

# Preparation of polymer-silicate hybrid materials bearing silanol groups and the apatite formation on/in the hybrid materials

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## Summary

The aim of this work is to develop a basic approach to the preparation of biologically active polymer-apatite composites with various mechanical properties. To this end, a type of hybrid materials bearing silanol groups were prepared from triethoxysilyl-terminated poly(oxyethylene) (PEG) and tetraethoxysilane (TEOS) by using the in-situ sol-gel process. The hybrid materials having different PEG contents were subjected to the biomimetic process for forming the bone-like apatite layer on/in the hybrid materials. It was found that a dense bone-like apatite layer can be prepared in an arbitrary thickness on/in the hybrid materials, indicating that the silanol groups introduced provide the effective sites for the apatite nucleation and formation. The polymer-apatite composites thus obtained are expected to have a great potential in the biomedical applications.

## Introduction

It has been established that the essential condition for artificial materials to bond to living bone is the formation of a biologically active bone-like apatite layer on their surfaces in the living body<sup>1</sup>. So far, various types of bioactive ceramics have been developed and used under rather loaded sites such as vertebrae and intervertebral discs<sup>2-5</sup>. However, the fracture toughness of the ceramics is lower and the elastic modulus is much higher than those of the natural bone. The bioactive materials with the mechanical properties analogous to those of the natural bone are desirable for development.

The organic polymer-apatite composite materials are of practical interest as a candidate for the bone-repairing material, since they are expected to exhibit the mechanical properties analogous to those of natural bone. In addition, they might be also useful even as soft tissue-repairing materials because they exhibit ductility as well as high compatibility even with soft tissues<sup>6</sup>.

Recently, we have developed a biomimetic process for forming a biologically active apatite layer of the desired thickness on the organic substrates at normal

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temperature and pressure<sup>7</sup>. We also suggested that the silanol groups and hydrated silica of the surface of the substrates provide the favorable sites for the apatite nucleation<sup>8</sup>.

In the present study, a type of polymer-silicate hybrid materials having silanol groups were prepared from triethoxysilyl-terminated poly(oxyethylene) (PEG) and tetraethoxysilane (TEOS) by using the in-situ sol-gel process<sup>9,10</sup> and then the apatite formation on/in the hybrid materials was investigated by using the biomimetic process<sup>7</sup>.

## Experimental

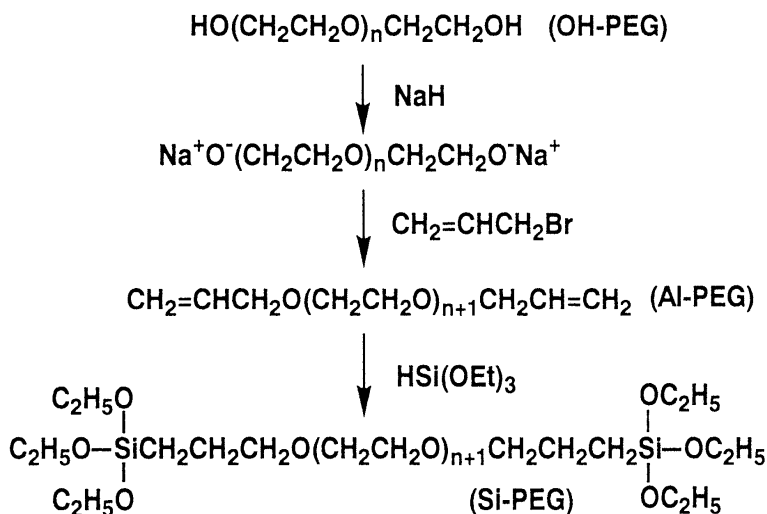
### Materials

Tetraethoxysilane (TEOS) and triethoxysilane (TrEOS) were obtained from Shin-Etsu Chem. Co. and used as received. A di(hydroxy) poly(oxyethylene)(OH-PEG) having a molecular weight of 600 was purchased from Tokyo Kasei Co.

The reagents used as solvents were refined, respectively, according to the relevant procedures. Sodium hydride(50% in paraffin) was obtained from Nacalai Tesque and refined with hexane before use. Distilled water was used throughout the experiments. All other reagents were used without further purification.

### Preparation of Triethoxysilyl-Terminated PEG

Triethoxysilyl-terminated PEG(Si-PEG) was prepared via allyl-group terminated PEG(Al-PEG) according to the method of Kohjiya et al<sup>9,11</sup>. The preparation reactions are shown in Scheme 1.



**Reaction Scheme 1**

The disodium salt of PEG was prepared at room temperature in dry tetrahydrofuran (THF) with an excess of sodium hydride under nitrogen. To this solution, allyl bromide was added and reacted at 70°C for 5h. Hydrosilylation of Al-PEG by TrEOS was carried out in toluene at 90°C for 48h using hexachloroplatinic acid ( $\text{H}_2\text{PtCl}_6(\text{IV}) \cdot 6\text{H}_2\text{O}$ ) as a catalyst (Speier's catalyst)<sup>9</sup>. The degree of reactions was estimated by <sup>1</sup>H-NMR.

