# Preparation of plated $\beta$-tricalcium phosphate containing hydroxyapatite for use in bonded inorganic-organic composites

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Calcium phosphate ceramics such as $\beta$-tricalcium phosphate ($\beta$-$Ca_3(PO_4)_2$, $\beta$-TCP) and hydroxyapatite ($Ca_{(10)}(PO_4)_6(OH)_2$, HAp) show excellent bioactivity for bone generation because their chemical composition is similar to that of bone [1–3]. The ceramics have been also used as a scaffold for fibroblast growth factor ($\beta$-FGF) [4], transforming growth factor-beta 1 (TGF-$\beta$ 1) [5], or bone morphogenic protein-2 (BMP-2) [6] showing a high affinity for those molecules. Such bioceramics have found widespread application in the medical field.

Recently, we demonstrated a sintered HAp particle coating on polymer substrates with covalent linkage for use in development of a percutaneous device. This technology is a unique approach to surface coating, and bioactivity of HAp such as cell-adhesiveness can be provided at the surface of underlying polymers substrates without the mechanical properties of the polymers being effected adversely [7–12]. To increase the interaction between ceramics particles and polymer substrate surface, a particle with a larger surface area for adhesion to the substrate surface is necessary. Nanometer-sized sintered HAp rods were therefore developed by an emulsion system and by this way increased surface interaction with the polymer [13, 14]. Although the interface stability of a composite consisting of nanometer-sized HAp particles and a polymer substrate was increased, aggregated inorganic particles were observed on the composite surface [9–11]. This is because the nanometer-sized ceramics aggregated easily with each other in the process of calcination or composite preparation.

To increase interface interaction between a ceramic and substrate as well as to prevent aggregation, a novel micrometer-sized and plated ceramic consisting of $\beta$-TCP and a calcium-deficient apatite was developed. The prepared ceramic was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), and transmission electron microscopy (TEM).

Dicalcium phosphate dihydrate ($CaHPO_4{\cdot}2H_2O$, DCPD, brushite) and calcium carbonate ($CaCO_3$) (Kanto Chemical Co., Tokyo, Japan), and monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2{\cdot}H_2O$, MCPM] (Wako Pure Chemical Industries Ltd, Osaka, Japan) were used as supplied. Calcium-deficient hydroxyapatite ($Ca_9HPO_4(PO_4)_5OH$, CDHA) showing plated morphology was prepared by modification of previous reports [15, 16]. Briefly, tetracalcium phosphate monoxide [$Ca_4(PO_4)_2O$, TCPM] as a source material was prepared initially from heating an equimolar mixture of DCPD and $CaCO_3$ at 1500 °C for 5 hr. Past-like DCPD was prepared from 25.2 g of MCPM and 10.0 g of $CaCO_3$ with 250 ml of distilled water stirring for 15 min. An amount of 3.44 g of precipitated DCPD was subsequently mixed with 3.66 g of milled TCPM with 2.4 ml of 0.25 mol/l $Na_2HPO_4$ as an accelerator solution

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0022–2461  

adjusted at pH 7.4. The molar ratio of TCPM/DCPD is 1/2. The mixture was poured into hollow cylindrical containers (12 mm of height and 6 mm of diameter) and then mixed for 1 min to form a paste. The container containing the calcium phosphate paste formed was covered with a paper sheet and aged for 3 days in air to gradually remove moisture content. The product was remove form the container and then crushed in a mortar. The crushed calcium phosphate was finally calcined at 800 °C with a heating rate of 10 °C/min for different time periods.

XRD measurements of all reagents were taken to determined the phase purity, indexing of peaks was carried out by means of measuring known samples together with reference to JCPDS card no. 9-347 for MCPM, 25-1137 for TCPM, 9-77 for DCPD, 46-905 for calcium deficient apatite and 9-432 for HAp (Joint Committee on Powder Diffraction Standard). SEM (JSM-6301F, JEOL, Tokyo, Japan) was used in order to observe the sample surface. XRD were recorded using a Philips PW1729 X-ray diffractometer (Netherlands) with Cu K$\alpha$ radiation. $\beta$-TCP content in the calcined product was calculated from ratio of the peak intensity of the XRD patterns as follows;

$$\beta\text{-TCP content}(\%) = [I_{\beta\text{-TCP}}/(I_{\beta\text{-TCP}} + I_{\text{Ap}})] \times 100$$

$I_{\beta\text{-TCP}}$: peak intensity of (0210)($2\theta = 31.16°$) in XRD of $\beta$-TCP

$I_{\text{Ap}}$: peak intensity of (211)($2\theta = 31.92°$) in XRD of CDHA

The working curve was determined using $\beta$-TCP (SIGMA-ALDORICH, JAPAN, Tokyo Japan) and HAp (APACERAM®, Pentax Co., Japan). Microstructure observation and electron diffraction was performed using transmittance electron microscopy (TEM, JEM-2000EXII, operating at 200 kV JEOL, Tokyo, Japan).

