Evaluation of the structure of Ag in thermal sprayed Ag-containing hydroxyapatite coatings

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In orthopedic surgery, infection after implantation is a serious complication. Silver (Ag) exhibits antibacterial activity and a broad antibacterial spectrum and is considered to exhibit low toxicity in the human body. In our previous work, we developed an Ag-containing hydroxyapatite (Ag–HA) coating process in which the coatings were applied to substrates using a thermal spraying system and subsequently evaluated the antibacterial properties of the coatings. In this study, we investigated the structure of Ag–HA coatings, especially the microstructure of Ag. Hydroxyapatite powder containing silver oxide (Ag2O) was used as a thermal spraying powder. The coatings were applied onto pure titanium disks by the thermal spraying method, followed by a vacuum heat treatment to crystallize the hydroxyapatite phase of the coatings. X-ray diffraction (XRD) patterns indicated that the Ag–HA coatings consisted primarily of hydroxyapatite and a small amount of Ag metal and that the amount of Ag metal increased with increasing amount of Ag2O in the thermal spraying powder. XANES spectra of the coatings were compared with the spectrum of Ag metal as a reference material, and the peaks associated with the Ag–HA coatings were similar to those of Ag metal; however, the edge peaks shifted to the low-energy side. These results suggested that the Ag in the Ag–HA coatings existed primarily as Ag metal.

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Key-words : Antibacterial, Silver, Hydroxyapatite, Thermal spraying, XRD, XANES

[Received April 7, 2015; Accepted June 8, 2015]

1. Introduction

In joint diseases such as osteoarthritis and rheumatoid arthritis, the progression of symptoms causes severe pains and may make walking difficult for a patient. As one of the therapies of such joint disease, the procedure of artificial joint replacement is used to replace all or part of the lesion with an artificial joint that consists of artificial materials for the purpose of recovering joint function. The number of joint replacement surgery cases increases every year, and there are currently 100,000 or more cases per year in Japan.1) On the intraosseous fixation of implants, cementless-type prostheses with surface treatments using hydroxyapatite as the coating agent are widely used to bond bone and implants directly.

Post-operative infection is one of the three major complications associated with artificial hip joint surgery and has become a serious issue not only perioperatively but also in the long term. The incidences of postoperative infection following artificial hip joint and artificial knee joint operations have been reported to be 0.2–0.6%2–5) and 2.2–2.9%,6,7) respectively. Consequently, studies of biomaterials with associated antibacterial properties have attracted attention; in particular, antibacterial coatings that confer antimicrobial properties to the implant itself have been extensively researched.6,9–13) Simultaneously, to provide new functionality to hydroxyapatite and other calcium phosphate, researchers are increasingly substituting various metal ions into hydroxyapatite and other calcium phosphate via wet synthesis processes.6,11–13) Hashimoto et al. synthesized Ag-substituted β-tricalcium phosphate (β-TCP) in which calcium ions were replaced with Ag+ ions via solid-state sintering; they reported that β-TCP doped with Ag+ ions showed a high antibacterial activity.17)

Ag exhibits high antibacterial activity and a broad antibacterial spectrum and is considered to exhibit low toxicity in the human body. We have therefore developed an Ag-containing hydroxyapatite (Ag–HA) coating using a flame spraying system, a type of thermal spraying method. Fundamental materials evaluations such as dissolution, antimicrobial activity, and cytotoxic activity tests have been performed.18,19) However, the physical and chemical states and the antibacterial mechanism of Ag in Ag–HA coatings have not yet been clearly explained. The state of Ag in Ag–HA coatings can not only provide information about its antibacterial activity and mechanism but also provide an index of the long-term stability of Ag–HA coatings.

In this study, we used a thermal spraying system to prepare Ag–HA coatings with various concentrations of Ag and investigated the local structures of the Ag+ ions by X-ray absorption fine structure analysis (XAFS).

