Applied Surface Science 329 (2015) 76–82

Contents lists available at ScienceDirect

Applied Surface Science

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

Hydroxyapatite/gelatin functionalized graphene oxide composite coatings deposited on TiO_2 nanotube by electrochemical deposition for biomedical applications

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ARTICLE INFO

Article history:
Received 28 September 2014
Received in revised form 9 December 2014
Accepted 16 December 2014
Available online 25 December 2014

Keywords:
Graphene oxide
Gelatin
Hydroxyapatite
TiO_2 nanotube
Electrochemical deposition
Cell culture

ABSTRACT

Graphene oxide cross-linked gelatin was employed as reinforcement fillers in hydroxyapatite coatings by electrochemical deposition process on TiO_2 nanotube arrays (TNs). The TNs were grown on titanium by electrochemical anodization in hydrofluoric electrolyte using constant voltage. Fourier transform infrared spectroscopy, X-ray diffraction, X-ray photoelectron spectroscopy, Field emission scanning electron microscopy equipped with energy dispersive X-ray analysis and biological studies were used to characterize the coatings. The corrosion resistance of the coatings was also investigated by electrochemical method in simulated body fluid solution.

1. Introduction

Titanium and its alloys are wildly and successfully used in producing implants for their good mechanical properties, bioactivity and corrosion resistance [1,2]. To achieve good bioactivity and anticorrosion properties, the surface of titanium often needs modifications, such as alkali treatment, anodic oxidation of TiO_2 and coatings [3,4]. The 'increased surface area' of nanotubular surface in TiO_2 provides a large number of active reaction sites for chemical reaction and improves the physical bonding strength by anchorage. It also enhances adhesion, growth and differentiation of the cells [5,6]. TNs was firstly prepared by Gong et al. on pure Ti substrate by anodization in an aqueous solution containing hydrofluoric (HF) acid [7]. Such structure of TNs induced the formation of hydroxyatite (HAp) [8] and enhanced the bond strength between the HAp layer and the Ti substrate [9]. HAp has attracted much attention in implant dentistry due to its unique properties, such as excellent bioactivity, biocompatibility and osteoconductivity [10,11]. Synthetic HAp coatings have better bioactivity and osteoconductivity compared to metal surfaces [12]. Among various preparation methods of HAp coatings, the electrochemical deposition is prominent due to successfully in producing biomimetic HAp coatings [13].

Although HAp has been successfully used in some field, its brittle nature impedes its applications under load-bearing conditions. The incorporation of reinforcing material like gelatin (Gel), chitosan, graphene oxide (GO), carbon nanotube, is applied to address this weakness and improve the corrosion resistance and bioactivity [14–17]. GO has been used in numerous biomedical areas, such as biosensing, drug delivery, biological imaging [18,19], because of their large surface-to-volume ratio, biocompatibility, good dispersibility in water and organic solvent and intriguing electrical and optical properties [20]. GO contains a range of reactive oxygen functional groups, which make it a suitable candidate for use in different applications through chemical functionalization [21]. Modifications of GO by chitosan and Gel through amidation because of the epoxy groups of GO and amino groups of chitosan and gelatin have been reported [22,23]. Gel, as a denatured collagen, has been recognized to promote cellular adhesion, proliferation and differentiation of cells for its superb bioactive [24]. Li et al. found the addition of GO could enhance the adhesion strength, corrosion

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http://dx.doi.org/10.1016/j.apsusc.2014.12.115
0169-4332/

protection of Ti substrate in simulated body fluid (SBF) solution [25] and promote the proliferation of MG63 cells [15]. In our previous work, strontium doped HAp and Gel composite coatings were successfully prepared using electrochemical deposition, and composite coatings have better cytocompatibility than pure HAp coating [17]. Lee et al.'s study also represented the improved biomimetic properties of HAp/Gel composite in vitro [26]. The Gel could induce the HAp nuclei by supplying abundant reaction sites such as carboxyl, which bind with calcium ions [27].

The current research aims to integrate the advantages of these materials by preparing a hydroxyapatite/gelatin functionalized GO composite coating on anodized titanium using electrochemical deposition, hence to improve the biological and mechanical properties of HAp coatings. The effect of Gel and GO incorporation on the crystallinity, composition, morphology, and corrosion protection performance of the GelGOHAp composite coating on anodized titanium was analyzed. In vitro biological affinity of the composite coatings was also investigated by culturing mouse calvarial cells (MC3T3-E1).

2. Materials and methods

2.1. Preparation of Ti sheets

The 10 mm × 10 mm × 0.9 mm medical pure titanium sheets (Non-Ferrous Metals Ltd., Baoji, China) were polished by SiC paper to 400 grit, cleaned following standardized procedure and further etched in acid solution (HNO_3:HF:H_2O = 3:1:10) for 20 s.