### Preparation of Hybrid Gels

Reaction conditions are listed in Table 1. An appropriate amount of Si-PEG and TEOS was dissolved in ethanol, and 0.5M hydrochloric acid was added, followed by refluxing the reaction mixture at 90°C for 1h. Then, the resulted viscous solution was poured into a Teflon mold and allowed to evaporate the solvents at 60°C for 24h. During evaporation of solvents, the sol-gel reaction was completed, and a transparent sample film was obtained. The infrared (IR) spectra were taken on a Jasco FT/IR 8000 spectrometer.

Table 1. Reaction and Characterization Data of Hybrid Gel Films<sup>a)</sup>

Sample code	Si-PEG (g)	TEOS (g)	EtOH (mL)	PEG/SiO <sub>2</sub> <sup>b)</sup> (wt/wt)	Appearance of film <sup>c)</sup>
A	0.85	0.45	3.4	3/7	Transparent
B	0.25	0.5	4.2	5/5	Transparent
C	0.50	0.3	3.4	7/3	Transparent

a) 1ml of 0.5M HQ was added as catalyst for each run.

Reaction conditions: reaction time = 1h; temperature = 90°C

b) Weight ratio of PEG/SiO<sub>2</sub> in the hybrid gel.

c) Appearance of films after evaporation of solvents. See text for further details.

### Apatite formation on/in Hybrid Gels

The biomimetic process was performed according to the method which we described in an earlier article<sup>7</sup>.

Simulated body fluid (SBF) with the inorganic ion concentrations ( $\text{Na}^+$  142,  $\text{K}^+$  5.0,  $\text{Mg}^{2+}$  1.5,  $\text{Ca}^{2+}$  2.5,  $\text{Cl}^-$  148,  $\text{HCO}_3^-$  4.2,  $\text{HPO}_4^{2-}$  1.0,  $\text{SO}_4^{2-}$  0.5 in mM) nearly equal to that of human blood plasma and 1.5SBF with the ion concentrations 1.5 times those of SBF were prepared by dissolving NaCl, NaHCO<sub>3</sub>, KCl, K<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O, MgCl<sub>2</sub> · 6H<sub>2</sub>O, CaCl<sub>2</sub> and Na<sub>2</sub>SO<sub>4</sub> in distilled water and buffered at pH 7.4 at 36.5°C with tris(hydroxymethyl)aminomethane [(CH<sub>2</sub>OH)<sub>3</sub>CNH<sub>2</sub>] and 1M-hydrochloric acid (HCl).

The sample films were soaked for various periods at 36.5°C in the SBF and 1.5 SBF, which were replaced every 2 days.

### *Analysis of apatite*

The surfaces of the substrates were analyzed by thin-film X-ray diffraction with Rint-1400 (Rigaku Co., Tokyo, Japan). The surface and a cross section of the specimens were coated with gold-palladium film and observed under a scanning electron microscope (SEM) attached with Energy dispersive X-ray analyzer (EDX) (Hitachi Co. Ltd., S-2500CX Tokyo, Japan).

## **Results and discussion**

### *Preparation of hybrid gels*

Among the methods for introducing the silanol groups into polymers, hydrosilylation is more favorable because of the formation of Si-C bonds, and not Si-O-C bonds that are subject to hydrolytic cleavage. Furthermore, taking into consideration the reaction conditions of the biomimetic process for forming the bone-like apatite layer in/or polymer gels, hydrophilic polymers have an advantage as an organic polymer component. In this work, we prepared di(allyloxy) poly(oxyethylene) (Al-PEG) from di(hydroxy) poly(oxyethylene) (OH-PEG), and then the hydrosilylation of Al-PEG with TrEOS was adopted to prepare the hydrophilic telechelic prepolymers in which silanol groups are coupled via Si-C bonds.

The sample Al-PEG was obtained with a yield of 75% and characterized by NMR and IR spectra. Figure 1 shows the <sup>1</sup>H-NMR spectrum of Al-PEG. Allylic protons a, b and c appear at around 4.0, 5.9 and 5.2ppm, respectively, showing that an almost quantitative conversion was achieved from hydroxyl to allyl groups.

Figure 2 shows the <sup>1</sup>H-NMR spectrum of the sample Si-PEG. It can be seen that allylic protons in Al-PEG disappeared, and the protons on ethoxy group appeared at around 1.2ppm (triplet, methyl) and 3.8ppm (quartet, methylene), being in agreement with the expected structure. The yield of Si-PEG was 70%. The IR spectra of Al-PEG and Si-PEG were in accord with the NMR results.

