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Osteoblast interaction with laser cladded HA and SiO_2-HA coatings on Ti–6Al–4V

Yuling Yang [a,b], Kaan Serpersu [b], Wei He [b,c,*], Sameer R. Paital [b], Narendra B. Dahotre [d,1]

[a] Department of Physics, Northeastern University, Shenyang 110004, China
[b] Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996, USA
[c] Department of Mechanical, Aerospace and Biomedical Engineering, The University of Tennessee, Knoxville, TN 37996, USA
[d] Department of Materials Science and Engineering, University of North Texas, Denton, TX 76207, USA

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ABSTRACT

In order to improve the bioactivity and biocompatibility of titanium endosseous implants, the morphology and composition of the surfaces were modified. Polished Ti–6Al–4V substrates were coated by a laser cladding process with different precursors: 100 wt.% HA and 25 wt.% SiO_2-HA. X-ray diffraction of the laser processed samples showed the presence of $CaTiO_3$, $Ca_3(PO_4)_2$, and Ca_2SiO_4 phases within the coatings. From in vitro studies, it was observed that compared to the unmodified substrate all laser cladded samples presented improved cellular interactions and bioactivity. The samples processed with 25 wt.% SiO_2-HA precursor showed a significantly higher HA precipitation after immersion in simulated body fluid than 100 wt.% HA precursor and titanium substrates. The in vitro biocompatibility of the laser cladded coatings and titanium substrate was investigated by culturing of mouse MC3T3-E1 pre-osteoblast cell line and analyzing the cell viability, cell proliferation, and cell morphology. A significantly higher cell attachment and proliferation rate were observed for both laser cladded 100 wt.% HA and 25 wt.% SiO_2-HA samples. Compared to 100 wt.% HA sample, 25 wt.% SiO_2-HA samples presented a slightly improved cellular interaction due to the addition of SiO_2. The staining of the actin filaments showed that the laser cladded samples induced a normal cytoskeleton and well-developed focal adhesion contacts. Scanning electron microscopic image of the cell cultured samples revealed better cell attachment and spreading for 25 wt.% SiO_2-HA and 100 wt.% HA coatings than titanium substrate. These results suggest that the laser cladding process improves the bioactivity and biocompatibility of titanium. The observed biological improvements are mainly due to the coating induced changes in surface chemistry and surface morphology.

1. Introduction

To date, a number of materials have been successfully used as implants for hard tissue replacement such as bone. Among them one class of materials has the ability to integrate with host tissue and promote strong bonding at the tissue–material interface. This genre of materials has been dubbed as bioactive materials. In contrast, the other class of materials that remain completely neutral without any toxicity or reaction with the surrounding tissue is called the bioinert materials. Titanium and its alloys, especially Ti–6Al–4V, are the most commonly used bioinert materials for both dental and orthopedic applications [1,2], owing to their excellent mechanical properties, biocompatibility, corrosion resistance, and tissue compatibility. As bio-implants, they adsorb protein from surrounding biological fluid to create a protein layer that will support cell growth [3]. Titanium and Ti–6Al–4V being completely neutral also make them the ideal choice for patients who may develop toxic reactions to other metal alloys.

Although, titanium is bioinert, an oxide layer forms almost immediately on the surface interacting with the surrounding biological fluid. This naturally forming oxide layer occurs as a result of titanium high reactivity with oxygen [4]. Due to its thin layer, and amorphous and porous structures, the oxide layer possesses a risk of dissolving titanium ions into the body plasma and therefore is not a sound barrier for such dissolutions [5]. However, the field of applications can be expanded if the bioactivity and biocompatibility of the titanium implants are improved through a bioceramic coating. The techniques most commonly used to provide a bioceramic coating on the titanium implants are dip coating [6], sol–gel [7], plasma-spraying [8–10], simultaneous vapor deposition [11], and laser deposition [12] procedures. The criteria for judging the success of these coating processes are whether they can achieve a high crystallinity in the coatings, good adherence between the coating and the metal substrate, control over coating thickness and the ability to coat porous and complex-shaped implants. Of these methods, laser cladding (LC) has gained extensive use due to its ability to achieve appropriate surface textures and

* Corresponding author at: 209 Dougherty Engineering Bldg., Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996, USA. Tel.: +1 865 974 5275; fax: +1 865 974 4115.
E-mail address: whe5@utk.edu (W. He).
[1] Formerly at Department of Materials Science and Engineering, The University of Tennessee, USA.

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surface chemistry and thereby improve the biocompatibility of metallic surfaces at the tissue–implant interface [13–16]. Further, the LC technique has also demonstrated distinct advantages which can be listed as follows [17]:

(1) localized heating which reduces thermal distortion and the size of the heat-affected zone;
(2) controlled levels of dilution;
(3) controlled shape of the clad within certain limits;
(4) controlled surface roughness;
(5) fine microstructures and metallurgical bonding;
(6) high deposition rate and flexibility of the process.