Fig. 1 shows an SEM image of the pre-calcined crushed calcium phosphate after storage for 3 days in air. Calcium phosphate of irregular plate-like morphology of 1–2 $\mu$m in length and 50–100 nm thickness was observed. Fig. 2 shows the XRD profiles of the pre- and post-calcined products. The XRD profile of the preheated calcium phosphate give reflections consistent with poorly crystalline calcium deficient apatite (d(Å) spacings of 3.44 (002), 2.81 (211), 2.78 (112), 2.72 (300), 2.26 (130), 1.94 (222), 1.84 (213) and 1.72 (004) corresponding to the hexagonal unit cell of calcium deficient apatite) (Fig. 2a). After calcination for 60 min at 800 °C, the XRD profile shows reflections consistent with crystalline $\beta$-TCP (d(Å) spacings of 3.45 (1010), 3.21 (214), 2.88 (0210), 2.76 (128), 2.61(220), 1.93 (4010) and 1.73 (2020) corresponding to the rhombohedral unit cell of $\beta$-TCP ) together of a small amount of HAp crystal as shown by a small reflection present at $d = 2.81$ Å (211) (Fig. 2b). Fig. 3 shows the $\beta$-TCP content in the calcium phosphate powder as plotted against calcination time at 800 °C. Zero of the calcined time means temperature in furnace increases by 10 °C/min up to 800 °C and simultaneously decreases to room temperature.

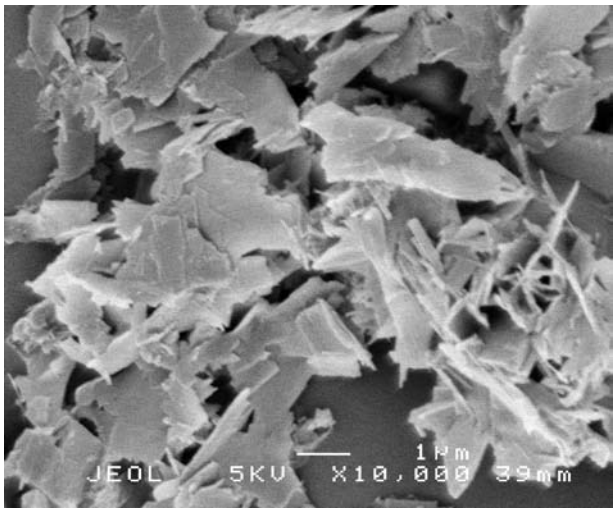


*Figure 1* SEM micrograph of plates of crushed calcium phosphate before calcination.

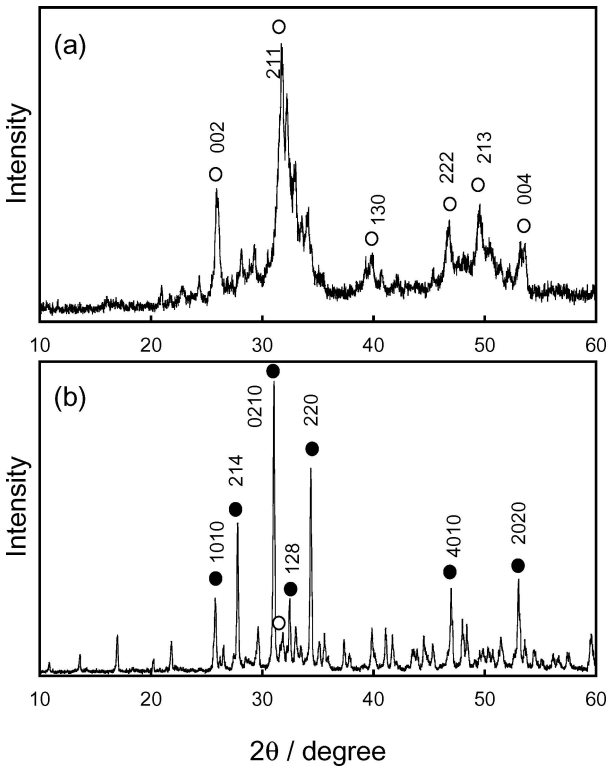


*Figure 2* XRD profiles of calcium phosphate products (a) before and (b) after calcination for 60 min at 800 °C. Open and closed cycles show peaks of HAp and $\beta$-TCP, respectively. Miller indices of HAp and $\beta$-TCP present in figure.

