Mechanical Strength Improvement of Apatite Cement Using Hydroxyapatite/Collagen Nanocomposite

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Abstract. The combination of tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$) and dicalcium phosphate anhydrous (DCPA; $CaHPO_4$) which are known as one system of apatite cements already used in the medical and dental application. In spite of several advantages of apatite cements, such as self-setting ability and biocompatibility, their mechanical strengths are still low. The aim of this study is to improve the mechanical strength of the TTCP-DCPA apatite cement using the hydroxyapatite/collagen nanocomposite (HAp/Col). The apatite cement powder was prepared using an equimolar TTCP and DCPA with addition of 10% and 20% of the HAp/Col. That without the HAp/Col was used as a control group. Each group was mixed with 1 mol/L $Na_{1.8}H_{1.2}PO_4$ aqueous solution at powder/liquid ratio of 0.5 and hardened at 37°C and 100 % of relative humidity for 24 hours. A setting time of the cement was evaluated using Vicat needle according to ISO 1566 for dental zinc phosphate cements. Morphology of the cements set were observed by the scanning electron microscopy (SEM), and crystalline phases were identified by the powder X-Ray diffractometry (XRD). The mechanical strength of the cement set was evaluated by the diametral tensile strength (DTS). The setting times of cements were the shortest for the cement with HAp/Col and the longest for the control. XRD patterns of the cement at 24 hours after mixing revealed that all cements changed into apatite from the mixture of TTCP and DCPA. The DTSs of cements were the highest for the cement with 20% HAp/Col and the lowest for the control with significant differences between the cement with 20 % HAp/Col and respective other two cements. The scanning electron micrographs of the surface and fracture surface of the cements suggested that the cement with HAp/Col showed denser structure in comparison to the control and the HAp/Col fibers and/or sheets covered the fracture surface. The HAp/Col would act as reinforcement fibers as well as an adhesive of apatite granules formed by the reaction between TTCP and DCPA. The setting time and mechanical strength of apatite cement was statistically significant improved by adding 20% HAp/Col.

Introduction

Apatite Cement (AC) which consists of an equimolar mixture of tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$) and dicalcium phosphate anhydrous (DCPA; $CaHPO_4$) has an excellent characteristic that similar to biological bone systems.[1] AC is based on dissolution and precipitation reaction that when powder phase in contact with aqueous solutions dissolve to a more stable calcium phosphate, leading to hardening of the cement and hydroxyapatite (HAp) precipitation.[1,2] In addition, they are bioactive, osteoconductive, and moldable that can easily use within the bone defect.

Although, AC has a lot of advantages, however AC are still far from the ideal for bone substitutes because their mechanical strength is lower compared to bone. On the other hand, particle size of the starting materials was also give an effect to the cement properties such as increase the

mechanical properties and shorter the setting time.[3,4,5] The final phase of AC is HAp which has characteristic as ceramics are still too brittle and crushed when implanted to bone.[6] These clinical problems with AC as a synthetic bone substitute are due to their remaining in bone for a long period.

The HAp cement collagen-reinforced self-setting prepared by Miyamoto *et al.* showed its higher biocompatibility and improved mechanical properties.[7] The hydroxyapatite/collagen (HAp/Col) composite have a bone-like nanostructure that reported by Kikuchi *et al.*[8] The HAp/Col nanocomposite obtained showed excellent biocompatibility and bio-integrative activities which similar to autogenous bone and much better than other artificial bone materials.[9] When HAp/Col formed a network structure in AC and a tight bonding to the apatite crystal, the mechanical strength could be increased. Furthermore, the HAp/Col and AC composite might be a desirable biomaterial which similar to bone structure composed of apatite crystals and collagen.

