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Calcium phosphate formation in gelatin matrix using free ion precursors of Ca^{2+} and phosphate ions

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ABSTRACT

Objectives. Hydroxyapatite (HAp)/gelatin (GEL) nanocomposite has been developed as a bone substitute. The nanocomposite formation in the GEL matrix is greatly affected by the reaction between Ca^{2+} and phosphate ions. The mineralization of GEL macromolecules was investigated through a co-precipitation of calcium phosphates (Ca-P) by using free ions of Ca^{2+} and phosphate ions, Pi. The purpose of this study was to prepare a dense HAp/GEL nanocomposite through a free ion production process.

Methods. Free ionic calcium, Ca^{2+}, was produced through electrodialysis process using a cation membrane (CMV). Triprotic acid ions were diffused through an anion membrane (AMV) from an aqueous solution of H_3PO_4. The HAp/GEL nanocomposite was prepared by the co-precipitation process. As a reference material for comparison, $Ca(OH)_2$ and H_3PO_4 were used for the preparation of a HAp/GEL nanocomposite.

Results. The dense compact body of dried Ca-P/GEL nanocomposite was obtained through the fine chemical reaction of Ca^{2+} and Pi. The free calcium ion Ca^{2+}, diffused from the CMV of the cation reactor greatly affected the formation of Ca–P phase. Phosphate ion species diffused through the AMV in the anion reactor definitely influenced the reaction with Ca^{2+}. For the formation of the Ca–P phase in the GEL matrix, the organic–inorganic interaction was analyzed using FT-IR. The crystal growth of HAp in the GEL matrix increased with the increase of GEL from XRD, FT-IR and TEM.

Significance. The mineralization reaction in GEL macromolecules was critically influenced by the free ions of Ca^{2+} and inorganic phosphate ions, Pi. The interaction between Ca^{2+} and Pi in the GEL matrix was very fine compared to the HAp/GEL nanocomposite prepared from $Ca(OH)_2$ and H_3PO_4 with the GEL. The dense compact body of HAp/GEL nanocomposite was obtained for an artificial bone application.

1. Introduction

Calcium is the most abundant mineral in the human body. The biologic effect of calcium is determined by the amount of ionized calcium [1]. Calcium flow in the bone is neutral and bone serves as an important storage point for calcium. Calcified tissue such as bone and teeth are basically considered as a biologically and chemically bonded composite between hydroxyapatite (HAp), $Ca_5(PO_4)_3(OH)$ nanocrystals and type-I collagen [2–4]. It has been shown [5] that the

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unusual tensile and elastic properties of bone result from specific organic–inorganic interactions.

In biological bone we can consider three structural factors: HAp nanocrystals, collagen (COL), and organic–inorganic interaction between HAp and COL. Being a major bone component, HAp has been widely used in the production of artificial materials for substituting the autogeneous bone [2,3]. We have recently developed a biocompatible nanocomposite using HAp and a gelatin (GEL) precursor [6,7]. During the co-precipitation as a biomimetic approach, a characteristic feature of this process is a dynamic reaction using active $Ca(OH)_2$ as a free Ca^{2+} ion precursor instead of $CaCl_2$ or $Ca(NO_3)_2$ which are used in the double diffusion process [8]. The double diffusion process has been used to study the crystal formation of HAp or octacalcium phosphate (OCP), $Ca_8H_2(PO_4)_6{\cdot}5H_2O$ [9,10] to understand the mineralization of biological bone.

In the development of HAp/GEL nanocomposite we have focused on the chemical coordination between the apatite crystallites and the GEL macromolecules as a matrix template. During the co-precipitation process, the calcium phosphate (Ca-P) phase is formed through the chemical reaction between Ca^{2+} and phosphate ions. $[Ca^{2+}]$ depends on the ion releasing kinetics of the $Ca(OH)_2$ powders in water. The average pH value was set as 8.0 to obtain HAp phase [10,11] in the mixture solution of GEL and phosphate ions. However, the pH values might be locally varied because of releasing rate of Ca^{2+} from $Ca(OH)_2$ in water. To reduce the local variation of the pH, the main reactor was vigorously stirred during the precipitation. We have prepared a highly reactive $Ca(OH)_2$ powders [6,7] to activate the releasing rate of Ca^{2+}. The pure $Ca(OH)_2$ contains no anionic elements such as Cl^- and NO_3^-. However, it was not easy to obtain a consistent alkalinity because pH depends on the dynamic releasing of Ca^{2+} from the $Ca(OH)_2$. The alkalinity is controlled by the slow releasing kinetics of Ca^{2+} from the swelled $Ca(OH)_2$. During the co-precipitation process, the resulted $[Ca^{2+}]$ is not same with the theoretically calculated value of $[Ca^{2+}]$. The releasing kinetics of Ca^{2+} from $Ca(OH)_2$ in water is based on a complicate chemistry because there are free Ca^{2+}, a calcium ion complex, and calcium ions solvated with H_2O molecules [12,13]. The control of dynamic process greatly affected the crystal morphology and organic–inorganic interaction of the resulted HAp/GEL nanocomposite slurries [6,7]. Here we report a co-precipitation process of Ca-P phase using free ions of Ca^{2+} and phosphate ions. The resulted formation of HAp/GEL nanocomposite was investigated by using XRD, FT-IR and TEM. Additionally we discussed the influence of triprotic acids [14,15] of H_3PO_4 for the formation of Ca-P phase. Free ions of Ca^{2+} and phosphate ions were produced through an electrodialysis process [16] using CMV and AMV as ion diffusion membranes, respectively.

