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Laser fabrication of Ag-HA nanocomposites on Ti6Al4V implant for enhancing bioactivity and antibacterial capability

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ABSTRACT

For titanium alloy implants, both surface bioactivity and antibacterial infection are the two critical factors in determining the success of clinical implantation of these metallic implants. In the present work, a novel nanocomposite layer of nano-silver-containing hydroxyapatite (Ag-HA) was prepared on the surface of biomedical Ti6Al4V by laser processing. Analysis using SEM, EDS and XRD shows the formation of an Ag-HA layer of about 200 μm fusion bonded to the substrate. Mineralization tests in simulated body fluid (SBF) showed that laser fabricated Ag-HA nanocomposite layer favors the deposition of apatite on the surface of the implants. Antibacterial tests confirmed that all Ag-HA nanocomposite layers can kill bacteria while a higher Ag content would lower the cytocompatibility of these coatings. Cell viability decreases when the Ag content reaches 5% in these coatings, due to the larger amount of Ag leached out, as confirmed by ion release evaluation. Our results reveal that laser fabricated Ag-HA nanocomposite coatings containing 2% Ag show both excellent cytocompatibility and antibacterial capability.

1. Introduction

As load-bearing materials, titanium alloys such as NiTi, pure Ti, Ti6Al4V and other Ti-based alloys are widely used for orthopedic implants, orthodontic materials and biomedical devices, owing to their high specific strength, good corrosion resistance and good biocompatibility [1–9]. However, as bone implants, Ti6Al4V alloys, as other metallic materials, shows some natural shortcomings. For example, they are bioinert and cannot exert positive influences on cell and tissue behaviors. As a consequence, both osteoblasts and new bone tissues cannot grow well on the surface of bare metallic implants. This will lead to poor osteointegration between implants and the surrounding tissues, and loosening of implants resulting in failure of metallic implants has been reported [10–13]. Therefore, it is necessary to activate the surface of implants through some surface techniques like plasma immersion ion implantation [14], micro-arc oxidation [15,16], thermal spray [17], chemical treatment [18], surface nano-functionalization [19], and laser surface treatment [20]. However, these only focus on the enhancement of surface biocompatibility or osseointegration, but cannot solve other implants-related problems like bacteria induced infection.

Metallic implants including titanium alloys do not possess intrinsic antibacterial capability. As is known, post-operative implant-related bacterial infection often results in failure of bone implants. Bacterial adhesion on metallic implants and subsequent formation of biofilms are crucial steps in bacterial infection and the related mechanism involves physico-chemical interactions or mediation from extracellular polysaccharides and lectin-like substances [21–23]. Thus, for ensuring the success of implantation, bacteria adhesion inhibition or direct bacteria killing on the implant surface is very important for successful implantation. Many techniques have been used to enhance the antibacterial function of metallic implants. Antibiotic coatings have been used to reduce implant-related bacterial infection by the release of antibiotics and nitric oxide (NO) from coatings [24]. Antibacterial peptide coatings are also employed to prevent biofilm formation at the site of the implant [25]. However, some varietal bacteria strains like the reported superbug NDM-1 have developed strong resistance against known antibiotics [26]. In view of these, an implant surface with self antibacterial activity is an advantage over treatments such as antibiotic corporations. Inorganic antibacterial nano-materials like silver and copper nanoparticles are widely used for killing of bacteria. It is believed that these positively charged metallic ions attach to the negatively charged bacteria cell wall causing cell lysis and death [27–29]. These nanoparticles and ions can also poison the surrounding cells at the same time if they are released into the human body in an appropriate amount, and tissue necrosis may occur [30–34]. The current strategy to employ these inorganic nano-antibacterial agents is to fix them on the surface of the implants to reduce their release or control their leaching rate, thus maintaining its self-antibacterial capacity while not inducing cytotoxicity on the interface between implants and their surrounding tissues. Among the common surface modification techniques, laser surface treatment possesses unique superiority, such as ease of process control, high energy input, and cleanliness [35,36].

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To solve the two aforementioned implants-related problems, one has to enhance the surface biocompatibility together with the self-antibacterial capacity of implants. This might be achieved by employing biocompatible inorganic carriers to hold these antibacterial agents. As the major component of bone mineral, hydroxyapatite (HA) is bioactive for osteoblasts adhesion, growth and proliferation as well as for bone tissue formation. Herein, we use nano-HA powders as the carrier of nano-silver to produce a novel Ag-HA nanocomposite layer with enhanced bioactivity and self-antibacterial capability on the surface of Ti6Al4V by laser surface technique.

2. Experimental procedures

2.1. Laser surface processing

Ti6Al4V samples of dimensions 60 mm × 30 mm × 5 mm were spark cut from 5-mm sheet (supplier: Xi'an Bossin Metal Technology Co. Ltd, China). Silver-hydroxyapatite (Ag-HA) composite powder containing different contents of Ag were prepared using a reduction method as described in detail in our previous work [37]. As reported in [37], the hydroxyapatite (HA) powders (Aladdin Reagent Corporation) had an average particle size of about 100 nm, and the Ag nanoparticles produced had a size range of 20–30 nm. The Ti6Al4V samples were ground with 60-grit sand papers to create a roughened surface for enhancing adhesion with preplaced powder. After roughening, the samples were ultrasonically washed in deionized water and cleaned with acetone. Pastes of Ag-HA composite powder containing 0 wt% Ag, 1 wt% Ag, 2 wt% Ag, and 5 wt% Ag were prepared by mixing the Ag-HA powder with PVA (polyvinyl alcohol), which acted as a binder. The Ag-HA paste was then painted with a brush on the Ti6Al4V samples. The thickness of the preplaced Ag-HA layer was controlled to 0.3 mm, as measured using a digital micrometer. In the present study the cladding material was introduced by the powder preplacement method instead of coaxial powder feeding since the process parameters in the latter are more difficult to control and powder wastage is usually high. In addition, because of the non-compact nature of the preplaced powder layer, absorption of laser energy is more efficient. Laser irradiation of the sample surface to achieve laser cladding was performed using a CW 2 kW Nd:YAG laser (wavelength 1.06 μm, LUMONICS, Model MW2000). The laser beam was transmitted by optical fibre and focused onto the samples by a BK-7 glass lens with a focal length of 80 mm. To prevent oxidation, argon flowing at a rate of 20 l/min was used as shielding gas. The quality of the layer formed in laser processing depends on the choice of the laser processing parameters, including laser power P, laser spot diameter D, and scanning speed V. These parameters will determine the laser fluence F, which is the laser energy incident on unit surface area of the sample. F is approximately related to P, D and V by (it is approximate for circular spot, while exact for square spot):

