

Nucleation of Hydroxyapatite on an Inert Polymer Surface by Covalent Linkage

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Previously, it has been believed that an inert polymer surface, *i.e.* silicone, cannot nucleate hydroxyapatite(HAp) due to having a low Lewis acid-base surface tension.¹ We propose a novel method for HAp nucleation on an inert polymer surface by covalent bonding. Initially, acrylic acid grafted silicone sheet and sintered spherical HAp particles with average diameter of 2.0 μm having amino groups were prepared. After formation of ammonium ionic bonds between both samples, they were reacted at 180 °C for 6h in vacuum to form covalent bonds. The HAp particles can be regarded as nuclei that bond to the carboxylated polymer surface via an amide bond. Furthermore, it is expected that a higher adhesive strength between HAp layer and a polymer surface be obtained.

Key words: hydroxyapatite, particle, silicone, surface modification, covalent linkage

INTRODUCTION

Hydroxyapatite(HAp)/silicone composites have been developed in many medical applications as reconstructive, and prosthetic materials for bone or soft tissue due to its osteoconductivity, elasticity and moldability. Wu *et al.* demonstrated that higher hydrophobic surfaces could not nucleate HAp due to low lewis acid-base surface tensions.¹ It has been therefore believed that an inert polymer surface, *i.e.* silicone, cannot act as nucleators.

This paper details a novel method for HAp-layer formation on silicone possessing an inert surface. The HAp layer formation was performed through the creation of covalent linkage using amino group modified HAp particles and a silicone substrate grafted polymer containing carboxyl groups. We can expect to not only develop a new technique for HAp nucleation on an inert

polymer surface but also generate a more stable composite consisting of a HAp layer and a polymer substrate bonded with a covalent linkage.

MATERIALS AND METHODS

Materials

Silicone sheet (0.3mm thickness, Sin-Etsu Polymer Co., Tokyo, Japan) was purified using a soxhlet extractor with methanol for 24h. Acrylic acid (AAc, Nakarai Tesque, Kyoto, Japan) was purified by vacuum distillation. Sintered hydroxyapatite particles with an average diameter of 2.0 μm made by Asahi Pentax Co. Ltd. used without further purification. γ -Aminopropyltriethoxysilane (γ -APS, Shin-Etsu Chem. Ind. Co., Tokyo, Japan) was used as received.

Measurement

Fourier transform infrared spectrometer (FT-IR)

spectra were recorded by Spectrum 2000, Perkin Elmer, USA. The composite was observed using SEM-- ABT-32, Topcon Co., Tokyo, Japan -- operating at 15 kV.

Surface treatment of HAp particles

5.0 g of HAp particles after drying at 120 °C for 24h were added into 250 ml of anhydrous toluene under nitrogen atmosphere in 300 ml of three-neck flask with a reflux condenser. 5.0 ml of γ -APS was injected into the reaction system, and then refluxed at 120 °C for 24h. The reactant was purified by centrifugation at 5,000 rpm at room temperature to remove unreacted silane coupling agent.

Determination of amino groups on HAp particles

Quantitative determination of amino groups on HAp particles was conducted by a sensitive spectrophotometric method with sulfo-succinimidyl-4-O-(4,4'-dimethoxytrityl)-butyrate (s-SDTB, PIERCE, IL, USA).²

Graft polymerization onto silicone sheet

Graft polymerization of AAc onto silicone sheet was conducted according to the method of Ikada *et al.*³ Briefly, a silicone sheet, which was already treated with corona discharge, was immersed into 10 wt% of AAc monomer aqueous solution in glass tubes. After the tubes were degassed and sealed, graft polymerization was conducted at 60 °C for 1h.

Preparation of HAp/silicone composite

Covalent linkage formation between AAc-grafted silicone sheet and amine groups modified HAp particles was performed through a solid phase condensation by heating. 150 mg of the modified HAp particles were added into 10 ml of pure water in a 20 ml glass tube, and then stirred rapidly. After a circular piece of AAc-

grafted silicone sheet 1.0 cm in diameter hanging from a stainless steel wire was immersed in the solution, the solution was stirred strongly for 1h at room temperature. After soaking, the silicone sheet with absorbed particles was washed using a large quantity of pure water. The particles covered sheet was, and then, heated at 180 °C for 6h in vacuum of 1 mmHg.