2. Experimental

Silver oxide powder (Ag2O) (Kanto chemical, Tokyo, Japan) and hydroxyapatite [Ca10(PO4)6(OH)2] powder (KYOCERA Medical, Osaka, Japan) were used as starting materials. The powder for spraying was prepared in the laboratory by mixing 3, 5, and 10 wt% of Ag2O powder and hydroxyapatite powder and shaking for 5 min in a plastic container. A commercially pure titanium plate was used. It was roughened by being sandblasted at pressure of 0.3–0.6 MPa with 180-grit aluminum oxide and...
subsequently cleaned ultrasonically in alcohol. The coatings were applied to the sandblasted surface of each titanium plate using a laboratory flame spraying system (Oerlikon Metco, Tokyo, Japan) using an acetylene torch under ambient atmosphere. The temperature of the flame produced by the acetylene-oxygen gas mixture used with the thermal spraying system was approximately 2700°C. The spraying powder was melted by the flame and deposited onto a titanium plate (Fig. 1). The sprayed thickness was approximately 20–60 μm. The coatings produced by spraying powder that included 3, 5, and 10 wt% of Ag2O are referred to as 3AgHA, 5AgHA, and 10AgHA, respectively. Subsequently, the coatings were vacuum heat treated at 600–700°C; these samples are referred to as 3AgHAH, 5AgHAH, and 10AgHAH, respectively. In addition, a hydroxyapatite coating containing no Ag2O was prepared as a control under the same conditions that were used to prepare the Ag-containing samples. The control sample fabricated without a vacuum heat treatment is referred to as 0AgHAH, whereas the one fabricated with a vacuum heat treatment is referred to as 0AgHA.

Samples were embedded in epoxy resin, and each spraying cross-section was polished with 4000-grit SiC paper. Cross-sections and surfaces of the coatings were observed by scanning electron microscopy (SEM) (S-3400N; Hitachi High-Technologies, Tokyo, Japan). After being detached from the titanium substrate and crushed into powder, the coatings with various Ag concentrations were dissolved in 30% nitrate, and the Ag concentration in each coating was quantitatively analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPS3100; SII Nanotechnology, Tokyo, Japan). X-ray diffraction (XRD) patterns of coatings before and after they were subjected to a heat treatment under vacuum were obtained using an X-ray diffractometer (EMPYREAN PW6000/00; Spectris, Tokyo, Japan) equipped with a Cu-Kα radiation source (45 kV and 40 mA) after the coatings were detached from their substrates and ground. Each powder was scanned from 20 to 50°. To investigate the local structure of Ag in the coatings (3AgHA, 3AgHAH, 10AgHA and 10AgHAH), XAFS measurements were conducted via the fluorometric method at beamline BL14B2 (SPring-8, JASRI) using 19Ge-SSD detectors. The monochromator was equipped with Si(311)crystals. Ag-K-edge X-ray absorption near edge structure (XANES) spectra and extended X-ray absorption fine structure (EXAFS) vibration spectra were obtained 5 times by quick scan mode in the range from 8.62 to 8.40° (25260–25915 eV). Ag foil, Ag3O4, AgO, and Ag2PO4 were used as reference samples.

3. Results and discussion

Cross-sectional SEM backscatter images of coatings before and after vacuum heat treatment are shown in Figs. 2 and 3, respectively. In 3AgHA and 3AgHAH, a tiny amount of fibrous particles approximately 1–5 μm long contained Ag in the hydroxyapatite coating. On the other hand, in 10AgHA and 10AgHAH, spherical particles with diameter of approximately 1–10 μm and spherical particles and fibrous particles with lengths of 10–20 μm were observed. The amount of these particles tended to increase with increased loading of Ag2O. The difference of size and shape of particles before and after heat treatment was not found. SEM backscatter images of surfaces of coatings after vacuum heat treatment are shown in Fig. 4. These images of surface of coatings show that Ag containing particles are plate-like structure. Many of these platy particles containing Ag were located parallel to the titanium substrate, which suggests that some Ag2O powder were melted on heat and then deposited parallel to the substrate in the hydroxyapatite coating during the thermal spraying process.

The Ag concentrations of the 3AgHAH, 5AgHAH, and 10AgHAH coatings were 0.10, 0.35, and 0.58 wt %, respectively. The Ag2O in most of the starting powders likely sublimed at high temperatures during the thermal spraying process. Furthermore, the coating of 0AgHAH that did not contain Ag was white and the 3AgHAH and 10AgHAH coatings were pale-yellow and brown, respectively.

The XRD diffraction patterns of coatings before and after vacuum heat treatment are shown in Fig. 5, and the patterns of each coating after the vacuum heat treatment shown in Fig. 6. The XRD samples of the coatings before the vacuum heat treat-
ment showed broad diffraction peaks because almost hydroxyapatite decomposed into amorphous phase by rapid cooling from high temperature in the thermal spraying process; however, peaks attributable to the Ag(111) and Ag(200) reflections of Ag metal were identified in the patterns of the samples with compositions that contained Ag₂O. Because the diffraction peaks attributable to Ag₂O, which was added to the spraying powder, were not detected, the aforementioned Ag metal was likely produced by decomposition of Ag₂O during the coating process. In addition, the intensity of Ag-metal peaks tended to increase with increased loading of Ag₂O. In contrast, in the XRD pattern of 0AgHAH after vacuum heat treatment, the broad diffraction peak became sharper and the crystallization of hydroxyapatite was confirmed. In the case of samples with compositions that contained Ag₂O, crystallization of hydroxyapatite was also confirmed after vacuum heat treatment and Ag(111) and Ag(200) peaks attributed to Ag metal were also confirmed. The diffraction peaks of Ag oxides or Ag compounds such as Ag₃PO₄ were not detected, irrespective of the amount of Ag₂O. Ag metal that was deposited during the spraying process was thought to not be affected by the vacuum heat treatment process.

