Increased Osteoblast Adhesion on Nano-rough Anodized Titanium and CoCrMo

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ABSTRACT

One approach to improve biological properties of current titanium and CoCrMo implant is to mimic the nanostructures of bone. Surface modification via anodization is popular to create such nanometer surface features on these metallic implants. The objective of the present study was to anodize titanium and CoCrMo and then determine osteoblast adhesion on such materials. Hydrofluoric acid was used as an electrolyte for anodization and different anodization conditions were applied for each metal. Osteoblast adhesion was determined by cell counting under a fluorescence microscope. The results demonstrated the ability to make nano-tube-like structures on anodized titanium compared to smooth surfaces before anodization. The inner diameters of the tubes were about 60 nm and the depths were limited to a few hundred nanometers. The original CoCrMo surface had micro-scale scratches and cracks probably due to mechanical processing but was mainly smooth at the nano-scale. On the contrary, the anodized CoCrMo possessed a very rough surface in the micro-scale as well as a porous structure within the nano-scale. For both titanium and CoCrMo, the results of osteoblast adhesion tests showed significantly ($p<0.01$) increased osteoblast numbers on anodized compared to unanodized metals; properties which make them useful for orthopedic applications.

Keywords: osteoblast, adhesion, titanium, cobalt-chromium alloy, anodization

1 INTRODUCTION

Titanium and cobalt-based alloys are two main groups of metallic materials successfully used in orthopedic applications. For example, primary hip and knee replacements have a ten-year success rate approaching 95 % and a fifteen-year success of about 85 to 90 % [1]. However, they still cannot meet all patients' demands, especially for younger implant patients. As a result, designing the next generation of bone implants with longer effective lifetimes is a principal field of research for investigators in the biomaterials field.

The surface of an implant plays a critical role in determining long term performance because only it is in direct contact with tissues and bodily fluids. Basically, there are two main factors to consider in promoting cell function: surface chemical composition and surface topography or roughness. Conventional orthopedic implants made from titanium and CoCrMo may possess macro-scale roughness to achieve mechanical fixation and mostly possess micro-scale roughness due to different types of mechanical treatments (e.g., grinding, polishing, blasting). On the other hand, the recent development of nanotechnology enables researchers to investigate the effect of nanoroughness or nanotopography on bio-implant interactions. By mimicking the nanostructure of natural bone, implants possessing nano-scale roughness have been shown to improve bone cell functionality at the tissue-implant interface [2-6]. For instance, it has been reported that compacts composed of nano-particulate metals (Ti, Ti6Al4V, CoCrMo) increased osteoblast (bone-forming cells) adhesion compared to conventional or micro-particulate counterparts [2].

Besides fabricating bulk materials, surface modification via anodization is an alternative way to create nanometer surface features on these metallic implants. The anodization techniques to create an oxide layer with nano-topographies on titanium surfaces has for traditional applications been well established by several research groups [7-10]; this allowed for the present study to apply these methods to modify CoCrMo surfaces. For the above reasons, the objective of the present in vitro study was to anodize titanium and CoCrMo and then determine osteoblast adhesion on such materials.

2 MATERIALS AND METHODS

2.1 Materials

Titanium foil (99.2 % pure, Alfa Aesar) was cut into 1 x 1 cm squares using a metal abrasive cutter (Buchler). CoCrMo samples (DePuy) were discs with diameters about 1 cm. Borosilicate glass (Fisher Scientific; 1.8 cm diameter) was used as a reference material in the present study. The glass coverslips were etched in 1 N NaOH (Sigma) for 1 hour at room temperature before use.

2.2 Anodization

The home-made electrochemical cell used in the present study had a two electrode configuration: a platinum cathode

and an anode (titanium or CoCrMo). Dilute hydrofluoric acid (0.5 wt%) was used as an electrolyte for anodization according to previous studies [7, 8]. Briefly, to anodize titanium, a constant voltage of 20V was applied to the electrodes and was maintained for 20 minutes while to anodize CoCrMo a voltage of 10 V was applied and maintained for 2 minutes. After anodization, all of the samples were rinsed, sonicated in acetone (Mallinckrodt), 70% ethanol (AAPER) and deionized water, and then sterilized in an autoclave (VWR) for 30 minutes.

