The Influence of Plasma-spraying Parameters on the Characteristics of Fluorapatite Coatings

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Abstract

This study was undertaken to evaluate the effect of plasma-spraying parameters, including spraying current and hydrogen content, on the properties of hydroxyapatite (HA) and fluorapatite (FA) coatings. It was found that FA coating displayed the less-molten to molten structure by variable spraying parameters, but the HA coatings show the molten structure in the present range of spraying parameters. By X-ray diffractionmetry (XRD), FA coatings showed better crystallinity than HA coatings. The index of crystallinity was found to be in the range of 65.3% to 91.1% for FA coating and 39.7% to 54.6% for HA coating. The difference of structure and crystallinity could be attributed to the thermal properties of HA and FA material. It is generally believed that amorphous phase in calcium phosphate coating would easily dissolve and lose long-term stability in clinical use. The results suggest that FA coating has the potential to show stability in long-term clinical use, and we can modify the characteristics of FA coatings by variable spraying parameters.

Keywords: Plasma-spraying, Fluorapatite coatings, Hydroxyapatite coatings, Crystallinity, Sprayed parameters

1. Introduction

Calcium phosphate ceramics are generally used as and considered to be bone substitute materials. For example, dense or porous hydroxapatite (Ca₁₀(OH)₆(PO₄)₄; HA) is successfully used for jaw augmentation and bone substitution in clinical application. In vivo, owing to having the same chemical and crystalllographic structure as the main inorganic component (apatite) of living bone, HA can bond physicochemically to bone with no fibrous tissue at the interface [1]. However, the brittleness, poor tensile strength, and poor impact resistance of HA materials limit their application as loading-bearing implants. Using HA as a coating on metallic substrate can combine the good biological properties of the HA layer while retaining the mechanical superiority of the metallic substrate. Various coating techniques have been used to deposit HA coating on Ti-alloy substrate. The well-known plasma spraying technique is the most commonly used. Excellent clinical results were reported for plasma-sprayed HAC on Ti-alloy substrate [2,3]. However, compositional and structural changes of HA may occur in the high velocity and temperature (as high as 30,000 K) of the plasma-spraying process. Long-term stability is still the major concern about the use of plasma-sprayed HA coatings [4]. Owing to the resorption and degradability of HA coating in a biological environment [5], the disintegration of the coating will decrease the mechanical properties and result in loss of the firm fixation between the implant and surrounding bone tissue.

Fluoride can be incorporated into HA to form chemically homogeneous distributions and stable crystals within bone and tooth enamel, respectively. For example, 0.04 wt. % to 0.07 wt. % of fluoride in apatite protects tooth enamel against the aggressive attack of oral environment [6]. The low solubility and acid resistance could improve stability of fluorapatite (Ca₁₀(PO₄)₆F₂; FA) by replacing the hydroxyl ion with a fluoride ion in biological environments. Several researchers have reported histological studies of FA coatings implanted into the femora of adult goats [7,8]. Direct bone apposition was observed at the interface of FA coatings [8], and the FA coatings did not show signs of degradation compared to the partial resorption of HA coatings after 12 and 25 weeks of implantation. By push-out test, the FA coating also showed similar bone bonding strength compared to HA coatings.

Several studies reported that the structural characteristics of HA coatings such as microstructure and crystallinity in the
coating vary with the spraying parameters, such as gas combination and flow rate, spraying power, and stand-off distance [5,9]. It was found that the biological responses of HA coating in vivo were dominantly affected by phase purity, crystallinity, and microstructure of HA coatings [10]. Similarly, the different plasma-spraying parameters would possibly influence the characteristics and biological responses of FA coatings. The aim of the present study was to vary two major parameters, spraying current and flow rate of secondary plasma gas, to modify the microstructure and crystallinity of FA coatings. In vitro, an osteoblast-like cell line, ATCC CRL-1543, was cultured on the as-sprayed FA coatings to evaluate the relation between coating characteristics and cytocompatibility in the early stage.