Therefore, LC is justifiably becoming a popular choice as a method for modifying surface properties [18–20], and lasers have been used to produce ceramic coatings on metallic materials for biomedical applications. In this way, the benefits of the bioceramics are combined with the mechanical performance of titanium implants. The most common examples of this type of combination are titanium coated with hydroxyapatite (HA) [8,9,11], and titanium with pseudowollastonite [21]. HA (chemical formula $Ca_{10}(PO_4)_6(OH)_2$) due to its chemical and crystallographic resemblance to bone and tooth minerals [22], has been widely studied as an artificial bone and tooth replacement material in orthopedic and dental implants. They are most commonly used as a coating of hard tissue implants, bone fillers, and for drug delivery. However, HA coated implants have the disadvantage that, in comparison with bioactive glasses and glass ceramics, the rate of bonding of bone tissue with bone implants is relatively low [23] and this has implications for prolonging the time required for patient recovery. Approaches toward improving the integration rates of HA with bone have included the incorporation of biological entities such as growth factors, proteins, and cells into the HA implant [24,25]. As an alternative to improve the biocompatibility of the implant, HA may also be chemically doped with small amounts of elements which are commonly found in physiological bone [26]. It was reported that trace elements of silicon and titanium in calcium HA ceramics and coatings can influence both the biological response of implant materials [27], and the crystallographic, mechanical and chemical properties of manufactured ceramics and coatings [28]. Best and co-workers have put their efforts in studying the development of silicate-substituted HA (Si-HA) [29–31], and demonstrated a significant increase in the amount of bone apposition and organization around Si-HA implants, illustrating their potential as bone graft materials [32]. Other researchers have reported the results of firing a stoichiometric HA precipitate to which SiO_2 has been added [33–35]. In these reports, SiO_2 was incorporated either by diffusion from an adjacent substrate [33] or by incorporation of SiO_2 into the precipitate using the thermal decomposition of a metallorganic additive [34,35]. There are also several reports by Thian and co-workers [36–38] where they have synthesized biocompatible silicon substituted hydroxyapatite composite coatings on Ti substrates by a magnetron co-sputtering technique. The authors reported that the presence of a thin Si-HA coating synthesized by this process stimulated the osteoblast attachment, proliferation and differentiation. So far, few published papers have reported laser cladding of SiO_2-HA on metallic alloys and corresponding bone cell response to such coatings.

In the present study, HA and SiO_2-HA coatings were prepared on Ti–6Al–4V substrates using a laser cladding (LC) technique. Here, a highly intense laser beam was used to melt the precursor (HA and SiO_2-HA) and the surface of the Ti–6Al–4V substrate to achieve a micro-textured, multi-phase coating as well as metallurgical bonding at the interface. The in vitro bioactivity was assessed by immersing the samples in a simulated body fluid (SBF). The in vitro biocompatibility, including cell attachment, cell viability, and cell proliferation was investigated by culturing of mouse MC3T3-E1 pre-osteoblast cell line.

2. Materials and methods

2.1. Precursor materials and laser cladding

Ti alloy (Ti–6Al–4V) plates, cut from the rolled sheets with a thickness of 3 mm and dimensions of $50\times50\ mm^2$ were used as substrates. They were polished using 30 μm grit silicon carbide (SiC) emery paper and sequentially rinsed with acetone to obtain a clean surface free from rust and grease. The HA ($Ca_{10}(PO_4)_6(OH)_2$) and silica (SiO_2) powders, obtained from Fisher Scientific, were used as the precursor materials. The HA and SiO_2 precursor powders had a spherical morphology with a unimodal distribution in the 10–30 μm range. The precursors (25 wt.% SiO_2-HA or 100 wt.% HA) were mixed in a water-based organic solvent (LISI W 15853) obtained from Warren Paint and Color Company (Nashville, TN, USA) and mechanically stirred for 25 min to get a viscous slurry. The slurry was then sprayed onto the polished and clean substrate coupons using an air pressurized spray gun. The sprayed coupons were dried in air to remove moisture. The thickness of the precursor deposit was maintained at 80 μm for all samples.

The laser source employed in this work was a pulsed Nd:YAG laser equipped with a fiber optic beam delivery system that operates in the infrared region with a wavelength of 1064 nm. A high power laser radiation obtained from the above source is directed to the precursor sprayed surface of the substrate and as the laser beam heats up the precursor and the surface of the substrate a molten pool is created on the metallic substrate. Rapid quenching of the molten pool takes place as the laser beam is scanned away from the irradiated area. The scanning is carried in a way such that overlapping (0.1 mm or ~11%) laser cladding tracks were laid to obtain a continuous coating. Thus, a coating with metallurgical bonding with the substrate is achieved. The laser processing parameters employed in the present work are listed in Table 1.

2.2. Bioactivity

In vitro bioactivity studies were performed by soaking the samples in a simulated body fluid (SBF) solution with ionic composition similar to that of the human plasma. The SBF solution is prepared by dissolving the reagent grade chemicals in the following order: NaCl (8.026 g), $NaHCO_3$ (0.352 g), KCl (0.225 g), $K_2HPO_4\cdot3H_2O$ (0.230 g), $MgCl_2\cdot6H_2O$ (0.311 g), $CaCl_2$ (0.293 g) and Na_2SO_4 (0.072 g) in distilled water (700 mL). The fluid was then buffered to pH 7.4 with tri-hydroxymethylaminomethane [$(CH_2OH)_3CNH_2$] (6.063 g) and hydrochloric acid (1 M, 40 mL) at 37 °C [39]. To study the bioactivity, the in vitro assays were carried out for a set of three samples from each processing condition. The samples were soaked in 10 mL of SBF solution in plastic containers. Soaking periods were varied for 1, 3, 5 and 7 days. The solution was refreshed every 24 h to maintain a pH value of 7.4, and the temperature was maintained at 37 °C during the soaking course. The samples following SBF immersion were dried in a vacuum desiccator for more than 12 h to remove any entrapped water molecules. The weight of samples before and after immersion in the SBF solution was measured to evaluate the HA deposition rate in SBF. The weight change measurements were carried out using a microbalance.

Table 1
Laser parameters used in this study.

Pulse width (ms)	1.0
Pulse repetition (Hz)	30
Pulse energy (J)	4.0
Spot diameter (μm)	900
Laser scan speed (cm/min)	75
Line spacing (mm)	0.1