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Fabrication of biopolymers reinforced TNT/HA coatings on Ti: Evaluation of its Corrosion resistance and Biocompatibility

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ABSTRACT

Titania nanotube (TNT) arrays were fabricated on Ti alloy by rapid breakdown anodization (RBA) method and chitosan (CS) - polyvinylpyrrolidone (PVP) biopolymers were codeposited on TNT followed by subsequent electrodeposition of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$,). The coatings were characterized by FE-SEM, EDX, XRD and AT-FTIR techniques. The mechanical, anticorrosion properties and biocompatibility of the coatings were evaluated. SEM analysis shows that among all coatings, the TNT/CS-PVP/HA coating possesses homogeneous surface and the incorporation of PVP into CS resulted in better morphology. The tentative mechanism for the deposition of CS-PVP biopolymers on TNT was proposed. Studies on the mechanical properties indicate that the addition of PVP into CS increase the hardness and adhesion strength of the coatings and the deposition of HA on TNT/CS-PVP coating further increase the mechanical strength. Similarly TNT/CS-PVP/HA coating shows good corrosion resistance, better fibroblast cell adhesion and low cytotoxicity behaviour than TNT and TNT/CS-PVP coatings.

1. Introduction

Titanium and its alloys have become excellent materials of choice for the production of long term prosthetic, cardiovascular and orthodontic implants compared to stainless steel and magnesium alloys due to their low toxicity, biocompatibility and good mechanical properties [1–3]. Even though these materials exhibit high corrosion resistance and high specific strength, the body fluid environment inevitably results in the release of noncompatible metal ions by the implants into the body. These released ions are found to cause allergic sand toxic reactions. To overcome the problem of ion release, many surface treatment techniques like anodic oxidation [4], plasma spraying [5], sol-gel method [6], electrophoretic deposition [7] and ion implantation [8] have been attempted to modify the topography and chemistry of titanium.

Another major problem is that the titanium and its alloys are bioinert materials, which can be covered by the host organism without being integrated with bone [9,10]. To overcome this shortage, HA has been applied as a coating on the metallic implants due to its similarity in chemical compositions, high biocompatibility and osteoconductivity to bone tissue of human [11,12]. To enhance the adherence of the HA coating on titanium alloy by reducing the thermal expansion mismatch of HA coating and metal substrate, several recent studies have introduced intermediate layers like single oxide coatings such as alumina [13] titania [14,15] composites [16,17], polymers [18,19] carbon nanotubes [20] between bioactive HA coating and metal substrate and provided some encouraging results. Among all the materials so far implanted into the body, polymers for bone generation must be biocompatible. In addition, they should be mutable, shapeable, or polymerizable in situ to ensure good integration in the defective area. As natural bone is an inorganic/organic composite, to mimic nature considerable research interests have been developed to fabricate composites such as CS/Ag/PVP [21], CNT/HA/CS [22], CS/graphene oxide [23], CS/silk fibroin [24] and CS/MgO [25] as synthetic bone materials. Such organic/inorganic composites have the potential for greater functionality and performance than pure organic and inorganic materials. CS has long been marked as a one of the most promising natural polymers exhibiting properties such as chemical inertness, biodegradability, biocompatibility, high quality film forming properties and low cost [26,27]. To improve its mechanical strength, a rigid polymer like pyrrole [28], polyethylene glycol [29] and PVP [30] is incorporated into the CS flexible matrix. PVP has attracted considerable interest due to its hydrophilicity, lubricity, anti-adhesive property and excellent biocompatibility and it has the capability to form bonding with CS [30]. A possible route to harness the excellent properties of TNT, CS, PVP and HA for applications is through incorporating them into composite materials. Recently, a unique electrode material

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comprised of highly ordered TNT achieved within a few minutes by RBA method, has been shown to possesses large surface area, good uniformity, conformability and high porosity. Compared to other methods, RBA method produces a thick TNT layer with a chemical bond between the oxide and the substrate that likely results in the enhanced adhesion strength. Its potent nanostructuring offers a microenvironment so that high quantity of polymer can infiltrate into the oxide TNT framework effectively.

So, an attempt was made in the present work to fabricate TNT/CS-PVP/HA multilayer film on Ti alloy through RBA and eletrodeposition methods. On this TNT, CS- PVP biopolymers and HA films were deposited layer by layer through electrodeposition method. The corrosion behaviour of this processed alloy in Ringer's solution was studied by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) techniques. The morphology and composition of the composite film existing on the Ti alloy surface were determined by SEM, EDX, XRD and AT-FTIR techniques. It is fair and reasonable to speculate that the integration of TNT, CS, PVP and HA would pave an ideal platform for the fabrication of bioimplant materials.

2. Experimental

2.1. Materials

Ti alloy sheet (purity 99%) of 0.5 mm thickness was purchased from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany. HF, HNO_3, NH_4Cl, NaOH, NaCl, $CaCl_2$, KCl, $Ca(NO_3)_2$, $(NH_4)_3PO_4$, CH_3COOH, PVP and CS (produced from shrimp shell), having more than 85% degree of acetylation were supplied by Aldrich Chemie, GmbH.