Next, we attempted to prepare hybrid gels from Si-PEG and TEOS at the molecular level by using the in-situ sol-gel process<sup>9,10</sup>. The reaction conditions for the preparation of hybrid materials are shown in Table 1. The resulting reaction mixtures were transparent and it was possible to pour the reaction mixture into a Teflon mold. The sample films were obtained by evaporating the solvents. All the sample films obtained were transparent and flexible, but the sample A having a high Si content was less flexible with slight cracks. The strength of the sample films obtained suggests that the sol-gel reaction (that is, the hydrolysis and condensation of TEOS and Si-PEG) was completed during the evaporation of solvent. In fact, the presence of Si-OH (silanol) groups was confirmed from IR spectra of the hybrid gel films obtained. The absorption bands characteristic of

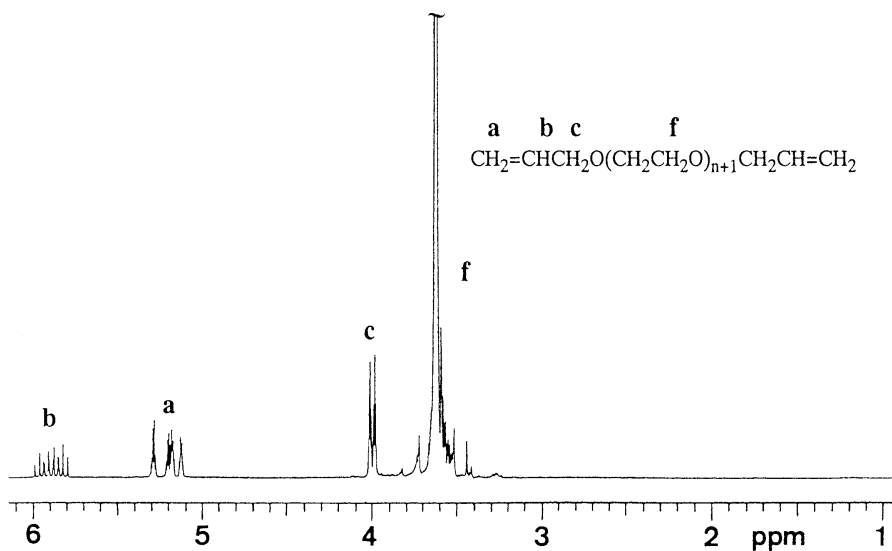


Figure 1.  $^1\text{H}$ -NMR spectrum of Allyloxy-terminated PEG in  $\text{CDCl}_3$

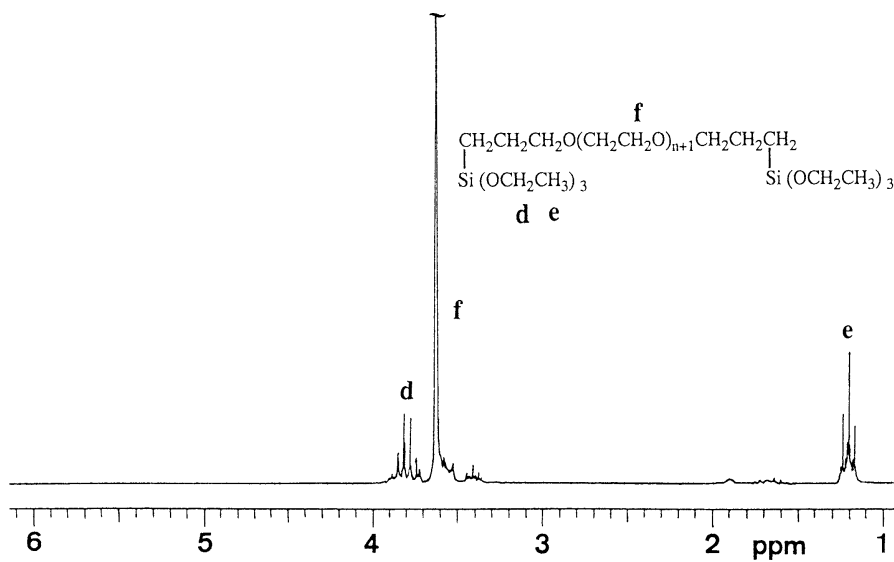


Figure 2.  $^1\text{H}$ -NMR spectrum of Triethoxysilyl-terminated PEG in  $\text{CDCl}_3$

Si-OH groups appear at around 840 and 3700  $\text{cm}^{-1}$ .

The resulted silanol groups are expected to act as the effective sites for the apatite nucleation in the subsequent biomimetic process. The presence of active silanol groups was also proved by the formation of apatite layer as described later.

