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Research Article

Fabrication of Apatite-Hyaluronic Acid Hybrids

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Abstract Apatite-polymer hybrids have been attractive as novel bone-bonding bioactive bone substitutes with mechanical performances analogous to those of natural bone. It is shown that carboxyl group (COOH) can induce the apatite nucleation in an environment of simulated body fluid (SBF). In the present study, we fabricated porous hyaluronic acid gels abundant in COOH group and investigated their apatite formation ability in SBF. Hyaluronic acid has been attractive for bone- and cartilage-repairing materials due to high biological compatibility. We attempted the preparation of bioactive organic-inorganic hybrids from Hyaluronic acid. It has been attractive for bone- and cartilage-repairing materials due to high biological compatibility. It is known that divinyl sulfone is a candidate of cross-linker to bridge the hydroxyl groups. The hydroxyl group of hyaluronic acid side chains were bridged by divinyl sulfone. Porous hyaluronic acid gels stable in aqueous environment were obtained by appropriate cross-linking. They formed the apatite on their surfaces in SBF, when they were priorly treated with $CaCl_2$ aqueous solution. In addition, trehalose, a kind of disaccharide, is found to increase the pore size of the porous hyaluronic acid. This suggests that the pore size and porosity can be controlled by addition of trehalose during fabrication of the scaffolds.

Keywords hydroxyapatite; hyaluronic acid; simulated body fluid (SBF); organic-inorganic hybrid

1 Introduction

Artificial materials are quite attractive for bone repair since problems of bone grafts such as the limit of supplement and contamination with disease factors can be solved. However, artificial materials implanted in the bone defects are generally encapsulated by fibrous collagen tissues and cannot integrate with the surrounding bone tissues. In contrast, several kinds of ceramics are known to directly bond to living bone [1,2]. They are called bioactive ceramics and widely used as important bone substitutes. However fracture toughness of ceramics is lower and elastic modulus is higher than that of natural bone. Organic-inorganic hybrids consisting of organic polymer and the apatite would be attractive as novel bioactive bone substitutes with mechanical performances analogous to those of natural bone.

Bioactive ceramics bond to bone through bone-like apatite layer formed on their surfaces by chemical reaction with body fluid. Biomimetic process inspired by the above mechanism has received much attention for fabricating apatite-polymer hybrids, where the apatite is deposited under ambient conditions on a substrate in simulated body fluid (SBF) or related solutions. As such functional groups, Si-OH, Ti-OH, Zr-OH, Ta-OH, Nb-OH, COOH and PO_4H_2 are known [2]. The apatite formation is significantly accelerated by the release of calcium ions (Ca^{2+}) from the material surfaces into the surrounding solution, since the release of Ca^{2+} increases degree of supersaturation of the surrounding body fluid with respect to the apatite [3].

Apatite-polymer hybrids have been attractive as novel bone-bonding bioactive bone substitutes with mechanical performances analogous to those of the natural bone. In the present study, we fabricated porous hyaluronic acid gels abundant in COOH group and investigated their apatite formation ability in SBF. Figure 1 shows the structure of hyaluronic acid. Hyaluronic acid has been attractive for bone- and cartilage-repairing materials due to high biological compatibility.

2 Materials and methods

2.1 Sample preparation

Powder of hyaluronic acid produced by *Streptococcus* was dissolved in ultrapure water at 1 mass%. Then divinyl sulfone (DVS) was added as a cross-linking agent to the

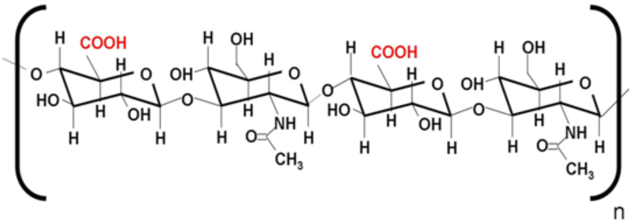

Figure 1: Structure of hyaluronic acid.

DVS content in mass%	10	20	30	40	50
Stability	X	X	X	Δ	O

O: not dissolved
Δ: partially dissolved
X: completely dissolved

Table 1: Dissolution of hyaluronic acid gels obtained by freeze drying after being treated with 1 M-$CaCl_2$.

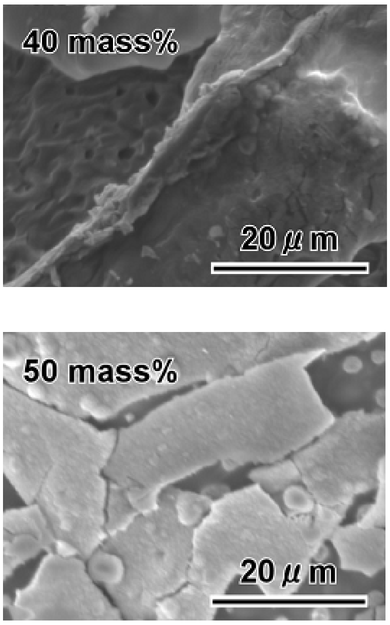

Figure 2: SEM photographs of the surfaces of the porous hyaluronic acid with various DVS contents, which were treated with 1 M-$CaCl_2$ and subsequently soaked in SBF for 7 d.

