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Greater osteoblast long-term functions on ionic plasma deposited nanostructured orthopedic implant coatings

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KEYWORDS

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ABSTRACT

Bioactive coatings are in high demand to increase the functions of cells for numerous medical devices. The objective of this *in vitro* study was to characterize for the first time osteoblast (bone-forming cell) long-term functions (such as proliferation and deposition of calcium containing mineral) on several potential orthopedic implant polymeric materials [specifically, ultrahigh molecular weight polyethylene (UHMWPE) and polytetrafluoroethylene (PTFE)] coated with nanostructured titanium using a novel ionic plasma deposition (IPD) coating process. UHMWPE is a widely used polymer in total knee and hip replacements, while PTFE is not, but it has been used in other orthopedic applications. The IPD coating process creates a surface-engineered nanostructure (with features usually below 100 nm) by first using a vacuum to remove all contaminants, and then guiding charged metallic ions or plasma to the surface of a medical device at ambient temperature. Results demonstrated that compared to currently used titanium and uncoated polymers, polymers coated with titanium using IPD significantly increased osteoblast proliferation and, most importantly, calcium deposition. In this manner, this study strongly suggests that IPD should be further studied for creating nanometer titanium surface feature coatings to enhance osteoblast functions necessary to increase orthopedic implant efficacy. © 2007 Wiley Periodicals, Inc. *J Biomed Mater Res*, 2008

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DIGITAL OBJECT IDENTIFIER (DOI)10.1002/jbm.a.31772 [About DOI](#)**ARTICLE TEXT****INTRODUCTION**

Bioactive coatings are in high demand to increase the functions of cells for numerous medical devices. For example, to improve the performance of conventional titanium-based materials for orthopedic applications (i.e., fabricated by traditional

metallurgy techniques and possibly surface-treated by mechanical methods such as grinding and polishing), hydroxyapatite has often been used as a coating.[1] By simulating the chemical composition of natural bone, hydroxyapatite coatings on titanium (Ti) greatly enhance osseointegration between the implant and juxtaposed bone.[2][3] Commercially, hydroxyapatite is coated on Ti-based metals through a high-temperature plasma-spray deposition process that transforms the initial nanocrystalline hydroxyapatite into micron grain size hydroxyapatite containing less crystalline calcium phosphates. Plasma-spray coating processes have, thus, often been criticized, since they are not versatile enough to handle a wide range of chemistries and frequently alter the properties of the starting materials to be coated. Specifically, plasma-spray deposition of hydroxyapatite results in phase transformations which may lead to the formation of highly soluble calcium phosphates that delaminate during clinical use.[1-3]

Furthermore, several groups have provided evidence that materials with nanometer surface features enhance bone formation compared to materials with conventional (or micron-scale) surface features.[4-8] Although these findings indicate that nanometer surface features are important to increase the cytocompatibility of currently used implants, traditional coating processes (like the aforementioned plasma-spray due to high heat) cannot uniformly create nanofeatures in orthopedic coatings to more effectively regenerate bone.

To obtain nanoroughness on a metal substrate, researchers have tried several techniques including the use of ultrafine-grained Ti (and other metals),[8][9] anodizing Ti,[10] and chemical etching of Ti; all have shown promise. In terms of coatings, in 2004, Ionic Fusion Corp. announced a novel vacuum surface modification process called ionic plasma deposition (IPD), which allows for the accurate control of material properties during coating procedures. Basically, the IPD process creates a surface-engineered nanostructure (with features usually below 100 nm) by first using a vacuum to remove all contaminants. High kinetic energies (a few hundred electron volts) then guide charged metallic ions or plasma to the surface of the medical device. The process runs at ambient temperature and can be supercooled when required, enabling a wide range of materials (i.e., Ag, Au, Ti, etc.) to be coated on a wide range of underlying materials (for example, metals, polymers, and ceramics).

Increased osteoblast adhesion has been measured on polymeric materials coated with either Au and Ti using IPD.[11] However, adhesion is just the first step in the successful osseointegration of an implant into juxtaposed bone. The objective of this present *in vitro* study was to determine long-term functions of osteoblasts (most importantly, calcium deposition) on two polymers of particular interest to the orthopedic implant field [specifically, ultrahigh molecular weight polyethylene (UHMWPE) and polytetrafluoroethylene (PTFE)] coated with Ti by IPD.