Materials and Methods

Preparation of apatite cement powder and HAp/collagen nanocomposite. Commercially TTCP powder (Taihei Chemicals Inc., Osaka, Japan) and DCPA powder (J.T. Baker Chemical Co., NJ, USA) was employed. The AC powder phase was composed of an equimolar mixture of TTCP and DCPA. In addition, the HAp/Col nanocomposite was prepared using $Ca(OH)_2$, H_3PO_4 (Reagent Grade, Wako Pure Chemicals Inc., Tokyo, Japan) and atelocollagen (Nitta Gelatin Inc., biomaterial grade, Japan) as starting materials while $Ca(OH)_2$ was synthesized from $CaCO_3$ (Alkaline Analysis Grade, Wako Pure Chemicals Inc., Tokyo, Japan) at 1050°C for 3 h by hydration process of CaO. Their composites were prepared using a previously described technique by a co-precipitation method.[6] For control, only AC was used, while others AC added by 10% and 20% HAp/Col, respectively. The AC + 10% HAp/Col and AC + 20% HAp/Col were lyophilized then freeze dry for 48 h. All the powder phase was grounded in a planetary ball mill (Fritsch 8 6560, Idar-Oberstein, Germany) with 95% ethanol at 400 rpm for 1 hour then drying overnight at 40°C, respectively.

Preparation of samples. AC powder phase included AC with HAp/Col was mixed with 1 mol/L $Na_{1.8}H_{1.2}PO_4$ as liquid solution at a liquid to powder ratio 0.5 (L/P ratio), respectively. After mixed, the paste obtained was packed into a Teflon mold with 6 mm in diameter and 3 mm in height. Both end of the mold was covered by a glass slide and clamped by a metal clip. The mold with the sample was then placed into an incubator kept at 37°C and 100% of relative humidity for 24 hours. After treatment, the set cement was immersed in the 99% ethanol for 3 min then dried in an oven at 80°C for 3 h.

Setting times measurements. Setting times of AC with HAp/Col and control were measured according to the International Standard 1566 (ISO 1566) for dental zinc phosphate cements. Briefly, a cement is considered set when 400 g weight loaded on Vicat needle with a tip of 1 mm diameter failed to make a perceptible circular indentation on the surface of the cement. Three replicate samples were measured (*n=3*).

Characterization of samples. Set cement samples were crushed into a fine powder and collected then characterized by mean of X-ray diffractometry (D8 Advance, Bruker AXS, Karlsruhe, Germany) with CuKα radiation operated at 40 kV of tube voltage and 40 mA of tube current.

Mechanical strength measurements. The mechanical strength of samples was examined in terms of diametral tensile strength (DTS). The diameter and height of each set cement samples were measured with a micrometer (MDC-25MU, Mitutoyo Co. Ltd., Kanagawa, Japan). The samples were crushed using a universal testing machine (AGS-J; Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 1 mm/min. DTS values were taken as average of at least 5 samples (*n=5*).

Morphological observation. The microstructure morphology of the fractured surface of the samples was performed by scanning electron microscope (SEM: S-3400N, Hitachi High-Technologies, Tokyo, Japan) at 10 kV of accelerating voltage after gold-palladium sputters coating (MSP-1S, Vacuum Device Co., Ibaraki, Japan).

Result and Discussion

Table 1 summarizes the setting time of cement consisting of AC (TTCP-DCPA), AC+ 10% HAp/Col, and AC + 20% HAp/Col mixed with 1 mol/L of $Na_{1.8}H_{1.2}PO_4$ solution with 0.5 L/P ratio, respectively. The setting time of AC+ 10% HAp/Col and AC + 20% HAp/Col were faster compared to AC. In other words, the setting times of cements with HAp/Col were the shortest and the longest for the control. However, the setting time between AC+ 10% HAp/Col and AC + 20% HAp/Col showed no statistically significant.

Table 1. Setting times of cement consisting of AC (TTCP-DCPA), AC+ 10% HAp/Col, and AC + 20% HAp/Col mixed with 1 mol/L of $Na_{1.8}H_{1.2}PO_4$ solution with 0.5 L/P ratio, respectively. At least 3 samples were used for setting time measurement.

