Biocatalysis and Agricultural Biotechnology 15 (2018) 364–369

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Biocompatible response of hydroxyapatite coated on near-β titanium alloys by E-beam evaporation method

S. Gnanavel[a], S. Ponnusamy[b,*], L. Mohan[c]

[a] Department of Biomedical Engineering, SRM Institute of Science and Technology, Chennai, Tamilnadu, India
[b] Center for Materials Science and Nano Devices, SRM Institute of Science and Technology, Chennai, Tamilnadu, India
[c] Surface Engineering Division, CSIR-National Aerospace Laboratories, Bangalore, Karnataka, India

ARTICLE INFO

Keywords:
Hydroxyapatite
E-beam evaporation
Corrosion
Ti-13Nb-13Zr

ABSTRACT

Biomedical implants such as dental and orthopedic implants fails due to poor mechanical and biological properties. In order to solve this problem, Electron beam (e-beam) evaporations is used as one of the methods to deposit the hydroxyapatite (HA) on Ti-13Nb-13Zr (near β titanium alloy) and the coated implant may play a significant role in increasing the mechanical and biological properties. In the present study, Ti-13Nb-13Zr was coated with hydroxyapatite (HA) by e-beam evaporation technique. The coated alloys were morphologically analyzed by FESEM and AFM, and it demonstrates that there is an increase in the growth of calcium phosphate layer. *In-vitro* corrosion behavior of the coated and uncoated titanium alloys were performed by electrochemical impedance spectroscopy (EIS) studies in simulated body fluid solution. The results show that the corrosion resistance of the hydroxyapatite-coated alloy higher than that of the uncoated alloys and it evident that the HA-coated alloy have better corrosion protection for the implant application. The bioactivity of the HA-coated composites were evaluated by Hanks' solution immersed them for seven days. The ratio Ca/P was increased gradually after soaking it for seven days. The cell viability results indicates that HA coated alloys support increase in the Osseo integration and it can be used for bone implant application.

1. Introduction

Biomedical alloys like titanium and stainless steel were used in orthopedic and dental application due to their good mechanical properties and corrosion resistance as it tis closer to the properties of bones (Das et al., 2008; Burns et al., 2009; Narayanan et al., 2009). Titanium and its alloy are relatively non-bioactive and lack rapid tissue integration which results in the subsequent development of interfacial fibrous tissue, finally leading to isolation of implants. Due to poor wear resistance and low surface hardness, pure titanium were limited application (Wang et al., 1999). Therefore, it is necessary to develop surface modification technologies that can improves the interfacial bonding of the alloy and bioactivity (Kuo and Yen, 2002; Shi et al., 2002; Thian et al., 2001). Hydroxyapatite ceramics, commonly utilized in bone implant because of its elemental compositions are equivalent to the bone. This type of ceramic material is to be added with host tissue and benefit high bonding at the bone tissue response. Hydroxyapatite ceramics production was accepted and considered as one of the bone implant alternate material (Bezzi et al., 2003; Fernández-Pradas et al., 2002;). But because of poor mechanical properties hydroxyapatite it was not utilized in implant application. Hence, HA coating on bioinert metal such as Ti-13Nb-13Zr alloy was developed to the excellent bioactivity of HA simultaneously. Many deposition methods have been utilized to modify the surface of the material. One of the deposition method was plasma spraying method, it is frequently used to produce a pristine hydroxyapatite layer on titanium metal. This type of hydroxyapatite deposition determines good biological properties in bone implants applications. It also enhance Osseointegration of the hydroxyapatite layer without any formation of fibrous tissue. However, there are disadvantages in plasma spray hydroxyapatite deposition particularly in the lifetime of the implants under mechanical properties. Different failure were also noticed such as discontinuity and break down of the deposition layer and its reduced mechanical stability, prompting an intense incendiary response. While trying to determine a solution these problems, different deposition method was proposed to deliver very thinly hydroxyapatite layer (200 nm) with more symmetric compounds. (Ramires et al., 2003; Sato et al., 2005). The electron beam deposited apatite film (HA and its fluoridated form) were much shown to be thin micrometer and to have the dense and homogeneous structure (Choi et al., 2000). Moreover, this method is the controllable coating

* Corresponding author.
E-mail address: suruponnus@gmail.com (S. Ponnusamy).

https://doi.org/10.1016/j.bcab.2018.07.014
Received 14 April 2018; Received in revised form 27 June 2018; Accepted 11 July 2018

thickness and also uniformly coated on the surface area. Commonly, an account of electron beam technique, a subsequent heat treatment process is essential to present in the thin films. In any case, the difference in the thermal properties between the coated surface and substrate, high thermal stress also increases cracking are produced inside the coating layer, bringing about coating failure and mechanical instability (Lee et al., 2005). In the e-beam method, the materials utilizations efficiency is relatively high compare with other coating methods. Another advantage of this method was coating film morphologically controllable. However, to our knowledge, near β titanium alloys of cell viability and corrosion studies is very less and these type alloys approved by FDA, US. In this study HA (hydroxyapatite) coated with Ti-13Nb-13Zr (ASTM F1713-08) substrate by the e-beam evaporation method is adopted and the results of coated materials are discussed.