Last to be mentioned here is the reactivity of triethoxysilyl groups at the PEG chain ends. In order to examine whether Si-PEG was truly incorporated into the network formed by TEOS, the solvent extraction of the hybrid materials was carried out using THF. Little weight loss was observed for all the sample materials, showing that triethoxysilyl groups at the PEG chain ends participated in the network formation during the in-situ sol-gel process.

### *Apatite coating*

At first, hybrid gels prepared as above were soaked in SBF solution at 36.5°C. It was found that the rate of apatite formation was too slow to examine the structure of apatite layers formed. In the present study, the apatite coating on the hybrid gels was carried out using 1.5 SBF solution.

Figure 3 shows the SEM photographs of the surfaces of the sample A subjected to the biomimetic treatment for 2 weeks. It can be seen that some materials are deposited densely on the polymer substrate. These deposited materials were identified as an apatite by thin-film X-ray diffraction. To be emphasized here is that the pretreatment for the apatite nucleation on the polymer substrates<sup>7)</sup> is not necessary for the silanol-containing polymer substrates prepared in this work.

The apatite layer formed by the present biomimetic process has an almost identical structure to the biologically active bone-like apatite layer which is formed on the surfaces of bioactive ceramics in vivo and is responsible for bonding to the living bone. Thus, it is expected that the apatite layer formed could exhibit very high bioactivity. Furthermore, the organic polymer-apatite composites can be prepared to process the mechanical property in a wide range from that analogous to the soft tissues to that analogous to the hard tissues, by varying on the volume ratio of the polymer apatite and overall three-dimensional structure of the composite. This indicates that the organic polymer-apatite composites obtained are also expected to be highly useful as bone-repairing materials as well as soft-tissue-repairing materials.

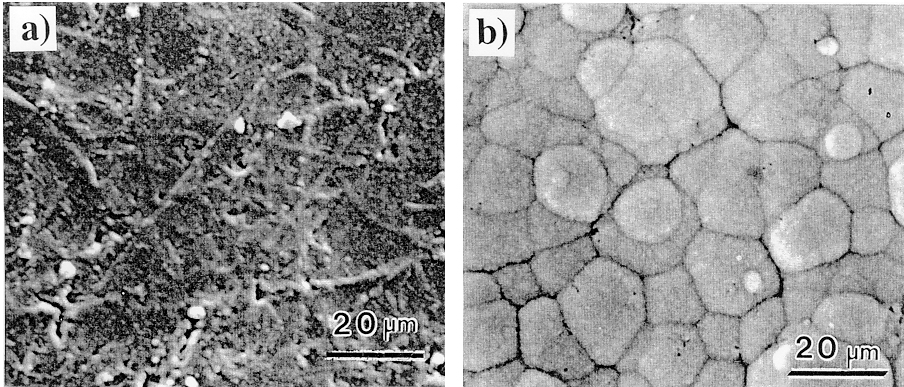


Figure 3. SEM photographs of the surfaces of the hybrid gel films prepared from the sample A. (a) before and (b) after soaking in 1.5SBF

Figures 4 and 5 show the SEM photograph of the cross section of the polymer-apatite composite prepared from the sample C and the distributions of Ca, P and Si elements in the composite, respectively. It can be seen that Ca and P elements are densely distributed on the surface of the polymer substrate film, but the apatites are formed even in the inner part of the polymer substrate film. These results are very attractive because they suggest that it is possible to prepare the polymer-apatite composites having morphology gradient by varying the structure of polymer substrates.

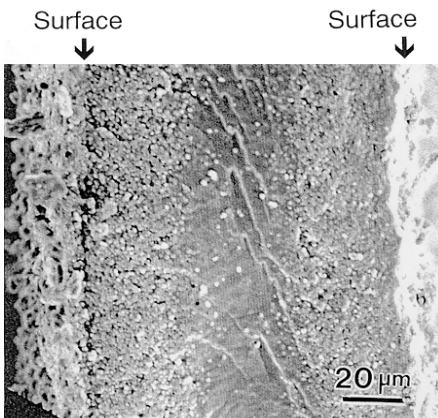


Figure 4. SEM photograph of the cross section of the specimen

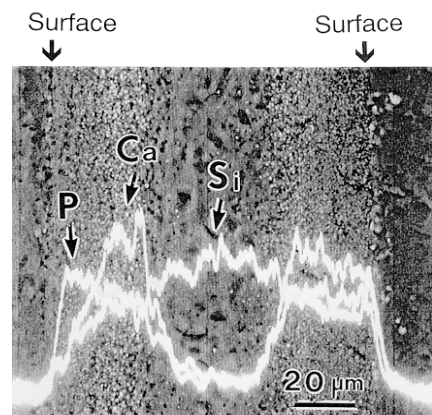


Figure 5. EDX profile on the section of the specimen

The adhesive strength of the apatite layer to the polymer substrates is one of the important factors for the practical applications. Such a study is currently under investigation. The details of these results will be reported in a subsequent paper.<sup>12)</sup>

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