2. Materials and methods

2.1 Powder preparation

FA and HA powders were obtained from commercial production of Showa (Showa, Tokyo, Japan). The powders were prepared by milling and stirring them in a polyethylene bottle with zirconia balls using ethyl alcohol as dispersant. The resulting slurry was vacuum dried, crushed and ground using a pestle and mortar. The powders were then granulated with 10 wt. % of aqueous poly(vinyl alcohol) (PVA) solution and sieved to the optimal particle size (100-125 μm). Finally, the granulated powders were heated at 600°C for 1 h, to volatilize the PVA binder, and sintered at 1000°C for 4 h, to consolidate the particles.

2.2 Atmospheric plasma spray

Two shapes type of bioinert Ti-6Al-4V alloy (ASTM F-136) were used as substrate. Plate specimens, measuring 22 mm (l) x 17 mm (w) x 3.3 mm (t), were used for structural characterization and disk plates, measuring 12.7 mm in diameter and 2 mm in thickness, were used for cell culture. Prior to spraying, their surface were degreased with dilute acid to remove surface native oxide, cleaned in acetone ultrasonically, and then grit-blasted with Al2O3 to rough the surfaces; the spraying parameters are shown in Table 1. Except for the spraying current and secondary plasma gas flow rate, other spraying parameters such as primary gas, powder carrier gas flow rate, powder feed rate and stand-off distance were fixed. High purity argon (Ar) at a flow rate of 5 l/min was used to carry the powder at about 25 g/min from a powder feeder to the plasma torch of a plasma spraying system (Plasma-Technik M-1100C). The spraying current (400, 500 and 600 amperes) and secondary plasma hydrogen gas (flow rate: 3, 6 and 9 l/min) served as variables. After six cycles of X-Y robot arm with plasma gun movement at constant transverse speed of 10 mm/sec across the specimen surface, the uniform coatings were deposited onto Ti-6Al-4V substrate, and the coating thickness depended on the spraying parameters. During spraying, the substrates were kept at a low temperature by compressed air cooling in order to prevent overheating. The FA and HA coatings, employed by parameter of 500 A and hydrogen gas flow rate of 6 l/min, are denoted FA56 and HA56, respectively.

2.3 Material characterizations

The phases of the powder and plasma-sprayed coating were identified by X-ray diffractometry (Rigaku D/MAX III V), using CuK radiation, operated at 30 kV, 20 mA with scan speed of 10/ min and step size 0.01°. To evaluate the degree of FA crystallinity, the relative index of crystallinity (IOC) was defined from the ratio of the main peak intensities of the FA coatings (Ic) and the FA powder (Ip) by the relation of IOC (%) = (Ic/IP) 100%. This method assumes that the IOC of the FA powder is 100%. The surface morphology of FA coatings was examined using a secondary electron image of a scanning electron microscope (SEM, Philips XL-40 FEG). The specimens were coated with a conductive layer of gold in a sputter coater to avoid charging effects.

2.4 Cytocompatibility

Human osteoblast-like cells (ATCC CRL-1543), derived from an human osteosarcoma (HOS) were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and maintained in a humidified, 5% CO2/balance air incubator at 37°C. Subculturing was performed with the use of a phosphate-buffered saline (PBS) with 0.05% trypsin solution. After sterilization, specimens (HA69, FA43, FA56, and FA69 coatings) were placed in 24-well culture plates (Nunclon™, Denmark). Osteoblast-like cells were also cultured in the tissue culture polystyrene served as a control group. To evaluate the cell growth on the specimens, each specimen was seeded with osteoblast-like cells (10,000 cells/well). After 3 and 6 hours, medium was pipetted put from the dishes, and the samples were washed three times with PBS and fixed with 2.5% glutaraldehyde at 4°C, 4% OsO4 at 37°C and 1% tannic acid at 4°C. The sample was dehydrated with a series of graded ethanol solution and immersed in hexamethydisilazane (HMDS SIGMA) for 10 minutes. Finally, after sputter-coating with gold, the specimens were observed by SEM. After culturing for 24 hours, the cell numbers on specimens were assessed as previously described [11]. In brief, MTT (Thiazolyl Blue Tetrazolium Bromide, Sigma, USA) solution was working with attached cells. The converted dye was solubilized in dimethyl sulfoxide (DMSO) solution, and then read by enzyme-linked immunosorbent assay (ELISA) reader. Analysis of one-way variance (ANOVA) was used to evaluate the significant difference between cell numbers on different kinds of coatings. Differences were considered significant at p < 0.05.