Estimation of covalent bond

In order to estimate the formation of amide bond, two kinds of samples were prepared. (1) A conjugation product of AAc homopolymer with γ -APS (polyAAc-co- γ -APS): 200 mg of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was added into AAc homopolymer having 0.15 mmol of carboxyl groups in 20 ml of pure water. After stirring for 30 min, 1.5 mmol of γ -APS was added into the system, and then reacted for 1h at room temperature. (2) A heating product of physical mixture of AAc homopolymer and the amino groups modified HAp particles (heating product of polyAAc-co-HAp-NH₂): a physical mixture containing AAc homopolymer and amine modified HAp particles by 3/10 of weight ratio was prepared. The physical mixture was then heated at 180 °C for 6h in a vacuum of 1.0 mmHg.

RESULTS

Introduction of amino groups onto HAp particles

Amino groups were introduced onto the HAp particles using an amino end group terminated silane-coupling agent. In the spectrum of original HAp particles (Fig. 1, A), bands at 600 and 575 cm⁻¹ represent ν_4 PO₄³⁻ in hydroxyapatite. Peaks at 963 cm⁻¹ reflect ν_1 PO₄³⁻ and 1100/1035 cm⁻¹ for ν_3 PO₄³⁻. Peaks at 1465/1412 cm⁻¹ were attributed to ν_3 CO₃²⁻ and 877 cm⁻¹ to ν_2 CO₃²⁻. Peaks of structural OH groups in the apatite lattice showed 3572 cm⁻¹ attributed to OH

stretching vibration and that at 632 cm^{-1} attributed to OH bending vibration. In the spectrum of the amino group modified HAp particles (Fig. 1, B), additional peaks at 2928 cm^{-1} indicating C-H stretching vibration and 1043 cm^{-1} attributed to Si-O stretching vibration, appeared. The amount of amino groups on the HAp particles determined using s-SDTB were $1.03\text{ }\mu\text{mol/g}$ ($SD=0.24$, $n=3$). The specific surface area of the original particle was $6.15\pm 0.08\text{ m}^2/\text{g}$.

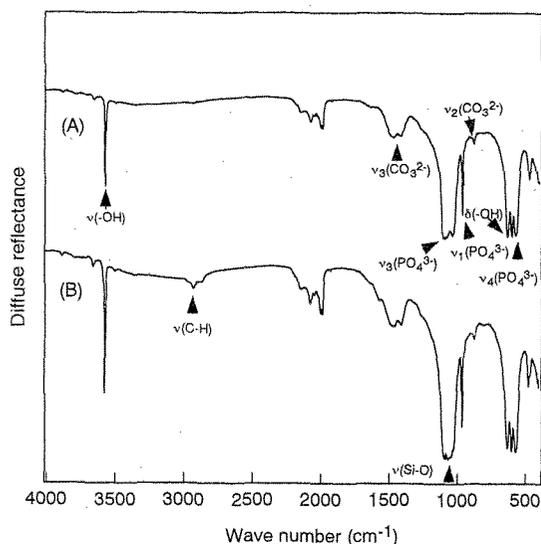


Fig. 1 FT-IR spectra of original HAp (A) and amino groups modified HAp (B) particles.

Graft polymerization onto silicone sheet

AAC-grafted silicone with $16.7\mu\text{g}/\text{cm}^2$ ($SD=5.1$, $n=4$) of an average grafting density was prepared the graft polymerization using corona discharge treatment. The graft density was almost same value that Ikada *et al* reported.³ We confirmed the accomplishment of the graft-polymerization by ATR FT-IR measurement.

SEM observation

The morphology of the surface of HAp particles/silicone composite was observed by SEM (Fig. 2). The silicone surface was smooth without any cracks. The spherical surface of modified particles seems to be also unchanged following modification.

The surface coverage ratio estimated by SEM was 64.5% ($SD=6.3$, $n=3$). The particles were scattered on the silicone surface in a partially closely-packed formation.

