Applied Surface Science 290 (2014) 448–457

Contents lists available at ScienceDirect

Applied Surface Science

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

Biomimetic novel nanoporous niobium oxide coating for orthopaedic applications

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ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form
21 November 2013
Accepted 21 November 2013
Available online 1 December 2013

Keywords:
Niobium oxide
Nanostructures
Hydroxyapatite
Corrosion test

ABSTRACT

Niobium oxide was synthesized by sol–gel methodology and a crystalline, nanoporous and adherent coating of Nb_2O_5 was deposited on 316L SS using the spin coating technique and heat treatment. The synthesis conditions were optimized to obtain a nanoporous morphology. The coating was characterized using attenuated total reflectance-Infrared spectroscopy (ATR-IR), X-ray diffraction analysis (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX), atomic force microscopy (AFM) and transmission electron microscopy (TEM) and the formation of crystalline Nb_2O_5 coating with nanoporous morphology was confirmed. Mechanical studies confirmed that the coating has excellent adherence to the substrate and the hardness value of the coating was excellent. Contact angle analysis showed increased hydrophilicity for the coated substrate. *In vitro* bioactivity test confirmed that the Nb_2O_5 coating with nanoporous morphology facilitated the growth of hydroxyapatite (HAp). This was further confirmed by the solution analysis test where increased uptake of calcium and phosphorous ions from simulated body fluid (SBF) was observed. Electrochemical evaluation of the coating confirmed that the crystalline coating is insulative and protective in nature and offered excellent corrosion protection to 316L SS. Thus, this study confirmed that the nanoporous crystalline Nb_2O_5 coating conferred bioactivity and enhanced corrosion resistance on 316L SS.

1. Introduction

Development of novel implant devices with appreciable performance and functionality is essential, in view of the ever increasing public demand. The materials developed for load bearing implants must be mechanically strong and must possess high resistance to corrosion and wear to prevent the loss of mechanical strength and release of metallic ions in the human body [1,2]. Further, the implantable material should have excellent biocompatibility as the formation of an interfacial bond between the tissue and the implant determines the success of early peri-implant healing [3]. In recent years, the importance of topography of implant materials is widely acknowledged and nanostructured features are favored as many fundamental biological processes occur on the nanoscale at the surfaces and interfaces in the human body [4]. The nanoscale topography has been reported to influence cellular interactions such as osteoblast adhesion and further biomineralization [5].

Of the various metallic materials used as orthopaedic implants, 316L SS is a preferred implant material due to its appreciable corrosion resistance, high mechanical strength and acceptable biocompatibility [6]. However, in the presence of aggressive body fluid, 316L SS is susceptible to localized corrosion in due time and releases metallic ions into the neighbouring tissues leading to adverse biological effects [7]. Application of bioceramic oxide coatings on 316L SS surface is an effective method to increase the corrosion resistance as well as biocompatibility of the material. Various ceramic oxide coatings such as TiO_2, SiO_2, ZrO_2, Nb_2O_5, calcium phosphate etc., have been studied for their biocompatibility [8–11]. Of these, Nb_2O_5 is a preferred coating material as it has extremely high corrosion resistance, is thermodynamically stable and biocompatible [12]. Further, it has been reported that, Nb is hypoallergic and is a safest metal as it is tolerated by the human body [13].

A nanostructured coating can be fabricated on 316L SS by sol–gel technique as this method offers the possibility of controlling the microstructure of the coated films. Sol–gel technique is highly versatile as it can yield powders, films, fibres, aerogels, xerogels etc. of high purity and homogeneity and it has the ability to coat large and complex area at relatively low temperatures [14,15]. Further, sol–gel based coatings offer good adhesion to metallic surfaces via chemical bonding [16]. Eisenbarth et al. [17] reported that niobium oxide coated cp titanium showed increased migration and adhesion of MC3T3-E1 cells compared to uncoated titanium. Rojas et al. [18] deposited amorphous niobium oxide film on 316L SS by magnetron sputtering and has reported that the coated substrates exhibited improved corrosion resistance. However, there are no reports

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http://dx.doi.org/10.1016/j.apsusc.2013.11.112

focusing the effect of a crystalline and nanostructured Nb_2O_5 coating on biocompatibility and corrosion resistance of 316L SS.

Hence in the present investigation, novel biomimetic nanoporous crystalline Nb_2O_5 coating was deposited on 316L SS surface by sol–gel methodology and spin coating technique. The coated specimens were characterized by various surface characterization techniques. The ability of the crystalline coating in increasing the corrosion resistance of 316L SS was evaluated by electrochemical techniques. The influence of the nanoporous morphology in increasing bioactivity was evaluated by *in vitro* bioactivity test.

2. Experimental procedure

Niobium ethoxide (NbE) (Sigma Aldrich), iso-propanol (i-PrOH) (Merck, India), Acetylacetone (ACA) (Merck, India) and polyethylene glycol 400 (PEG) (Merck, India) were used as the starting materials without further purification. The molar ratio of each chemical in the sol was 1:0.2:30:0.02 (NbE: ACA: i-PrOH: H_2O). Iso-propanol was mixed with ACA and PEG and the solution was stirred vigorously at 80 °C for 15 min. Later, NbE–i-PrOH solution was added and vigorously stirred for another 30 min. Subsequently, H_2O was added and the mixture was allowed to stir vigorously at 80 °C for another 3 h. The resultant clear sol was aged for 8 h to facilitate gelation.