CDHA with a Ca/P ratio of 1.5:1 will convert to pure $\beta$-TCP on heating at temperatures over 800 °C (Equation 1), but at a ratio of 1.5:1 to 1.67:1 a mixture of $\beta$-TCP and HAp is obtained [17]. Thus under the reaction conditions used, the CDHA formed has a Ca/P ration between 1.5:1 and 1.67:1 which resulted in a mixed phase product.

$$Ca_9HPO_4(PO_4)_5OH \rightarrow 3\beta\text{-}Ca_3(PO_4)_2 + H_2O \quad (1)$$

Fig. 4 shows SEM image of the product calcined for 60 min at 800 °C. The product largely consisted

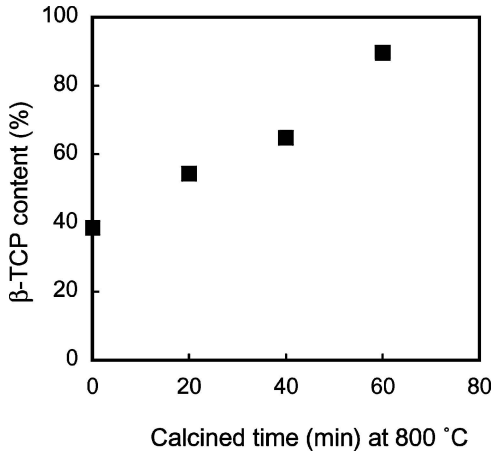


*Figure 3* $\beta$-TCP content in the calcium phosphate powder plotted against calcination time at 800 °C.

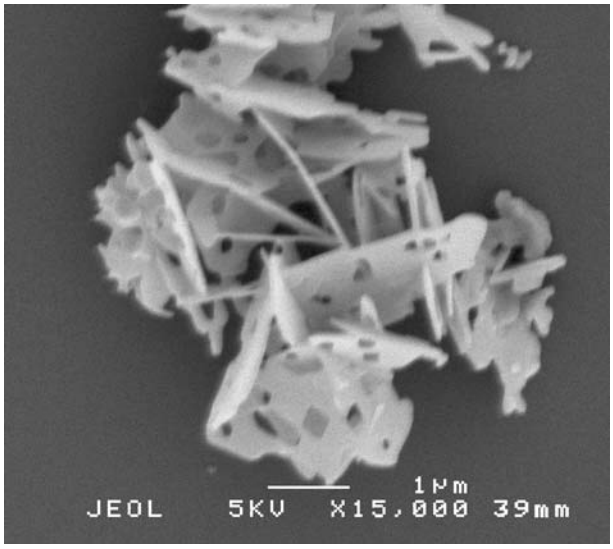


*Figure 4* SEM micrograph of calcium phosphate plates calcined for 60 min at 800 °C.

of porous plated crystals of 0.5–2 $\mu$m in length and 50–100 nm in thickness, holes when present were 100–300 nm in diameter. The calcined crystals were generally more smoother in appearance than the pre-calcined plates. The holes in a plate of $\beta$-TCP crystal were generated due to a decrease in crystal mass and volume with increasing calcination at 800 °C.

The plates produced are expected to be more suitable for coating of a polymer surface since they provide a larger surface area for adhesion to a substrate surface. The plated calcium phosphate also contains hydroxyl groups due the minor presence of HAp, this functional group can react with alkoxysilyl or isocianate groups by covalent bonding. Thus it is possible to prepare an inorganic/organic composite with the plated ceramics prepared herein, employing a novel hybrid system by using graft-polymers having alkoxysilyl or isocyanate groups in the branches [9, 10]. Also surface calcium ions of the plated $\beta$-TCP can be utilized to develop a composite, for example poly(4-methacryloyloxyethyl trimellitate anhydride) possessing dicarboxyl groups in the branches shows and ionic interaction with cationic ions [11].

In conclusion, a porous plated $\beta$-TCP containing a low proportion of HAp was prepared by calcinations of a prepared CDHA at 800 °C, the plated morphology of a source DCPD being broadly retained in the CDHA intermediate and subsequent $\beta$-TCP product. The content of the HAp phase in the plated calcium phosphate could be controlled by calcinations time at 800 °C. The plates of $\beta$-TCP containing of HAp are expected to be more suitable for coating of a polymer surface due to the larger surface area of adhesion to the substrate. We are now developing a novel inorganic/organic composite using the inorganic plates.

## Acknowledgments

Authors thank CREST of JST (Japan Science and Technology) and Dr. A. Matsuda for assistance with measurement of XRD.

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*Received 9 August*
*and accepted 17 November 2004*