2.3 Materials Characterization

Samples after cleaning and drying were imaged using a JEOL JSM-840 Scanning Electron Microscope and a Hitachi S4800 Field Emission Scanning Electron Microscope (FE-SEM) for ultra-high magnifications. All samples were sputter-coated with AuPd before imaging using a HUMMER I sputter-coater for 3 min.

2.4 Osteoblast Adhesion Tests

For cell adhesion tests, human osteoblasts (ATCC, population number 7~8) at a density of 3500 cells/cm^2 were seeded onto a 12-well cell culture plate containing each sample in 2 ml Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin (all chemicals from Gibco). The samples were then incubated under standard cell culture conditions for 4 hours. After that time period, non-adherent cells were rinsed away by deionized water while the adherent cells were fixed in fomaldehyde, stained by Hoescht 33258 dye (Sigma) and counted in five random fields under a fluorescence microscope (Leica).

2.5 Statistical Analysis

All experiments were carried out in triplicate and repeated three different times. Numerical data were analyzed using standard analysis of variance (ANOVA) techniques; statistical significance was considered at $p < 0.01$.

3 RESUTLS

3.1 Anodized Titanium with Nano-tubular Structures

The results of the present study demonstrated the ability to make nano-tube-like structures on anodized titanium compared to surfaces before anodization (Figure 1(a) to (d)). The original titanium surface was micro-rough (Fig 1(a)) but pretty smooth within the nano-scale (Fig. 1(b)). After anodization, the outermost titanium layer was etched away and the grain boundaries looked sharp (Fig. 1(c)). Within each grain, ordered nano-tubular structures could be seen (Fig. 1(d)) and the inner diameters of the tubes were about 60 nm estimated from the SEM images. The depths of such nano-tubes were limited to a few hundred nanometers according to findings from a previous study using Atomic Force Microscopy studies [3]. The main composition of this film was titanium dioxide [7].

3.2 Anodized CoCrMo with Nano-porous Structures

After anodization, the CoCrMo surface was covered by a grey layer. The composition of the newly-formed layer was thought to be a mixture of chromium oxide, cobalt oxide and molybdenum oxide. Furthermore, it was seen that the original CoCrMo surface had micro-scale scratches and cracks probably due to mechanical processing (Fig. 1(e)) but was rather smooth at the nano-scale (Fig. 1(f)).

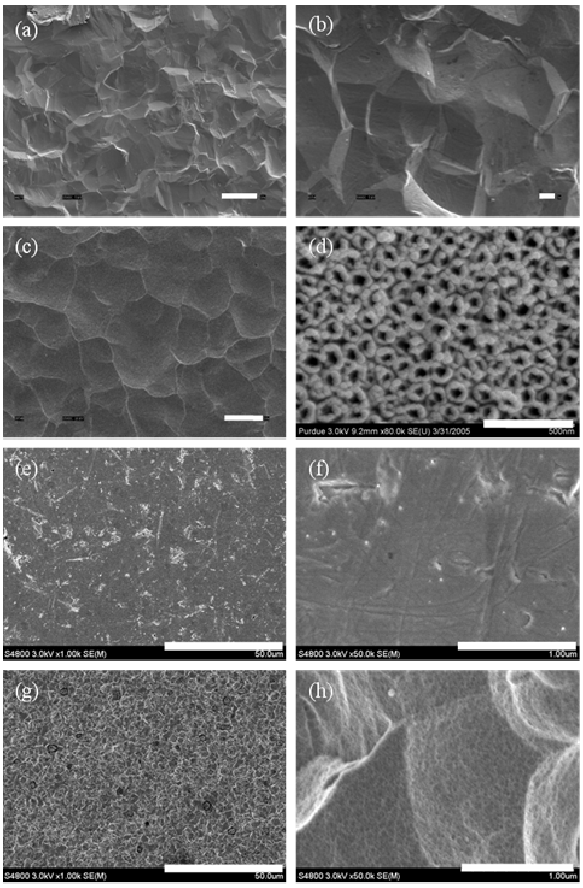

Figure 1: SEM micrographs of unanodized titanium, bar = 10μm in (a), bar = 1 μm in (b); anodized titanium bar = 10 in (c), bar = 500 nm in (d); unanodized CoCrMo, bar = 50 μm in (e), bar = 1 μm in (f); and anodized CoCrMo, bar = 50 μm in (g), bar = 1 μm in (h).

