Surface Microstructure and Bioactivity of Hydroxyapatite and Fluorapatite Coatings Deposited on Ti-6Al-4V Substrates Using Nd-YAG Laser

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Abstract

Hydroxyapatite (HA) and fluorapatite (FA) coatings were deposited on Ti-6Al-4V substrates with an Nd-YAG laser and then immersed in simulated body fluid (SBF) for up to 21 days to evaluate their bioactivity. Prior to SBF immersion, the coating layer of the HA specimen had a coral-like structure, and was mainly composed of Ti, CaTiO3, TiO2, Al2O3, and Ca3P2O7, whereas that of the FA specimen had a dense cellular-like structure, and was mainly composed of Ti, CaTiO3, TiO2, Al2O3, and residual FA. The Ca/P ratios of the HA and FA coating layers were 7.61 and 2.12, respectively. After 21 days of immersion in SBF, only a very small amount of precipitates, mainly consisting of CaCO3, with some hydroxycarbonated apatite (HCA) and HA, formed on the HA coating layer, whose Ca/P ratio retained a high value of 6.34. In contrast, a dense accumulation of granulated precipitates, mainly consisting of HCA, formed on the FA coating layer after just 7 days of SBF immersion, with a corresponding Ca/P ratio of 1.63. The SBF immersion test shows that FA coatings produced via an Nd-YAG laser cladding technique on a Ti-6Al-4V substrate have better bioactivity than that of their HA counterparts.

Keywords: Hydroxyapatite (HA), Fluorapatite (FA), Nd-YAG laser cladding, Simulated body fluid (SBF), Hydroxycarbonated apatite (HCA)

1. Introduction

Due to lifestyle changes and improvements in medical science, the average human life expectancy has increased notably over the past 60 years [1]. At the same time, the occurrence of aging-associated diseases has also increased, in particular cardiovascular disease, cancer, Alzheimer’s disease, and arthritis, which is the most common [2]. In severe cases, replacing the afflicted joint with an artificial implant may be the only method for easing a patient’s discomfort. The immobilization of artificial implants to the host tissue is generally achieved using cementsless methods [3]. In such methods, the implant (typically made of titanium or one of its alloys) is coated with a thin layer of bioceramic material such as hydroxyapatite (HA, Ca10(PO4)6(OH)2) in order to induce the formation of chemical bonds between the implant and the surrounding bone tissue following implantation. However, the high temperature generated in the coating process destabilizes the HA structure. Consequently, significant phosphorus loss occurs due to evaporation, and thus the Ca/P ratio of the coating increases significantly beyond the ideal value of 1.67 [4,5]. In addition, HA coating layers contain a large number of highly dissolved phases (e.g., TCP, CaO, TP), which gradually decompose when exposed to human body fluid. As these phases decompose, the coating layer gradually flakes away from the substrate surface, resulting in the release of deleterious metallic ions into the body and the eventual failure of the implant [6-8].
The stability of HA coatings can be improved by adding additional components to the coating material. For example, in [9], the fluoride ion (F\textsuperscript{–}) in HA was replaced by hydroxide in the OH\textsuperscript{–} lattice position, resulting in the formation of a new biocompatible coating material designated as fluorapatite (FA, Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}F\textsubscript{2}). Compared to HA coatings, FA coatings have improved porosity and a lower dissolution rate and are therefore better suited to surgical implant applications [10–12]. However, the bonding quality of the coating layer used for medical implants depends not only on the elemental composition of the coating material, but also on the method used to deposit the coating on the substrate. Among the various coating techniques available (e.g., sol-gel deposition, plasma spraying, and laser cladding), laser cladding has received particular attention due to its applicability to three-dimensional (3D) processing, high yield rate, and high metallurgical bonding strength [7]. A review of the literature reveals that the laser cladding process is most commonly applied to HA coatings but only limited number of studies were done on other coating materials [13–15]. Moreover, most of the related studies address the mechanical properties of the coating layer rather than the bioactivity properties [16]. That is, the literature contains scant information regarding the microstructural characteristics of laser-clad FA coatings. No systematic comparison of the bioactivity properties of HA and FA coatings has been conducted. Accordingly, the present study utilizes an Nd-YAG laser system to clad HA and FA coatings on Ti-6Al-4V substrates. The coatings are then immersed in simulated body fluid (SBF) for up to 21 days in order to compare their bioactivity properties.

