# Development of a Bone-Repairing Material Through Hybridization of Biodegradable Polymer with Bone-like Apatite

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Abstract: Hybrids consisting of biodegradable polysaccharide scaffold coated with bone-like apatite are expected to show both biodegradability and bone-bonding ability, i.e. bioactivity. This study is focused on a coating method of bone-like apatite on alginate. In this study, we proposed modification of alginate with silanol groups (Si-OH), followed by a treatment with calcium chloride solution. The modified alginate gel was successfully coated with bone-like apatite in a biomimicking solution to body fluid. Bone-like apatite layer was successfully coated on the surface of the modified alginate. This technique enables us to produce the hybrids consisting polymer and bone-like apatite with biodegradability and bioactivity for bone repair.

Keywords: apatite, biodegradability, alginate

## Introduction

Hybrids consisting of bone-like apatite and biodegradable organic polymers, such as polysaccharides and proteins, are attractive materials for bone repairing, since they are expected to show both bone-bonding ability, i.e. bioactivity and mechanical properties similar to those of natural bone. Kokubo *et al*. proposed a biomimetic process using a simulated body fluid (SBF)[1,2] to coat a hydroxyapatite layer onto organic substrates.[3] This process can produce a polymer substrate covered with a so-called bone-like apatite, which contains carbonate ions and show low crystallinity. Such bone-like apatite shows high biological affinity when implanted into bony defects and can achieve tight bonding to living bone. In the biomimetic process using SBF, both the existence of a specific functional group effective for heterogeneous nucleation of apatite, and an increased concentration of calcium ions in the surrounding fluid are needed. Previous studies reported that carboxyl (COOH)[4] and silanol (Si-OH)[5] groups can provide heterogeneous nucleation sites for apatite.

Alginate is known as a biodegradable polymer rich in carboxyl groups. An alginate gel crosslinked covalently with ethylenediamine (EDA) is applicable to artificial skin and drug delivery systems[6], and therefore regarded as a candidate of scaffold materials for tissue regeneration. In this study, novel process for fabrication of alginate scaffold coated with bone-like apatite layer. Calcium ions were incorporated into an alginate gel cross-linked covalently with EDA, and the apatite deposition on the scaffold was conducted in SBF or 1.5SBF, which has 1.5 times the ion concentrations of SBF. Incorporation of silanol groups and calcium ions into alginate was also attempted to examine its effect on apatite deposition in order to investigate the fundamental conditions for fabrication of apatite-alginate hybrids.

## Materials and methods

A 1% sodium alginate solution was mixed with ethylenediamine (EDA) and/or 3-aminopropyltriethoxysilane (APES), which is a silane coupling agent. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC×HCl) and N-hydroxysuccinimide (HOSu) were used for promotion of the dehydration condensation between carboxyl groups in alginate and amino groups in the reactants. The reagent concentrations for synthesis of alginate gels are given in Table 1. The mixed solutions were kept in polypropylene containers at room temperature to form gels. After gel formation, gels were washed with an aqueous solution with 143 mmol/L of sodium chloride and 2.5 mmol/L of calcium chloride. The gels were then washed with ultrapure water and subsequently freeze dried. Rectangular specimens with dimensions of about 10 ´ 10 ´ 4 $mm^3$ were cut from the dried gels. The dried gels were soaked in a 1 mol/L $CaCl_2$ solution at 36.5 °C for 24 hours, and then gently washed with ultrapure water. The specimens were then soaked in SBF or 1.5SBF, whose concentrations are given in Table 2, at 36.5 °C. After soaking for 7days, the specimens were rinsed with ultra-pure water. Specimens before and after soaking in SBF or 1.5SBF were observed under a scanning electron microscope (SEM) equipped with an energy dispersive X-ray microanalyzer (EDX). The surfaces of the specimens were characterized using thin-film X-ray diffraction (TF-XRD).

