Self-protective Oxide Nano-Coatings for Enhanced Surface Biocompatibility of Titanium

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ABSTRACT

The biocompatibility of an implant material depends on the bulk physical properties in addition to the surface properties. In biomedical engineering and industry Ti and Ti-alloys are very popular biological implant material for their bulk physical properties and strength to weight ratio resembling those of nature bone. It is possible to modify the surface properties of titanium for enhanced surface biocompatibility. The main objective of the this study is to engineer a smart Ti-based prosthesis surface by self induced chemically modified titanium oxide nano-film by the chemical mechanical polishing process (CMP). This new process applied on bio-implants aims at significantly reducing the out-diffusion of Ti and other metallic impurities from prosthesis in contact with body fluids and tissue and simultaneously enhancing the surface mechanical, chemical and biological properties. CMP technique enables the growth of a thicker and denser self-protective native oxide on Ti and Ti alloy samples, while simultaneously inducing a controlled surface roughness. It is demonstrated that the Ti based dental implants with self-protective oxide induced surfaces help minimize chemical and bacterial reactivity in addition to Ti ion dissolution while promoting their biocompatibility through surface patterning. The studied self-protective oxide films can also be utilized for many additional applications including bio-sensors.

INTRODUCTION

Biomaterials are widely used for dental prostheses, orthopedic devices, cardiac pacemakers and catheters [1]. A search of the Sciencedirect® data base with the key words “metallic implants” produced 23,625 results, a testimony both of the importance of this topic, research efforts but also of the many problems that are yet to be resolved. Generally, Ti and its alloys are favored as bio implants due to their surface characteristics, which promoting biocompatibility [2]. However, the surface of titanium maybe contaminated during casting or surface structuring due to its highly reactive nature, which in turn lessen the biocompatibility and the mechanical properties at the tissue/bio implant interface [3-4]. Patients with bio-implants face on the average of 4% infection risk and this ratio goes up to 40% for ventular support implants. In the case of infection, both medical treatment and the time spent in a medical institution would result in considerably high expenses. In short, it is very critical to promote cell growth and limit infection risk where the bioimplants are exposed to the live tissue.

The main factors that promote the biocompatibility of implant materials are the increase of surface roughness and formation of a surface oxide film. The surface structuring techniques such as sand blasting and chemical etching tend to cause contamination on the implant surface, while the high temperature plasma coating or laser texturing result in increased cost. Usually, the surfaces of Ti and Ti alloys are protected by a native oxide layer, which also increases the metal biocompatibility. Unfortunately, this oxide layer is too thin to be an effective diffusion barrier lasting for the many years of the prosthesis usage. There are several studies where the oxide layer thickness was increased by implementing various oxidation techniques typically by wet etching. However, the formed oxide layer was less dense, presented porous structures and an increased surface roughness that resulted in larger amounts of wear debris. Consequently, alternative techniques to increase the thickness of the native oxide and maintain its properties are needed. In this project, CMP technique is proposed as an alternative for surface treatment of bioimplants, which can enable surface texturing through removal of nanometer, scale films and spontaneously form a protective oxide film on the titanium surface.

In CMP process, the top film surface of the metal is exposed to the chemicals in the slurry, which is made of submicron size particles and corrosives. Generally, CMP is used to form very smooth surfaces but in our earlier studies we have demonstrated that by changing the slurry particle size and the pad material properties, it is possible to generate controlled roughness on the polished surface [5]. The protective nature of the oxide film enables planarization in semiconductor applications and it is believed to help reduce the contamination on the surface of the bioimplants and infection risk by stopping the chemical reactions. It has been shown in literature that the application of CMP on Ti films has been successful in terms of creating a smooth surface and a TiO_2 oxide film [6]. However, the formed oxide film after CMP has not been fully characterized for its protective nature other than the passivating properties of the Ti/TiN films in semiconductor CMP applications [7]. The effect of oxidation of Ti films on cell activity improvement as well as hydroxyapatite formation is well known [8-11]. Yet, the studied oxide films are typically formed through forced oxidation at very high temperatures and are very thick and porous. These films are much thicker than the self-protective oxide film of Ti, which is known to be only a few nanometers. It is likely that the porosity on the oxide films would not stop the chemical attack on the base titanium metal or alloy and leaves them prone to contamination. In summary, it is aimed to create an engineered surface on Ti based bio implants with self-protective surfaces to minimize chemical and bacterial reactivity, while promoting their biocompatibility through surface patterning.