2. Experimental procedure

HA and FA powders were respectively mixed with polyvinyl alcohol (PVA, (C\textsubscript{2}H\textsubscript{4}O)n) binder material in a 1:1 weight ratio and then stirred until a slurry-like consistency was obtained. Substrates for the laser cladding process were prepared by machining Ti-6Al-4V alloy with the composition shown in Table 1 into thin sheets with dimensions of 100 mm x 60 mm x 3.8 mm, grinding their surfaces using 400-grit SiC paper, and then cleaning them in acetone and ethanol. The substrates were then coated with a 0.8-mm-thick layer of HA-PVA or FA-PVA slurry using the pre-place method. All the pre-placed specimens were dried in an oven under atmospheric conditions at 100 °C for 40 min. Once the pre-placed layer was dry, the samples were processed using an Nd-YAG laser (Rofin Sinar, ROFIN CW025, 2500W) set in continuous wave mode with a 740-W output power. The cladding process was performed in an Ar-shielded atmosphere (Ar flow rate: 25 l/min) with a 5° laser incident angle, a 15-mm positive defocus length, and a 300 mm/min cladding speed. The laser spot size was about 3 mm in diameter. The microstructures of the clad specimens were observed via SEM (JEOL JSM-6390LV). The Ca/P ratio of each coating was calculated by measuring the atomic percentages of Ca and P within the structure using an energy-dispersive X-ray spectrometer (EDS)-equipped SEM. In addition, the phases within the coatings were identified using XRD (Cu Kα radiation, Rigaku D/max III.V). The XRD patterns were collected in a 20 range from 20° to 70° with a scanning rate of 2°min\textsuperscript{–1}.

Table 1. Chemical composition of Ti-6Al-4V.

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>V</th>
<th>Fe</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-6Al-4V</td>
<td>6.1</td>
<td>4.24</td>
<td>0.152</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The clad specimens were cleaned, sterilized, and soaked in a standard SBF solution prepared in accordance with Kokubo’s protocol by dissolving appropriate quantities of the relevant reagent-grade chemicals in deionized water [17]. The ion concentrations in the SBF and human blood plasma are listed in Table 2. Following immersion for 7, 14, and 21 days at 37 ± 0.1 °C, the samples were removed from the SBF solution and the nucleation and growth of apatite were examined using SEM and EDS.

Table 2. Ion concentrations in SBF and human blood.

<table>
<thead>
<tr>
<th>(mM)</th>
<th>Na\textsuperscript{+}</th>
<th>K\textsuperscript{+}</th>
<th>Ca\textsuperscript{2+}</th>
<th>Mg\textsuperscript{2+}</th>
<th>Cl</th>
<th>HCO\textsubscript{3}\textsuperscript{–}</th>
<th>HPO\textsubscript{4}\textsuperscript{2–}</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBF</td>
<td>142.0</td>
<td>5.0</td>
<td>2.5</td>
<td>1.5</td>
<td>148.8</td>
<td>4.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Blood</td>
<td>142.0</td>
<td>5.0</td>
<td>2.5</td>
<td>1.5</td>
<td>103.0</td>
<td>27.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1 Microstructures of clad specimens

Figure 1 shows cross-section optical microscopy images and a dilution ratio schematic diagram of the clad specimens. Using the current process conditions the fusion zone of the clad samples form two types of layers namely the coating layer (CL) and the transition layer (TL). The dilution ratios (B/(A+B) × 100%) of all specimens are between 30-50%. Since a previous study [18] has shown that the CL and the TL have obvious differences in composition, where the composition of
the CL is closer to that of the coating material and that of the TL is closer to that of the substrate, the variation of composition was used as an index for the comparison of dilution between coatings.

Studies [19,20] have reported a thick bioceramic coating on top of a metal substrate with negligible dilution between the substrate and the coating, resulting in a sharp substrate-coating interface, which is the weakest zone and can lead to premature failure of the coating in vivo. An ideal coating should have an intermediate metal-ceramic region with a compositional gradient at the interface [21], which is the case for the coatings in this study.

The as-received Ti-6Al-4V substrate sheet was rolled and annealed. Its microstructure is shown in Fig. 2. The substrate is composed of an α phase precipitation (lighter parts) and a residual β phase (gray parts). Figure 3 shows the microstructural morphology of the surface and cross section of the HA and FA specimens after laser cladding. Figure 3(a) reveals that the coating surface of the HA specimen is slightly rougher than that of the FA specimen. It can be seen clearly that the CL in the HA specimen has a loose coral-like structure, whereas that in the FA specimen has a relatively dense cellular-like structure. The FA sample has a finer grain structure than that of the HA sample, which may also result in better structural strength. The differences of the two microstructural arrangements can be attributed to the interatomic bonding strength and thermal properties of the two coating materials. Since the electro-negativity of FA is higher than that of HA, FA not only has better chemical stability and biocompatibility than those of HA, but also higher interatomic bonding strength [22]. Consequently, during the laser radiation process FA was reported to have a more stable structure than that of HA [22-26]. Moreover, the coefficient of thermal expansion (CTE) values of HA, FA, and Ti-6Al-4V are $13.3 \times 10^{-6}$, $9.1 \times 10^{-6}$, and $8.8 \times 10^{-6}$ K$^{-1}$, respectively. Therefore, the CTE difference between Ti-6Al-4V and HA is $4.5 \times 10^{-6}$ K$^{-1}$, which is much larger than that of Ti-6Al-4V and FA. A greater difference in CTE gives rise to a greater interfacial stress during the heating and cooling stages of the laser cladding process, and affects the adhesion strength of the coatings. Therefore, FA may have higher coating adhesive strength with the Ti-6Al-4V substrate than that of HA.