2.2. Synthesis of TNT coating by RBA

The sample material was Ti alloy with size of 1.0 cm × 1.0 cm and a thickness of 0.5 mm. Prior to anodization, the samples were ground with sandpaper up to 1200 grit and then chemically etched in a mixture of HF, HNO_3 and H_2O in the ratio 1:4:5 for 30 s followed by rinsing with deionised water and ethanol for 20 min sequentially and then dried. The anodization was performed in a two-electrode configuration connected to a DC power supply with Ti alloy as the anode and graphite foil (1 cm × 1 cm) as cathode at a potential of 20 V for 10 min in an electrolyte containing 0.3 M NH_4Cl in a thermostatically controlled manner [31,32].

2.3. Synthesis of TNT/CS-PVP composite films

To the CS solution (1 g in 1% acetic acid), various concentrations (2 – 6 wt %) of PVP were gradually added and the solution was ultrasonicated for 30 min before electrodeposition for uniform distribution. The deposition was performed in an individual cell using a three electrode configuration in which anodized titanium served as the cathode and platinum electrode acts as an anode. A saturated calomel electrode (SCE) was used as the reference electrode and it was carried out in potentiostatic mode by applying a potential of -2.5 V vs. SCE for 45 min using an electrochemical system CHI 760 C (CH instruments, USA). The inter-electrode distance and deposition area were maintained at 10 mm and 1 cm^2.

2.4. Electrodeposition of HA coating

Stoichiometric HA nanoparticles for electrodeposition were prepared by wet chemical method. Briefly, 0.6-M ammonium phosphate solution was added slowly into 1.0-M calcium nitrate solution at 70 °C. The pH of the solution was adjusted to 6 by adding NaOH. Stirring was performed for 8 h at 70 °C and then 24 h at room temperature and this suspension was used for deposition of HA [33]. The electrodeposition of HA was carried out using the same set up as mentioned for the deposition of biopolymers. Here TNT/CS-4%PVP coated substrate was used as working electrode. 0.1 M NaCl was added in order to improve the conductivity of the electrolyte. Before electrodeposition, the suspensions were ultrasonicated for 30 min to achieve a homogeneous dispersion. HA nanoparticles were electrodeposited for 30 min under constant potential at–1.5 V vs Ag/AgCl [34].

2.5. Characterization of the fabricated coatings

The microstructural and morphological characterization of coated samples was carried out using Scanning Electron Microscope (Hitachi, Japan, S-3400 N) and subsequently the elemental composition of the coatings were analyzed by Energy dispersive spectroscopy (EDS). The phase analysis was done using XRD (Philips PAN analytical Xpertpro, PW3040/60). Fourier transform infrared - attenuated total reflectance (AT-FTIR) spectroscopy 400 PerkinElmer infrared spectrometer in the wavenumber of 400–4000 cm^{-1} was used to investigate the functional groups present in the deposited coatings. Microhardness was measured by Vickers microhardness method on automatic microhardness tester LM 247 ATLECO (LECO Corporation, St. Joseph, MI) at 25 g load and 10 s dwell time. Thickness of the coating was evaluated using Dermitron thickness tester. For assessing the adhesion of the composite coating qualitatively on Ti substrate, a standard test method (SCOTCH Tape method, ASTM D 3359-02) was used. The corrosion studies were carried out in Ringer's simulated body fluid (SBF-the aqueous solution containing 8.60 g/l NaCl, 0.33 g/l $CaCl_2$ and 0.3 g/l KCl) at 37 ± 0.1 °C using Electrochemical workstation (CHI instruments, 760model). A conventional three electrode setup was used for electrochemical measurement, with Pt as counter electrode, standard Ag/AgCl as reference electrode and anodized Ti/Ti as the working electrode. The exposed area of working electrode was maintained at 1 cm^2. OCPT measurements were monitored for all coatings with the exposure time of 1 hr. Similarly tafel measurements were performed by applying potential in the range of +2 V to -2 V with a scan rate of 1 mV/s. Likewise, the impedance measurements were performed by applying a sinusoidal wave of 10 mV to the working electrode at a frequency range of 0.1 MHz–10 MHz.

2.6. In vitro biocompatibility studies

2.6.1. Cell culture

Mouse fibroblasts (L929) cells were obtained from National Centre for Cell Science (NCCS), Pune, India and were cultured in minimal essential media (Hi Media Laboratories) supplemented with 10% Fetal Bovine Serum (FBS), Streptomycin (100 U/mL) and Penicillin (100 U/mL) (Cistron laboratories). The cell culture was then incubated under the humidified atmosphere (CO_2) at 37 °C. The samples under examinations were sterilized in an autoclave at 120 °C for 2 h and placed in 24 well cell culture plates. After the stipulated time period (2 days), the coated samples were washed twice with phosphate buffer saline (1X PBS, pH 7.4). The cells were detected by live/dead staining. The amount of 200 μL of dye mixture (100 μL/mg Acridine Orange (green fluorescence in live cells) and 100 μL/mg ethidium bromide (red fluorescence in dead cells) in distilled water) were mixed with 2 ml cell suspension (30,000 cells/ml) in 6-well plate. The suspension was immediately examined and viewed under olympus inverted fluorescence microscope (Ti-Eclipse) at 200 × and 400 × magnification.