Table 1. Plasma spraying parameters employed for preparing hydroxyapatite and fluorapatite coatings.

<table>
<thead>
<tr>
<th>Spraying parameter</th>
<th>Measurements</th>
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<tbody>
<tr>
<td>Primary gas (Ar), flow rate (l min⁻¹)</td>
<td>41</td>
</tr>
<tr>
<td>Secondary gas (H2), flow rate (l min⁻¹)</td>
<td>3, 6, 9</td>
</tr>
<tr>
<td>Powder carrier gas (Ar), flow rate (l min⁻¹)</td>
<td>5</td>
</tr>
<tr>
<td>Powder feed rate (g min⁻¹)</td>
<td>25</td>
</tr>
<tr>
<td>Spraying current (Amp)</td>
<td>400, 500, 600</td>
</tr>
<tr>
<td>Stand-off distance (cm)</td>
<td>7.5</td>
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<tr>
<td>Transverse speed (mm sec⁻¹)</td>
<td>10</td>
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3. Results

The morphology feature of HA coatings with different spraying parameters were examined by SEM (Figure 1). The surface morphologies were distinguished as follows: when the spraying current was 400 A, three kinds of specimen showed a molten coating with some unmelted powder. The amount of molten powder decreased with the increase of hydrogen content. The HA49 coating consisted of accumulated splat and microcrack, resulting from the thermal contraction during rapid cooling rate. When the spraying current was 500 A, HA53 specimen showed a molten coating with some unmelted powder. HA56 and HA59 specimens showed the more molten coatings consisted of accumulated splats, microcrack and a few partially molten droplets. When the spraying current was enlarged from 400 A and 500 A to 600 A, HA66 and HA69 specimens displayed the molten coating consisting of well-flattened and accumulated splats. The major difference between these nine coatings was the extent of molten, attributed to either the heat transfer ability or the level energy content exerted by combinations of plasma atmosphere and the power used.

Figure 2 shows the SEM surface morphology of FA coatings. We can observe that the spraying parameters changed...
the microstructure. When the spraying current was 400 A, there was major distinctive difference in the surface morphology of FA coatings sprayed by variable hydrogen content. The surface of FA43 coating was mainly covered with unmolten powder, which indicated the powerful plasma arc was insufficient to fully melt FA powder to deposit onto the substrate. When the hydrogen current increased to 6 or 9 l/min at the current of 400 A, the specimens showed a partially molten coating with some unmelted powder. At the spraying current of 500 A, the surface morphology of FA coating also depended on the hydrogen content. The surface of FA53 specimens was mainly covered with unmelted particles with less content of flattened splat. Although the FA59 specimens mainly consisted of accumulated splat, unmelted particles were observed on the surface of FA coatings. When the spraying current was raised to 600 A, the un-melted particles were observed on surface of three FA coatings with variable hydrogen contents. FA69 specimens contained molten coating consisting of accumulated splats.

The diffractograms of HA coatings with variable spraying parameters are shown in Figure 3. The peaks shown in the diffractogram match well with the standard phase (Ca_{10}(PO_{4})_{6}(OH)_{2}, JCPD No. 9-432). However, by comparison, the diffractograms of HA powder and HA coatings, the differences in the crystallinity become evident. As illustrated in Table 2, the relative index of crystallinity (IOC) of sprayed HA coatings decreased with the increasing spraying current and hydrogen content. It was found that the IOC values were 54.6% for HA43 and 39.7% for HA69.