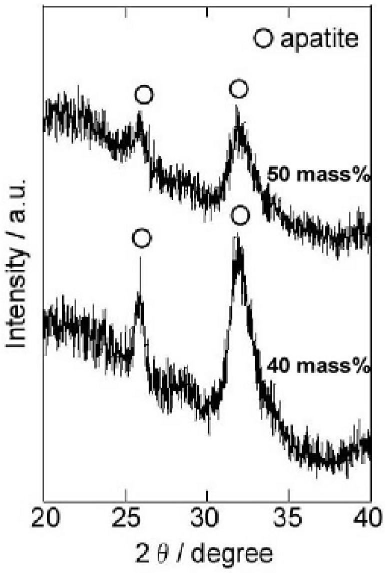

Figure 3: TF-XRD patterns of the surfaces of the porous hyaluronic acid with various DVS contents, which were treated with 1 M-$CaCl_2$ and subsequently soaked in SBF for 7 d.

solution at 10 to 50 mass% against the hyaluronic acid. The solutions were poured into polystyrene container, and dried in a vacuum freeze drier at −80 °C. For some specimens, trehalose was added to the hyaluronic acid solution with (trehalose)/(hyaluronic acid) mass ratio from 0 to 100 mass%.

2.2 Soaking in SBF

The obtained samples were then soaked in 1 M (= $mol{\cdot}m^{-3}$) of $CaCl_2$ aqueous solution at 36.5 °C for 24 h. They were soaked in 30 cm^3 of SBF with inorganic ion concentration nearly equal to those of human blood plasma at 36.5 °C for various periods. Surface structural changes of the specimens after soaking in SBF were analyzed by using scanning electron microscopic (SEM) observation and thin-film X-ray diffraction (TF-XRD).

3 Results and discussion

For all the compositions, porous membranes were obtained after freeze-drying. Table 1 shows the dissolution of obtained hyaluronic acid gels by freeze drying after being treated with 1 M-$CaCl_2$. Dissolution was suppressed by DVS addition at 40 mass% or more to the hyaluronic acid. Figure 2 shows SEM photographs of the surfaces of the porous hyaluronic acid treated with 1 M-$CaCl_2$ and subsequently soaked in SBF for 7 d. Spherical particles formation was detected on all the hybrids after the soaking. Figure 3 shows TF-XRD patterns of the surfaces of the porous hyaluronic acid treated with 1 M-$CaCl_2$ and soaked in SBF for 7 d. They gave broad peaks assigned to hydroxyapatite with low crystallinity at 26 ° and 32 ° in 2θ. This means that the spherical deposits observed under SEM are low-crystalline apatite. Figure 4 shows SEM photographs of the surfaces of the porous hyaluronic acid as a function of trehalose content. We can see that the pore size has a tendency to increase with increasing trehalose content.

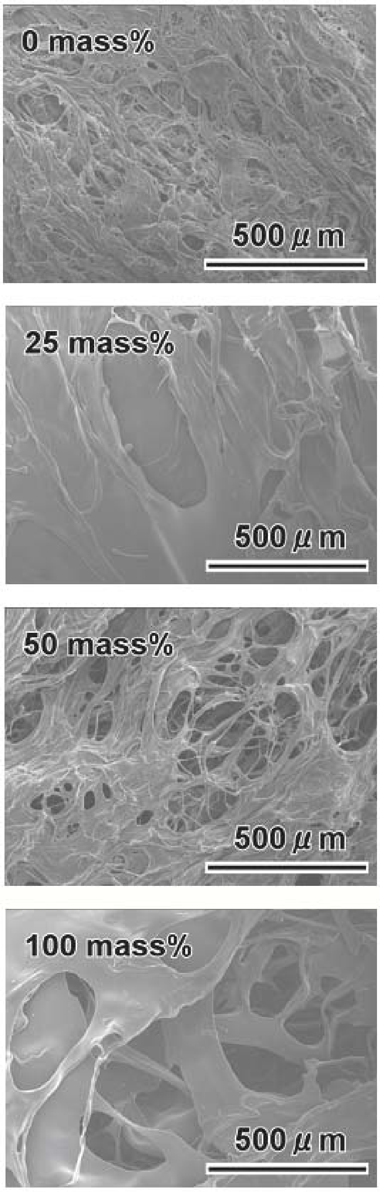

Figure 4: SEM photographs of the surfaces of the porous hyarulonic acid as a function of (trehalose)/(hyaluronic acid) mass ratio.

The results in Figures 2 and 3 indicate that the porous hyaluronic acid can be fabricated through covalent cross-linking with DVS and that they form the apatite on their surfaces after soaking in SBF, when they were priorly treated with $CaCl_2$ solution. These hybrids are expected to be useful not only bone substitutes but also scaffolds for bone tissue engineering, since hyaluronic acid is easily fabricated into meshes and sponges.

In addition, trehalose addition affects the pore size of the porous hyaluronic acid. The trehalose is known to increase the mean particle size of the ice during freezing [4]. The pore size would be changed according to the change in ice growth rate. The results mean that the pore size of the polymer scaffolds can be appropriately controlled by the addition of the trehalose.

4 Conclusions

Porous hyaluronic acid gels stable in aqueous environment were obtained by appropriate cross-linking. They formed the apatite on their surfaces in SBF, when they were priorly treated with $CaCl_2$ aqueous solution. It was also found that the pore size of the porous hyaluronic acid can be controlled by the addition of the trehalose The obtained hybrids are believed to show bone-bonding ability in the living body.

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