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Decreased Fibroblast and Increased Osteoblast Functions on Ionic Plasma Deposited Nanostructured Ti Coatings

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Abstract Bioactive coatings are in high demand to control cellular functions for numerous medical devices. The objective of this in vitro study was to characterize for the first time fibroblast (fibrous scar tissue forming cells) adhesion and proliferation on an important polymeric biomaterial (silicone) coated with titanium using a novel ionic plasma deposition (IPD) process. Fibroblasts are one of the first anchorage-dependent cells to arrive at an implant surface during the wound healing process. Persistent excessive functions of fibroblasts have been linked to detrimental fibrous tissue formation which may cause implant failure. The IPD process creates a surface-engineered nanostructure (with features usually below 100 nm) by first using a vacuum to remove all contaminants, 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 silicone, silicone coated with titanium using IPD significantly decreased fibroblast adhesion and proliferation. Results also showed competitively increased osteoblast (bone-forming cells) over fibroblast adhesion on silicone coated with titanium; in contrast, osteoblast adhesion was not competitively increased over fibroblast adhesion on uncoated silicone or titanium controls. In this manner, this study strongly suggests that IPD should be further studied for biomaterial applications in which fibrous tissue encapsulation is undesirable (such as for orthopedic implants, cardiovascular components, etc.).

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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, which 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. 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].

Moreover, hydroxyapatite coated on traditional orthopedic implants using plasma spray is not known to decrease the functions of fibroblasts (a cell well known to contribute to fibrous tissue encapsulation which can decrease orthopedic implant efficacy). In fact, fibrous encapsulation by excessive fibroblast functions on hydroxyapatite implants coated by plasma spray deposition is a common mode of

implant failure. Clearly, materials and associate coating properties are needed which increase functions of osteoblasts (bone forming cells) and simultaneously decrease functions of fibroblasts.

One promising classification of materials which may simultaneously increase functions of osteoblasts and decrease functions of fibroblasts are nanophase materials [4–13]. Nanophase materials are materials with at least one dimension <100 nm that mimic the natural surface roughness of bone. 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) usually cannot create nanofeatures in orthopedic coatings to more effectively regenerate bone.

Ways that have been used to create nanoroughness on a metal substrate to promote bone cell functions include the use of ultrafine-grained Ti (and other metals) [8, 9], anodizing Ti [10], and chemical etching of Ti. In terms of coatings, in 2004, Ionic Fusion Corp. announced a novel vacuum surface modification process called ionic plasma deposition (IPD), which allows for 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 100 eV) 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).

This novel coating process (IPD) has previously been shown to increase the functions of osteoblasts (such as adhesion, proliferation, and the deposition of calcium-containing mineral) on polymers coated with metals [14, 15]. In particular, promoted functions of osteoblasts have been measured on ultra high molecular weight polyethylene (UHMWPE), polytetrafluoroethylene (PTFE) and silicone coated with either gold or Ti using IPD [14, 15]. However, no evidence exists concerning in vitro functions of fibroblasts on these novel nanostructured coatings. Thus, the objective of this in vitro study was to characterize fibroblast functions on one particular polymer (silicone) coated with nano Ti using the IPD system.

Materials and Methods

Substrates

Silicone was modified using the IPD deposition process. Raw materials were purchased off the shelf from McMaster-Carr. This process, preformed in a vacuum, creates controllable nanometer surface features to mediate cell attachment. Energy levels of 500 eV were used to control the properties of the depositing Ti allowing for low temperature (~30 °C) deposition onto the various substrates. Ti 6–4 was obtained from Process Materials Inc. 90% of the depositing Ti was <100 nm in diameter. Uncoated samples were used as controls. Moreover, commercially obtained conventional (micron) grain size Ti (Osteonics) was used as a reference.

After the coatings were completed, all samples were cleaned with deionized water in an aqua-sonicator 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.

The surface roughness of the samples of interest to the present study was determined using Field Emission Scanning Electron Microscopy (LEO).