$$F \approx P/(V \times D) \quad (1)$$

When the laser fluence is too low, the preplaced layer is not totally melted and the layer is not tightly bonded to the substrate. On the other hand, when the laser fluence is too high, excessive melting of the substrate occurs and the layer is excessively diluted by the substrate material. Thus preliminary trials using different values of P, D and V were carried out to determine the feasible set of parameter values, which were ultimately selected to be: $P = 300$ W, $D = 3$ mm and $V = 5$ mm/s. The laser fluence F was about 20 J/mm^2, according to Eq. (1).

These set of values were used in forming a single clad track on the sample. To treat the whole sample surface, adjacent melt tracks with an overlapping ratio of 50% were carried out with the aid of the computer-aided control in the system. Such a ratio is quite common in laser surfacing to obtain a good balance between surface homogeneity and processing efficiency. When the overlapping ratio is too low, the laser treated surface is inhomogeneous, featured by a wavy topography. When the overlapping ratio is too high, the cladding efficiency is low and overheating may also occur. After laser processing, the samples were ultrasonically cleaned and rinsed with deionized water. The corresponding laser treated samples were labeled as HA, 1% Ag-HA, 2% Ag-HA, and 5% Ag-HA. Triplicate samples of each composition were used in the tests.

2.2. Microstructural analysis

2.2.1. X-ray diffraction (XRD)

Structural analysis of the coating was carried out on a Siemens D500 X-ray diffractometer with a copper K_α X-ray source operated at 40 kV and 30 mA. The XRD patterns were acquired in the 2θ range of 10°–80° at a step increment of 0.05°, and a scan time 0.5 s was used.

2.2.2. Scanning-electron microscopy (SEM)

The elemental contents of the nanocomposite were determined by energy dispersive X-ray spectroscopy (EDS) (average of five measurements from different areas). The surface morphology and cross-section of the HA coatings were examined by scanning-electron microscopy (SEM, JEOL-820 and JSM-6510LV).

2.2.3. Immersion tests

In the simulated body fluid (SBF) immersion test, triplicate samples of 5% Ag-HA and HA samples were used to obtain statistical averages. The SBF which mimics human body fluids is composed of 7.996 g/l of NaCl, 0.35 g/l of $NaHCO_3$, 0.224 g/l of KCl, 0.228 g/l of $K_2HPO_4 \cdot 3H_2O$, 0.305 g/l of $MgCl_2 \cdot 6H_2O$, 0.278 g/l of $CaCl_2$, 0.071 g/l of Na_2SO_4, as well as 6.057 g/l $(CH_2OH)_3CNH_2$. The samples were immersed separately in 25 ml of SBF in polypropylene (PP) bottles with a pH value of 7.42. The PP bottles were closed tightly and incubated in a thermostatic chamber at 37 ± 0.5 °C for 5 days and 21 days, respectively. All the bottles were shaken gently for a few seconds every 2 days.

2.3. Biological analysis

2.3.1. Evaluation of antibacterial capacity

To investigate bacterial adhesion behaviors on the laser fabricated Ag-HA coatings, clinical isolated *Staphylococcus aureus* bacteria were used in the bacteria culture. Brain heart infusion broth (BHI) was prepared to culture *S. aureus* cells and BHI agar was also prepared for the growth of the single colony before growing an overnight culture. The BHI broth powder and agar powder had a concentration of 7.4 g/200 ml and 9.4 g/200 ml, respectively. 200 ml of distilled water were added to each bottle and mixed. The solutions were sterilized by autoclaving at 121 °C for 15 min. About 20–25 ml of the agar solution was poured into the culture dish to allow solidification and formation of an agar plate. The streaking plate isolated single colony was incubated for 24 h at 37 °C. Then, one single colony was taken and put into the BHI broth. The overnight culture was grown at 37 °C in a temperature control shaker (Thermo Forma, Thermo Fisher Scientific Inc, Waltham, MA, USA) operated at 250 rpm for 16–20 h, unless otherwise specified.

The number of bacteria in the overnight culture was recorded. As the overnight culture contained 10^{10}/ml bacteria (*S. aureus*), it was diluted to 10^8/ml to test bacterial adhesion on the laser fabricated Ag-HA and the untreated sample. 20 μl BHI with 10^8/ml bacteria, which was about 2×10^6 bacteria, were added to the surface. After incubation for 1 h, the unattached bacteria were removed by rinsing 3 times in 1 ml of PBS. The adherent bacteria were then removed from the surface by sonication. 1 ml of 0.01% Tween 80 in 0.01 M PBS was used to detach the bacteria from the surface and mixed with the sample for 1 min sonication. Afterwards, the suspension containing the bacteria was collected. The sample surface was rinsed 3 times again with 1 ml of PBS. Some of the suspension was diluted 50 times. In addition, 50 μl of the