2. Materials and methods

2.1. Deposition procedure

Commercially available near β titanium substrate (Ti-13Nb-13Zr) with the dimension of 10 mm × 10 mm × 3 mm was prepared as the substrate and then polished with a grit silicon carbide paper. The Ti-13 Nb-13Zr substrate was washed ultrasonically in ethanol with distilled water about for ten minutes. The substrate was kept on the sample holder in a vacuum chamber and again washed. The substrate depicts by argon gas with 40 min with operated 90 V with a two-ampere current. The target material HA was purchased from (Sigma Aldrich chem co, India). Pellet was formed using pellet pressing machine with the thickness of 10 mm. The pellet was sintered at 1200 °C for 6 h. Prior to the deposition of the Ti-13Nb-13Zr substrate was cleaned using acetone and kept in ultrasonicator for 30 min with Millipore water. The substrate (Ti-13Nb-13Zr) placed in the substrate holder with a distance between target and substrate holder were15 cm. Diffusion pumps was used to create a vacuum with a pressure of 5×10^{-2} Torr for deposition. The thickness of the coating were monitored by thickness control system. The rate of deposition is 10 A°/s. The thin films with a thickness of 90 nm were deposited on Ti-13Nb-13Zr substrates by E-beam evaporation (Lee et al., 2007).

2.2. Hanks' solutions

In-vitro immersion test was conducted by the coated and uncoated samples by immersing them simulated body fluid solution (SBF) for seven days at a temperature of 37 ± 1 °C. The 40 ml of freshly prepared body fluid (Hanks' solution) which is equivalent to that human blood (0.42 g = KCl_2, 9 g = $NaCl_2$, 0.21 g = $NaHCO_3$, 0.25 g = $CaCl_2$, 0.063 g = KH_2PO_4 and glucose) at pH 7.5 (Al-Mobarak et al., 2011) was used as the SBF solution for this study.

2.3. Corrosion test

Corrosion test for the HA-coated substrates were performed using electrochemical device supplied by the BIOLOGIC system, United Kingdom. The experiment was carried in 200 ml of body fluid solution. The coated substrate was treated as working electrode. Platinum and SCE (saturated calomel electrode) was utilized as a counter and reference electrode respectively. To maintain the stability of the samples they were soaked in body fluid solution for about 60 min. Once stabilized EIS (electrochemical impedance spectroscopy) test was conducted in the frequency ranging from 100 kHz to 10 MHz. A Ten millivolt alternative current was applied on the E_{ocp} (open circuit potential). After each experiment, the impedance data were presented in the Bode plot. The data obtained were curve fitted and analyzed using Zsimpwin program to get suitable equivalent circuit parameter. After the electrochemical experiment, the system was allowed to attain open circuit potential and the electrode was linearly polarized from a starting voltage of 200 mV below the OCP value to 2500 mV on the positive side. 1 mV/s rate was maintained as the linear potential. The Tafel plot was obtained from the potentiodynamic polarization experiment in the form of voltage versus log (i) plot. The corrosion voltage (E_{cor}) and corrosion current (I_{cor}) are understood from Tafel plot. The corrosion current is obtained by using stern-Geary Eq. (1) (Gnanavel et al., 2018; Singh et al., 2011).

$$I_{corr} = (\beta_a \times \beta_c)/2.3R_p(\beta_a + \beta_c) \quad (1)$$

where, β_a and β_c – Slopes of the anodic and cathodic parts of the Tafel Plot

R_p - Polarization resistance.

2.4. Cell culture

3T3 (mouse fibroblast) cell line were cultured in liquid Dulbecco's Modified Eagle's Medium(DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37 °C.

2.5. MTT assay

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were purchased from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3–4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were supplied by received from Sigma, (USA), 1 × PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were purchased from Tarson (India). The metal sample was tested for in vitro cytotoxicity, using 3T3 cells by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured 3T3 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 0.5×10^6 cells/ml cells/well (1 ml) into 24-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24 h at 37 °C. The wells were washed with sterile Phosphate buffered saline (PBS) and treated with various concentrations of the metal sample in a serum-free DMEM medium and the cells were incubated at 37 °C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (50 µl of 5 mg/ml) was added into each well and the cells incubated for another 2–4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT was aspirated off the wells and washed with 1 × PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (200 µl) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability was calculated using GraphPad Prism 6.0 software (USA).

3. Results and discussion

3.1. X-ray diffraction studies

An XRD pattern obtained for the nearβ titanium substrate e-beam coated with HA ceramics is presented in Fig. 1. The highest peak in these X-ray diffraction patterns compares the angle 34.41°, 39.60°, 52.09°, 55.03°, 75.58° and 76.15° well matches with hydroxyapatite when filed as per JCPDS file # (90432). The value of 2θ = 38.16° and 69.52° were confirm the titanium substrate (with reference of JCPDS file No 44129). The presence of the hydroxyapatite in the deposition mimic the bone mineral and provide the excellent biological properties such as the implant-bone growth factor (Mohan et al., 2012). The particle size of the near β titanium alloys was calculated by Scherrer formula,

$$d = 0.9\lambda/\beta \cos\theta$$

Where, λ wave length of X-rays source and β–FWHM (full wave half