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Pulsed laser deposition of hydroxyapatite on nanostructured titanium towards drug eluting implants

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ABSTRACT

Titania nanotubes grown on titanium substrates by electrochemical anodization in glycerol–ammonium fluoride–water system were used to develop efficient drug carrying implants upon coating hydroxyapatite (HA) ceramic. The nanostructured surfaces achieved by anodization were caped with HA crystallites by pulsed laser deposition. The implant substrates were studied for their drug carrying capacity using gentamicin as a model. The nano-tubular surface with HA coating had better drug loading capacity of about 800 μg/cm^2 gentamicin while the bare anodized substrate carried less than 660 μg/cm^2. The HA coating alone stored as low as 68 μg/cm^2 and released the drug within the initial burst period itself. The ceramic coated anodized substrates were found to be more efficient in controlled delivery for longer than 160 h with a drug release of 0.5 μg/cm^2 even towards the end. The substrate with nanostructuring alone delivered the whole drug within 140 h. This study proposes the application of laser deposition of HA over nanostructured titanium, which proves to be promising towards controlled drug eluting bioceramic coated metallic prostheses.

1. Introduction

The application of bioceramics has ever since been very promising as substitutes in bone and orthopedic surgical interventions [1–3]. Since these ceramics cannot be used for load bearing implants, the use of metallic implant devices with bioceramic coatings is of particular interest among clinicians in orthopedic and dentistry as an aid to support the patients to perform their normal duties at an early stage [4,5]. Even if the implant materials are biocompatible and able to osseointegrate at a later stage, chances of infection in and around the implant is a serious clinical problem. To avoid this, local delivery systems for particular drug moieties were suggested. These practices lead to sought for the need of implantable devices with drug release properties. The most systems used in orthopedic and bone substitute for drug delivery includes calcium phosphate ceramics [6,7]. Calcium phosphate ceramics include various forms with different Ca/P ratios differing in their specific surface area, crystal structure, porosity etc. Calcium hydroxyapatite (HA), tricalcium phosphates and silicon containing calcium phosphate glasses are well known for their bioactive properties. These materials when placed in bone tissue were shown to promote bone formation and bond to bone as a result of the gradual change at the ceramic surface by dissolution, precipitation and ion-exchange reactions which results in a carbonate containing, calcium-deficient HA with small crystal dimensions. The bioactive behavior was then achieved by cellular activities, protein absorption, and other biological phenomena at the material–tissue interface [2,8–10]. Dense, stoichiometric (Ca/P = 1.67) HA ($Ca_{10}(P0_4)_6(OH)_2$ is the more stable of the calcium phosphates, which *in vitro* shows a very low rate of precipitation on the ceramic surface [11]. These calcium phosphate ceramics are limited in use for non-load bearing situations as the lack of strength leads to fatigue fracture and fail under load-bearing situations. This problem leads to focus much towards the development of bioceramic coated metal implants, which would combine the desired strength characteristics imparted by the metal and bone adaptability imparted by the HA or bioactive glass coating [12–14].

The requirement for biological fixation in which the prosthetic components become firmly attached to the bone tissue without the use of conventional bone cement leads towards the need for bioceramic coating of calcium phosphates, particularly of HA onto load bearing metallic prostheses. Among the techniques presently available to produce HA coatings, the most commercially viable one is the plasma or arc spraying technique. Being high temperature processes, and since having poor quality reproducibility of thermal processing techniques, alternate methods are sought for [15–17]. Pulsed laser deposition (PLD) technique using UV laser was shown to produce various bioceramic coatings including stoichiometric HA onto metallic substrates under controlled experimental conditions [18–20]. These HA coated metal implants should be superior in function to the presently available metal implants, which eventually fail and cause alveolar bone loss. Ever since the development of implantable systems or prostheses the improvement towards better clinical performance was challenging because in spite of the general surgical efforts, the growth of bacterial colonization of the implant during initial healing time lead to septic prosthesis loosening

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and further complications [21,22]. To reduce the risk of infection, low systemic concentrations of local antibiotics with a high local bactericidal antibiotic concentration at the region of interest may be of special attraction. The concept of controlled local delivery of proteins, growth factors or drugs consists of a biologically active agent such as a drug incorporated in a polymeric or ceramic matrix [23,24]. The objective is to deliver the drug to the intended site at the therapeutic level extending to a specified duration.

