Apatite Deposition on Hyaluronic Acid Gels in Biomimetic Conditions

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Abstract Bioactive ceramics are used for bone-repairing owing to attractive features such as direct bone-bonding in living body, because they have the ability to bond directly to bone. However, there is limitation on clinical applications due to their inappropriate mechanical properties performances such as higher brittleness and lower fracture toughness than 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. We attempted preparation of apatite-polymer hybrids from hyaluronic acid. It has been attractive for bone- and cartilage-repairing materials due to high biological compatibility. The hydroxyl groups in side chains of the hyaluronic acid were bridged by divinylsulfone. Dense films were obtained after drying at ambient pressure, whereas porous specimens were formed by freeze-drying. They were treated with 1 M-CaCl₂ aqueous solution for 24 h. Ability of apatite formation on the specimens was examined in vitro using simulated body fluid (SBF, Kokubo solution). The specimens formed the apatite in SBF within 7 d, when they were previously treated with CaCl₂ solution.

Key words: Hyaluronic acid, Organic-inorganic hybrids, SBF, Apatite, Biomimetic process, Bioactivity

1. INTRODUCTION
The bone is an important organ that supports our bodies, protects internal organs and enables smooth movement. Our bone is sometimes damaged due to disease, accidents and aging. Therefore bone diseases can remarkably decline our quality of life (QOL). Bone graft such as autograft and allograft has been used. However they have problems such as limit of supplement or contamination with disease factors. Therefore development of artificial materials suitable for bone repair is desired. However, artificial materials implanted into bone defect are generally encapsulated with a fibrous tissue of collagen.

Bioactive ceramics have been studied extensively for clinical applications including bone repair, because they have the ability to bond directly to bone [1]. However, there is limitation on clinical applications due to their inappropriate mechanical properties performances such as higher brittleness and lower fracture toughness than natural bone. Therefore 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 abundant in functional groups able to deposit the apatite in simulated body fluid (SBF) or related solutions [2-4]. As such functional groups, Si-OH [5], Ti-OH [6], Zr-OH [7], Ta-OH [8], Nb-OH [9], COOH [10] and PO₄H₂ [10-11] are known. The apatite formation is significantly accelerated by the release of calcium ions (Ca²⁺) from the material surfaces into the surrounding solution, since the release of Ca²⁺ increases degree of supersaturation of the surrounding body fluid with respect to the apatite [5].

In the present study, we fabricated porous hyaluronic acid (Hya) gels abundant in COOH group and investigated their apatite formation ability in SBF. Figure 1 shows structure of hyaluronic acid. Hyaluronic acid has been attractive for bone- and cartilage-repairing materials due to high biological compatibility [12].
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2. MATERIALS AND METHODS

Powder of hyaluronic acid produced by *Streptococcus* was dissolved in ultrapure water at 1 mass%. Then divinylsulfone (DVS) was added as a cross-linking agent to the 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 or ambient pressure at 40°C.

The obtained samples were then soaked in 1 M (=kmol·m⁻³) of CaCl₂ aqueous solution at 36.5°C for 24 h, and subsequently in SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0 and SO₄²⁻ 0.5 mM) at 36.5°C for 7 d. 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

Dense membranes were obtained after dried by ambient pressure, whereas porous membranes were obtained after freeze-drying. Table 1 shows dissolution behavior of the hyaluronic acid gels obtained by freeze drying after treated with 1M-CaCl₂. Complete dissolution in CaCl₂ solution was suppressed when DVS content was 40 mass% or more. Therefore the apatite formation in SBF was examined for the specimens with DVS content of 40 mass% or more.

Table 1. Dissolution behavior of hyaluronic acid gels obtained by freeze drying of after treated with 1M-CaCl₂

<table>
<thead>
<tr>
<th>DVS/Hya / mass%</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>O: Not dissolved, : Partially dissolved, X: Completely dissolved</td>
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</table>

Figure 2 shows SEM photographs of the surfaces of the freeze dried hyaluronic acid gels with various DVS content, which were treated with 1M-CaCl₂ and subsequently soaked in SBF for 7 d. Spherical particles formation was detected on all the hybrids after soaking in SBF for 7 d. Figure 3 shows TF-XRD patterns of the surfaces of the freeze dried hyaluronic acid gels.

![Fig. 2 SEM photographs of the surfaces of the freeze dried hyaluronic acid gels with various DVS content, which were treated with 1M-CaCl₂ and subsequently soaked in SBF for 7 d.](image)
which were treated with 1M-CaCl₂ and subsequently soaked in SBF for 7 d. The TF-XRD patterns of the samples 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.

4. DISCUSSION

We can see that organic-inorganic hybrids stable in aqueous solutions can be obtained at appropriate DVS content. This means that cross-linking is essential for preparation of scaffolds which are gradually resorbed in body environment.

The results in Figs. 2 and 3 indicate that the prepared hyaluronic acid gels form the apatite on their surfaces after soaking in SBF, when they were priorly treated with CaCl₂ solution. COOH groups would act as heterogeneous nucleation site of the apatite. On the other hand, Ca²⁺ ions incorporated into the gels by the CaCl₂ treatment would release from the gels into SSP, and enhance the apatite nucleation by increasing degree of supersaturation of the surrounding fluid with respect to the apatite. The apatite formation on the specimens without CaCl₂ treatment is not examined in this study. However the apatite formation ability would be significantly enhanced by the treatment, judging from the previous result that shows validity of the CaCl₂ treatment for the apatite formation on polyglutamic acid hydrogels abundant in COOH group in SBF [13].

The specimen cross-linked with 50 mass% of DVS formed a little finer apatite particles than that cross-linked with 40 mass% of DVS (See Fig. 2). It is known that sulfonic groups also have apatite nucleation ability in body environment [14]. Therefore the sulfonyl group in DVS would also play a role of site of the apatite nucleation to give fine apatite particles.

5. CONCLUSION

Hyaluronic acid gels were obtained by appropriate cross-linking. They formed the apatite on their surfaces in SBF, when they were priorly treated with CaCl₂ aqueous 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.

References


Fig. 3 TF-XRD patterns of the freeze dried hyaluronic acid gels, which were treated with 1M-CaCl₂ and subsequently soaked in SBF for 7 d.

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