Figure 2. Microstructure of as-received Ti-6Al-4V sheet.

3.2 Ca/P ratio of coating layers for as-laser-cladded specimens

Pure HA and FA both have a Ca/P ratio of around 1.67. However, following deposition on a Ti-6Al-4V substrate, the Ca/P ratio of both materials was greatly changed due to the effects of the binding material and the processing conditions [26-28]. The Ca/P ratios on the surface of the CLs in the laser-clad HA and FA specimens are 7.61 and 2.12, respectively. In other words, the Ca/P ratio of the FA specimen is much closer to the ideal value of 1.67 than that of the HA specimen. The large difference in the Ca/P ratios of the two coating specimens is due principally to the different crystal structures of the two materials. In HA, the distance between the OH atoms and the other atoms is larger than that in FA. At temperatures in the range of 700–1200 °C, the OH bonds are easily broken and become detached from the HA structure. This causes a rapid evaporation or oxidation of the P element in the HA structure [13], and leads to a high Ca/P ratio. Under high-energy-density laser processing, such reactions are more drastic. Figure 4 presents the EDS analysis results for the TL in the HA specimen. It can be seen that the TL is composed mainly of P, O, Ti, and Al. Ti and Al are the main components of the substrate (Ti-6Al-4V), whereas P and O are the main elements

Figure 3. Morphologies of as-laser-cladded in HA and FA specimens: (a) clad surface morphology and (b) cross-sectional morphology.

Figure 4. (a) SEM image and (b) EDS analysis results for transition layer in HA specimen.
in the HA coating material. In other words, the migration of P ions from the CL to the TL also contributes to the high Ca/P ratio of the CL. Figure 5 presents the EDS analysis results for the TL in the FA specimen. It can be observed that the TL contains only a small amount of P. Thus, it is inferred that most of the P is retained in the CL layer. Consequently, for a given laser output power, the FA cladding layer has a more stable structure than that of the HA layer and maintains a Ca/P ratio closer to the ideal value of 1.67 [29]. The EDS line scanning results in Fig. 6 further reveal that the CL has less P than that of TL in both HA and FA specimens, which shows that after laser cladding there is a prevalent evaporation of P on the CL layer in comparison to that of the TL layer. This is one of the main causes for the CL having a higher Ca/P ratio than that of the TL in all specimens.

![Figure 5](image1)

Figure 5. (a) SEM image and (b) EDS analysis results for transition layer in FA specimen.

![Figure 6](image2)

Figure 6. Comparison of P content in CL and TL of (a) FA- and (b) HA-coated specimens after laser cladding by EDS line scanning method.

### 3.3 Microstructure and bioactivity of SBF-immersed specimens

Figures 7 and 8 show the CL surface microstructures of the HA and FA specimens after SBF immersion. After immersion in SBF for only 7 days, a large number of granular precipitates were noticed on the CL surface of the FA specimen, whereas only a very small number of precipitates formed on the HA surface, which shows that the FA specimen has better bioactivity than that of the HA counterpart. Both the number and the density of the granular precipitates increased when the SBF immersion time was increased to 14 days and 21 days.

![Figure 7](image3)

Figure 7. Surface morphologies of HA coatings after SBF immersion for (a) 7, (b) 14, and (c) 21 days.

![Figure 8](image4)

Figure 8. Surface morphologies of FA coatings after SBF immersion for (a) 7, (b) 14, and (c) 21 days.

Figure 9 shows an SEM image and the corresponding EDS analysis results for an FA specimen immersed in SBF for 14 days. It can be seen that the specimen surface is composed mainly of O, Ca, and P. Thus, it is inferred that the granular precipitates on the FA surface are bone-like apatite induced via the allelochemics of the Ca and P ions released from the Ca$^{2+}$ and PO$_4^{3-}$ components in the SBF [30]. In general, specimens with higher Ca and P ion concentrations readily form apatite when exposed to SBF [31,32]. Of the cladding specimens prepared in the present study, the FA specimen has higher concentrations of Ca and P in the CL, and therefore prompts a greater precipitation of the bone-like apatite phase, which therefore results in better bioactivity in SBF than that of the HA counterpart.
Energy-keV.