Our approach was to incorporate gentamicin as a model drug into the HA coated titanium implant substrate. Bone regenerative materials loaded with antibiotics for controlled and sustained release were shown to protect augmentation procedures from infection during the resorption phase of bone substitute. Also, the nano and micro level porosities present in the composition determines the loading capability and release kinetics under *in vitro* and *in vivo* conditions [25]. Gentamicin sulphate was the choice as a model candidate drug because it belongs to the aminoglycoside antibiotic family. Gentamicin is an efficient bactericide against gram negative bacteria and can penetrate bone tissues as well. It has been extensively used in orthopedics in combination with bone cements [23,25]. Also, it is very effective to prevent micro organisms which are responsible for bone infections. Furthermore, because gentamicin and other antibiotics are water soluble, they can diffuse from the filled site to protect the surrounding bone from infection. Even though there are few reports showing that gentamicin loaded titanium implants significantly improved infection prophylaxis compared to unloaded titanium implants, other studies are mainly based on biomimetic and sol–gel methods of incorporating drug into bulk calcium phosphate ceramics [26,27]. Successful incorporation of different antibiotics such as vancomycin and tobramicin were also reported into comparatively thick and less adherent biomimetic HA coating followed by their *in vitro* studies [7,28,29]. These studies show release time of antibiotic less than six hours which is the minimum time for effective protection after implantation. Our objective is to attain release kinetics *in vitro* from HA coated implantable titanium substrate for longer duration with sufficient delivery. To this, we developed a method based on the concept of nano-drug delivery using titania nanotubes grown by anodization [30–32]. Anodic oxidation was used to create regular arrays of oxide nanotubes on metals such as zirconium, niobium, tantalum and titanium. The titania nanotube layer has excellent optical, electrical and catalytic properties. Deposits of bioceramics such as HA were developed for implant applications over these anodized surfaces. The surface nanostructuring can improve the adhesion of the upper bioactive layer and also promote nucleation of calcium phosphates [33–35]. HA deposited onto the anodized titanium substrate can act as a reservoir towards the controlled delivery of gentamicin at the site of implantation. The HA coating provides dual function i.e. acts as a capping material as well as a bioactive surface to promote osseointegration at later stages. Here we propose such a hybrid system to provide better control over drug release kinetics which can be an added advantage for bioceramic coated implantable prostheses.

2. Materials and methods

2.1. Substrate preparation

Titanium substrates were divided into three groups: HA coated titanium, titanium substrate with nanostructured titania tubes grown by anodization and nanostructured titanium with HA coating. Implantable grade Ti6Al4V (ASTM F1108, Manhar Metal Supply Corporation, Mumbai, India) alloys were used as the study substrate. Titanium substrates were machined into discs of dimension 20 × 15 × 2 mm and mechanically polished (Buehler-Ecomet 3 Variable speed Grinder-Polisher, Buehler Ltd, U.S.A) with series of SiC papers and finally with suspensions of alumina having particle size up to 0.05 microns to get uniform surface roughness. The substrates were subjected to ultrasonic cleaning in deionized water, ethanol and finally in acetone. A schematic of nanostructuring of titanium by electrolytic anodic oxidation process for drug loading and HA coating is depicted (Fig. 1).

2.2. Anodization

Nanotubes of TiO_2 were grown on polished Ti6Al4V substrates by electrochemical anodization. The cleaned samples were chemically etched in 1:1 V/V HNO_3 and HF (Merck, India). The electrolyte for anodization was based on glycerol (S.d.Fine-Chem Ltd. India) and deionized water in a 1:1 weight ratio. 0.5 wt% NH_4F (Merck, India) was added to the glycerol–water system kept at 25 °C and magnetically stirred at 700 rpm for 2 h prior to anodization to ensure the complete and homogeneous mixing. A polished and cleaned sample of titanium was used as the cathode, while the chemically etched Ti substrate was placed as anode at 4 cm apart from cathode. Anodization was performed at a constant voltage of 20 V with continuous magnetic stirring for 60 min and then rinsed with deionized water. The anodized samples were then heat treated at 450 °C for 2 h in ambient atmosphere as it is known to help crystallization of TiO_2 [36].

2.3. Preparation of dense HA target

HA powder was prepared in-house by a wet precipitation technique using $Ca(NO_3)_2.4H_2O$ and $NH_4H_2PO_4$ (Rankem, India) at pH 11 and 80 °C. HA target disc was prepared from calcined (at 300 °C) and pulverized powder of particle size around 125 microns. Powder compaction was done at 200 MPa in a cold isostatic press (Cold Isostatic Press, EPSI, Belgium) and subsequent sintering at 1100 °C for 2 h [19]. The sintered target having 96% theoretical density was mechanically polished by silicon carbide papers and cleaned ultrasonically in distilled water, acetone and then dried in hot air.

2.4. The PLD process

The PLD of HA was performed using the third harmonic of (λ = 355 nm) Nd-YAG laser (Quanta systems, Italy). A stainless steel deposition chamber with all essential components as vacuum and temperature controllers, inlet for working gas, rotating target and substrate holders were used for the PLD process (Excel Instruments, Mumbai, India). The deposition process and laser was automated using a deposition controller (Screw deposition controller version 2.2; Dynamic Control Systems, Mumbai, India) [37]. The laser delivering an output of 2 W was focused to the sintered HA target at an angle of 45°. The deposition was done by keeping the substrate at 400 °C under an oxygen pressure of the order of 10^{-3} mbar. HA was deposited for a period of 1 h over each titanium substrate. HA coating was done for the two groups of polished and anodized titanium substrates.

2.5. Structural and morphological characterization

The surface profile was obtained using Talysurf CLI 1000 (Taylor Hobson, UK) with the software Talymap Gold. The anodized and HA deposited titanium were analyzed using X-ray diffractometer (Brucker D8 advance, Germany) to identify the phase and to examine the crystalline nature. The samples were scanned between 2θ = 20–50° for HA coating and 20–70° for anodized samples using Cu-Kα1 radiation at a voltage of 40 kV and 30 mA current. Presence of different functional groups in HA samples and drug loaded samples were confirmed by Fourier transform infrared spectroscopy (FTIR) (FT/IR 6300; Jasco; Japan). The spectra were collected by KBr pellet method. Samples were prepared by mixing the powder collected by scratching the surface of coated samples with optical grade KBr powder and making a pellet of it; while pure KBr pellet was used as the background. The spectra were recorded at a resolution of 4 cm^{-1} and scanned between 400 and 4000 cm^{-1}. Surface of anodized