MATERIALS AND METHODS



Substrates

Substrates [specifically, ultrahigh molecular weight polyethylene (UHMWPE) and polytetrafluoroethylene (PTFE)] were modified with a Ti coating using the IPD deposition process. Raw materials were not medical grade and were purchased off the shelf from McMaster-Carr. IPD was conducted at Ionic Fusion, Corp. This process, performed in a vacuum, creates controllable nanometer surface features to promote cell attachment. Energy levels of 150-500 eV were controlled depending on the properties of the depositing materials (specifically, Ti), allowing for low temperature (~30°C) deposition onto the various polymer substrates. Ti was obtained from Process Materials. Ninety percent of the depositing materials were less than 100 nm in diameter while the remaining 10% was greater than 100 nm but less than 1 μm . Uncoated polymer samples were used as controls. Moreover, commercially obtained conventional grain size Ti (Osteonics) was used as a reference.

After the coatings were completed, all samples were cleaned with deionized water in an aquasonicator with 70% ethanol for 10 min. These cleaned substrates were dried in an oven at 65°C and exposed to UV light for 1 h.

Material characterization

Samples were characterized by scanning electron microscopy according to standard techniques[11] at various magnifications.

Cytocompatibility tests

Human osteoblasts (CRL-11372, American Type Culture Collection, population numbers 2-4) were used in the cell adhesion experiments in this study. All substrates of interest were rinsed with phosphate buffered saline (PBS; 1 \times strength) before seeding the cells. The cells were cultured on the substrates in Dulbecco's modified eagle medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Hyclone) with an initial seeding density of 3500 cells/cm². Cells were then allowed to proliferate on the substrates under standard cell culture conditions (37°C temperature, 5% CO₂ and 95% humidified air) for 1, 3, and 5 days; media was changed every other day.

After the prescribed time period, the cell culture medium was aspirated from the wells and the substrates were gently rinsed with PBS three times to remove any nonadherent cells. The cells were then fixed with a 4% formaldehyde solution (Fisher) and stained with DAPI (Sigma). The cell numbers were counted and images taken under a fluorescence microscope (Swiss).

For long-term cell experiments, osteoblasts were seeded at a cell density of 50,000 cells/sample and were cultured in DMEM supplemented with 10% FBS, 1% P/S, 2.16×10^{-3} g/mL β -glycerophosphate, and 5×10^{-5} g/mL ascorbate for 7, 14, and 21 days. At the end of the prescribed time periods, cells were lysed using three freeze-thaw cycles. In order to determine the amount of calcium-containing mineral that had been deposited by osteoblasts, substrates were then soaked in 10N hydrochloric acid (J.T. Baker) overnight to dissolve the calcium mineral deposits. These supernatants were then collected and tested for calcium content using a Calcium assay (Sigma Diagnostics; Procedure No. 587) following the manufacturer's instructions.

Statistics

Statistical analysis was performed using standard analysis of variance techniques coupled with a Duncan's Multiple Range test. All experiments were run in triplicate with at least three replicates; $p < 0.01$ was considered statistically significant.

RESULTS



Materials characterization

SEM analysis revealed that the Ti coated onto the polymers of interest to the present study possessed numerous nanometer features (Figs. 1 and 2). This result confirmed that of a previous study,[11] which also demonstrated highly nanostructured roughness of Ti coated onto polymers using IPD. In contrast, uncoated polymers of interest to this study were smooth at the nanometer resolution (Figs. 1 and 2). Previous studies showed that the conventional Ti used in this study was also smooth at the nanometer level.[11] Diagonal striations in the uncoated UHMWPE are machining lines.

