

## Regeneration of a Micro-Scratched Tooth Enamel Layer by Nanoscale Hydroxyapatite Solution

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Hydroxyapatite (HAp)-based materials have attracted considerable attention on account of their excellent stability and recrystallization. Nanoscale HAp powders with a mean particle size of 200 nm were used to regenerate the enamel layers of damaged teeth. An artificially scratched tooth was immersed in a nanoscale HAp powder suspension in d.i. water (HAp of 70 wt%) at 37 °C for a period of 1~3 months. SEM and AFM showed that the scratched surface was ultimately inlaid with HAp after three months and the roughness increased from 2.80 to 5.51. Moreover, the hardness of the neo-generated HAp layer on the crown was similar to that of the innate layer.  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions from the HAp powders dissolved in d.i. water were precipitated on the tooth to produce cemented pastes on the enamel surface due to its high recrystallizing characteristics, resulting in a hard neo-regenerated HAp layer on the enamel layer. This nanoscale HAp powder solution might be used to heal decayed teeth as well as to develop tooth whitening appliances.

**Key Words:** Hydroxyapatite (HAp), Human tooth, Regeneration,  $\text{Ca}^{2+}$  ion,  $\text{PO}_4^{3-}$  ion

### Introduction

Ceramics have attracted considerable attention for biomedical applications in the field of cell interfacing, scaffolding, transplants and supports on account of their high mechanical strength and biocompatibility as well as their long term chemical inertness in complicated biological environments.<sup>1-7</sup> Bio-applicable ceramics can generally be classified into bio-activated ceramics that are normally embedded or transplanted in humans to interface with the host biostructures *via* strong chemical bonding, and biochemical ceramics that play important roles in filtration, absorption, adhesion, and catalysis. In particular, hydroxyapatite (HAp), which is composed of calcium phosphates,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , with a similar chemical composition to calcified tissues, *i.e.*, teeth and bones, has been used as transplant composites with other biocompatible materials, such as natural or biologically sustainable polymers or other ceramics in bone regeneration. In addition, they have also been used as artificial supports for physically deprived jaws or collar bones. The main merits of HAp ceramic are its remarkable biocompatibility and bio-inertness with the interface of biological moieties. Implant supports in dental surgery are prepared by nanoscale composites consisting of organic and inorganic compounds with apatite, and exhibit coincident osteoconduction with coaptated bones to support the ingrowth of capillaries, perivascular tissues and osteoprogenitor cells from the host into an implant or graft.<sup>4-11</sup> Bones are precisely constructed biological composites consisting of nanoscale apatite and collagen. Therefore, the physical or chemical preparation of nanoscale artificial HAp is quite attractive to biomaterial engineers for assembling bio-mimicking structures.<sup>12-14</sup>

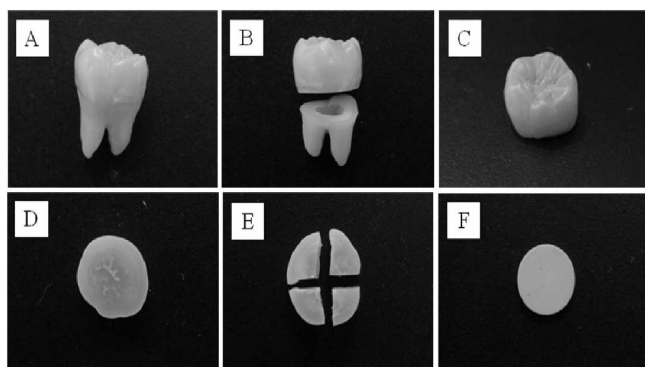
Teeth are one of unique bones in the human body that are crucial in their functionality as well as cosmetic appearance, and are composed mainly of HAp. The tooth enamel is com-

pletely different from bone tissue and has quite different in function from it. In the living body, the tooth enamel has little contact with the cells except for contact at the epithelial junction which forms the hemidesmosome.<sup>15,16</sup> Micro-scratches on the tooth surface can induce dentalgia and deteriorate the nervous system in the dentine. In dental implants and caries, various medical remedies have been used depending on the level of periodontal damage in patients using, for example, autogenous bones, allogeneic or xenogeneic bones, and even metal and plastic fasteners. Although these treatments are quite effective and stable in the long-term, there are some limitations with autogenous bones, immune system and infection. The metallic fasteners or supports require replacement and reinforcement every 2~3 years to prevent secondary infections from the interface between the gums and treated biomaterials, where the low biocompatibility of metallic materials cannot play a role.