![Figure 3. X-ray diffraction patterns of hydroxyapatite coatings by variable parameters. HA43 means HA coating prepared by spraying current and hydrogen content were 400 (amp) and 3 (l min	extsuperscript{-1}), respectively.](image)

Table 2. Results of index of crystallinity (%) of plasma-sprayed hydroxyapatite coatings.

<table>
<thead>
<tr>
<th>Spraying current (amperes)</th>
<th>Hydrogen content (l min	extsuperscript{-1})</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>400</td>
<td>54.6</td>
</tr>
<tr>
<td>500</td>
<td>54.4</td>
</tr>
<tr>
<td>600</td>
<td>53.0</td>
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The XRD patterns for FA coatings with different spraying currents and hydrogen contents are shown in Figure 4. The results show that FA coatings contain the peaks matching well with the standard phase (Ca_{10}(PO_{4})_{6}F_{2}, JCPD No. 34-11). As shown in Table 3, the crystallinity of the sprayed FA coatings decreased with increasing spraying current and hydrogen content. It was found that FA43 coating showed the highest IOC values (91.1%) compared to the lowest value (65.3%) of FA69 specimen, deposited with the highest spraying current and hydrogen content.

![Figure 4. X-ray diffraction patterns of fluorapatite coatings by variable parameters. FA43 means FA coating prepared by spraying current and hydrogen content were 400 (amp) and 3 (l min	extsuperscript{-1}), respectively.](image)

Table 3. Results of index of crystallinity (%) of plasma-sprayed fluorapatite coatings.

<table>
<thead>
<tr>
<th>Spraying current (amperes)</th>
<th>Hydrogen content (l min	extsuperscript{-1})</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>400</td>
<td>91.1</td>
</tr>
<tr>
<td>500</td>
<td>75.7</td>
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<tr>
<td>600</td>
<td>72.9</td>
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The results of FTIR on the surfaces of HA coatings prepared by variable parameters are shown in Figure 5. The results indicate the presence of phosphate groups at 595 and 1088 cm	extsuperscript{-1} and hydroxyl ions at 628, 3576, and 3658 cm	extsuperscript{-1}. As shown in Figure 6, the FTIR spectral analysis of FA coatings shows the vibration peaks of phosphate groups at 596, 968 and 1090 cm	extsuperscript{-1} and hydroxyl ions at 3548 cm	extsuperscript{-1}. In comparison to HA coatings, the relative intensity of hydroxyl ions in FA coatings is very weak. The presence of hydroxyl ions in FA coating could be attributed to physical adsorption from the surrounding environment.

![Figure 5. FT-IR spectra of hydroxyapatite coatings by variable parameters. HA43 means HA coating prepared by spraying current and hydrogen content were 400 (amp) and 3 (l min	extsuperscript{-1}), respectively.](image)
After 3 h of culture, the cell morphology on the surface of specimens was observed by SEM, as shown in Figure 7. On the well-melted morphology of HA69, FA56 and FA69 coatings, the cells displayed spherical shape with no apparent sign of filopodia. In contrast, the cells showed flattened morphology on the surface of FA43 coatings with main content of unmelted particles. Figures 8(a)-(d) represent the micrographs of cells on all four coatings after 6-h culture, respectively. On the surfaces of HA69, FA56 and FA69 coatings, the cell morphology started to spread and demonstrated filopodia extending from the central area. However, well-flattened morphology was observed on the surface of FA43 coatings. The results indicate that the microstructure of sprayed coating would influence the initial stage of cell behavior. As shown in Figure 9, the cell numbers on the surface of each specimen were evaluated by MTT assay after culturing for 24 hours. Although FA43 showed the highest cell number, indicated by the optical density, there was no significant difference among four kinds of coatings by ANOVA analysis.

