Restoration of Tooth Enamel Using a Flexible Hydroxyapatite Sheet Coated with Tricalcium Phosphate

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Abstract

Restoration and protection of tooth enamel are of great importance in operative and conservative dentistry. Using a Pulsed Laser Deposition (PLD) method, we have successfully created a freestanding flexible double-layered sheet composed of a Hydroxyapatite (HAp) layer (4 m in thickness) coated with a Tricalcium Phosphate (TCP) layer (500 nm). In order to apply the newly developed HAp/TCP sheet to the restoration and protection of tooth enamel, the adhesive characteristics of the HAp/TCP sheet on the enamel were evaluated mechanically and microstructurally in the present study. The adhesive strength (5.7 MPa) between the HAp/TCP sheet and enamel was markedly higher than that (1.9 MPa) between the mono-layered HAp sheet and enamel. The electron microscopic observation revealed that HAp/TCP sheet was widely fused with the enamel. Therefore, the double-layered HAp/TCP sheet can be used as a material to promote the repair of tooth eruption and to maintain healthy dentine.

Keywords: Hydroxyapatite sheet; Tricalcium phosphate; Pulsed laser deposition; Artificial enamel; Tooth restoration

Introduction

The primary function of teeth is to prepare food for digestion by mastication. Therefore, dental integrity is significantly important for overall health. For a long while, dental materials have been essential components of the effective treatments for dental diseases. As the demand for healthy teeth increases, new materials in restorative dentistry have been advanced progressively. Metals, alloys, composite, and ceramics have been extensively used for tooth restoration and protection [1]. However, most dental materials need to be replaced due to the time-related deterioration. More adequate therapeutic methods by means of useful biomaterials are strongly required in restorative dentistry. Dental enamel is a rigid and hard substance that covers the tooth crown and protects the tooth from decay. Harmful chemicals and temperatures are insulated by the layer of the enamel. In contrast with bone fractures that can be naturally repaired in human body in most cases, the extreme damages of tooth enamel are hardly restored or regenerated. The enamel has no living cells, and therefore its healing capabilities are inferior to the other biological tissues. The cause of dental hyperesthesia is loss of the enamel on tooth crowns. In the hyperesthetic condition, a sharp pain is generated from exposed tooth dentine in response to stimuli. A well-accepted mechanism for the hyperesthesia is the hydrodynamic theory which is related to a rapid outward flow of fluid contained in dentinal tubules. This fluid movement in the tubules creates a pressure change that stimulates pulpal nerves and results in pain. Dental hyperesthesia is a common disease experienced in clinical dentistry. To cure a hyperesthetic tooth, exposed dentinal tubules were covered by organic compounds in order to achieve an immediate nerve blockage of pain-producing stimuli. Previous clinical studies [2,3] demonstrated the effectiveness of the covering of resin materials in the short term management of the hyperesthesia. However, it is difficult to intrinsically regenerate tooth enamel by attaching the resin cement. This cement is frequently detached from tooth surface. In addition, the resin materials contain a substance that causes an allergic response. To overcome such disadvantages, the development of novel materials is important for a new strategy of pain management in hyperesthesia. Hydroxyapatite (HAp) is one of the most attractive materials for the reconstructing of biological hard tissues, because its chemical composition is resemble to that of natural bones and teeth in mammals. This resemblance leads to an excellent and bioactivity and biocompatibility. Therefore, synthetic HAp in forms such as powders, beads, or blocks is extensively used for various biomedical applications [4,5]. In dentistry, metallic implants are often coated with HAp to alter the surface properties. Furthermore, tooth defects or cavities are filled with HAp materials. However, it is impossible to repair tooth enamel by attaching HAp powders or particles. The surface quality of tooth inhibits to unify the materials into the enamel. Thus, to date, there have been no efficient biomaterials for the treatment of hyperesthesia.

In our previous study, a freestanding HAp sheet has successfully developed by a pulsed laser deposition (PLD) method and a film isolation technique [6]. Then, we have been doing a series of experimental studies on the development and application of HAp ultrathin films and sheets [7-9]. In these previous studies, the HAp sheet was directly attached to the surface of human tooth enamel. Biomechanical and microstructural evaluations showed that the sheet and enamel were not adhered completely at the interface between them. Thus, the enhancement of adhesiveness of the HAp sheet is essential for the initial mechanical integrity as well as the long-term remineralization at the sheet-enamel interface. The repair of tooth enamel is of great importance, in particular for the development of a new treatment for hyperesthesia in dentistry. Using a pulsed laser deposition method and thin film isolation techniques, we have successfully created a freestanding flexible double-layered sheet composed of a HAp layer coated with a tricalcium phosphate (TCP) layer. Our final goal was to apply the newly developed double-layered HAp/TCP sheet to the restoration and protection of tooth enamel. In the present study,

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the adhesive properties of the HAp/TCP sheet on the enamel were evaluated crystallographically, mechanically, and microstructurally, and were compared to those of the mono-layered HAp sheet.

