

Organic-Inorganic Hybrids of Hydroxyapatite with Chitosan

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Abstract In tissue engineering, a scaffold helps determine 3-dimensional morphology, increases cell survival, provides initial mechanical stability, supports tissue ingrowth, aids in the formation of tissue structure. Chitosan is the partially deacetylated form of chitin that can be extracted from crustacean. It degrades in the body to non-harmful and non-toxic compounds and has been used in various fields such as nutrition, metal recovery and biomaterials. Hydroxyapatite, a major inorganic component of bone, has been used extensively for biomedical implant applications and bone regeneration due to its bioactive, biodegradable and osteoconductive properties. The application, however, of hydroxyapatite is limited due to own brittleness. Since the natural bone is a composite mainly consisted of organic collagen and inorganic hydroxyapatite, many efforts have been made to modify hydroxyapatite by polymers. In this study, organic/inorganic hybrids were fabricated solid-liquid phase separation and a subsequent freeze-drying process. The microstructure, mechanical properties, and bioactivity of the scaffolds with various contents of hydroxyapatite were studied. The structure of the scaffolds prepared was macroporous and interconnected. The compressive mechanical properties such as compressive modulus and yield strength were improved according to the increase of hydroxyapatite contents mixed with chitosan. After 7 days of sample immersion in a simulated body fluid, for scaffolds containing hydroxyapatite, numerous bonelike apatites were formed on the surfaces of the pore walls. This study suggests that desirable pore structure, mechanical properties, and bioactivity of the hybrid scaffolds might be achieved through controlling the ratio of hydroxyapatite and chitosan.

Introduction

In tissue engineering, a scaffold helps determine 3-dimensional morphology, increases cell survival, provides initial mechanical stability, supports tissue ingrowth, aids in the formation of tissue structure [1]. Chitosan(CTS), made of glucosamine and *N*-acetylglucosamine units linked by 1-4 glycosidic bonds, stemming from the shells of crustaceans and exoskeletons of insects. The biopolymer is comprised of glucosamine and *N*-acetylglucosamine, and the latter is a moiety of glucosaminoglycans. Therefore, the biologic origin of aminopolysaccharide exhibits multiple bioactivities, for example, low toxicity and biocompatibility, biodegradability, antimicrobial, and wound-healing properties [2].

Hydroxyapatite(HA), a major inorganic component of bone, has been used extensively for biomedical implant applications and bone regeneration due to its bioactive, biodegradable and osteoconductive properties [3]. It is available in dense blocks, porous blocks, and granules. The application, however, of HA is limited due to own brittleness. Since the natural bone is a composite mainly consisted of organic collagen and inorganic HA, many efforts have been made to modify HA by polymers, such as collagen, gelatin, chitosan, chitin, a block copolymer of poly-(ethylene glycol) and poly(butylene

terephthalate) (PEG/PBT), polyhydroxybutyrate (PHB), and polylactide (PLA), which have been developed to fix bone fractures. Among these polymers, the biopolymers have received much attention in the field of medical applications, due to their excellent biocompatibility and biodegradability [3].

HA/CTS composite combines their advantages and has gained much attention from biologists and biomaterial scientists [4]. In this study, organic/inorganic hybrid scaffolds were fabricated by solid-liquid phase separation and subsequent sublimation of solvent based on HA. The microstructures and mechanical properties of the scaffolds were controlled by varying content of HA. The bioactivity was evaluated after scaffolds were immersed into SBF during 7 days.

Methods

HA and CTS with minimum 85 % deacetylated were purchased from Sigma-Aldrich. The HA/CTS hybrid scaffolds were prepared by solid-liquid phase separation and a subsequent freeze-drying process. Scaffolds were typically obtained as follows. CTS solutions with concentrations of 2 wt% were prepared by the dissolution of CTS in a 0.2 M acetic acid. The mixture was stirred to obtain a homogeneous polymer solution. The HA powders were then added to the prepared solution to make an inorganic/organic mixture. HA was dispersed in the CTS solution with various concentrations ranged from 10 to 70%. The final composition of the composite foam was determined by the concentration of the CTS solution and HA content in the mixture. The mixture was then rapidly transferred into a freezer at a preset temperature of -70°C to solidify the solvent and induce a solid-liquid phase separation. The solidified mixture was maintained at that temperature for 5 h, and the frozen mixture was then transferred into a freeze dryer. The samples were freeze-dried at 6 mTorr for at least 3 days to remove the solvent. Lyophilized scaffolds were rehydrated and equilibrated in 2 M NaOH solution and then washed with deionized water. The sample treated was freeze-dried again to yield a HA/CTS hybrid scaffold.

The morphological characterization of the scaffold structure was carried out with a SEM (S 800, Hitachi, Japan), and the cross section of scaffolds were previously coated with gold under an argon atmosphere. Mechanical properties of the prepared hybrid disk with 6 mm diameter were determined by a universal testing machine (4465, Instron, USA) with a 5 kN load cell. Compressive modulus was calculated as the slope of the initial linear portion of the stress-strain curve. The yield strength was determined from the cross point of the two tangents on the stress-strain curve around yield point.

