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Dual nanofibrous bioactive coating and antimicrobial surface treatment for infection resistant titanium implants

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ABSTRACT

Failures of biomedical implants due to implant-related infections and implant loosening remains a major concern in orthopaedic fixations. The current work aims to address the issues by examining the effect of dual interaction *i.e* surface modification and surface coatings on orthopaedic implant materials, *i.e.* commercially pure titanium (cpTi). The cpTi surface was initially modified with piranha solution (H_2SO_4 + H_2O_2) to create an antibacterial surface. Further, the biological properties similar to bone tissue were improved by electrospun coating on the piranha treated substrate with poly(ε-caprolactone)(PCL)/hydroxyapatite (HA) composite nanofibers. The PCL/HA composite nanofibers have been characterized using SEM, XRD, EDS contact angle measurements, and FTIR spectroscopy. The coating adhesion of PCL/HA on cpTi was evaluated by cross-cut tape test (ASTM D3359-09). The newly fabricated substrates showed favourable properties and higher wettability. The antibacterial tests on piranha treated nanostructured substrates also confirmed a substantial reduction in bacterial growth over large areas. Cellular interactive responses such as adhesive and proliferation of osteosarcoma MG-63 cell lines has also demonstrated that presence of PCL/HA electrospun coating on the modified surface have improved the biological properties. The currently developed piranha treated and PCL/HA nanocomposite coated cpTi substrates seems to be a promising method to obtain both antibacterial and bioactive titanium surfaces.

1. Introduction

Commercially pure titanium (cpTi) is a well-established orthopaedic implant material for its exceptional combination of properties including, bioinertness, biocompatibility, chemical stability, and excellent mechanical properties with high corrosion resistance [1]. The biocompatibility of cpTi with the bone is mainly due to its direct ability to form a chemically stable protective titanium oxide (TiO_2) layer on the surface [2]. Though, despite of these exceptional properties, failure of titanium implants is still a major obstacle and occurs very regularly due to implant loosing and implant-associated bacterial infections. Unfortunately, the thin TiO_2 layer (1.5–10 nm) as existing form on the surface lacks sufficient mechanical strength and can be one of the reason behind poor implant stability [3,4]. Also, bacterial colonization during post-surgery and after surgery leading to not only implant failure, but also have long-term deleterious side effects. Bacterial infection on to the implant materials occurs when adhesion of bacteria materializes on to the surface resulting in the formation of biofilms which consecutively offers resistance to antimicrobial agents [5]. Prevention of implant infection while surgery and formation of strong bonding between the bone and implant should be addressed in an effective way. So, in order to avoid any contrary effects, biocompatibility, antibacterial properties, and surface properties of Ti implants with bone interface were further improved by modifying it by chemical treatment, mechanical treatment and surface coating [6,7]. By doing so, it has been well demonstrated that the surface modified Ti implant material has a better interfacial reaction with osteoblast cells with a controlled tissue-healing [8]. It is a well-recognised fact that the first interaction between the implant surface and biological environment determines the overall stability of the implant. At the implant-bone interface, bioactivity of the implant, cellular response towards surrounding tissue can be significantly altered by the surface chemistry and also by the topographical features such as roughness. It has been proved that modified Ti-implants tend to adhere to the bone more by '*micromechanical anchorage*' due to its surface roughness [9]. Therefore, to achieve desired biological response on the implant, extensive research on modifying the

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surface has been carried out. A variety of mechanical or chemical surface pre-treatments including grit blasting, alkaline treatment (NaOH), acid treatment (HF, HNO_3, H_2SO_4), dual acid etching (HCl/H_2SO_4), and H_2O_2 etching have been proposed to achieve better cellular interactions with bone and also to enhance the adhesion between the implant and the bone tissue [10–13]. It is also being observed that, by subjecting the material to various kinds of surface treatments, nano-topographical changes can be induced on the surface which possesses an exceptional capacity to support the cells in their early phase of culture, i.e., protein adsorption, attachment and adhesion [14,15].

In this view, it is found that, chemical etching performed by piranha solution on cpTi induces homogenous sponge-like networks (nanopits) across the surface, similar to other acid surface treatments [16]. Interestingly, treating cpTi chemically by this particular solution is demonstrated to possess superior antibacterial properties by hindering bacterial adhesion in its early stages and also enhanced biological response [17,18] which is not found in other acidic/basic treatment strategies. Surface roughness and topography of the sample can be manipulated by changing the exposure time (min to h) of piranha solution, the treatment temperature (20 °C to 80 °C) and the solution composition (1:1 to 7:3). The details about variations of piranha solutions reported in literature is tabulated in Table 1. After removing the foreign contaminants from the surface and also by forming stable TiO_2 layer, Nanci et al. have demonstrated that immobilizing bioactive molecules onto piranha treated titanium can be advantageous at initial stages of tissue regeneration. Oliveira et al. demonstrated that *in vitro* osteogenic potential of calvaria-derived cells have been accelerated by piranha oxidization which subsequently enhance bone-like nodule formation.[8,19] However, for long duration implants (12–15 years), a robust bonding between the implant/bone interfacial areas is critical.