On the contrary, the anodized CoCrMo possessed a very rough and porous surface in the micro-scale (Fig. 1(g))

as well as a porous structure within the nano-scale (Fig. 1(h)). The irregular shaped particles embedded within the surface were likely the breakdown points during anodization.

3.3 Increased Osteoblast Adhesion

For both titanium and CoCrMo, the results of osteoblast adhesion tests showed significantly ($p<0.01$) increased osteoblast numbers on anodized compared to unanodized metals (Figure 2). Specifically, 33 % more osteoblasts attached to the anodized titanium compared to unanodized titanium while 24 % more cells attached to anodized CoCrMo surfaces compared unanodized CoCrMo after 4 hours.

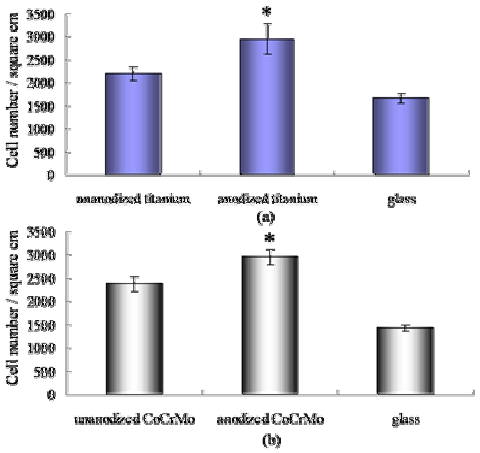

Fig 2: Increased osteoblast adhesion on anodized titanium (a) and CoCrMo (b) compared to their unanodized counterparts. Data = mean $\pm$ SEM, n =3; $p < 0.01$ compared to respective unanodized surfaces and glass.

4 DISCUSSION

The study of titanium anodization is still on-going and improvements of the nano-tube arrays are being developed. For example, the depth of the tubes now formulated could be a few micrometers [9] and the tubes could be selectively grown after implementing some imprinting steps [10]. These findings could be useful to optimize nano-tube properties according to their bio-performance in the future. In the present study, well-developed parameters to produce nano-tubular structures on titanium were used. For CoCrMo, its anodization parameters were varied and finally set based on observations in the present experiments.

The surface morphology of anodized titanium was as expected and it contained arrays of nano-tubes. In contrast, the thin layer formed on CoCrMo surface after anodization had a highly porous topography and no ordered tubular structures appeared. This difference may be attributed to the reaction mechanism for titanium and CoCrMo; it could also be due to the use of non-optimized CoCrMo anodizing parameters. However, nano-porous structures on anodized CoCrMo were achieved in this study.

Our preliminary study demonstrated very promising results for designing better orthopedic implants, considering that osteoblast adhesion is a prerequisite for subsequent functions (such as proliferation, differentiation, and finally deposition of a new bone matrix). Although it is hard to eliminate the influence of chemical composition in the present study, it is believed that the interaction between proteins (nano-scale)/cells (micro-scale) and implant surfaces could be mainly altered by decreased surface feature size. Similar studies on osteoblast function on anodized aluminum showed greater osteoblast adhesion, proliferation, and calcium deposition on nano-pore alumina arrays [4, 5] compared to smooth alumina substrates without chemistry differences. This is, in part, supporting our findings that nanometer surface roughness as created by anodization is a critical factor mediating cell-surface interactions.

Necessarily, future studies on osteoblast long-term function are needed to confirm the advantages of bringing nano-features onto implant surface. A more precise characterization of surface properties before and after anodization is also desired in the future, which would provide the underlying mechanisms of enhanced osteoblast functionality.

5 CONCLUSION

In the present study, anodization was applied to titanium and CoCrMo for producing nano-scale features on their surface. Greater osteoblast adhesion was demonstrated on both anodized titanium and CoCrMo compared to their original counterparts. This result suggests that surface modification of titanium and CoCrMo via anodization might be an inexpensive and efficient way to produce better orthopedic implants.

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