In this paper, nanoscale HAp powders were used to substitute for the above described insufficient periodontal materials and to regenerate a scratched tooth surface using a HAp sol though its regeneration mechanisms. This technique is quite useful for the repair of decayed teeth through micro-cleavage and in the development of tooth whitening appliances.

### Experimental Section

A 635-mesh ( $\leq 20 \mu\text{m}$ ) HAp powder (HAp Tech. Inc., Korea), which shows excellent biocompatibility with hard calcified tissues and is similar to the mineral components of bone, was used in this study. For the regeneration experiments on the surface of damaged teeth, a tooth pulled from the paradentitis of a healthy adult male patient was cut into four pieces to produce identical experimental conditions and reduce the number of uncontrollable factors in terms of the materials, such as different chemical compositions in each

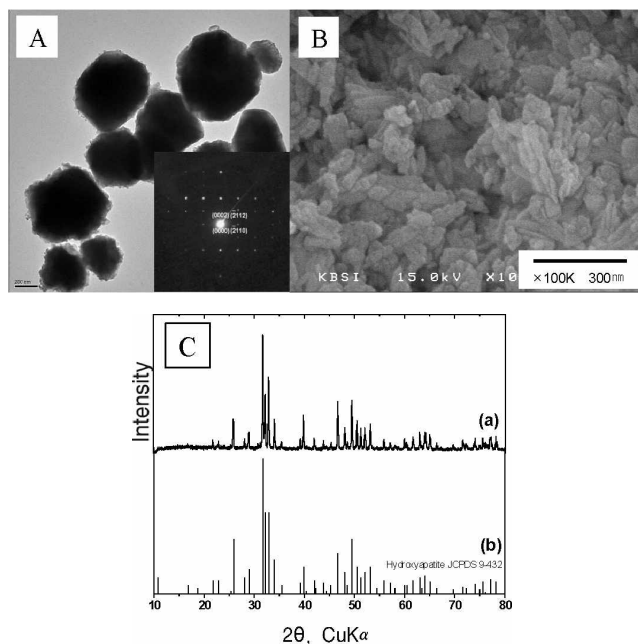
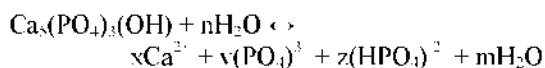


**Figure 1.** Images of four pieces of a cut tooth pulled from the paradentitis of a healthy adult male patient and an artificial tooth. (A) pulled out tooth, (B) the root of the tooth dissected, (C) The crown dissected, (D) after polishing the cross-section of the crown, (E) the quadrisectioned crown. (F) artificial tooth.

person (Figure 1). The following gives details of the preparation of the tooth samples: first, the tooth root was separated using a diamond cutter. The crown of the tooth sample was polished with sandpaper (#2000) followed by a 0.5  $\mu\text{m}$  alumina paste, and then cleaned by ultra-sonication in ethanol to remove the remnants generated from polishing. The polished sample was then cut into four pieces that were cleaned by ultra-sonication in ethanol. The four prepared samples were immersed in a sterile HAp solution for 0, 1, 2, and 3 months, respectively. The HAp solution was prepared from 70 wt% HAp powder in d.i. water. The samples were stored in tightly silica-capped glass bottles in an isothermal oven at 37  $^{\circ}\text{C}$  to prevent exposure to the air or solvent evaporation, which might induce unexpected infections and micro-corrosion of the surface of the scratched tooth samples.

The quantitative and qualitative properties of the HAp powder were characterized using Rigaku Miniflex II X-ray diffractometer measurements with Cu K $\alpha$  radiation generated at 30 kV and 15 mA between  $10^{\circ} < 2\theta < 80^{\circ}$  at a scan speed of 4 deg/min. The X-ray diffraction (XRD) patterns were analyzed and compared with the Joint Committee on Power Diffraction Standards (JCPDS) card (Figure 2). Inductively Coupled Plasma-Optical Emission spectroscopy (ICP-OES, OPTIMA 3300 DV, PERKIN-ELMER, USA) and energy dispersive spectroscopy (EDS HORIBA 6853-H, HORIBA, Japan) were used to characterize the HAp powder and examine the change in Ca and P concentration on the tooth surface. Scanning electron microscopy (SEM, HITACHI S-4200, HITACHI, Japan) and atomic force microscopy (AFM, XE-120, PSIA, Korea) were used to observe the tooth enamel surfaces, *i.e.*, before and after the HAp treatment.