4. Discussion

Plasma-sprayed HA coatings on various orthopaedic prostheses and dental implants have been found successfully in clinical results since the mid-1980s. However, the long-term stability of HA coatings is still the main clinical concern regarding the durability of implant fixation. It is generally believed that coating with lower crystallinity and higher content of amorphous phase would more rapidly dissolve and further prompt the faster rate of bone growth. The degradation of HACs is known to produce disintegrated HA granules causing third-body wear and induce osteolysis [12,13]. Røkkum et al. found calcium phosphate particles embedded within the polyethylene surface retrieved from the osteolysis and acetabular loosening HA-coated hip arthroplasties [13]. The microstructure, mechanical property, phase, composition, porosity, and crystallinity of HA coating, which vary according to the plasma spraying parameters used, have been evaluated in previous study [9]. It has been suggested that the low crystallinity and high amorphous phase
in HA coatings will increase the solubility of the coatings [14] and hence decrease their mechanical properties, including loss of the firm fixation between implant and bony tissue. It has been shown that from a technological point of view, HA is not an ideal material for use in the plasma-spraying process. For instance, dehydroxylation of the HA can occur in the plasma-spraying process. Fluoride ion substitution for hydroxyl groups in the apatite lattice has been found to be irreversible [15]. It is suggested that incorporation of fluoride ions into the apatite structure increases the thermal stability compared to HA. Pure FA is known to have a lower bio-resorption rate than HA [16], and retains comparable biocompatibility to HA in terms of its fixation to bone and bone ingrowth.

Several investigations have been undertaken on the plasma-spraying parameters of HA coatings. However, there is no report on the plasma-spraying parameters of FA coating in relation to the properties and biological responses. From SEM observation of varied spraying parameters, the surface morphology of FA coatings can be categorized into three types: (1) molten coating consisting of accumulated splats (major); (2) partially molten coating consisting of accumulated splats (major) and unmelted particle (minor); (3) partially molten coating with main content of unmelted particles. In comparison to FA coating, HA coatings display the well-melted structure, with flattened and accumulated splats. The mechanism for feedstock powder in plasma jet for sprayed HA coatings has been proposed by many researchers [17,18]. Cheang and Khor suggested that outside skin (shell) of a feedstock powder is molten and the core is unmelted, depicting that insufficient heat was transferred to melt powder completely during plasma-spraying process [18]. The rationale of well-melted HA coatings and less-melted FA coatings with lower spraying current and hydrogen content is thought to be due to the thermal properties of both materials. The molten temperatures are 1614 and 1630°C for HA and FA, respectively [16,19]. The thermal conductivities are 0.013 and 0.007 Wcm⁻¹K⁻¹ for HA and FA, respectively [20,21]. The less-molten FA coating could be attributed to higher molten temperature and lower thermal conductivity.

In comparison to FA material, the molten droplet of HA particle with highly heat-affected shell volume comes into contact with the substrate upon droplet spreading, which will form the amorphous phase. This could be confirmed by the results of crystallinity of HA and FA coatings in this study. As shown in Tables 2 and 3, all FA coatings demonstrated higher crystallinity than the HA coatings. Gross et al. found that hydroxyl-deficient feedstock HA material will more likely give rise to an amorphous phase compared to high hydroxyl-content powder [22]. The result suggested that the loss of hydroxyl in plasma jet would induce the formation of amorphous phase during the rapid solidification from the high cooling rates in the plasma spraying process. The feedstock particle sizes also affect the formation of the amorphous phase. A smaller particle size permits higher heat transfer, loss of hydroxyl ions and more molten particles. In this study, the particle size of HA and FA powders ranged from 100 to 125 m. This particle size distribution is larger than in other studies. For example, Gross and Berndt generally used the 5–40 m powder to investigate the factor of spraying parameters on the properties of HA coatings [17]. They also indicated that smaller particle size lead to highly molten droplets and was associated with an increase in amount of amorphous phase. In this study, both large particle size and thermal property were important factors affecting the microstructure and crystallinity of FA coatings. It is easier to modify the microstructure and crystallinity of FA coating than those of HA coatings. For example, nine HA coatings displayed a similar or the same surface morphology, but nine FA coatings showed a deviation in morphology from molten to less melted state, dominantly influenced by the spraying parameters. The influence of spraying parameters on the properties of HA coating has been well studied by many researchers [22–25]. However, the effect of plasma-spraying parameters on the properties of FA coatings still needs to be investigated because this is more relevant to the optimal FA coating for clinical use from material aspects.