Cell Assays

Fibroblasts (CRL-2317, American Type Culture Collection, population numbers 2–4) and osteoblasts (CRL-11372, American Type Culture Collection, population numbers 2–4) were used in the cell experiments in this study. All substrates of interest were rinsed with phosphate buffered saline (PBS) (1X 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/cm^2 of substrate surface area. Some experiments were performed with fibroblasts alone and some by simultaneously seeding fibroblasts and osteoblasts (prestained with different fluorescent markers; Molecular Probes) to ascertain competitive cell adhesion. Cells were then allowed to adhere on the substrates under standard cell culture conditions (37 °C temperature, 5% CO_2 and 95% humidified air) for 4 h. 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 non-adherent cells. For the experiments with fibroblasts alone, the adherent cells were then fixed with a 4% formaldehyde solution (Fisher) and stained with a Hoescht 33258 dye (Sigma). For competitive fibroblast and osteoblast experiments, cells were directly visualized at the conclusion of the experiment using fluorescence microscopy. The cell numbers were counted under a fluorescence microscope (Swiss).

Similar experiments were conducted to determine long-term fibroblast density with the exception that fibroblasts were cultured for 1, 3, and 5 days. Media was changed every other day. Cells were fixed stained, and counted in a similar fashion to that described above.

All experiments were conducted in triplicate at least three different times.

Results

High Degree of Nanometer Surface Roughness for Silicone Coated with Ti Using IPD

Results of the present study demonstrated the expected high degree of nanometer surface roughness of silicone coated with Ti using IPD (Figs. 1, 2). In contrast, uncoated silicone did not possess a high degree of nanometer surface roughness.

Decreased Fibroblast Functions on Silicone Coated with Ti Using IPD

Results of this in vitro study showed for the first time significantly decreased fibroblast adhesion (Figs. 3, 4), decreased competitive fibroblast compared to osteoblast adhesion (Fig. 5), and decreased fibroblast density after 1, 3, and 5 days on silicone coated with Ti using IPD compared to any of the other substrates of interest (Figs. 6, 7). Half the number of fibroblasts were counted on silicone coated with Ti compared to uncoated silicone after 4 h. In addition, four times more osteoblasts competitively adhered to silicone coated with Ti compared to fibroblasts after 4 h. Of particular interest is that the number of fibroblasts decreased only on silicone coated with Ti using IPD from 1 to 3–5 days of culture. Such data provide strong evidence of the ability of silicone coated with Ti using IPD to inhibit fibroblast function while promoting competitive osteoblast function.

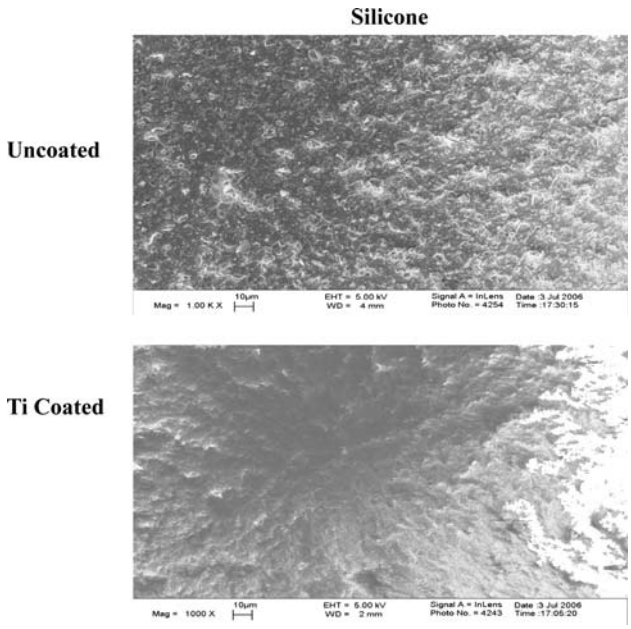


Fig. 1 Low magnification scanning electron micrographs of uncoated and ionic plasma deposited (IPD) Ti on silicone. Numerous nanometer features were present on IPD coated Ti. Bars = 10 μm

