Hydroxyapatite film coated on poly-L-lactic acid by Aerosol Deposition Method

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The new apatite coating method, aerosol deposition method (ADM), was carried out on poly-L-lactic acid (PLLA) substrates by a following process. Hydroxyapatite (HAp) particles formed on aerosol flow by mixing with a helium carrier gas with the velocity of about 300 m/s were accumulated on PLLA substrates via impact adhesion at room temperature. Subsequently the thus prepared HAp coating film on PLLA by ADM was treated by heating. Then it was assessed by in vitro simulated body fluid (SBF) test. As a result, it was proved that the HAp film coated on PLLA by ADM with the heat treatment has bioactivity and that bonelike apatite was formed on the substrate. It is expected that the HAp film fabricated by ADM on PLLA with the heat treatment can be used in clinical orthopedics.

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1. Introduction

Currently, biodegradable polymers with the surfaces induced apatite-forming ability are applied to clinical orthopedics and their demands are greatly developed. The biodegradable polymer, for example, poly-lactic acid (PLA), poly-glycolic acid, and poly-(ε-caprolactone) (PCL) coated with bone-like apatite on their surfaces, are useful as scaffolds for bone tissue regeneration. Since apatite is a major inorganic component of natural bone, it shows osteoconductivity and good biocompatibility. Hence biodegradable polymers are used as pins or screws for bone bonding materials without extirpating operation.

Various coating techniques, involving Biomimetic,1–6 alternate soaking,7,8 spray coating,9 laser deposition,10 coupling,11 and low-temperature precipitation12 process have been developed over the past two decades to deposit hydroxyapatite (HAp) on the surfaces of the polymer materials.

Besides A. Oyane et al., has reported that simple surface modification of poly (ε-caprolactone) to induce its apatite-forming ability, that is PCL surface treatment to form oxygen-containing functional groups which was subsequently dipped alternately in an alcoholic solution containing calcium ions and one containing phosphate ions to deposit apatite precursors on the surface. The surface modified PCL samples formed a dense and uniform surface bone-like apatite layer after immersion in a SBF for 24 h.13,14 They also obtained the good consequences using ethylene-vinyl alcohol copolymer (EVOH) plate and knitted EVOH fiber.15

In this present study, a new HAp coating process, aerosol deposition method (ADM), has been developed with a maximum deposition rate of 25 μm/min for 5 mm² area, and does not need any vacuum chambers or expensive lasers involving the laser-assisted laser ablation (LALA).10 This process has also the advantage that apatite formed in the simulated body fluid (SBF)17–20 is very similar to bone apatite on this HAp coating film: the bone-like apatite contained a few % of CO₂⁻⁻ ion and slightly amount of Cl⁻, Na⁺, and Mg²⁺ ions; Ca/P atomic ratio was decreased compared to that for stoichiometric apatite, resulting in non-well crystallized apatite. We proved that the HAp film coated on PLLA by ADM has bioactivity through in vitro assessments using the SBF test.

2. Experimental procedure

2.1 The HAp film coated on a biodegradable polymer substrate by ADM

We employed HAp particles (HAP-200, Ca₁₀(P0₄)₆(OH)₂, mean particle size of 5–20 μm, specific surface area of 5–10 m²/g, bulk volume of 4.0–6.0 mL/g, Taihei chemical industrial Co., Japan) as bioactive ceramics and PLLA (Lacty, 10 × 10 × 2.5 mm², Shimadzu Co., Japan) as a polymer substrate. Figure 1 shows a schematic drawing of the ADM coating system used in this study.20 The HAp particles were mixed with helium gas to form an aerosol flow in an aerosol chamber. The flow was transported through a tube nozzle, and was accelerated and ejected from the nozzle into a deposition chamber, which was evacuated by a rotary pump with a mechanical buster. The gas consumption was 10 L/min. The substrate was placed at a distance of 10 mm from the nozzle, and was bombardied with the HAp particles, forming a film on the substrate. The HAp coating process on PLLA was carried out at room temperature. Subsequently the HAp film on PLLA was kept at 200°C for 20 min.