The qualities of FA coatings determine the biological responses and long-term stability, such as coating resorption, bone ingrowth, and mechanical fixation. Schwartz and Boyan have mentioned that material properties such as surface energy, composition, roughness, and topography are believed to be of critical importance at the implant-tissue interaction [26]. As shown in Figures 7 and 8, the results of SEM observation indicate no significant difference could be distinguished among the morphologies of cells on the HA69, FA56 and FA69 coatings. However, the cells showed more flattened morphology on FA43 coating than on other specimens. In this study, the factors that influenced the cell responses of the plasma-sprayed coating included its composition, phase, crystallinity, and surface morphology. By XRD analyses, HA and FA have the same apatite phase and similar composition except for the existence of fluoride in FA. Although some documents indicate that an amorphous HA coating may be more beneficial for early bone ingrowth than a coating with high crystallinity [10], it is suggested that crystallinity did not influence the cell behavior at early cell phase in this study. The surface morphology or microstructure of coating could induce the different cellular responses during the early phases. At early cell attachments (3 h), cells exhibited round shape on molten coatings of HA69, FA56 and FA69 specimens compared to more flattened morphology on partially melted coatings of FA43 specimen. As shown in Figure 9, there was no significant difference in the level of cell number for HA69, FA43, FA56, and FA69 specimens, implying that a change of the cell morphology on sprayed coatings in the present range studied could induce a similar cell number during the early phase (1-d culture).

After plasma spraying occurred, the crystallinity of the HA decreased because of the high temperature and the rapid cooling rate. The amorphous phase has a high dissolution rate in aqueous solution, and this will decrease the mechanical properties and result in loss of the firm fixation between the implant and surrounding bone tissue. How to decrease the
amorphous phase is very important for the long-term application of plasma-sprayed coatings. In this study, FA coatings with molten structure also showed high crystallinity. Bhadang and Gross investigated the microstructure, crystallinity, surface characteristics and solubility of HA and FA coatings manufacturing by flame sprayed method [27]. Their results indicated that FA coating is highly crystalline and offers the potential for lower mineral ion release by dissolution. Although FA coating has the potential to show chemical stability in long-term clinical use, it is also possible to manufacture the different properties of FA coating by variable parameters. The different FA coatings would present diverse properties, by which it is possible to induce dissimilar biological responses. In the present study, we analyzed the crystallinity and surface morphology of FA coatings. As to further study, the relation between coating properties and biological responses still needs further investigation.

5. Conclusion

The relationships between spraying parameters and coating characteristics were systematically evaluated. FA coatings with specified coating characteristics could be obtained by varying spraying parameters. The conclusions of this study can be stated as follows: (1) Coating characteristics including crystallinity and morphology were affected by spraying parameters. The crystallinity of HA and FA coatings decreased with the increase of spraying current and hydrogen content. (2) Substitution of the hydroxyl ion by fluoride in the HA lattice makes the calcium phosphate more thermally stable. During plasma spraying process, FA material showed more thermal stability than did HA material. HA coatings contained less crystallinity and better melting of the coating. In contrast, FA coatings revealed higher crystallinity and less melting of the coating. The results suggest that FA coating would reveal more long-term stability than HA coating in clinical use. (3) From the in vitro experiments, the divergence of cell morphologies on four different coatings could be attributed to the topography of sprayed coatings. However, there was no significant difference in the level of cell number among the four kinds of specimens. The relation between coating properties and biological responses, especially in long-term biological stability, still needs further investigation.

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References