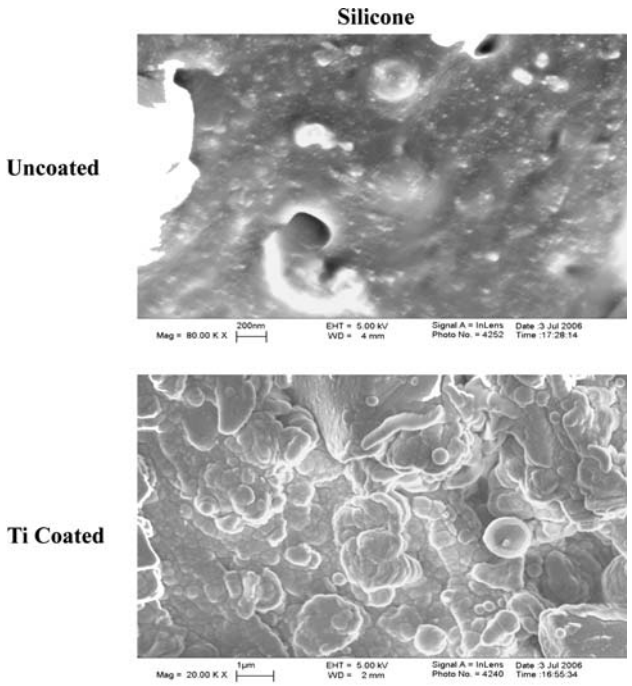


Fig. 2 High magnification scanning electron micrographs of uncoated and ionic plasma deposited (IPD) Ti on silicone. Numerous nanometer features were present on IPD coated Ti. Bars = 200 nm for top and 1 μm for bottom

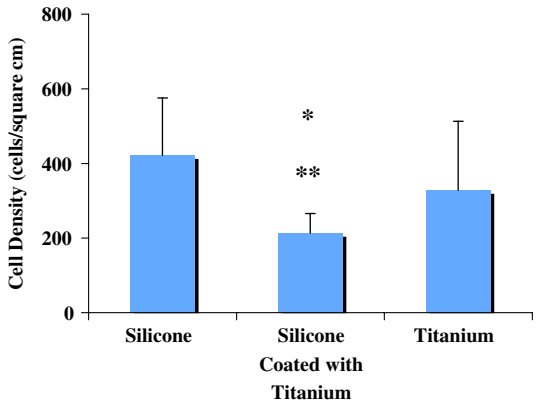


Fig. 3 Decreased fibroblast adhesion on silicone coated with Ti using ionic plasma deposition after 4 h. Data = mean ± STDEV, $n = 3$; *$p < 0.01$ (compared to silicone alone) and **$p < 0.01$ (compared to currently-used Ti)

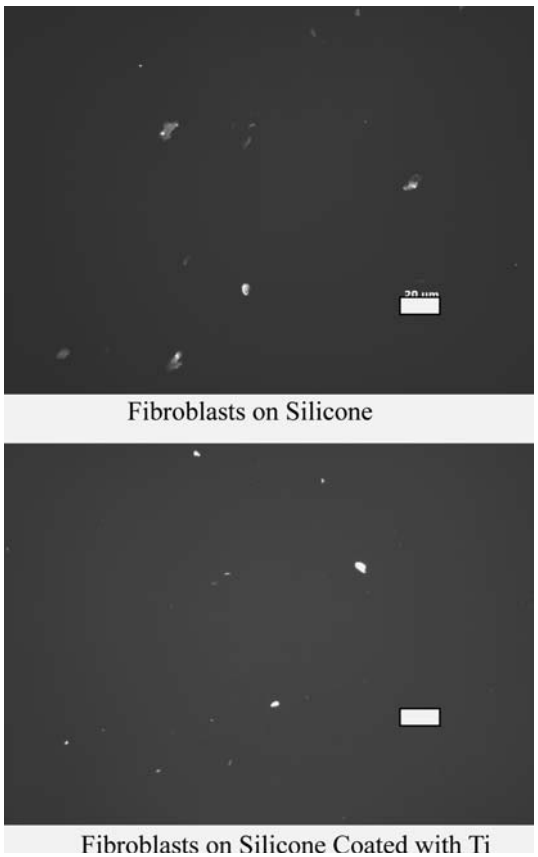


Fig. 4 Fluorescent microscopy images of decreased fibroblast adhesion on silicone coated with Ti using ionic plasma deposition. Bars = 20 μm