Liquid	Powder	Setting time (min)
$Na_{1.8}H_{1.2}PO_4$	AC + 10% HAp/Col*	2:41 ± 0:05
$Na_{1.8}H_{1.2}PO_4$	AC + 20% HAp/Col*	2:52 ± 0:12
$Na_{1.8}H_{1.2}PO_4$	AC	3:45 ± 0:04

n=3; *p<0.05

Figure 1 shows the XRD pattern of set cement consisting of AC, AC + 10% HAp/Col, and AC + 20% HAp/Col mixed with 1 mol/L of $Na_{1.8}H_{1.2}PO_4$ solution, respectively. XRD patterns of the set cement after 24 hours confirmed that all cement from the mixture of TTCP, DCPA, and HAp/Col were fully transformed to apatite. This XRD result revealed that the transformation to apatite was not inhibit by adding HAp/Col.
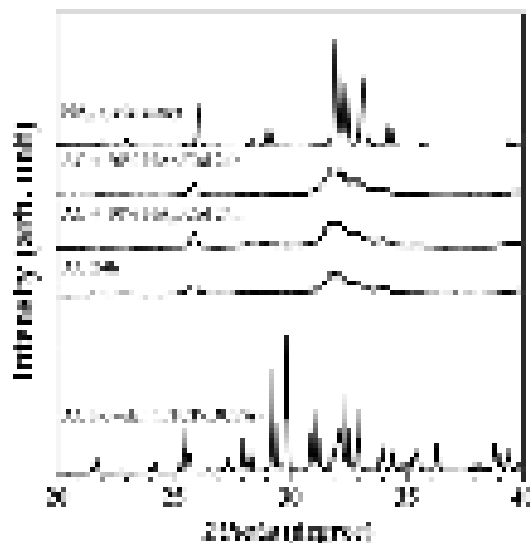

Fig. 1. XRD patterns of set cement consisting of AC, AC+ 10% HAp/Col, and AC + 20% HAp/Col mixed with 1 mol/L of $Na_{1.8}H_{1.2}PO_4$ solution, respectively. HAp pattern was also showed as a reference.

Figure 2 shows the SEM images of the surface and fracture surface of the set cement. The SEM images of the surface and fracture surface set cements of the control showed more pores area than HAp/Col. In other words, the set cement with HAp/Col showed a dense structure in comparison to the control and the HAp/Col fibers and/or sheets covered the fracture surface. The HAp/Col would act as reinforcement fibers as well as an adhesive of apatite granules formed by the reaction between TTCP and DCPA.
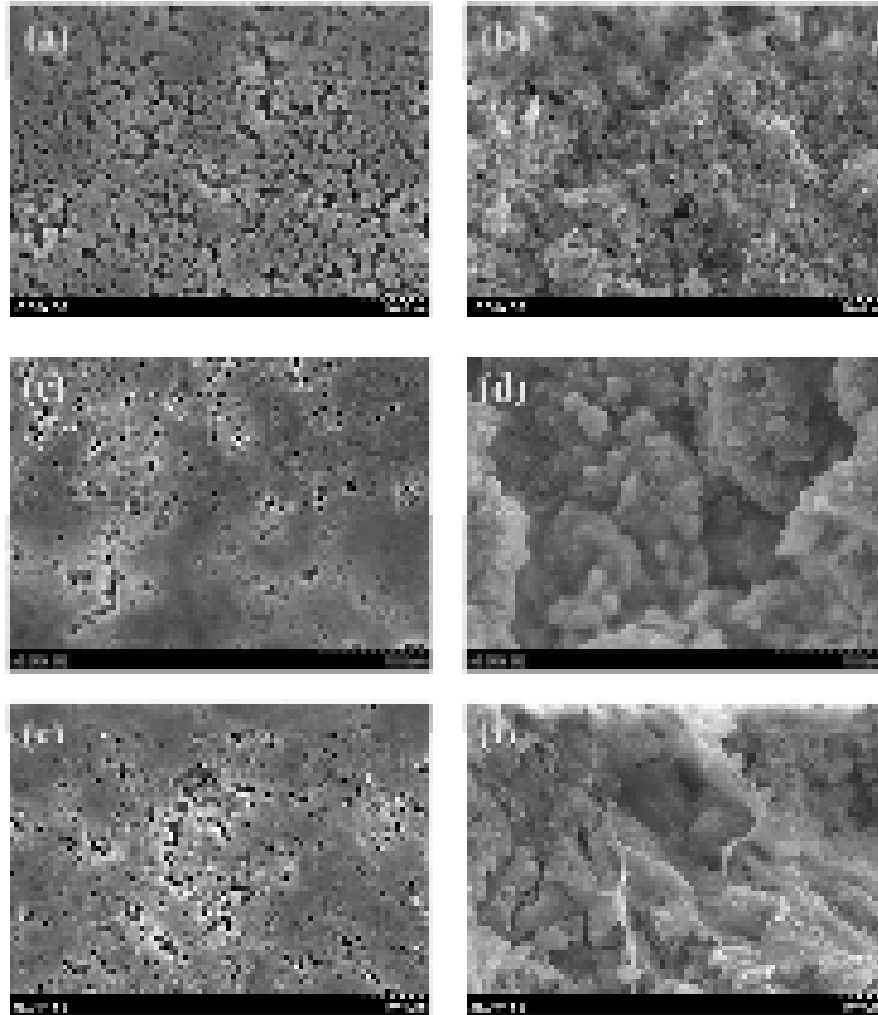

