Colloidal β-TCP Facilitates Synthesis of Collagen Nano-composite

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ABSTRACT

Preparing collagen composites is recognized as difficult because it has been believed that composites cannot be synthesized without complicated processing. However, we found that the alkaline colloidal β-TCP facilitates synthesis of collagen composites. Many collagen molecules were tightly polymerized to form stable collagen fiber in a β-TCP/collagen composite by catalysis of the -P-O-P- polyphosphate chain generated. Cross-linking between collagen NH^+ amino groups and -P-O-P-, and an increase of bonding strength between Ca^{++} and $RCOO^-$ of the collagen molecules were identified in the composite. These chemical reactions due to the colloidal β-TCP might be crucial in the synthesis of collagen composites.

Keywords: collagen, β-TCP, biocomposite

β-TCP have been intensively investigated as bone substitutes because of their biodegradable and high-osteoconductive properties. Even though many studies have suggested biodegradable β-TCP have better osteoconductivity than hydroxyapatite (HA), synthesis of the β-TCP /collagen composite remains problematic. The specific surface area of CP particles that can conjugate organic molecules can be increased by a reduction of particle diameter. CP particles sizes have been generally reduced (average diameter 3μm) by agate-ball milling followed by ultrasonically pulverization [1]. To facilitate chemical reactions between CP particles and collagen molecules, colloidal β-TCP and HA (pH 11.3) with submicron diameters (0.17-0.20μm) were prepared by discharging in modified body fluids [2].

To investigated the conformational changes between the colloidal CPs and type-I collagen solution (pH 3.0), Fourier transformed infrared (FTIR) was used in this study.

After 30min processing, the height of the amide-A peak [3] of β-TCP /collagen was remarkably higher than that of the HA/collagen composite (Fig.1). Type-I collagen has a triple-secondary helical structure of collagen is produced by strong hydrogen bonding, which is indicated by the high amide-A band of collagen, and these reactions are directly associated with the supercoiled triple-helical conformation of collagen fibers [4]. It has been reported that the intensity of amide-A is sensitively dependent on the extent of the polymerization of collagen molecules, which is critically important for the bone mineralization process [5,6].

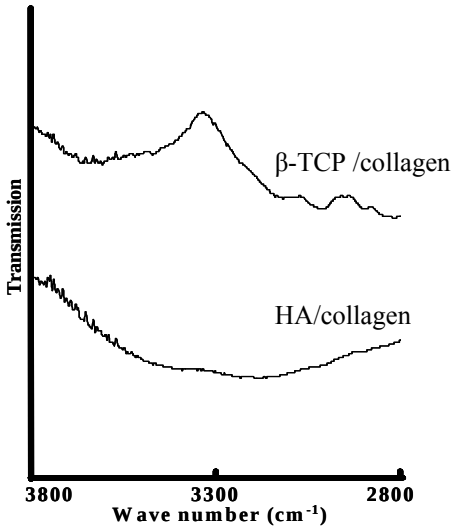

Fig.1 Amide-A spectra of each composite

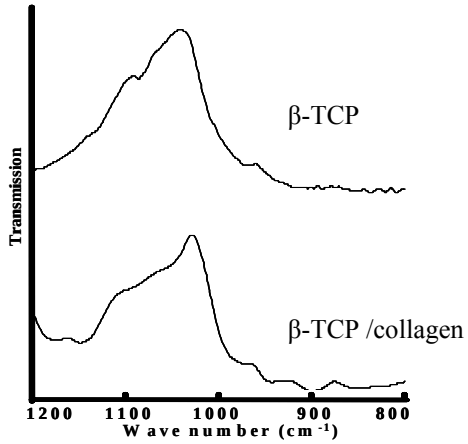

Fig.2 The phosphate vibration mode of β-TCP

Surprisingly, the phosphate vibration mode of β-TCP shifted to 1025 cm^{-1}, clearly indicating that the -P-O-P- polymerization chain (Fig.2) was produced [7] only in β-TCP /collagen after mixing.

Polyphosphate has been employed as a catalyst in organic chemistry [8,9]; thus the -P-O-P- polymerization chain might have an important role in forming stable collagen fibers in β-TCP /collagen composites.

To clarify the cross-linking between those inorganic components and the stable collagen fibers generated, an X-ray photoelectron spectroscopy (XPS) analyzer was employed. Since the secondary peak at 134.8eV, indicating NH^+-phosphate binding [10], was detected only on the β-TCP/collagen, the -P-O-P- polymerization chain was tightly cross-linked to the collagen NH^+ groups in this composite.

Ca2p spectra reveal similar doublets with $Ca2p_{1/2}$ and $Ca2p_{3/2}$, typical for the Ca^{++} state in inorganic calcium phosphate compounds [9] were detected on both composites. The energy position of a Ca2p doublet is increased with cross-linking between CP nanocrystal and collagen molecules because the bonding strength of Ca^{++}-COO^- is higher than that of inorganic Ca^{++}- PO_4^{3-} . The XPS spectra showed the electron binding states of Ca of the colloidal CPs, which were coordinated with both the inorganic PO_4^{3-} and RCOO- groups of the collagen molecules. Further, since the energy positions of N1s and Ca2p of the β-TCP/collagen were significantly higher ($p<0.01$) than that of HA/collagen, the bonding strength between the collagen RCOO- groups and Ca^{++} of β-TCP was also much increased.

In this study, many collagen molecules were polymerized together to form stable collagen fibers in the β-TCP/collagen composite. Furthermore, since the cross-linking between collagen NH^+ amino groups and the -P-O-P- polymerized chain, and bonding strengths between Ca^{++} and $RCOO^-$ of collagen fibers were much increased in the composite, it can be expected that the β-TCP/collagen composite has high biological stability without polymeric reinforcement.

Thus, we believe that this colloidal β-TCP has an important role to play in the synthesis of the spatial structure manipulation of collagen composites.

METHODS

Preparation of collagen composites

3ml of the colloidal CPs were dispersed in the same quantity of collagen solution (8mg/ml, pH 3.0, Type-I collagen BM, Nitta Gelatin). Subsequently, each mixture was stirred for 30sec in a polypropylene centrifuge tube.

FTIR analysis

Colloidal CPs were dried and stored for 24h, and then prepared as KBr pellets for FTIR analysis. Pure β-TCP and HA powder (Wako) were used as standard reference materials.

In addition, 10μl of each composite was immediately put between ZnSe windows, and analyzed by a FTIR analyzer (FT/IR-660, JASCO) in a vacuum. The conformational changes of each mixture over time were monitored for 30min. A measuring resolution of $4cm^{-1}$ and iterations were performed for 200 times in the range 400-$4000cm^{-1}$ to characterize the various functional groups.
All data were confirmed at least six times in repeated investigations (n=6).

XPS analysis

Each dried composite was analyzed by a XPS device (ESCA-3400, SHIMADZU). Findings were analyzed statistically by ANOVA (n=6). High-resolution spectra of Ca2p, O1s, P2p, C1s, and N1s were analyzed using Mg Kα radiation, applying 20mA emission current and 8kV accelerated voltage. Binding energies for each spectrum were calibrated using a C1s spectrum of 285.0 eV. The results were expressed as the mean ± standard deviation (SD) of six specimens (n=6), and analyzed statistically by Student's t-tests. Significant differences were considered to exist when $p<0.01$.

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