The surface of 316L SS specimens of size 30 × 15 × 3 mm were abraded with silicon carbide papers up to 400 grit, washed thoroughly with double distilled water and degreased with acetone. Then the specimens were etched with a mixture of 15% HNO_3 and 5% HF for 2 min to remove the surface oxides, washed thoroughly with double distilled water, ultrasonically degreased with acetone and finally dried at 40 °C. The composition of 316L SS is given in Table 1. The specimens were then spin coated with the as prepared sol at a rotation speed of 2000 rpm for 2 min. The Nb_2O_5 coated specimens thus obtained were dried in air at 60 °C to facilitate further gelation and condensation. The dried specimens were then sintered at 500 °C for 1 h at a slow heating rate of 2 °C min^{-1}. Calcination at slow heating rate enables oxide conversion and removal of solvent and residual organics. Moreover, the oxides could crystallize during the sintering process.

The changes that occur during thermal treatment were analyzed by carrying out thermo gravimetric analysis in a nitrogen atmosphere, using Netzsch STA 409 instrument. The heating rate was fixed at 10 °C min^{-1}. The specific surface area of Nb_2O_5 was determined using Brunauer–Emmett–Teller (BET) analysis by nitrogen adsorption–desorption isotherms at liquid nitrogen temperature using a Quantachrome quadrawin version 5.02 instrument. The ATR-IR spectra of Nb_2O_5 coated 316L SS before and after *in-vitro* bioactivity test was recorded in the 4000–400 cm^{-1} range on a Perkin Elmer FT-IR spectrometer Spectrum Two with UATR Two Accessory and KBr window. The X-ray diffraction patterns of Nb_2O_5 coating on 316L SS before and after bioactivity test was recorded with a Pan Analytical X-pert pro diffractometer using Cu $K\alpha$ radiation (λ = 0.15406 nm) with 40 kV and 30 mA, at a scan rate of (2θ) 0.02° over the range 10–80°. The average crystallite size of Nb_2O_5 was determined from the most intense X-ray peak by Scherrer's equation [19].

$$\text{Crystallite size} = \frac{k\lambda}{\beta \text{Cos}\theta} \quad (1)$$

Table 1
Composition of 316L stainless steel.

Element	Cr	Ni	Mo	Mn	N	C	Fe
Wt%	17.20	12.60	2.40	1.95	0.02	0.03	Balance

where k is a constant related to the crystallite shape (0.89) and β is the full width of half maximum (FWHM) of the diffraction peak. λ and θ represent the wavelength and diffraction angle of the X-rays, respectively.

The surface morphology of the coatings on 316L SS before and after *in vitro* bioactivity test was characterized by scanning electron microscope (SEM) on a Hitachi Model-S 3400 with an accelerating voltage of 5–30 kV. The three dimensional topography of the coating was examined by atomic force microscopy (AFM) on Agilent Technology's Pico LE SPM in contact mode with silicon cantilevers with force constants 0.02–0.77 N m^{-1}. The microstructures of the coated films scratched off the substrates were examined using high resolution transmission electron microscope (HRTEM) (FEI TecnaiTM G2 F20 (S-TWIN). Hydrophilicity of the uncoated and Nb_2O_5 coated 316L SS was studied with three test liquids, namely, ultrapure water, glycerol and diiodomethane using the contact angle meter (OCA 15EC, Data physics instruments, Germany). Drops of the test liquid were delivered onto the specimen surface by a syringe with a set drop volume of 10 μL at a dosing rate of 1 μL s^{-1}. An average of 10 readings was taken for each sample. The surface energy, E_s was calculated from the contact angle values using the following equation [20]:

$$E_s = E_{vl} \cos\theta \quad (2)$$

where E_{vl} is the surface energy between water and air under ambient condition, (*i.e.*, 72.8 mJ m^{-2} at 20 °C) for pure water and θ is the static contact angle.

The uncoated and Nb_2O_5 coated 316L SS were soaked in simulated body fluid (SBF) under static conditions for a period of 14 days to assess their ability to favour apatite deposition. Simulated body fluid (SBF) was prepared according to the procedure proposed by Kokubo et al. [21]. In addition, sodium azide was added to SBF to avoid bacterial growth. On alternate days, SBF was renewed to avoid precipitation. The temperature of the samples immersed in SBF was maintained at 37 °C, similar to human body temperature throughout the experimental time. Solution analysis was carried out to assess the coatings ability to aid HAp growth. Changes in the concentration of calcium and phosphate ions in the solution were determined using ICP-OES analysis after 7 and 14 days to assess the coatings ability to aid HAp growth.

The thickness of the coated films on 316L SS were measured using the Elcomaster thickness meter. Tape adhesion measurement was performed on Nb_2O_5 coated 316L SS according to ASTM D 3359. Six parallel score lines were made with a separation of 1.0 mm; further six score lines were scribed perpendicular to the original score lines. For each individual specimen, 25 grids were generated. Adhesive tape was placed on the grids using a soft eraser; the tape was then removed with a firm and steady pulling action. The equation given below was used for evaluating the percentage of adhesion remaining:

$$\text{AR}\,\% = (n/25) \times 100 \quad (3)$$

where AR represents adhesion remaining and n is the average number of squares of undetached coating [22]. Hardness of the coating was studied using Vickers Micro hardness Tester with a loading force of 50g for the duration of 5 s. For both coated and uncoated substrates, hardness was measured for 10 times and the average value was taken.

The elastic modulus of the coating was determined perpendicular to the surface based on elastic recovery of Knoop indentation. The measurement was carried out by indenting a Knoop indenter (Wolpert Wilson, Model 402 MVD, USA) for 10 s at a load of 100 N.