Buckley et al. synthesized Ag/Ag₃PO₄ and hydroxyapatite composites by mixing hydroxyapatite powder and AgNO₃ using a wet process and subsequently heating the mixtures at 500°C. In the present study, the formation of Ag₃PO₄ was not observed, which suggests that Ag metal produced by the decomposition of Ag₂O during the coating process was thermostable during the subsequent vacuum heat treatment. Additionally, Rameshbabu et al. have reported that Ag⁺ ions can be doped at concentrations as high as 3 mol% into hydroxyapatite by a wet process and that its chemical structure remains stable during processing at temperatures of 750°C or below; however, they also observed that Ag metal precipitated when the composites were treated at temperatures of 800°C or above. Also, Jadalamnagar et al. reported that Ag⁺ ions could be substituted at concentrations as high as 5 mol% into hydroxyapatite via a new sol–gel method involving a semi-permeable membrane. In the present study, because a small amount of Ag was present in all of the coatings, shifts of the XRD peaks of hydroxyapatite could not be detected. Therefore, the substitution of Ag into hydroxyapatite could not be confirmed in the present study.

The XANES spectra of the Ag-K edge of various reference samples and of each Ag-HA coating before and after vacuum heat treatments (3AgHA, 3AgHAH, 10AgHA and 10AgHAH) are shown in Fig. 7. The XANES spectra of two different coatings with different Ag concentrations were similar to the XANES spectrum of Ag foil as a reference sample, irrespective of whether the samples were subjected to a vacuum heat treatment. The radial distribution function obtained by the Fourier transform-
tion of the EXAFS vibrations of 3AgHA, 3AgHAH and Ag foil are shown in Fig. 8, and 10AgHA, 10AgHAH and Ag foil are shown in Fig. 9. The peak positions at \( R = 2.2 \) Å or greater in the spectra of all Ag–HA coatings exhibited a good fit with the corresponding peaks in the spectrum of Ag foil, which suggests that the Ag atoms in Ag–HA coating were in the same state as those in Ag metal regardless of content of Ag₂O and heat treatment. These XAFS results suggest that the chemical state of most of the Ag atoms in the Ag–HA coatings were similar to the Ag atoms in Ag metal, and consistent with the previously discussed XRD results.

The superimposed XANES spectra of the Ag–K edge of 3AgHA, 3AgHAH, and Ag foil as a reference sample are presented in Fig. 10. In comparison with the spectrum of Ag foil, the positions of the absorption edge peaks of 3AgHA and 3AgHAH were shifted to the low-energy side by approximately 2 eV. In addition, the difference in the absorbance in the spectrum of 3AgHA was greater than that in the spectrum of 3AgHAH. These results suggest that the surface chemical state of the Ag metal particles was changed by the vacuum heat treatment.

Although the color of the hydroxyapatite powder used in this study was white and that of Ag₂O was black, the obtained 3AgHAH exhibited a pale-yellow color. This difference in color implies that 3AgHAH contained Ag₃PO₄ because Ag₃PO₄ is bright-yellow. However, the presence of Ag₃PO₄ was not detected by the XRD or XAFS measurements, suggesting that the pale-yellow color of 3AgHAH was due to other factors. A more detailed investigation is necessary.

4. Conclusion

We used a thermal spraying process to prepare Ag–HA coatings with various Ag concentrations and subsequently investigated the physical and chemical states of the Ag in the coatings by XRD and XAFS. The obtained XANES spectra and Fourier-transform spectra of the EXAFS vibrations indicated that most of the Ag in the thermal sprayed coatings was composed of Ag metal, suggesting that the coatings contained no Ag compounds such as Ag₃PO₄.

Acknowledgements

The authors gratefully acknowledge Dr. Mitsutaka Sato of Tohoku University and the students of Osaka Prefecture University. In addition, the XAFS measurements were performed at beamline BL14B2 in the Spring-8 facility with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2014A1567).

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