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Preparation of bone-implants by coating hydroxyapatite nanoparticles on self-formed titanium dioxide thin-layers on titanium metal surfaces

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ABSTRACT

Preparation of hydroxyapatite coated custom-made metallic bone-implants is very important for the replacement of injured bones of the body. Furthermore, these bone-implants are more stable under the corrosive environment of the body and biocompatible than bone-implants made up of pure metals and metal alloys. Herein, we describe a novel, simple and low-cost technique to prepare biocompatible hydroxyapatite coated titanium metal (TiM) implants through growth of self-formed TiO_2 thin-layer (SFTL) on TiM *via* a heat treatment process. SFTL acts as a surface binder of HA nanoparticles in order to produce HA coated implants. Colloidal HA nanorods prepared by a novel surfactant-assisted synthesis method, have been coated on SFTL *via* atomized spray pyrolysis (ASP) technique. The corrosion behavior of the bare and surface-modified TiM (SMTiM) in a simulated body fluid (SBF) medium is also studied. The highest corrosion rate is found to be for the bare TiM plate, but the corrosion rate has been reduced with the heat-treatment of TiM due to the formation of SFTL. The lowest corrosion rate is recorded for the implant prepared by heat treatment of TiM at 700 °C. The HA-coating further assists in the passivation of the TiM in the SBF medium. Both SMTiM and HA coated SMTiM are noncytotoxic against osteoblast-like (HOS) cells and are in high-bioactivity. The overall production process of bone-implant described in this paper is in high economic value.

1. Introduction

Metallic biomaterials are commonly used as orthopedic implants to replenish bone defects, owing to their high fracture toughness and high tensile strength when compared to those of other implant materials such as ceramics and polymeric materials [1]. Stainless steel [2], titanium metal (TiM) [3] and titanium metal alloys (TiMA) [4] are widely used to manufacture these metallic medical prostheses (implants). Apart from stainless steel, TiMA and TiM have favorable properties, such as corrosion-resistance, non-toxicity to the human body, and also the latter materials possess required mechanical strength in order to function as orthopedic implants, surface modification is usually required to overcome corrosion, to improve bioactivity and to increase osteo-integration properties of these materials [5]. As such, in order to enhance the biocompatibility of metallic implants, biocompatible ceramic coatings are combined with polymers in such a way to disperse ceramic nanoparticles in biologically inert polymer matrices so as to mimic the structure of the natural bones [6,7]. However, biocompatible, ceramic-coated, metallic implants could also undergo corrosion, after implantation, if the biocompatible ceramic coatings have porous structures due to phase changes that would take place during the coating process. This would result in weak bonding between biocompatible ceramics and metals [8]. It is, therefore, vital to use a few nanometer thick layer of a binder material, such as TiO_2, MgO, ZrO_2 and Al_2O_3, between the ceramic coating and the metal surfaces [9–11].

Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] nanoparticles are widely used as biocompatible ceramics on metallic implant surfaces [12–17]. HA nanoparticles are the major and most abundant material in human hard tissues. Synthetic HA nanoparticles, that mimic natural HA, are widely used as biocompatible coatings on implant surfaces to repair and substitute defects in human bones. The composition (Ca:P molar ratio = 1.67:1) and the crystallinity of the synthetic HA nanoparticles are very

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similar to those of the biological apatite. These synthetic HA nanoparticles have good biocompatibility, bioactivity and osteoconductivity [18–23]. In a previous publication, we described a systematic approach to prepare both spherical and needle-like hydroxyapatite nanoparticles with controlled size, shape and the crystallinity [24]. In the present study, we have modified that preparation procedure with the help of Triton X-100, a nonionic surfactant, in order to produce colloidal HA nanorods with uniform and controlled particle size distribution. Herein, the surfactant micelles prevent particle aggregation in order to keep HA particles in the nanometer-scale [25]. The synthesized HA nanorods have been deposited on TiM surfaces in order to imitate the properties of natural bone to the metallic implant. In order to do so, we have systematically investigated the composition of the TiM surface due to heat-treatment for 1 h at different temperatures, ranging from 300 °C to 800 °C. The morphology of the TiM surfaces is observed through scanning electron microscopy (SEM) and elemental composition mapping. Ti on the surface of TiM converts into TiO_2 by reacting with atmospheric oxygen which is known as self-formed TiO_2 thin layer (SFTL).The optimum temperature to obtain due to the reaction of TiM surface with atmospheric oxygen is investigated. The deposited HA nanorods on SFTL have been converted into spherical nanoparticles and they are well bound to the surface after the sintering process of prepared implant.

