PREPARATION OF A BIOACTIVE CHITOSAN/CALCIUM SILICATE NANOCOMPOSITE

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Abstract

A chitosan/calcium silicate nanocomposite was synthesized by sol-gel method and evaluated its bioactivity in simulated body fluid (SBF). Chitosan was covalently bonded with phtalic anhydride and subsequently reacted with 3-isocyanatopropyl triethoxysilane (Si-Chitosan) in dimethylformamide. Following this, the Si-Chitosan was hydrolyzed and condensed with tetraethyl orthosilicate and calcium nitrate tetrahydrate to yield a nanocomposite. The gelation was carried out for one week at 70 ^{o}C in a covered Teflon mold with a few pinholes and then dried under vacuum at room temperature for two days. The bioactivity of the chitosan/calcium silicate nanocomposite was evaluated by examining the apatite forming capacity in SBF. The surface microstructure and functional groups of the products were analyzed by field emission scanning electron microscopy and Fourier transformed infrared spectroscopy, respectively. The crystal phases of the specimen before and after the bioactivity testing were analyzed by thin film X-ray diffractometry. Newly developed chitosan/calcium silicate nanocomposite showed apatite-forming capacity in the SBF within one week of soaking. The potential application of this chitosan/calcium silicate nanocomposite will be a bone grafting material due to its good bioactivity and biocompatibility.

1 Introduction

A bioactive ceramic has a nano-sized carbonate apatite forming capacity in simulated body fluid (SBF) [1] and *in vivo*. Resultantly, it becomes to have osteoconductivity. However, it has serious drawbacks such as low fracture toughness and high elastic modulus compared to those of natural bone.

A synthetic polymer has good properties for biomaterial applications such as high fracture toughness, malleability, and controllable biodegradability. However, it has no osteoconductivity at all and provokes severe inflammations *in vivo*. Thus, it has a limitation to be used as a bone grafting material.

Thus, the mixture of two materials is desirable to obtain good carbonate apatite forming capacity, suitable mechanical and biodegradable properties. In fact, many kinds of bioactive ceramic and synthetic polymer composites have been developed for this purpose. The HAPEX® is a representative composite which is composed of micron-sized bioactive

hydroxyapatite particles and synthetic polyethylene matrix [2]. It has been successfully applied to ossicular replacement prostheses [3].
However, some problems originating from different wettabilities have been found when mixing ceramic and polymer phases. One problem is phase separation that occurs at the interface of the two phases because the ceramic is hydrophilic whereas the synthetic polymer is generally hydrophobic; this phase separation results in a weakening of the composite's mechanical properties. Another problem is the low degree of dispersion of the bioactive ceramic particles in the polymer matrix. To solve these two problems, nanocomposite has been developed, which can suppress phase separation and improve the degree of dispersion [4].
For last decades, there have been many investigations to make new polymer/ceramic nanocomposites for the potential application as a bone grafting material. The poly(ε-caprolactone)/calcium silicate nanocomposite is a biodegradable one [5-9] while non-degradable ones are poly(methylmethacrylate)/calcium silicate [10, 11], poly(dimethylsiloxane) (PDMS)/calcium silicate [12], PDMS/calcium silicate/titania [13-15], poly(tetrametylene oxide) (PTMO)/calcium silicate [16, 17], PTMO/titania [17], PTMO/calcium silicate/titania [18] nanocomposites. However, inflammations or foreign body reactions were observed to occur *in vivo*.
The purpose of this work is to develop a new bioactive and biocompatible chitosan/calcium silicate nanocomposite, which has a capacity to induce the low crystalline carbonate apatite on its surface in the SBF. A chitosan is known to have excellent biocompatibility, non-toxicity, biodegradability, and inherent wound healing property [19-21] compared to synthetic polymers. However, unfortunately, it does not have an osteoconductivity. In this work, the bioactivity was given to a chitosan by the chemical reaction with the calcium silicates at the molecular level.

2 Materials and testing methods

The chitosan/calcium silicate nanocomposite was prepared by the sol-gel method. The chitosan powder (Kumho Hwasung) was purified three times with a conventional method. Subsequently, it was dissolved in N,N-dimethylformamide (Aldrich) and then reacted with phtalic anhydride (Aldrich) with constant stirring at 120 ^{o}C for twenty four hours under dry Ar atmosphere. The weight ratios of the reactants were chitosan 1, phtalic anhydride 2.8. From now on, it will be referred to as P-Chitosan. The P-Chitosan was collected as precipitates from deionized water and then washed with ethanol using Soxhlet's apparatus to remove unreacted phtalic anhydride at 70 ^{o}C for three days.
The 10g P-Chitosan was reacted with 12.5g 3-isocyanatopropyl triethoxysilane (IPTS; Aldrich) with triethylamine (Aldrich) as a catalyst and dry N,N-dimethylformamide as a solvent. The reaction was carried out at 70 ^{o}C for four hours with constant stirring under dry Ar gas. It was purified via repeated precipitation in cold methanol for three times and then freeze dried for three days. The chitosan modified with silane coupling agent will be referred to as Si-Chitosan from now on.
The Si-Chitosan was dissolved in N,N-dimethylformamide with tetraethyl orthosilicate (Aldrich), and calcium nitrate tetrahydrate (Aldrich) in dry Ar atmosphere. The weight ratio between Si-Chitosan and tetraethyl orthosilicate was 80:20 while the molar ratio between tetraethyl orthosilicate and calcium nitrate tetrahydrate was 1 : 0.15. Then, hydrochloric acid and deionized water were added into the Si-Chitosan/tetraethyl orthosilicate/calcium nitrate tetrahydrate mixture and then reacted for thirty minutes. The molar ratios among tetraethyl orthosilicate, hydrochloric acid, and water were 1 : 0.01 : 12. The hydrolysis following condensation reaction (gelation) was carried out for one week at 70 ^{o}C in a Teflon® mold

