commercially available but the particle size and shape being no resemblance to the nanorod-like crystals seen in enamel. Such nanorod structures of the correct size and composition were nucleated and grown from supersaturated aqueous solutions of HA and examined by using TEM, EDS, and FTIR. These nanorods were then modified with the surfactant docusate sodium salt (AOT), which mimicked the biological role played by amelogenin in the assembly of hydroxyapatite crystals into the enamel prisms as described above. Langmuir–Blodgett films comprised of these nanorods assemblies were created at a water/air interface and characterized by using TEM and AFM. The results of these experiments will allow us to have a better understanding of biomineralization and will permit the design of biomimetic materials and therapeutic agents with the applications in both the biomedical and material science fields.

2. Materials and methods

2.1. Enamel crystals

Individual crystals from specific stages of enamel development were obtained from the mandibular incisors of 4 week-old male Sprague Dawley rats. Particles of enamel were microdissected using the beginning of the white opaque enamel as a marker for the onset of the maturation stage as described previously by Robinson and Hiller [28,29]. All developing enamel was removed from each tooth and the tissue pooled according to developmental