The devised surface modification technique is simple and cost-effective compared to the other techniques. It uses readily and freely-available and inexhaustible atmospheric oxygen and gives a uniform and pinhole-free TiO_2 coating on TiM. The TiO_2-coated TiM is termed surface-modified titanium metal (SMTiM). There are several techniques to coat HA on TiO_2 surfaces. These include dip-coating, sol–gel methods, template assisted electrohydrodynamic atomization, electrophoretic deposition, thermal spraying, sputter-coating, pulsed laser ablation, dynamic mixing, ion-beam-assisted-deposition, *etc.* [5,26–33]. However, most of these methods are associated with drawbacks, such as, uneven coating, involvement of high-costs, low bond strength between TiO_2 and HA, and decomposition of HA into other calcium phosphates and uncontrollable coating thickness [30,31]. Therefore, development of a low-cost coating technique which is free from the above obstacles is required. In this regard, spray pyrolysis could be utilized to fabricate uniform layers of interconnected nanoparticles on many substrate surfaces, both on the small scale and for large areas. However, in the conventional spray pyrolysis technique, a wide-range of particle sizes are usually obtained due to the aggregation of small particles. To avoid such a distribution of particle sizes, Bandara et al. [34], have designed and developed a novel technique known as the atomized spray pyrolysis (ASP) technique. The developed ASP instrument has a unit to convert particle aggregates to finer particles and to select only the finer particles for the deposition and to redirect the remaining unbroken particles back to the feed suspension. We have utilized this technique for the preparation of a few nanometer thick films of various technologically important materials on various substrate materials [34–36] and as such, we have made use of this same simple, yet a technologically advanced technique to coat HA nanoparticles on SMTiM surfaces. By following this approach, we have successfully prepared TiM prostheses with a binder TiO_2 layer and exposed HA layer on the TiO_2 layer. The formation of SMTiM species is followed by using electrochemical impedance spectroscopy and the corrosion behavior of the bare TiM, SMTiM, and HA coatings on SMTiM in a simulated body fluid medium (SBF) is investigated electrochemically using Tafel Plots [37–39]. The cytotoxicity and bioactivity experiments of each coating show that the prepared implants are nontoxic and bioactive in order to use as bone-implants.

2. Materials and methods

2.1. Materials

All chemicals used were of 99% purity and were used without further purification. The chemicals were purchased from Sigma-Aldrich. Water used was doubly distilled. Medical grade 99.99% Ti metal plate (density = 4.506 g cm^{-3}) was purchased from Goodfellow Cambridge Ltd. UK.

2.2. Preparation of dispersion of HA nanoparticles

HA nanoparticles were formed by reacting calcium sucrate with ammonium dihydrogen orthophosphate as we have reported elsewhere [24]. However, this method was modified using Triton X-100 in order to produce colloidal HA. Herein, 0.5 M calcium sucrate solution (100.0 mL) was prepared by dissolving 2.80 g of CaO in 0.5 M sucrose solution. 5 drops of Triton X-100 were added to the 0.5 M calcium sucrate solution while stirring in order to form micelles. 0.3 M ammonium dihydrogen orthophosphate (100.0 mL) was added drop-wise to the calcium sucrate solution, by maintaining the Ca/P mole ratio at 1.67 in the final mixture. The mixture was then stirred for 12 h and the suspension thus obtained was collected by centrifuging. The collected HA particles were washed with 5 aliquots of 50.0 mL of distilled water in order to remove Triton X-100 and were dispersed in 50.0 mL of water. Part of the synthesized HA nanoparticles was used for characterization before coating on TiM. The mean crystallite size (D) of HA was calculated using Debye–Scherrer formula [$D = (0.89\,\lambda)/\beta \cos\theta$, where, λ — Cu Kα wavelength, β — full width at half-maximum of the HA (211) line of the XRD pattern, θ — diffraction angle]. 1.0 mL of the HA dispersion was diluted to 20.0 mL, using distilled water and it was used as feed suspension to fabricate HA on SMTiM surfaces using the ASP technique.

2.3. Preparation of simulated body fluid

Na_2SO_4 (0.071 g), $MgCl_2 \cdot 6H_2O$ (0.305 g), $NaHCO_3$ (2.268 g), KCl (0.373 g), $Na_2HPO_4 \cdot 2H_2O$ (0.178 g) and NaCl (6.547 g) were separately dissolved in 500 mL of distilled water. 15.00 mL of each of 1 M HCl and $CaCl_2 \cdot 2H_2O$ (0.368 g) was added to the mixture. The temperature was raised to 37 °C and buffer (N-tris(hydroxymethyl)aminomethane) was added. The pH was adjusted to 7.4 using 1 M HCl and the final volume to 1000 mL using deionized water [40,41].

2.4. Preparation and characterization of metallic-implants

A TiM plate was cut into (25 mm × 50 mm) pieces and prior to experiments, all pieces of TiM were cleaned ultrasonically, using acetone, ethanol, and distilled water, successively, and dried at 60 °C. TiM pieces were heated separately at 300 °C, 400 °C, 500 °C, 600 °C, 700 °C and 800 °C temperatures for 1 h, five pieces per each temperature, respectively in order to form TiO_2 thin films on TiM. Morphologies of samples were analyzed using scanning electron microscope (SEM), (FESEM, Zeiss Sigma VP). The elemental composition of Ti and O on TiM surfaces was analyzed using Energy-dispersive X-ray (EDX) spectroscopy. X-ray diffraction (XRD) patterns of the TiM and SMTiM were obtained using a Siemens D5000 X-ray powder diffractometer. After electrochemical analyses, it was found that 700 °C is the best heating temperature to obtain lowest corrosion rate with better SFTL (*Vide infra*). TiM plates which were modified at 700 °C, were heated at 250 °C, and HA coatings were performed using ASP technique. Herein, the modified ASP technique was used to make thin-films of HA on metallic implant surfaces by breaking agglomerated particles in the suspension. First, the precursor solution (HA colloid) was directed under high speed to an atomizing chamber using pressurized air which can make aerosol droplets. Then, the aerosol droplets of the precursor solution were allowed to collide with a round shape target (Teflon spherule). Hence, the agglomerated particles were broken into individual particles and were separated from large aggregates. In this technique, the aerosol containing large aggregates which have not broken down into individual particles during the atomization step were recycled into the precursor solution. Separated fine particles were deposited on the hot substrate surface through the spray nozzle, which was placed just above the hot substrate surface