Under certain conditions, ion exchange is allowed with other identical ions in solid materials. HAp is a well-known material in which  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions can be exchanged with solid materials, *e.g.*, bone and teeth. In these experiments, the ionization equation of HAp powder in d.i. water can be written as follows:



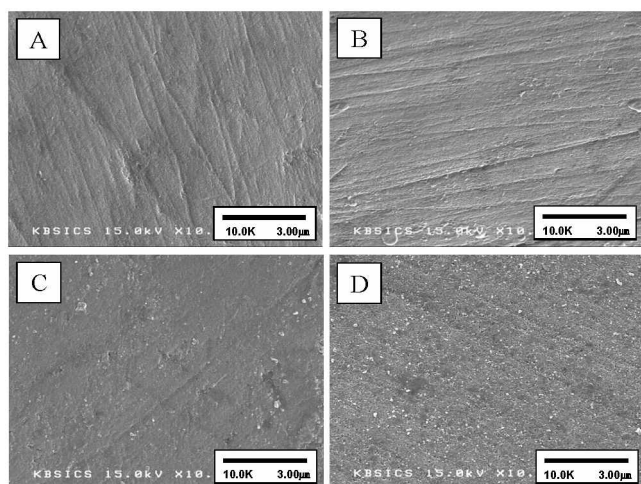
**Figure 2.** TEM and SEM images and XRD patterns of the HAp powder. (A) TEM image and diffraction pattern of the HAp powder, (B) SEM image ( $\times 100\text{K}$ ). (C) XRD data of (a) HAp prepared, and (b) standard peak of HAp provided by JCPDS.

Ionized  $\text{Ca}^{2+}$  in a concentrated HAp solution can reach equilibrium with ionized  $\text{Ca}^{2+}$  on the surface of the immersed tooth samples. This can result in ion exchange and the adsorption of  $\text{Ca}^{2+}$  on the surface of the tooth sample, resulting in tooth regeneration. An artificial tooth made from HAp with > 90% of the composition of a human tooth was used to observe the adsorption mechanism between the HAp solution and enamel surface (Figure 1F). A sintering process was operated to produce an artificial tooth at a pressure of 3000-kgf/cm $^2$  at 1350  $^{\circ}\text{C}$  for 2 h. The artificial tooth was cleaned using an ultra-sonicator in ethanol.

The hardness test (FM-700, Future Tech, Japan) of the artificial tooth was carried out once every week with the samples immersed in a 70% HAp solution at a 10 gf/mm $^2$ . After polishing in series with sandpapers (# 400, 600, 800, 1000, 1200, 1500, 2000) and a 0.5  $\mu\text{m}$  alumina paste, the samples cleaned in ethanol were dried at 60  $^{\circ}\text{C}$ . The surface morphology of the tested areas was observed by optical microscopy.

## Results and Discussion

**EM and Instrumental Analysis of HAp Powder.** TEM and XRD revealed the HAp powder to have a mean size of 200 nm with a polycrystalline structure. FESEM was used to examine the surface of the HAp powders. The HAp powder separated from a micro mesh consisted of agglomerates of nanoscale HAp powders, 60 nm in length and 30 nm in diameter, with a cylindrical shape. The majority of the agglomerated powders dispersed with sequential ultra-sonication. XRD showed that the general peaks and intensities of the HAp powders at 31.8 $^{\circ}$  2 $\theta$  corresponded to the standard data for HAp in the JCPDS card (# 9-432). ICP-OES analysis confirmed that the weight