Discussion

Ionic plasma deposition is a versatile technique that can be used to coat different medical devices with 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, etc. [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, properties of the coatings are improved and are highly controllable at the nanometer level.

Due to prior studies [4–8], one important property in material coatings to create to increase osteoblast functions is nanometer surface features. That is, due to the importance of nanometer features in promoting bone cell functions and decreasing fibroblast functions, another key advantage of IPD is that the original particle size, chemistry, and crystallinity can be retained due to the low heat presented during the coating application. Clearly, this allows IPD to create nanotopographies on conventional

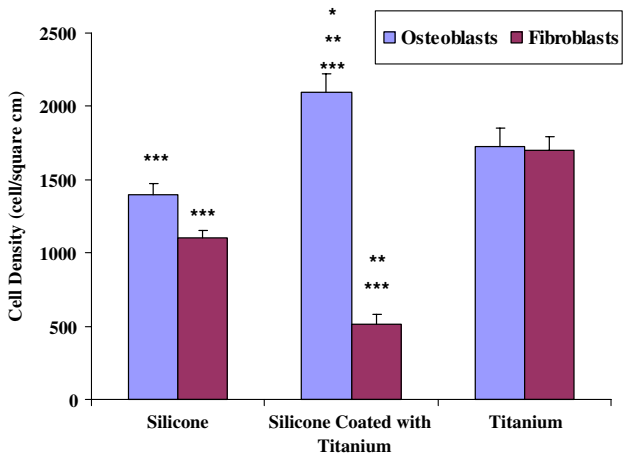


Fig. 5 Increased selective osteoblast density on silicone coated with Ti using ionic plasma deposition after 4 h. Data = mean ± STDEV, $n = 3$; $*p < 0.01$ (compared to fibroblast adhesion on respective sample); $**p < 0.01$ (compared to respective cell adhesion on silicone alone); and $***p < 0.01$ (compared to respective cell adhesion on Ti)

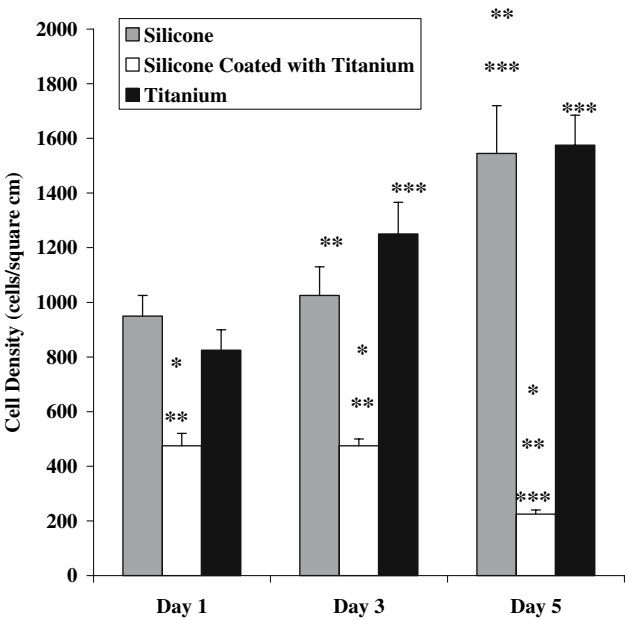
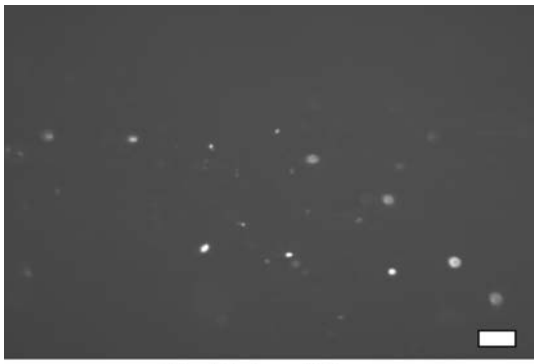