Fig. 2. SEM of morphology the surface (a,c,e) and fracture surface (b,d,f) of the set cement. Images (a) and (b) were AC, (c) and (d) were AC + 10% HAp/Col, (e) and (f) AC + 20% HAp/Col.

Figure 3 shows the DTS values of set cements after treatment for 24 h of AC, AC + 10% HAp/Col, and AC + 20% HAp/Col. The average DTS values of AC was 4.42 ± 1.06 MPa, AC + 10% HAp/Col was 5.03 ± 0.99 MPa, and AC + 20% HAp/Col was 7.22 ± 0.59 MPa. The DTS value of AC + 20% HAp/Col was the highest than the others and there was statistically significant. Higher mechanical strength in AC + 10% HAp/Col and AC + 20% HAp/Col might be due to low porosity of the samples associated with the high content of HAp/Col that improving the interconnectivity of the two material. Since the HAp/Col incorporated in this cement, thus could increase the mechanical strength of the cement.
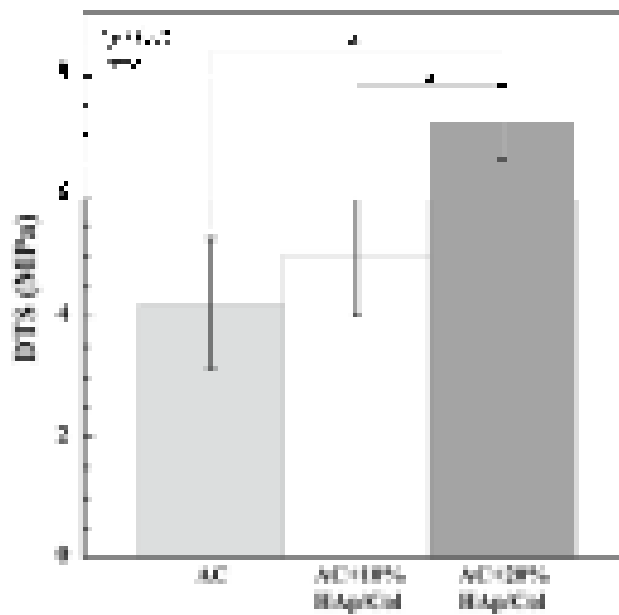
Fig. 3. DTS values of set cements after treatment for 24 h of AC, AC + 10% HAp/Col, and AC + 20% HAp/Col. At least 5 samples were evaluated for DTS.

Conclusion

The mechanical strength of AC was successfully increased by incorporated the HAp/Col nanocomposite into AC. The presence of HAp/Col nanocomposite had a complementary effect to improve the mechanical properties of the cement. The HAp/Col nanocomposite not only increased the mechanical strength of AC but also make the setting time shorter. The mechanical strength was statistically significant improved by adding 20% HAp/Col.

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