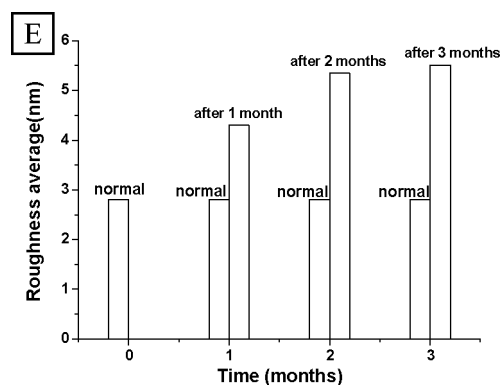
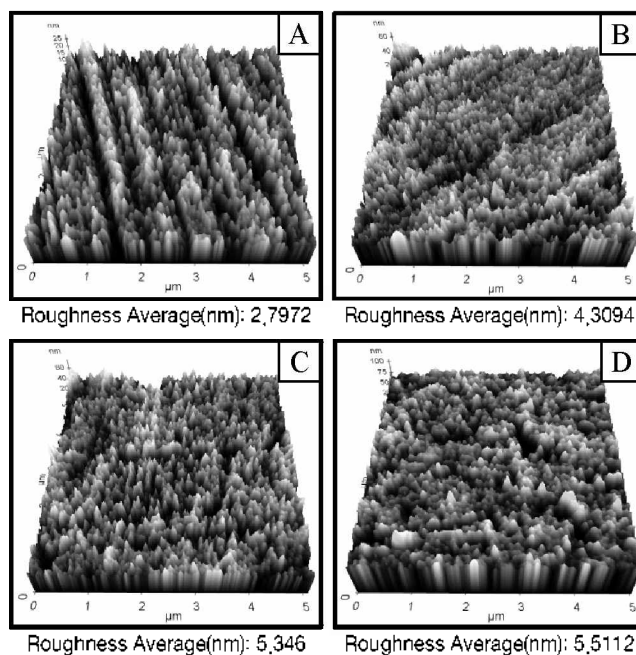


**Figure 3.** SEM images of the Tooth-HAp matrix ( $\times 10K$ ). (A) normal tooth, after (B) 1 month, (C) 2 months, (D) 3 months. The white dots on the surface in Figure 3C and D were confirmed to be recrystallized HAp powder.

percentage of CaO and  $P_2O_5$  were 57.0 wt% and 41.4 wt%, respectively. EDS showed that the molar ratio of Ca and P was 1.67. The quantitative analysis of the composition confirmed that the prepared HAp powder was similar to natural HAp (Table 1).

**Observation of Micro-scratches on the Tooth Enamel Immersed in HAp Sol.** A tooth artificially micro-scratched with sandpaper and a micro-polisher was cut to produce 4 samples. Three of the quartered samples were immersed, respectively, in a 70% HAp solution for 1, 2 and 3 months. The enamel surface of the micro-scratched tooth was observed by SEM. Figure 3 shows SEM images of the tooth enamel after immersion. Compared with Figure 3A, which shows the non-immersed sample, the scratched lines of three immersed samples immersed for different times had disappeared. In particular, after 3 months (Figure 3B), it was difficult to find scratched lines on the enamel surface. The white compounds on the surface in Figure 3C and 3D were characterized as recrystallized HAp powders. This suggests that  $Ca^{2+}$  ions dissolved from the HAp powder in the solution state interacted with the tooth surface, which concurs with the morphology and roughness determined by AFM.

Figure 4 shows that the roughen surface by scratching was inlayed with the HAp solution, and the roughness increased from 2.80 to 5.51. This shows that the scratched ravine on the enamel layer was filled with the HAp in where the  $Ca^{2+}$  and



**Figure 4.** AFM images of the tooth-HAp matrix. (A) normal tooth, after (B) 1 month, (C) 2 months, (D) 3 months, (E) The average roughness of the tooth-HAp matrix by AFM.