3. Ca/P ratio of coating layer surface after SBF immersion

Figure 10 shows the change in the Ca/P ratios of the two specimens over the duration of the SBF test. It can be seen that the Ca/P ratio of the HA specimen reduces only very slightly following immersion in SBF. After an immersion time of 21 days, the Ca/P ratio only indicated a slight reduction from 7.61 to 6.34, i.e., far higher than the ideal value of 1.67. The high value of the Ca/P ratio following SBF immersion indicates that the HA coating has poor bioactivity. In other words, apatite is not readily formed on the HA coating surface when exposed to SBF. In contrast, the Ca/P ratio of the FA specimen has a value of 1.63 after an immersion time of just 7 days. The Ca/P ratio remained virtually unchanged when the immersion time was increased to 14 days and 21 days, respectively. Note that a similar result was reported in [30] for the in vitro formation of apatite on the surface of HA/polyetheretherketone biocomposites. It is noted that the final value of the Ca/P ratio for the FA specimen is lower than the ideal value of 1.67, which suggests that the apatite layer on the FA coating surface is Ca-deficient, with properties consistent with those of bone-like apatite [33].

For the FA as-laser-clad specimen, its CL composition can be determined from the 0-day XRD pattern in Fig. 11(a), which reveals that the CL is composed of Ti, CaTiO$_3$, TiO$_2$, Al$_2$O$_3$, and residual FA, where Ti and CaTiO$_3$ show relatively strong peaks. CaTiO$_3$, TiO$_2$, and Al$_2$O$_3$ are all products from decomposed or melted FA with the Ti-6Al-4V substrate [34], and they are all biocompatible. Some research has indicated that CaTiO$_3$ can enhance the precipitation of apatite on the surface [35] and increase osteoblast adhesion on materials [36].

Figure 11(a) clearly shows that all the peaks decreased with immersion time. FA gradually disappeared and transformed into hydroxycarbonated apatite (HCA,Ca$_{10}$ (PO$_4$)$_3$(CO$_3$)$_3$(OH)$_2$) from the 7th day, with the HCA peak getting stronger with immersion time thereafter. HCA was continuously deposited as a bone-like apatite precipitate on the specimen surface during immersion in SBF, as discussed in Section 3.3. Its formation mechanism starts from the positively charged Ca$^{2+}$ ions on the specimen surface, which act as nucleation sites for attaching with negatively charged (PO$_4$)$^{3-}$ and (CO$_3$)$^{2-}$ ions, with crystallized HCA gradually formed at the end [36]. The HCA layer that formed on the surface of the coatings was conducive to the attachment of proteins such as collagen, fibronectin, and vitronectin [37]. This in turn enhances the osteoblast adhesion of the coatings and improves the bonding strength between the implant and the surrounding tissue [38].

For the HA as-laser-clad specimen, its CL composition can be determined from the 0-day XRD pattern in Fig. 11(b), which shows that the CL is composed of Ti, CaTiO$_3$, TiO$_2$, Al$_2$O$_3$, and Ca$_3$P$_2$O$_7$. The original HA was fully decomposed into Ca$_3$P$_2$O$_7$, and reacted with the Ti-6Al-4V substrate to form CaTiO$_3$, TiO$_2$, and Al$_2$O$_3$. Some research has indicated that CaTiO$_3$ can enhance the precipitation of apatite on the surface [35] and increase osteoblast adhesion on materials [36].

Figure 10. Ca/P ratios of FA and HA coatings following immersion in SBF.

3.5 X-ray diffraction patterns of CL surface before and after SBF immersion

Figure 11. XRD analysis results for (a) FA and (b) HA specimens for various immersion times.
**4. Conclusion**

HA and FA coatings were deposited on Ti-6Al-4V substrates with an Nd:YAG laser and then immersed in SBF for up to 21 days to evaluate their bioactivity. Following the Nd:YAG laser cladding process, the FA specimen had a finer and denser microstructure than that of the HA counterpart, which may improve the interfacial and structural strength. The surface of the post-clad HA specimen was composed of Ti, CaTiO3, TiO2, Al2O3, and Ca3P2O7, and that of the post-clad FA specimen was composed of Ti, CaTiO3, TiO2, Al2O3, and FA. The Ca/P ratios of the cladding layers in the HA and FA specimens were 7.61 and 2.12, respectively. The higher Ca/P ratio in the HA specimen is due to evaporative losses of P during the cladding process and the migration of P ions to the transition layer. After immersion in SBF for only 7 days, a very small number of precipitates, mainly consisting of CaCO3 with some HCA and HA, formed on the HA surfaces, whereas a large number of granular precipitates, mainly consisting of HCA, appeared on the surface of the FA specimen. Moreover, the Ca/P ratio of the HA specimen surface slightly decreased (i.e., from 7.61 to 6.34) following prolonged (21 days) immersion in SBF, whereas that of the FA specimen decreased from 2.12 to 1.63 after just 7 days of immersion. These results clearly reveal that the FA specimen has better bioactivity than that of the HA counterpart.

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**References**