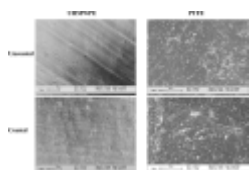


Figure 1. Low magnification scanning electron micrographs of uncoated and ionic plasma deposited (IPD) nanotitanium on UHMWPE and PTFE. Numerous nanometer and micron features were present on IPD coated nanotitanium on polymers. Bars = 10 μ m for both uncoated samples, 20 μ m for UHMWPE coated with nanotitanium, and 10 μ m for PTFE coated with nanotitanium.

[Normal View 67K | Magnified View 335K]

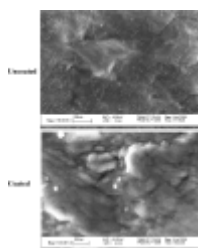


Figure 2. High magnification scanning electron micrographs of uncoated UHMWPE (top) and ionic plasma deposited (IPD) nanotitanium on UHMWPE (bottom). Numerous nanometer features were present on IPD coated nanotitanium on UHMWPE. Bars = 200 nm.

[Normal View 34K | Magnified View 145K]

Cytocompatibility characterization

More importantly, results of this *in vitro* study showed that compared to the respective uncoated polymer samples, osteoblast density after 1, 3, and 5 days increased on the two polymer substrates (UHMWPE and PTFE) coated with nanoparticulate Ti (Figs. 3-5 for days 1, 3, and 5, respectively). Osteoblast density was greater on all samples after each time period coated with nanoparticulate Ti using IPD compared to currently used micron grain size Ti. Osteoblast density also increased from days 1 to 5 for the nano Ti-coated samples. Osteoblast density was similar after 5 days on the UHMWPE and PTFE coated with nano Ti. Figures 6 and 7 highlight differences in cell numbers on the substrates of interest to the present study.

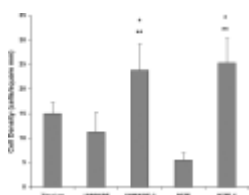
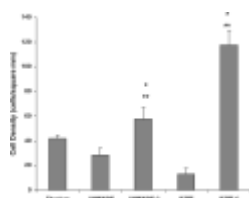


Figure 3. Increased osteoblast density on UMMWPE and PTFE coated with nano Ti after 1 day. Data = mean + SD, $n = 3$; * $p < 0.01$ (compared to respective uncoated polymer sample) and ** $p < 0.01$ (compared to currently used Ti, labeled as Titanium). C, coated with nano Ti using IPD. [Normal View 12K | Magnified View 27K]

Figure 4. Increased osteoblast density on UMMWPE and PTFE coated with nano Ti after 3 days. Data = mean + SD, $n = 3$; * $p < 0.01$ (compared to respective uncoated polymer sample) and ** $p < 0.01$ (compared to currently used Ti, labeled as Titanium). C, coated with nano Ti using IPD.



[Normal View 12K | Magnified View 25K]

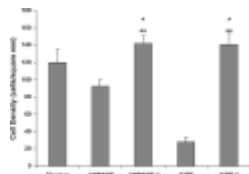


Figure 5. Increased osteoblast density on UMMWPE and PTFE coated with nano Ti after 5 days. Data = mean + SD, $n = 3$; $*p < 0.01$ (compared to respective uncoated polymer sample) and $**p < 0.01$ (compared to currently used Ti, labeled as Titanium). C, coated with nano Ti using IPD. [Normal View 13K | Magnified View 27K]

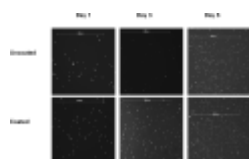


Figure 6. Fluorescent microscopy images of increased osteoblast density on UMMWPE coated with nano Ti. Bars = 100 μm . [Normal View 35K | Magnified View 107K]

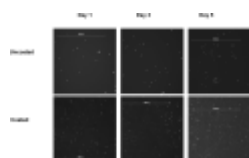


Figure 7. Fluorescent microscopy images of increased osteoblast density on PTFE coated with nano Ti. Bars = 100 μm . [Normal View 27K | Magnified View 93K]

Similar to osteoblast density results after 1, 3, and 5 days of culture, deposition of calcium containing mineral by osteoblasts was significantly greater for the nano Ti-coated polymers compared to respective uncoated polymers and currently used Ti after 7, 14, and 21 days (Fig. 8). Mineral deposition also increased with time when osteoblasts were cultured on the nano Ti-coated polymers. Mineral deposition by osteoblasts was similar after each time point on nano Ti-coated UHMWPE compared to nano Ti-coated PTFE.