2. Experimental methods

2.1. Production of free ions of Ca^{2+} and phosphate using ion exchange membranes

As a schematic design (Fig. 1), there are a main reactor for the co-precipitation reaction, a cation reactor for the Ca^{2+} diffusion, and an anion reactor for the diffusion of phosphate ions. We used SELEMION membranes donated by Asahi Glass Engineering in Japan. The ion reactor was separated into two compartments using a membrane. CMV and AMV were used for the cation reactor and anion reactor, respectively. In the cation reactor, one compartment is filled with Ca^{2+} solution, which was diffused from $Ca(NO_3)_2$ solution in the second compartment. In the anion reactor, one compartment is filled with $H_xPO_4^{3-y}$ solution that was diffused from the H_3PO_4 solution in the second compartment. The dissociated phosphate ions, Pi are $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} [14,15]. Diffusion through the membrane was driven by the DC current potential. The DC current potential was controlled by a digital DC power supply (BK Precision 1697, USA). In the cation compartment, DC power was automatically adjusted with the increase of $[Ca^{2+}]$. The voltage and current values from the power supply circuit were automatically adjusted with the concentration variation in the reactor. In this experiment the proper voltage range was between 10 and 30 V. The diffusion kinetics depends on the current density between both electrodes. Pt electrodes were used to obtain the higher electric density.

Ion exchange membranes [16] are thin films of polymeric chains containing electrically charged functional sites. These selectively charged membranes can separate ions. If the membrane is positively charged, only anions will be allowed through, and it is called an anion-exchange membrane. Similarly, negatively charged membranes are called cation-exchange membranes. SELEMION membrane is the ion exchangeable membrane without an actual hole. The imaginary temporary hole [17] of SELEMION membrane is 20–50 A°. Normally, non-chargeable species cannot pass through membrane. AMV and CMV are not charge selective membranes. Therefore, variously charged ions may pass through. The effect of triprotic ions in aqueous H_3PO_4 is not same as real moving ion with molecular H_2O. It is not easy to characterize the concentration difference between $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} at present. It will be further reported later.

2.2. Raw materials, sample preparation and characterization

The precursors were $Ca(NO_3)_2{\cdot}4H_2O$ (Alkaline analysis grade, F.W. 236.15 Aldrich, USA), H_3PO_4 (AP grade, Aldrich, USA) and Gelatin (Unflavored, Natural Food, Canada). The amount of $Ca(NO_3)_2{\cdot}4H_2O$ and H_3PO_4 was 23.615 and 11.53 g for 1 l of double distilled deionized (DI) water, respectively. In cation and anion reactor the liquid volume of each compartment was set as 1 l. When the pH of Ca^{2+} compartment in the cation reactor was above 10, the Ca^{2+} solution was pumped into the main reactor. Measured amounts of GEL powders were dissolved in the aqueous solution of the $H_xPO_4^{3-y}$ at 37 °C for 2 h according to the designated schedule before the co-precipitation. The slurry for HAp/GEL nanocomposite was prepared by the simultaneous titration method using peristaltic pumps (Masterflex, Cole-Parmer, USA), a water bath (Boekel, USA), and a pH controller (Bukert 8280H, Germany). The temperature and pH of the reaction solution in the vessel were set and maintained at 37 °C and 8.0, respectively. The aqueous solution of Ca^{2+} and aqueous $H_xPO_4^{3-y}$ solution with the GEL were gradually added to the reaction vessel using the peristaltic pumps. The temper-