The human bone extracellular matrix (ECM) is designed with rich hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] nanoparticles and collagen nanofibers. Hence, many research efforts have been dedicated for the advancement of nanostructured surfaces with biological properties closer to human bone matrix. Owing to the properties of HA, *i.e.* chemical inertness, structural stability, and superior osseointegration with the implant surface, many traditional and well-known methods have explored for coating various methods for injecting HA onto the metallic surfaces *viz* ion beam sputtering, flame spraying, plasma spraying, HVOF spraying, chemical vapour deposition, dip coating, electrophoresis and electrochemical deposition [20,21] to increase the osteointegration ability with the bone. Polymer-ceramic nanocomposites have superior application in dental and orthopaedic implants in line with their unique physical and chemical properties. Ceramic coating by electrospinning is an effective technique for nanostructure depositions from polymeric-ceramic homogeneous solutions. Fabrication of composite nanofibers by electrospinning exhibits several advantages *viz* large surface area to volume ratio and porous structure mimicking the natural extra cellular matrix. Furthermore, superior control the over the electrospinning process makes it easier to control thickness of the coating, composition (PCL to HA ratio) and morphology of the deposited layers.

To best of available literature, no attempts have been made to study the effect of piranha treatment on coating adhesion. Therefore, we have studied the dual active combination of surface modification and bioactive coating by treating the surface of cpTi with piranha solution and then applying an electrospun coating with PCL/HA respectively to achieve the bioactivity and antimicrobial properties on the implant.

2. Materials and methods

2.1. Materials

The cpTi substrates of grade 2 (MIDHANI, Hyderabad, India) were cut into square pieces (10 mm × 10 mm) of 2 mm thick. These substrates were polished with series of SiC abrasive papers (400, 1000, 1500, 2000 grit), cleaned ultrasonically in acetone, anhydrous ethanol and distilled water for 20 min, then dried at 50 °C in a hot oven overnight.

2.2. Surface treatment of cpTi

The polished samples are then treated with piranha solution (concentrated sulphuric acid H_2SO_4 + hydrogen peroxide H_2O_2) at 3:1 ratio for 2 h. The substrates were then rinsed thoroughly with deionized water and dried in air over night.

2.3. Preparation of PCL/HA composite and electrospinning

Poly-caprolactone (PCL) with molecular weight 80 KDa, chloroform, and methanol (Sigma-Aldrich, India) were used as starting chemicals. Hydroxyapatitie (HA) was synthesized in-house by microwave synthesis technique as per a previously reported procedure [26] and used as the secondary phase ceramic component. In a typical experiment, 8 wt% PCL was dissolved in 3 ml chloroform and 1 ml methanol mixture and kept for stirring for 24 h. Then 20 wt% of synthesized HA was added gently into solution under stirring conditions. The mixture was then dynamically stirred at room temperature for 48 h to obtain a homogeneous polymer/ceramic solution for electrospinning. The polymer/ceramic solution was loaded into a 5 ml syringe equipped with a 22-gauge needle made of stainless steel. The needle was connected to a custom made high-voltage supply (NE1000, New Era Pump Systems Inc., USA). The solution was fed at the rate of 500 µl/h with a voltage of 12 kV. The electrospun nanofibers (PCL and PCL/HA) were allowed to collect on cpTi plates (annealed and piranha treated), kept at a distance of 10 cm between the tip of the needle and the collector. After pre-

Table 1
Piranha treatments on biomedical titanium and its alloys.

Sl. No	Piranha Solutions	Material	Observations	Ref
1	H_2SO_4 and 30% aq. H_2O_2 at 25 °C for 2 h.	cpTi	Covalent immobilization of bioactive organic molecules	[19]
2	H_2SO_4 + 30% aq. H_2O_2 1:1 ratio etched at 25, and 80°C	cpTi	Reduction in bacterial adhesion was observed on piranha-treated substrates.	[17]
3	50:50 Mixture of H_2SO_4 and 30% aq. H_2O_2	cpTi	Produced nanotextured TiO_2 layer on the Ti surface.	[22]
4	H_2SO_4 and 30% aq. H_2O_2 for 4 h.	cpTi	Produced bioactive nanotopography titanium surface and had positive affect on early stages of *in vitro* osteogenesis.	[16]
5	H_2SO_4 and 30% aq. H_2O_2 at 3:1, 1:1, 1:3 ratios and etching at 5, 25, 50, and 80°C	cpTi	Solution richer in H_2SO_4 provide a more efficient of nanostructuring at lower temperatures.	[23]
6	H_2SO_4 conc + 30% H_2O_2.aq. (7:3 ratio) at 20°C	cpTi and ultrafine grain cpTi	Study of etching time (15 min − 24 h) on surface roughness on titanium and ECAPed titanium	[24]
7	H_2SO_4 + 30% H_2O_2 for 4 h (Cp Ti) and Ti alloy for 1 h at room temp.	cpTi & Ti–6Al–4V	Nanotextured surfaces by Ti-based surfaces has rapid effect on the cells	[8]
8	H_2SO_4 + 30% H_2O_2. Etched at 15 min, 30 min, 1 h, 2 h, and 4 h	Ti–6Al–4V	Study the physicochemical characteristics of nanopitted surfaces on Ti6Al4 V alloy	[25]
9	H_2SO_4 and 30% aq. H_2O_2 at 1:1 ratio	Titanium-coated sensors	Protein adsorption studies	[18]