EXPERIMENTAL DETAILS

CMP and bacteria growth analyses were conducted on titanium foils with 1 mm thickness and 99.6% purity (TI000430) obtained from Goodfellow Cambridge Limited. The original foil, which was 300x300 mm in size, was cut to 14 x 14 mm pieces to fit to the holder of the CMP tool. The original sample surface considered as baseline for the experimentation was annealed. In order to compare to the properties of the original surface against the surfaces prepared through CMP, polishing was conducted by using a desktop Tegrapol- 31 polisher and 5% weight alumina (Al_2O_3) slurry with 50nm particle size. Slurries were prepared at pH 4 using nitric acid through ultrasonicating long enough by repeated pH adjustment until the slurry was fully stabilized. CMP tests were conducted at 70 N downforce which is equivalent to a 7.88 psi pressure on the used sample size. Initial samples were polished using a Suba IV subpad stacked under a polytex buff pad to obtain a very smooth surface. In addition, two sizes of sand paper (silicon carbide 150C and P320) were used in place of the polishing pad to create the micro structures through CMP. All CMP testing except for the first sample were conducted by using H_2O_2 as oxidizer. Samples ran with the polymeric CMP pads and abrasive papers were polished for 2 minutes with ~3% oxidizer addition to promote chemical activity. Material removal rates were calculated through weighing the samples pre and post polish by a sensitive balance to the fourth digit after zero. All samples were cleaned in ultrasonic bath with pH 4 water for 5 minutes and dried with nitrogen gas before they were characterized. Same experimental conditions in 3D level applied to dental implant samples obtained from Mode Medikal Limited.

All samples were characterized for wettability through contact angle measurements with simulated body fluid (SBF) with a KSV ATTENSION Theta Lite Optic Contact Angle Goniometer using the sessile drop method. Five drops were measured on each sample. The drop image was stored by a camera and an image analysis system calculated the contact angle (Θ) from the shape of the drop. The microstructures of specimens were examined using a Nanomagnetics Atomic Force Microscope (AFM) with contact mode and the surface roughness values were recorded on 10 x 10 μm scan area. X-Ray Diffraction analysis were conducted on bare Ti sample and CMP applied sample in the absence of H_2O_2.

To evaluate the bone response Hydroxyapatite Attachment (HA) growth test were implemented. According to literature, HA sol was prepared using Ca and P routes such as calcium nitrate ($Ca(NO_3)_2$) and diammonium hydrogen sulphate ($(NH_4)2HPO_4$). Deposition process on pure titanium was carried out by dipping method uniformly. The HA deposits were investigated by AFM, optical microscope and the HA growth was evaluated through weight differences pre and post the coating procedure.

The infection resistance analyses were conducted through bacteria growth analyses. Titanium plates were sterilized with autoclave (120ºC, 20min.) before the microbiological analysis. Cronobacter Sakazakii (Gram-) was used as bacteria species and 100μl microorganisms from the nutrient broth microbial stock were spread on nutrient agar plates in sterile conditions. After the cultivation of bacteria, sterilized Ti plates were placed into each plate and incubated at 37 ºC. The bacteria density was observed over 1, 3 and 7 days. Bacteria growth was quantified by measuring the thickness of the colonies grown on the sides of the plates through photographs taken on the samples. L929 fibroplast cells amplified in the laboratory for the proliferation of the soft tissue cells on the samples. Cells seeded directly onto the well of the plastic plate, which include samples, placed on the bottom layer of the well plate. The nutrient medium was changed every 3 days. After 15 days of incubation period, cell morphology was assessed as 10^4 cell/cm^2 by counting under microscope with thoma lamel.

RESULTS AND DISCUSSION

CMP applied samples tests results show obvious difference characteristics on the material surface. After CMP process samples surface properties for the selected polishing conditions on the titanium plates are given in Table 1. It can be seen that the material removal rates on the samples polished on the polymeric pads were low and particularly the CMP test without oxidizer resulted in negligible material removal. On the other hand, using the abrasive papers resulted in much higher removal rates although the chemical component of the process was elevated by increased oxidizer concentration and the mechanical abrasion was limited by reduced polish times on the samples. Contact angle values reflecting on the wettability of the surface. The high

Table 1.Wettability and surface morphology of the Ti samples according to CMP conditions.

Samples	**Contact Angle Θ (deg)**	**Remove Rate (μm/min)**	**Comments**
As received	83.89 ±2.79	N/A	Porous oxide surface
CMP without H_2O_2	44.74± 3.18	~0	Titanium exposed
CMP with 3% H_2O_2	32.43± 5.25	0.11	CMP'ed smooth surface
CMP with 3% H_2O_2 + 45μm Ab.P	54.34±7.19	29.85	Fine grid abrasive paper rough surface
CMP with 3% H_2O_2 + 90μm Ab.P	65.53±6.37	35.99	High grid abrasive paper rougher surface

contact angle value on the untreated sample can be explained through the different nature of the surface since the original sample was anodized creating a very porous and thick oxide layer. On the CMP induced samples, the contact angles were higher as the surface roughness was increased through induced micro level roughness. Once the surface polish has started, the anodized oxide film started to be removed. Without the oxidizer in the slurry, the material removal rate was negligible yet the exposed titanium metal resulted in a change in the contact angle measurements. This is due to the fact that the surface energy of the fresh exposed titanium is higher and this leads to increased wettability response on the surface. Once the fresh surface of titanium is exposed, the effect of roughness on the contact angle response starts to dominate. As can be seen in Table 1, surfaces with an expected smoother finish, such as in the case of CMP application in the presence of oxidizer resulted in more wettability and hence a lower contact angle and the surfaces with the induced micro roughness (such as the samples polished with abrasive papers) resulted in a higher contact angle. When the surface is buffed with CMP without the chemical component provided with the H_2O_2 addition, the porous surface oxide was removed partially. CMP process with the oxidizer present at 3%wt exposed the titanium surface and finally polishing with the abrasive papers induced major surface scratching while exposing the titanium.