Fig. 6 Decreased fibroblast density on silicone coated with Ti using ionic plasma deposition after 1, 3, and 5 days. Data = mean ± STDEV, $n = 3$; $*p < 0.01$ (compared to silicone alone at the same time point); $**p < 0.01$ (compared to currently-used Ti at the same time point); and $***p < 0.01$ (compared to previous time point on the same substrate)

materials to improve their bioactivity properties, as this study demonstrated. Previous studies have shown that ceramics and polymers with nanostructured surface features decrease fibroblast functions compared to currently used nanometer smooth implant surfaces [4–8].



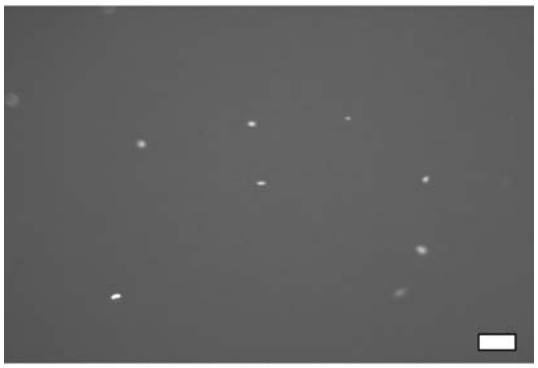


Fig. 7 Fluorescent microscopy images of decreased fibroblast density after 5 days on silicone coated with Ti using ionic plasma deposition. Bars = 20 μm

Such results have consequences not only for orthopedic applications, in which as discussed the selective promotion of osteoblast functions are desirable, but also for any implant device in which fibrous tissue encapsulation is undesirable. For example, for numerous cardiovascular applications (such as catheters, stents, grafts, etc.), increased fibrous tissue formation decreases the efficacy of a device. The present results of decreased fibroblast functions on silicone coated with one specific chemistry (Ti) shows promise for all of these implant applications.

At this time, though, it is unclear what properties of the coatings enhanced osteoblast adhesion (such as a change in wettability, chemistry, and/or nanometer surface features). For example, silicone is a hydrophobic material which may have been transformed through Ti coatings into hydrophilic materials to influence cell adhesion. However, as mentioned, when compared to traditional Ti (or micron grain size Ti) which possesses the same chemistry as the polymers coated with Ti, decreased fibroblast adhesion was measured; this suggests the possibility that nanometer roughness alone decreased fibroblast adhesion on the coated samples.

Importantly, 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 [11]. Authors speculated in those studies that hydrophilicity is enhanced on Ti with nanometer surface features due to the increased presence of surface defects compared to conventional Ti [11]. Those studies continued to show greater initial adsorption of hydrophilic proteins (specifically, vitronectin) and subsequently greater osteoblast functions (from adhesion to the deposition of calcium containing mineral on nanograined Ti) and decreased fibroblast functions [12, 13]. More studies, though, are needed for the presently described IPD process to determine specifically what properties selectively enhanced osteoblast and decreased fibroblast adhesion on the coated materials. None-the-less, 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 competitive osteoblast functions while at the same time decrease fibroblast functions known to contribute to fibrous tissue encapsulation harmful for orthopedic implant success. 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 desirable decreased fibroblast attachment and growth on silicone coated with Ti; this is in contrast to uncoated silicone or Ti, thus, demonstrating the strong potential IPD has at increasing conventional medical device efficacy for a number of biomedical applications (such as for orthopedic implants, cardiovascular components, etc.).

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