SBF with a pH of 7.4 at 36.5°C , buffered with 50 mM tris(hydroxymethyl)-aminomethane and 45 mM hydrochloric acid, was prepared by the dissolution of reagent-grade chemicals in distilled water. The inorganic ion concentrations (mM; Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 148.0, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5) were close to those found in human blood plasma. The specimens of the scaffolds were immersed in conical tube with 30 ml of SBF solution. All the immersions took place at 37°C without stirring. After 7 day exposure periods, the specimens were removed from the SBF solution, rinsed in deionized water, and freezing-dried. The changes of the scaffold before and after 7 days of sample immersion in a SBF were analysed by SEM and X-ray diffractometer (Rint 2000, Rigaku, Japan).

Results

The microstructures of the HA/CTS hybrid scaffold with ratio of 70/30 is shown in Fig. 1 compared to that of pure CTS scaffold. Both CTS scaffolds with and without HA were interconnected with open macropores. The hybrid scaffold showed higher interconnectivity due to its bimodality in pore size; macropores of around $200\ \mu\text{m}$ and a lot of micropores. The surface of the pure CTS scaffold was smooth, whereas the morphology of the hybrid scaffold was rough because of the introduction of HA.

The HA/CTS scaffold exhibited excellent elasticity as shown in Fig. 2. This means that the brittleness of HA scaffold could be improved by combining with CTS.

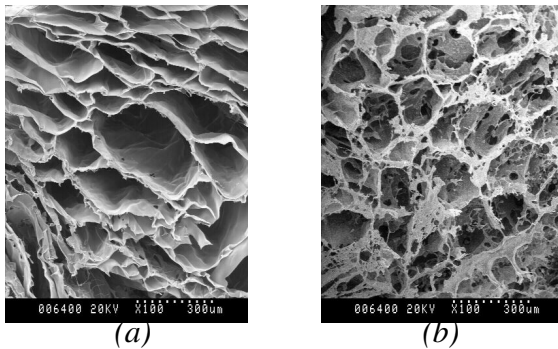


Fig. 1. SEM photos of (a) CTS scaffold and (b) HA/CTS hybrid scaffold with weigh ratio of 70/30.

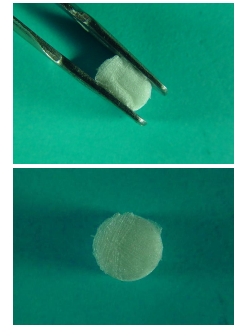


Fig. 2. Elasticity of HA/CTS hybrid.

Fig. 3 shows a series of compression stress-strain curves of HA/CTS hybrid scaffolds with various mixing ratios. The initial linear region defines the compressive modulus of the scaffolds. Pure CTS scaffolds were flexible, but weak. The compressive modulus and the yield strength as a function of the mixing ratio are shown in Fig. 4 and 5. As can be seen, both the compressive modulus and the yield strength were greatly improved with the addition of HA. The compressive modulus was increased ca. 4 times from 0.45 to 1.73 MPa. The yield strength was increased more than 3.5 times from 0.07 to 0.25 MPa.

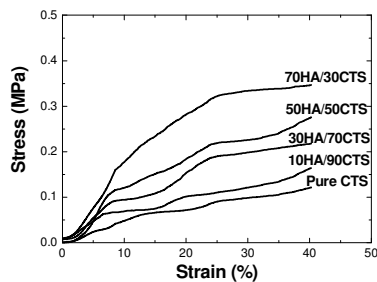


Fig. 3. Stress-strain curves

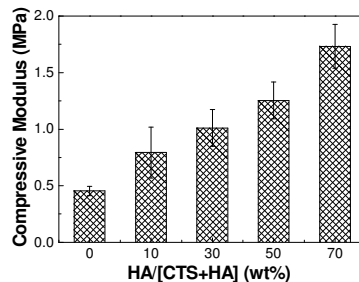


Fig. 4. Compressive modulus

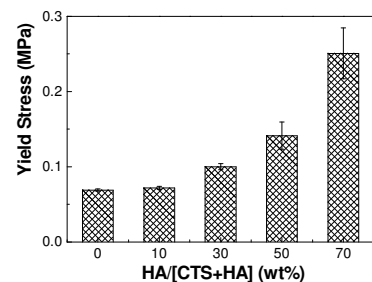


Fig. 5. Yield strength

As seen in Fig. 6, the SBF test of the hybrid scaffolds indicated that the formation of the bonelike apatite layer on the surface of pore walls was observed after 7 days of immersion. The more HA contents were added, the more bonelike apatites were formed.