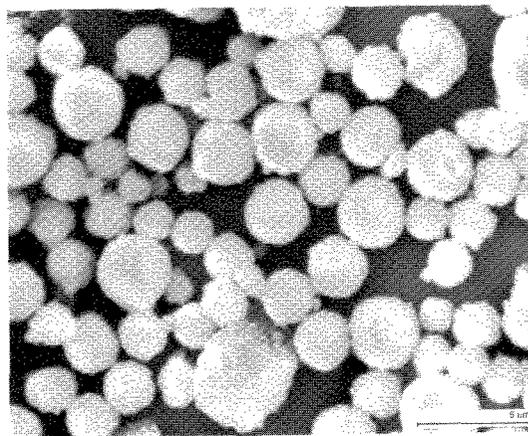


Fig. 2 SEM observation of HAp particles coating silicone material (x 5,000).

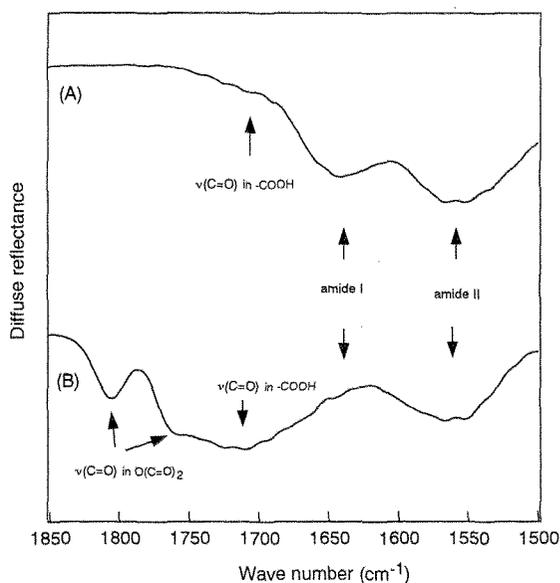


Fig. 3 FT-IR spectra of polyAAC-co- γ -APS (A) and heating product of polyAAC-co-HAp-NH₂ (B).

Estimation of covalent bond

We could not recognize the covalent bond between the HAp particles and AAC-grafted silicone sheet directly. In the spectrum of the heating product of polyAAC-co-HAp-NH₂ (Fig. 3, B), the bands of carbonyl groups in anhydride attributed to $1805/1755\text{ cm}^{-1}$, that of C=O of free carboxyl groups attributed to 1710 cm^{-1} ,

that of amide I attributed to 1640 cm^{-1} , and that of amide II attributed to 1560 cm^{-1} were observed.

DISCUSSION

There have been several kinds of reports for the donation of organic functional groups onto calcium phosphate ceramics by covalent linkages using functional groups terminated organic reagents. In this study, the amino donated HAp particles having average diameter of $2.0\text{ }\mu\text{m}$ was characterized by XPS and FT-IR, and also the population of amino groups via surface area was determined by a spectrophotometric method. In the FT-IR spectrum of amino groups modified HAp particles (Fig. 1, B), it was confirmed that the coupling agent bonded with the HAp particles by covalent linkage due to the appearance of new peak at 1043 cm^{-1} attributed to Si-O stretching vibration.⁴

Amino group population on the HAp particle surface was calculated to be about one molecule per 10 nm^2 from the amount of amino groups on the particles and the specific surface area. Nishizawa *et al.* reported that the donation ratio of a silane coupling agent onto calcium phosphate ceramics was almost same value over 24 h of the reaction time at $120\text{ }^\circ\text{C}$, and the value was calculated to be 30-40 molecules per 10 nm^2 by carbon elementary analysis.⁴ It was thought that the difference of the amino groups donation-ratios in these inorganic materials might depend on the analysis methods and the properties of surface structure.

In the observation of the surface of the composite, the particles that overlap might adsorb by ionic interaction. It was difficult to prepare a monolayer surface without three-dimensional aggregation of the particles. In the FT-IR spectrum of amino groups modified HAp particles (Fig. 1, B), a new peak at 1573 cm^{-1} was assumed to be NH_3^+ groups that interact with the HAp particle surface. Because the band of 1598

cm^{-1} attributed to free amine groups of γ -APS was absent,⁵ and there may remain a large virgin area of the HAp surface according to the value of the population of amino group. Thus, it was thought that amino modified particles might readily to aggregate.

In conclusion, it was estimated that amide bonds were generated between carboxyl groups of AAc graft-polymer and amino groups of the modified HAp particles. It could be expected that the adhesive properties in HAp coating silicone material can be further improved. The composite of this study can potentially be developed into elastic osteoconductive and percutaneous devices in medical fields.⁵

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