covered with a Parafilm® having a few pinholes. Hereafter, the as-prepared specimen will be referred to as CS-Chitosan.
The bioactivity of the CS-Chitosan was assessed by evaluating its capability to form low crystalline carbonate apatite on its surface in the SBF. Non-sterilized disk-shaped specimen 12 mm in diameter by 1 mm in thickness were cut, polished with #400 abrasive, washed with deionized water, dried in vacuum at room temperature, and then soaked in 30 mL of the SBF at 36.5 ^{o}C for one week. After soaking, they were removed from the fluid and gently rinsed with deionized water, and then dried at room temperature.
The change of functional groups during each step of the reactions were analyzed by Fourier transformed infrared spectrometry (FTIR; Nexus, Thermo Nicolet). For FTIR spectroscopy measurements, the pulverized specimens were diluted 150-fold with KBr powder and the background noise was corrected with pure KBr data. The microstructures of the specimens before and after the bioactivity testing were observed by a field emission scanning electron microscopy (FE-SEM; S-4700, Hitachi). The crystal phases present in the specimens before and after the bioactivity testing were analyzed by thin film X-ray diffractometry (TF-XRD; D8 Discover, Bruker) with an angle of 2^{o} to the direction of incident X-ray beam.

3 Results

Figure 1 shows the chitosan/calcium silicate nanocomposite made by the sol-gel reaction. The color was dark brown and showed hard fracture behavior. No phase separations or locally different colors were found in the specimen and it implies that it was a monolith.

Figure 1. Photograph of the as-prepared chitosan/calcium silicate nanocomposite.

Figure 2 shows the FTIR spectra of the pure chitosan and chitosan/calcium silicate nanocomposite. After the sol-gel reaction with Si-Chitosan and tetraethyl orthosilicate with calcium nitrate tetrahydrate under the acidic condition, siloxane linkage and silanol group were newly formed. It implies that the chitosan modified with silane coupling agent was covalently bonded with calcium silicate network through siloxane linkages.
Figure 3 shows the FE-SEM photographs of the chitosan/calcium silicate nanocomposite before and after soaking in the SBF for one week at 36.5^{o}C. Flake like tiny low crystalline carbonate apatite crystals (confirmed by TF-XRD, data now shown here) were observed to

form on the entire surface of the chitosan/calcium silicate nanocomposite. On the contrary, the pure chitosan film did not show low crystalline carbonate apatite forming capacity within the testing period. It means the newly developed chitosan/calcium silicate nanocomposite had bioactivity whereas the pure chitosan had not.

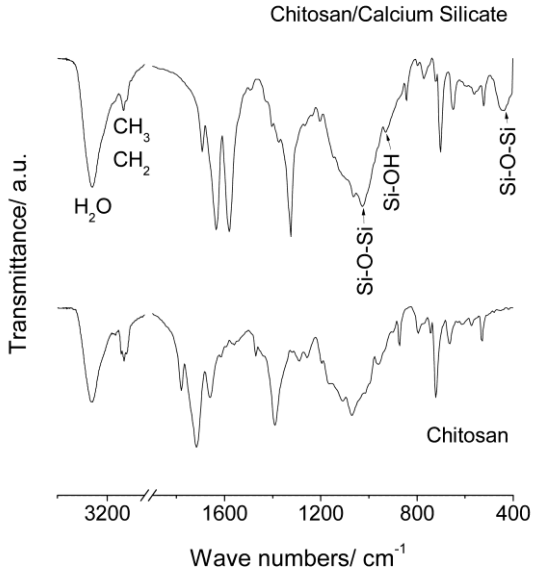

Figure 2. FTIR spectra of the pure chitosan and chitosan/calcium silicate nanocomposite made by sol-gel reaction.

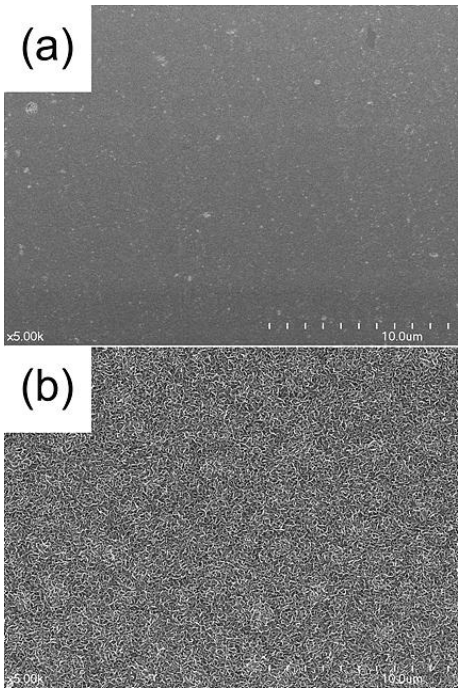

Figure 3. FE-SEM photographs of the chitosan/calcium silicate nanocomposite (a) before and (b) after soaking in the SBF for one week at 36.5°C.

4 Conclusions

A bioactive and biocompatible chitosan/calcium silicate nanocomposite was successfully synthesized through the sol-gel method. The chitosan powder was modified with phtalic anhydride to be dissolved into organic solvent and then modified with silane coupling agent.

Subsequently, it was reacted with tetraethyl orthosilicate with calcium nitrate tetrahydrate through sol-gel reaction. The chitosan/calcium silicate nanocomposite showed hard fracture behavior and showed good low crystalline carbonate apatite forming capability in the SBF. It means it has a good potential for the application as a bone grafting material due to its bioactivity and biocompatibility. Further study will be given to evaluate the exact properties of this newly developed nanocomposite for the biomedical applications.

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