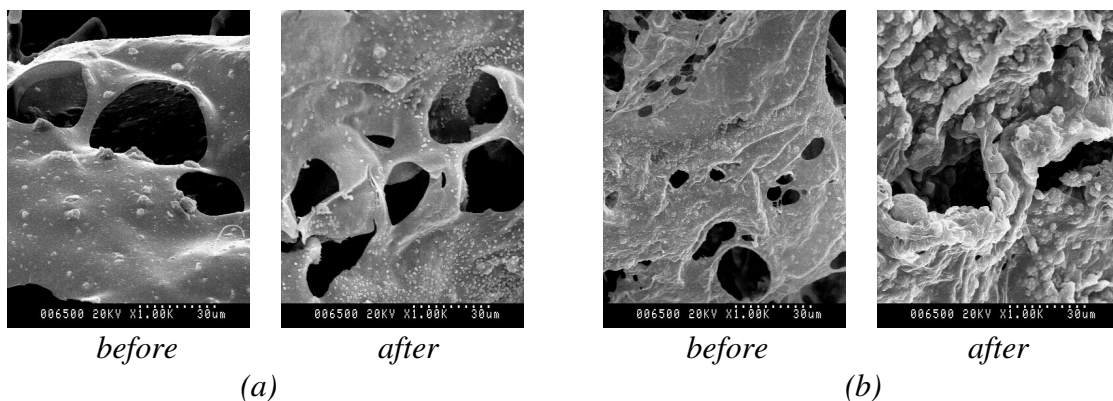


Fig. 6. SEM photo of HA/CTS hybrid scaffolds with weight ratios of (a) 30/70 and (b) 70/30 before and after 7 days of immersion in SBF.

Fig. 7 (a) and (c) show XRD patterns of the hybrid scaffolds with HA content before immersion in SBF. It can be seen from the patterns that the peak at 20.8° (2θ) which represents the CTS crystallinity

becomes flatter with the increase in the HA content. On the contrary, peaks which mean the HA crystallinity gets more narrow and more intensive. It means the addition of the HA powder makes the crystallinity of the CTS matrix lower than before the addition. As seen in both fig. 7 (b) and (d), the intensity of the characteristic peaks for the CTS and the HA after immersion became lower than before immersion, because the apatite mineral layers were on the surfaces of scaffolds after 7 days of immersion.

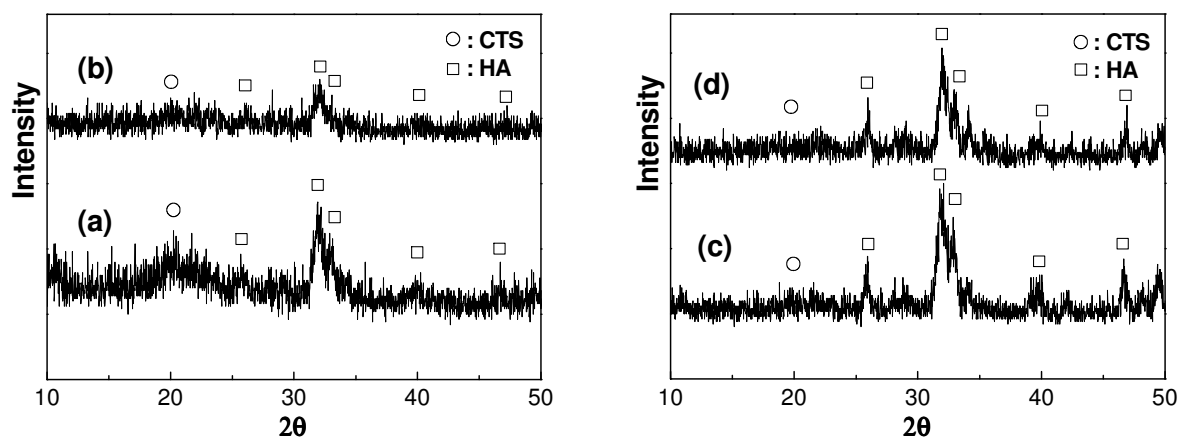


Fig. 7. XRD patterns of HA/CTS hybrid scaffolds with weight ratios of (a) and (b) 30/70, and (c) and (d) 70/30 before and after soaking in SBF

Discussion and Conclusion

In this study, organic/inorganic hybrids were fabricated through controlling the ratio of CTS and HA by solid-liquid phase separation and a subsequent freeze-drying process to combine excellent properties of polymer and bioceramics. The hybrid scaffolds showed a bimodal porous structure with macropores and micropores, which were interconnective and open. The morphology of the hybrid scaffolds was rougher than that of CTS scaffold. The increase of both the compressive modulus and the yield strength of the hybrids were observed with the increase of incorporated HA. The SBF test indicated that as the amount of HA content which was added into the CTS matrix increased, the amount of bondlike apatites which were formed on the surfaces of the hybrids increased. Especially, HA/CTS hybrid scaffold with weight ratio of 70/30 represented not only excellent mechanical properties, but also good bioactivity. The HA/CTS hybrid scaffolds has a potential for use as a tissue substitution materials.

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