2.2. Preparation of TiO_2 nanotube

The anodization cell consisted of a 250 mL plastic breaker contained 5 wt% HF solution with a 15 mm × 15 mm × 0.1 mm platinum foil (cathode) and a Ti sheet (anode) that were 4 cm apart. The anodization process was conducted at a constant voltage of 20 V for 60 min. Ti sheets were washed thrice in deionized water and dried at room temperature after anodization.

Lastly, some anodized Ti sheets were annealed in air at 450 °C for 3 h, with a heating rate of 15 °C min^{-1} to obtain the desired structure.

2.3. GelGOHAp formation on TiO_2 nanotube

The aqueous dispersion of GO sheets was carried out following the literature procedure. Aqueous suspensions of GO (2 mg mL^{-1} solution) were prepared by 40 min sonication of GO. A 10 mg mL^{-1} aqueous gelatin solution was prepared by dissolving 100 mg of gelatin in 10 mL deionized water at 60 °C for 2 h. Aqueous suspensions of Gel–GO were prepared by adding 2 mL aqueous suspensions of GO into the 10 mL solution of gelatin, and were stirred magnetically for 24 h at room temperature.

The deposition of GelGOHAp composite coating was conducted by a three-electrode cell system, with a platinum foil as anode, an anodized Ti sheet as cathode and a saturated calomel electrode (SCE) as reference electrode. The LK2005A (Tianjin, China) electrochemical workstation was used. The electrolyte was prepared by dissolving 0.042 mol L^{-1} $Ca(NO_3)_2$, 0.025 mol L^{-1} $NH_4H_2PO_4$ in deionized water and adding 3 mL aqueous suspension of Gel–GO, the pH value of electrolyte was adjusted to be 4.3 using ammonia solution. Deposition process parameters were as follows: constant current destiny, 0.85 mA/cm^2; temperature of the electrolyte, 65 °C; distance between work electrode and counter electrode, 2 cm; and deposition time, 2100 s. Subsequently, the obtained coatings were treated in 0.1 mol L^{-1} NaOH for 2 h. Finally, all coatings were gently rinsed in deionized water and dried at the room temperature.

3. Characterization

3.1. Surface morphology and microstructure analysis

X-ray diffraction (XRD) characterization of the coatings was performed using Cu Kα radiation at 40 kV and 30 mA at room temperature in the θ–2θ scan mode (X'Pert Pro DX1000, China). The surface morphologies of the samples were observed by a field emission scanning electron microscope (FESEM) (JSM-7500F, Japan) equipped with energy dispersive X-ray (EDX), whereas the chemistry of the coatings was investigated by Fourier transform infrared spectroscopy (FTIR) (NICONET NEXUS 670, USA). X-ray photoelectron spectroscopy (XPS) spectra were obtained by using a VG ESCALAB MKII X-ray photoelectron spectrometer (VG Scientific Ltd., UK) with Al Kα radiation. Survey spectra were recorded for 0–1350 eV binding energy range.

3.2. Bond strength test

Tensile tests were performed with an electronic universal testing machine (INSTRON-5567) to determine the bond strength between the coatings and the substrate. One side of the obtained sheets was fixed to the testing stage. The other side (GelGOHAp/TiO_2 or HAp/TiO_2 coating) was attached to the cylindrical stubby glue, and cured 48 h at a room temperature before testing. The pull speed of the stub was 1 mm min^{-1} until failure occurred. Bond strength of the coating to substrate was calculated from the fracture load and the reported bond strength was the average value of four samples.

3.3. Polarization curve test

The anodic polarization experiment was carried out to access the anticorrosive characteristics of the composite coatings in SBF at 37 °C with the LK2005A electrochemical workstation. SBF was prepared according to Kokubo and Takadama's paper [28]. A platinum, titanium specimens and saturated calomel electrode (SCE) were used as the counter, working and reference electrode, respectively. Before testing, the sample was immersed in SBF electrolyte for 2 h to be stable. Anodic polarization studies were measured at a scan rate of 5 mV/s in the potential range between −0.8 and 1 V. Every experimental was repeated three times.

3.4. Cell viability test

The biocompatibility of the samples was evaluated by culturing osteoblasts (OB; MCT3T3-E1) cells on various coatings. The following three groups of samples were used: GelGOHAp, HAp, and pure Ti. OB was cultured in α-minimal essential medium (Hyclone) supplemented with 1% penicillin–streptomycin solution (Gibco) and 10% fetal bovine serum (Hyclone). Cell culture was maintained in a 5% CO_2, 95% air humidified atmosphere at 37 °C, and culture medium was replaced every 2 days. Before experiment, the samples were sterilized at 121 °C for 25 min.

Cell morphology was observed using FESEM after 1 day of culture. First, the medium was taken out and the cells were fixed 2.5% glutaraldehyde for 2 h. Second, the cells were dehydrated with graded ethanol/water solutions (from 25% to 100%). Finally, cells were critically point dried using liquid CO_2 and gold-sputtered before FESEM observation.

Cell viability was evaluated by the MTT (1, 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay after 4 days of culture. Basically, cells with the density of