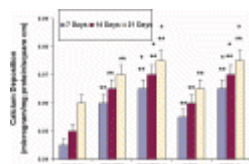


Figure 8. Increased osteoblast calcium mineral deposition on UMMWPE and PTFE coated with nano Ti after 7, 14, and 21 days. Data = mean + SD, $n = 3$; $*p < 0.01$ (compared to respective uncoated polymer sample) and $**p < 0.01$ (compared to currently-used Ti, labeled as Titanium). C, coated with nano Ti using IPD. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.] [Normal View 21K | Magnified View 50K]

DISCUSSION



IPD is a versatile technique that can be used to coat medical devices with (and of) diverse chemistries. Using conventional deposition methods (such as plasma-spray deposition), numerous problems exist such as poor adhesion strength, inability to maintain starting nanoparticle size, change of coating material crystallinity, and so forth.[1][2] However, in the IPD coating process, ions of the depositing material are accelerated to ensure that they have proper energy to coat the specific medical device at room temperature. As a result, the initial nanoparticle size of the coating materials can be preserved.

Because of prior studies,[4-8] one important property in material coatings to create is nanometer surface features. That is, owing to the importance of nanometer features in promoting bone cell functions, another key advantage of IPD is that the original particle size, chemistry, and crystallinity can be retained owing to the low heat presented during the coating application. Clearly, this allows IPD to create nanotopographies on conventional materials to improve their bioactivity properties, as this study demonstrated.

Specifically, to test whether such bioactive properties resulting from surface nanometer features are still present in the IPD-coated materials, osteoblast long-term function assays were conducted for the first time here. These results continued the promise of earlier studies showing increased osteoblast adhesion on polymers coated with either Ti or Au using IPD. [11] In this study, both osteoblast density after 1, 3, and 5 days and deposition of calcium containing mineral after 7, 14, and 21 days increased on two polymer chemistries popular to the medical device field (UHMWPE and PTFE) when coated

with nano Ti using IPD.

At this time, though, it is unclear what properties of the coatings enhanced osteoblast functions: such as a change in wettability, chemistry, and/or nanometer surface features. For example, the surface energy of UHMWPE and PTFE may have been altered through the nano Ti coatings; this is currently being investigated. However, as mentioned, when compared to traditional Ti (or micron grain size Ti) which possesses the same chemistry as the polymers coated with nano Ti using IPD,^[11] increased osteoblast long-term functions were measured; this suggests the possibility that nanometer roughness alone increased osteoblast functions on the nano Ti-coated polymer samples.

A change in nanometer roughness is also related to changes in wettability since previous studies have shown lower aqueous contact angles on Ti composed of nanometer compared to micron grain sizes (and consequently, nanometer compared to micron surface roughness).^[12] Authors speculated in those studies that surface energy is changed on Ti with nanometer surface features due to the increased presence of surface defects compared to conventional Ti.^[12] In an attempt to elucidate a mechanism of why this may be occurring, studies have also demonstrated greater initial adsorption of hydrophilic proteins (specifically, vitronectin) and subsequent osteoblast functions (from adhesion to the deposition of calcium containing mineral) on nanograined Ti.^{[13][14]} More studies, though, are needed for the presently described IPD process to determine specifically what material properties enhanced osteoblast functions on the coated materials. Nonetheless, this study provides strong evidence for the continued investigation of IPD for orthopedic applications.

CONCLUSIONS



Nanotopography or nanoroughness of an implant surface is desirable to improve initial cell-implant interactions. With respect to implant coatings, IPD is an efficient method to deposit nanostructured coatings onto versatile materials, including metals and polymers. The current study represents the first which demonstrated increased osteoblast long term functions (including the deposition of calcium-containing mineral) on various polymers coated with nano Ti by IPD; thus, demonstrating the strong potential IPD has in modifying conventional medical devices to be bioactive for orthopedic applications.

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