**Materials and Methods**

**Preparation of HAp and HAp/TCP Sheets**

The freestanding hydroxyapatite sheet was obtained by a thin film fabrication process using the PLD method and the thin film isolation technique reported in our previous paper [9]. In the present study, a HAp sheet coated with TCP thin layer and a mono-layered HAp sheet were produced by the PLD method using a KrF excimer laser. The TCP layer was used as adhesion promoter in order to reduce the period required to fuse with tooth enamel. First, the HAp sheet of 4 μm in thickness was obtained in the same conditions as that reported previously [9]. The sheet was crystallized by post-annealing at 800°C for ten hours. Then, a TCP thin layer of 500 nm in thickness was deposited on the HAp sheet under an oxygen gas atmosphere at 0.1 Pa at room temperature. Figure 1 shows the obtained HAp/TCP sheet.

**Adhesion of HAp and HAp/TCP sheets on tooth enamel**

An extracted human tooth was sectioned in the tooth root crown area, and the enamel was exposed. After the section was polished with water-resistant sand papers, we adhered the HAp and HAp/TCP sheets to the enamel surface using a calcium phosphate aqueous solution with a pH of 5.5. After drying the sheet, artificial saliva (Saliveht, Teijin Pharma Co.) was sprayed on it. This process was repeated every day. The HAp and HAp/TCP sheets on the enamel surface are shown in Figure 2.

**Crystallographic, mechanical, and microstructural characterization of HAp and HAp/TCP sheets attached to tooth enamel**

The crystallinities of the HAp and HAp/TCP sheets attached to tooth enamel were evaluated using an X-ray diffraction (XRD) instrument (Ultima IV, Rigaku Co.) at 5 days after attachment. The XRD analyses were also conducted for the HAp sheet itself and tooth enamel before attachment. At 5 days after attachment, quasi-static tensile tests were carried out to quantify the adhesive strength between the sheet and tooth enamel. A stainless steel rod of a diameter of 3 mm was glued on the sheet using epoxy adhesive. The rod was attached to the jig of a tensile tester (EZ-test, Shimadzu Co.) having a universal joint (Figure 3). Tensile load was applied to the specimen at the tensile rate of 0.5 mm/min until failure occurs. The tooth specimens attached the HAp and HAp/TCP sheets were embedded in a polymethylmethacrylate resin. Then, the specimens were polished with water-resistant sand papers until the interface between the sheet and enamel was exposed. Observation of the interface structure was performed by a low vacuum scanning electron microscope (Miniscope TM-1000, Hitachi High-Technology Co.).

**Results and Discussion**

The phase and crystallographic features analyzed by XRD demonstrated that the HAp sheet was highly crystallized (Figure 4). Moreover, the remarkable peaks of calcium oxide were observed in the XRD pattern of the sheet. There were high intensities of the (002) and (004) peaks in the XRD pattern of tooth enamel (Figure 5), indicative of the c-axis orientation. The XRD patterns at 5 days after attachment are given in Figures 6 and 7 separately for HAp and HAp/TCP sheets, respectively. There were tendencies to moderate the peaks of calcium oxide in the both sheets at this point. These results suggest that the HAp and HAp/TCP sheets were structurally unified in part to tooth enamel within a few days.
Figure 8 shows the stress-displacement relation of the HAp and HAp/TCP sheets adhered to tooth enamel. The adhesive strength between the HAp/TCP sheet and tooth enamel and that between the HAp sheet and the enamel were 5.7 MPa and 1.9 MPa, respectively. A previous report has shown that the bonding strength between HAp coated titanium and bone tissues is approximately 3.5 MPa [10]. This value is comparable to the adhesive strength between the sheet and enamel in the present study. Therefore, we can say that the HAp and HAp/TCP sheets are attached tightly to tooth enamel. In a previous study [11], the adhesive strength of HAp films deposited on titanium alloy can be improved by increasing the roughness of the underlying substrate surface. Therefore, the tooth surface morphology may be important for the adhesive strength of the HAp and HAp/TCP sheets attached to the enamel. The effects of tooth surface morphology on the adhesive strength should be investigated to improve the mechanical integrity of the sheets for dental applications. The adhesive strength of the HAp/TCP sheet was markedly higher than that of the HAp sheet (Figure 8). This result indicates that the thin TCP layer on the HAp sheet is efficacious in order to increase the adhesive strength in a short bonding duration. Figure 9 shows scanning electron micrographs of the interface structure between the sheet and enamel. There were adhesion layers at the interface for both HAp and HAp/TCP sheets. It is likely that the adhesion layers have important roles in the adhesive properties of the sheets. In addition, the adhesion layer of the HAp/TCP sheet was wider than that of the HAp sheet, indicative of the strong adhesion at the interface between the HAp/TCP sheet and enamel. Previous reports [12-14] demonstrated that TCP possesses the high degradability in biological environment. Therefore, TCP may provide an ideal condition for the repair of tooth enamel. Biochemical and ultrastructural studies should be conducted to know the mechanisms of the remineralization process at the adhesion layer of the HAp/TCP sheet attached to tooth enamel.

Conclusion

We newly developed a flexible HAp sheet coated with TCP using a pulsed laser deposition method. The ultrathin layer of TCP on the HAp sheet has an important role in the mechanical integrity at the sheet-enamel interface. The double-layered HAp/TCP sheet can be used as a material to promote the repair of tooth eruption and to maintain healthy dentine.

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References