$PO_4^{3-}$  ions in HAp solution were bonded to the enamel surface to produce apatite nuclei during the immersion period of 1~3 months. However, this was quite unclear using only the SEM images to determine if the filled HAp powder was adhered temporarily without chemical or ionic bonding. In order to determine if the HAp had completely adhered to produce new osteoplastic layers by recrystallization of the HAp powder, it is necessary to observe the mechanical properties of the inlaid layers. A micro-indentation test was performed to determine

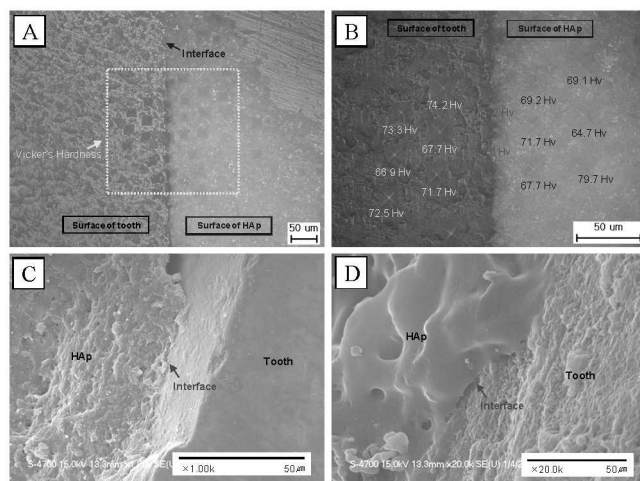
**Table 1.** ICP-OES and EDS results

#### ICP-OES

Analysis Elements (wt %)	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> O	TiO <sub>2</sub>	SrO	P <sub>2</sub> O <sub>5</sub>	Ignition loss	CaO+P <sub>2</sub> O <sub>5</sub>
Analysis date	0.13	0.10	57.0	0.02	0.01	0.14	0.10	0.02	41.4	1.08	98.4

#### EDS

Element	O	P	Ca	Total	Ca/P
Atomic (%)	69.56	11.41	19.03	100.00	1.6685



**Figure 5.** Optical images of the interface area between the tooth enamel and HAp cemented layer using optical microscopy and micro-indentator. (A) the tested area of the Vickers hardness (left: tooth enamel, right: cemented layer,  $\times 200$ ), (B) magnified image ( $\times 500$ ) of the yellow box in figure 4A. SEM image of the interface area magnified to  $\times 1K$  (C) and  $\times 20K$  (D).

the hardness of the new-adhered layers on the scratched ravines of the tooth enamel layers.

**Micro-indentation Test for Hardness.** Three samples were used to measure the Vickers hardness. The newly generated layers were observed optical microscopy and pressed to make diamond dents at precise positions, and the cross lines of the dents were measured using a micro measurer attached to the hardness tester. The calculated average hardness of each sample immersed for 1, 2 and 3 months was 283.36, 283.60, and 311.96 HV, respectively. The hardness of the normal tooth enamel not immersed was 315.59 HV. After one or two months, the hardness was lower than that of the normal enamel. However, after three months, the hardness of the neo-generated HAp layers on the crown was similar to that of the normal tooth. Figure 5 shows optical and SEM images of the interface between the tooth and regenerated HAp layer. The hardness test of the interface area in Figure 5A and B show that the Vickers hardness increased up to 150% from the tooth or HAp layer to the interface. It is possible that ionized HAp compounds deposited onto the scratched areas to produce novel osteoplastic layers and  $\text{Ca}^{2+}$  ions. Moreover, the  $\text{PO}_4^{3-}$  ions from the HAp powders dissolved in the d.i. water were agglomerated to produce cemented pastes on the tooth enamel surface, resulting in reinforced neo-regenerated HAp layers.<sup>17-21</sup> The layer at 1-2 months may not have been firmly established but the hardness of the 3 month-immersed sample showed a value comparable to that of a normal tooth.

### Conclusion

Nanoscale HAp powders were used to regenerate the enamel layers of a damaged tooth. The HAp chunks from the micro-mesh (# 635) had a diameter  $< 20 \mu\text{m}$ , and each chunk consisted of nanoscale HAp powders, 60 nm in length and 30

nm in diameter, with a cylindrical shape. AFM showed that the scratched surface was inlaid with HAp and the roughness increased from 2.7972 to 5.5112. Moreover, the hardness of the neo-generated HAp layers on the crown was similar to that of the normal tooth after three months immersion in the biological environment ( $37^\circ\text{C}$ ). The hardness of the interface was higher than both the tooth and HAp layers. This suggests that the ionized  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions from HAp powders dissolved in d.i. water can produce cemented pastes on the enamel surface, resulting in neo-regenerated HAp layers. This might be suitable for the long term prevention of tooth decay through micro-cleavage, and the development of teeth whitening appliances.

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