## Results and discussion

All the above-mentioned modifications for alginate induced the formation of stable gels. It has been revealed that alginate modified with EDA jellifies due to the cross-links produced by EDA. The alginate modified with only APES also formed a gel. It is considered that the APES bonded to alginate were hydrolyzed and the resultant silanol groups were condensed with those derived from other bonded APES molecules. These results indicate that APES was combined to alginate. Figure 1 shows the TF-XRD patterns of the surfaces of the samples after soaking in SBF or 1.5SBF for 7 days. The bone-like apatite was formed on AG-S in SBF and 1.5SBF, and on AG-ES in 1.5SBF. However, it was not formed on the AG-ES in SBF and on AG-E in SBF and 1.5SBF. Apatite-forming ability was induced on the alginate gels by incorporation of APES. This is caused by induction of heterogeneous nucleation of apatite by silanol groups derived from APES. It was found from EDX results that the Si content in AG-S were lager than AG-ES. This suggested that AG-S contained

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larger amount of silanol groups than AG-ES and this gave higher apatite-forming ability of AG-S than that of AG-ES. Figure 2 shows SEM photographs of a cross-section of AG-S soaked in $CaCl_2$ solution followed by soaking in SBF for 7 days. Apatite covered not only the top surface but also the inside of AG-S. Modification of alginate with silanol groups and calcium ions can induce apatite deposition both on its surface and within its structure in a biomimetic solution such as SBF or 1.5SBF. These findings support the proposition of using a biomimetic process for the fabrication of organic polymers coated with hydroxyapatite, using a polyuronic acid as a substrate for the deposition of bone-like apatite crystals.

## References


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Table 1 Concentrations of reagents in alginate solution for synthesis of alginate gels

| Notation | Concentration / mol·L$^{-1}$ | | | |
|---|---|---|---|---|
| | APES | EDA | EDC·HCl | HOSu |
| AG-E | 0 | $0.75\times10^{-2}$ | $0.98\times10^{-1}$ | $1.5\times10^{-2}$ |
| AG-ES | $1.5\times10^{-2}$ | $0.75\times10^{-2}$ | $1.95\times10^{-1}$ | $1.5\times10^{-2}$ |
| AG-S | $1.5\times10^{-2}$ | 0 | $0.98\times10^{-1}$ | $1.5\times10^{-2}$ |

APES: 3-Aminopropyltriethoxysilane
EDA: Ethylenediamine
EDC·HCl: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride
HOSu: N-Hydroxysuccinimide

Table 2 Ion concentrations of simulated body fluid (SBF), 1.5SBF and human blood plasma

| Ion | Concentration / mmol·L$^{-1}$ | | |
|---|---|---|---|
| | SBF | 1.5SBF | Plasma |
| $Na^+$ | 142.0 | 213.0 | 142.0 |
| $K^+$ | 5.0 | 7.5 | 5.0 |
| $Mg^{2+}$ | 1.5 | 2.3 | 1.5 |
| $Ca^{2+}$ | 2.5 | 3.8 | 2.5 |
| $Cl^-$ | 147.8 | 221.7 | 103.0 |
| $HCO_3^-$ | 4.2 | 6.3 | 27.0 |
| $HPO_4^{2-}$ | 1.0 | 1.5 | 1.0 |
| $SO_4^{2-}$ | 0.5 | 0.8 | 0.5 |

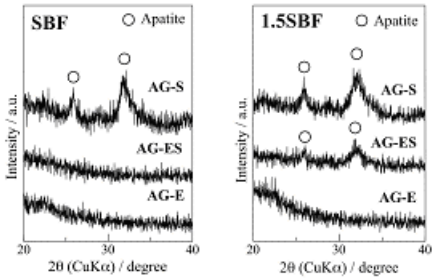


Fig. 1 TF-XRD patterns of the surfaces of gels soaked in $CaCl_2$ solution followed by soaking in SBF or 1.5SBF for 7 days.

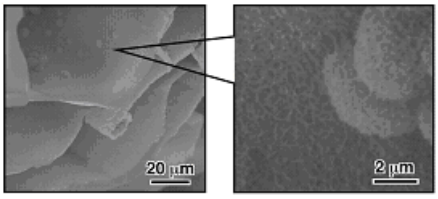


Fig. 2 SEM photographs of a cross-section of AG-S soaked in $CaCl_2$ solution followed by soaking in SBF for 7 days.