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spectra were set with a Fourier transform infrared spectrometer (FT-IR, FT-720, HORIBA Ltd., Japan). FT-IR

The SBF was prepared from reagent grade chemicals (Nacalai Tesque, Inc., Japan), strictly in the following order: NaCl, NaHCO₃, KCl, K₂HPO₄·6H₂O, hydrochloric acid, CaCl₂, and Na₂SO₄ dissolved into distilled water in a beaker. The SBF was buffered at pH 7.4 at 36.5°C with trihydroxymethyl-amino-methane and hydrochloric acid, and then it was diluted to 1000 mL with distilled water. The inorganic composition of the SBF emulates that of human blood plasma, as shown in Table 1. No precipitation was observed during the fluid preparation. The specimens, the HAp films coated on PLLA by ADM, were soaked in 50 mL of SBF in polystyrene bottles at 36.5°C in an incubator for 7 d.

2.3 Characterization of hydroxyapatite powder and apatite coating film before and after immersion in the SBF

Analysis of surface HAp film coating on PLLA by ADM with heat treatment and HAp film after immersion in the SBF were examined by a scanning electron microscope (SEM, JSM-5500, JEOL, Japan) and an X-ray diffractometer (XRD, Rint2100, Rigaku Co., Japan). CuKα radiation with a wavelength, λ of 0.154 nm was used for analysis; the operating conditions were 40 kV and 40 mA. XRD with 0.01 degree stepping and 20 seconds of count time was performed in the 2θ range of 20–60 degree. The lattice constants a and c were estimated using XRD spectra of miller indices (310) (222) (312) (213) (321) (410) (402) and (004) assuming a hexagonal structure. The lattice constant a and c were determined by Rietveld method with Jade5.0 (Materials Data, Inc. U.S.A.).

Energy-dispersive analysis was carried out raw HAp powder, HAp coating film on PLLA by ADM with heat treatment and HAp film after immersion in the SBF with an Energy dispersive X-ray analysis (EDX, Oxford Instruments, England).

Chemical analysis was used for a Fourier transform infrared spectrometer (FT-IR, FT-720, HORIBA Ltd., Japan). FT-IR spectra were set with a resolution 4 cm⁻¹ and revealed all the major peaks in a range of 400–4000 cm⁻¹. The background was used for pellet, equal to weighted KBr powder pressed into a die. Quantitative analysis of CO₃²⁻ ion was calculated by standard curve of FT-IR from internal standard method.

3. Results

3.1 SEM observation

Figure 2 shows SEM micrographs for the surface of the HAp films coated on PLLA by ADM with heat treatment before and after immersion in the SBF for 7 d. It can be seen from Fig. 2(c) and (d) that a dense and uniformed bone-like apatite layer was formed after the SBF test. The HAp film was fabricated about 30 μm on PLLA substrate by ADM, after that it partial was implanted into the PLLA substrate by heat treatment and became about 10 μm thickness from PLLA substrate. Finally the HAp film became about 20 μm thickness during soaking in the SBF.

3.2 EDX analysis

The HAp stoichiometry, the Ca/P atomic ratio of the HAp powder decreased from 1.69 to 1.55 by the HAp film coating on PLLA by ADM with heat treatment. Figure 3(c) shows that Na, Mg and Cl were detected from spectra of EDX element quality analysis. The Ca²⁺ ions in the HAp film deposited in the SBF are well known to be replaced with small amount of Mg²⁺ and Na⁺ ions,31) namely indicating bone-like apatite.

3.3 XRD analysis

Figure 4 shows XRD patterns of PLLA as a substrate (a), the HAp powder (b), the HAp film coated on PLLA by ADM with heat treatment (c), and the HAp film after immersion in the SBF for 7 d (d). According to these results, a hexagonal structure shown in a stoichiometric apatite was retained after coated by ADM. In addition, it was observed that the peaks for the HAp film were shifted to be higher degrees and were broadened compared to those of the HAp powder. Moreover, those peaks were more shifted and broadened after the SBF test.

3.4 FT-IR analysis

Figure 5 shows FT-IR absorption spectra of HAp powder (a), HAp film coating on PLLA by ADM with heat treatment (b), HAp film after immersion in the SBF for 7 d (c). Peak assignments were based on the previous report by H. M. Kim et al. The peaks ascribed to CO₃²⁻ ion were observed at about 873, 1420 and 1456 cm⁻¹, beside the main peaks ascribed to the apatite for all the spectra.32)
The HAp powder contains 3.8% of CO$_3^{2-}$. The HAp powder and the HAp film fabricated by ADM on PLLA did not change the CO$_3^{2-}$ ion content; however, the HAp film immersed in the SBF was increased to be 4.0% of CO$_3^{2-}$.