In order to quantify the surface roughness of the polished surfaces, AFM analyses were conducted on the samples. AFM measurement of the original untreated titanium plate and CMP conducted with the polymeric pad in the presence of 3% H_2O_2 resulted in surface roughness values of 118.77 nm (RMS) and 78.05 nm, respectivelly. The sample that has been buffed with the slurry without the oxidizer, however, maintained a similar roughness to the original samples (117.11 nm) as consistent with the negligible material removal rates obtained in the absence of the oxidizer. It is clear that the original sample has a very porous surface that can be attributed to the anodization on the surface. After CMP treatment to observe the oxide compounds of the surface ,XRD analyses applied on samples. Using oxidizer during the CMP process promote an amorphous oxide layer on the surface and its hide the titanium peaks which obviously seen from the analyses result [12] when the surface exposed in the absence of oxidizer.

In order to analyze the biocompatibility of the prepared surfaces controlled bacteria growth analyses were conducted. Results are published previously [12] and It is clearly seen that the surfaces intentionally scratched tend to accumulate more bacteria colonies as compared to the smoother surfaces. Particularly the sample processes through proper CMP process allowed the least amount of bacteria growth around the titanium plate and oxidizer used samples enable to the limit of the growth during the evaluation process. HA attachment test show an increased amount which with the respect of increasing surface roughness and less amount obtained from the

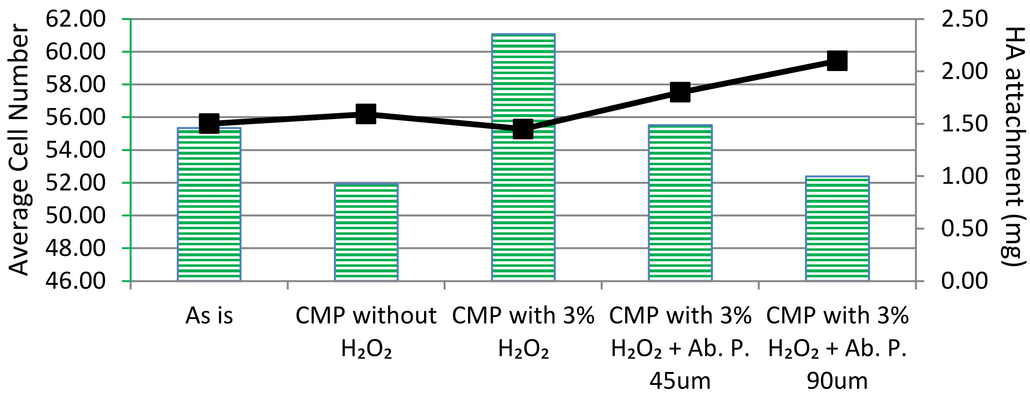

Figure 1. HA attachment evaluation of the titanium samples according to RMS and CMP conditions.

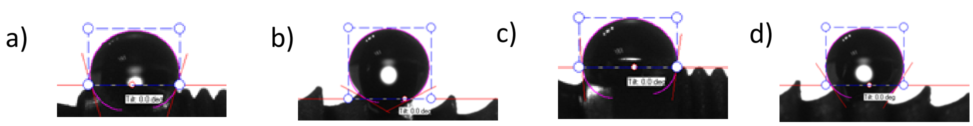

Figure 2. Wettability analyses on different part of dental implant with as received (a & b) and CMP induced with the presence of oxidizer (c & d).

smoothest sample (Figure 1) which is CMP applied in the presence of oxidizer with soft polymeric pad used. The fibroblast type L929 cell behavior show a difference growth behavior according to the microscale roughness. Samples surface which is the most rough one have least amount of cell attachment on it against long odds .We estimate the main reason of this response is the sharp edge of the surface topography which cause to rapture of the cell when they try to attach to the surface. On the other hand oxidizer using during the CMP process promote the cell growth. As the main approach we applied CMP process to a dental implant in 3D manner. CMP applied dental implant sample wettability analyses showed accordance results with the titanium plate. The wettability behaviour of the implant surface's different regions showed changes according to step height of the screws, which is given in the Figure 2.

CONCLUSIONS

In this study, CMP proces is proposed as an alternative technique to altering the surface roughness features of the titanium surface to enable more biocompatible surfaces by simultaneously forming a self-protective nano oxide layer.Besides our results we suggest that the reason of the constant bacteria growth on the CMP performed samples in the presence of an oxidizer.Wettability analyses through contact angle measurements were shown to be a valid and easy approach to detect the surface roughness that affects the bio-activity on the surfaces.

Biocompatibility analyses conducted through cell attachment also show differance in the cell growth by increased surface structuring. Hydroxyapatite growth also show an increase with surface roughness forming a uniform surface roughness on all the samples.3D CMP design is under development for the implant polishing, as a part of this study.

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