4. Discussions

The SBF has been well known to reliably reproduce in vivo bone-like apatite formation on bioactive materials in vitro, and provided significant information on the mechanism of apatite formation on these bioactive materials.\textsuperscript{17}–\textsuperscript{29} It is apparent from the results described above that the present HAp coating process (ADM), gives a dense and uniform apatite layer on biodegradable polymer substrate (PLLA).

As Fig. 4(c) shows, the crystallinity of HAp was retained throughout ADM and heat treatment. Further, Fig. 4(d) shows the deposited on the HAp film can be bone-like apatite because of broadened peaks. The broad apatite peaks indicate low crystallinity of apatite formed on the HAp film. A broad band peak centered at 31.8 degree is attributed to bone-like apatite, and an additional peak at 25.8 degree is due to a non-well crystallized apatite with a preferred (002) orientation.\textsuperscript{35} However the composition of the HAp was significantly changed after the HAp coating process on PLLA by ADM. In this method, the HAp powder is pre-processed by ball milling. Pre-processing intends to improve deposition rate. Ball milling of the HAp powder would inflict strain and defects on the powder particle, so that it changes the microstructure of the consolidated body.\textsuperscript{34,35}

The Ca/P atomic ratio or lattice constants a and c for the HAp-200 powder decreased through the film coating ADM process from 1.69 to 1.55 or from 9.465 to 9.437 Å and from 6.903 to 6.886 Å, respectively. The chemical composition of HAP-200 is Ca$_{10.36}$[PO$_4$]$_{6.30}$[CO$_3$]$_{0.60}$[OH]$_{0.40}$·1.2H$_2$O,\textsuperscript{36} whereas the stoichiometric apatite is given by the chemical formula of Ca$_{10}$[PO$_4$]$_6$(OH)$_2$, with a Ca/P atomic ratio of 1.67. In the case of
the stoichiometric apatite, the lattice constants a and c are 9.42 and 6.88 Å, respectively. When CO$_3^{2-}$ ions are substituted for PO$_4^{3-}$ ion sites of apatite, on the contrary, the constant a becomes larger than 9.42 Å and the constant c smaller than 6.88 Å.\(^{31,36}\) Since HAP-200, used in this study, was prepared by hydrolysis of a mixture of CaHPO$_4$ and CaCO$_3$, it contains 3.8% of CO$_3^{2-}$ ions.\(^{36}\) HAP-200 contains CO$_3^{2-}$ ions in the chemical formula and CO$_3^{2-}$ ions are substituted for PO$_4^{3-}$ and OH$^-$ ion sites of apatite, so that the lattice constants a and c are quite larger than those for the stoichiometric apatite. The lattice constants a for the HAP powder, the HAP coating films, and the HAP film after immersion in the SBF were determined as 9.465, 9.437, and 9.412 Å, and those of c were also determined as 6.903, 6.886, and 6.871 Å, respectively. Accordingly, it is proved quantitatively that the lattice constants a and c were shrunk by ADM process and more shrunk by soaking in the SBF. The shrinking of the lattice constants of the HAP film were caused by impact against a substrate or their own particles during the deposition with ADM process. The lattice constants of the HAP film after the SBF test were shrunk because of forming a dense and uniform bone-like apatite onto the HAP film.

5. Conclusions

The PLLA, a biodegradable polymer, acquired an osteoconductivity and good biocompatibility on its surface by a new HAP coating process (ADM). The HAP film was fabricated on the surface of PLLA by ADM, and the PLLA with the HAP film was subjected to a heat treatment, preventing the film from being peeled from the substrate through washing with distilled water after the SBF test. It was proved that the apatite layer on the HAP film fabricated by ADM was successfully formed to be a dense and uniform bone-like apatite during soaking in the SBF. This result shows that the film is useful as a scaffold for bone regeneration. It is expected that the HAP film coated on PLLA by ADM with the heat treatment can be used in clinical orthopedics.

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References