



Full length article

Titanium surface characteristics, including topography and wettability, alter macrophage activation



Kelly M. Hotchkiss^a, Gireesh B. Reddy^a, Sharon L. Hyzy^a, Zvi Schwartz^a, Barbara D. Boyan^{a,b}, Rene Olivares-Navarrete^{a,*}

^a Department of Biomedical Engineering, School of Engineering, Virginia Commonwealth University, Richmond, VA, USA

^b Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Georgia Institute of Technology, GA, USA

ARTICLE INFO

Article history:

Received 19 August 2015

Received in revised form 12 November 2015

Accepted 2 December 2015

Available online 7 December 2015

Keywords:

Macrophages

M1 activation

M2 activation

Surface roughness

Wettability

Immune response

Titanium

ABSTRACT

Biomaterial surface properties including chemistry, topography, and wettability regulate cell response. Previous studies have shown that increasing surface roughness of metallic orthopaedic and dental implants improved bone formation around the implant. Little is known about how implant surface properties can affect immune cells that generate a wound healing microenvironment. The aim of our study was to examine the effect of surface modifications on macrophage activation and cytokine production. Macrophages were cultured on seven surfaces: tissue culture polystyrene (TCPS) control; hydrophobic and hydrophilic smooth Ti (PT and oxygen-plasma-treated (plasma) PT); hydrophobic and hydrophilic microrough Ti (SLA and plasma SLA), and hydrophobic and hydrophilic nano- and micro-rough Ti (aged modSLA and modSLA). Smooth Ti induced inflammatory macrophage (M1-like) activation, as indicated by increased levels of interleukins IL-1 β , IL-6, and TNF α . In contrast, hydrophilic rough titanium induced macrophage activation similar to the anti-inflammatory M2-like state, increasing levels of interleukins IL-4 and IL-10. These results demonstrate that macrophages cultured on high surface wettability materials produce an anti-inflammatory microenvironment, and this property may be used to improve the healing response to biomaterials.

Statement of significance

Metals like titanium (Ti) are common in orthopaedics and dentistry due to their ability to integrate with surrounding tissue and good biocompatibility. Roughness- and wettability-increasing surface modifications promote osteogenic differentiation of stem cells on Ti. While these modifications increase production of osteoblastic factors and bone formation, little is known about their effect on immune cells. The initial host response to a biomaterial is controlled primarily by macrophages and the factors they secrete in response to the injury caused by surgery and the material cues. Here we demonstrate the effect of surface roughness and wettability on the activation and production of inflammatory factors by macrophages. Control of inflammation will inform the design of surface modification procedures to direct the immune response and enhance the success of implanted materials.

© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The goal of many dental and orthopaedic implants is complete integration of the implant with host bone with limited adverse effects in the surrounding tissue [1]. After implantation into the body, the cascade of events initiated by immune cells after interacting with the material surface determines the fate of a

biomaterial [1–3]. Many studies have focused on how material surface modifications can promote stem cell differentiation toward osteoblasts [4,5]. However, before osteoblasts can arrive and begin forming bone, the inflammatory response must be resolved. Activation of the immune system controls the initial response to the implanted material and affects its long-term survival and integration. Immune cells release factors in response to their interaction with the biomaterial surface to condition the microenvironment surrounding the implant controlling the immune response. Continued immune system activation can lead to chronic inflammation that can result in the breakdown of healthy tissue surrounding

* Corresponding author at: VCU School of Engineering, Department of Biomedical Engineering, 401 W. Main Street, E1254, Richmond, VA 23284, USA.

E-mail address: ronavarrete@vcu.edu (R. Olivares-Navarrete).

the implant [1]. However, a lack of inflammatory response will leave the debris from implantation to remain and affect the integration of the material and generation of new tissue [6,7].

Neutrophils, platelets, and macrophages migrate from blood vessels to the wound site. There, they produce growth factors, chemokines, and cytokines that recruit additional immune cells to the site, and in a normal wound healing response, induce phagocytosis of the damaged cells/tissue and stimulate the wound healing process [1]. Macrophages are responsible for the initial immune response, inflammation, and maintaining tissue homeostasis [3,8,9]. The ability of a material surface to control the reaction of these cells will influence the host's initial response to the device, and ultimately decide the integration of the material.

Two macrophage phenotypes have been established: the classical pro-inflammatory M1 and the alternative anti-inflammatory, wound healing M2 [4,9–11]. Classical M1 polarization is generated by activation of the naïve macrophage by interferon gamma ($\text{INF}\gamma$) and lipopolysaccharide (LPS). The alternative M2 activation results from cell stimulation by interleukin (IL)-4 and IL-13 released from the cells of the adaptive immune system [9,12]. Macrophage activation is characterized by the profile of cytokines and growth factors released by the cells into their microenvironment. The M1 activation is a pro-inflammatory response responsible for rapid immune activation in the presence of microbiological or other acute threats [13], characterized by high levels of interleukin IL-1 β , IL-6, and TNF α [9,10,13]. M2 activation is a wound healing and tissue remodeling response marked by anti-inflammatory cytokines IL-10, IL-4, IL-13, and TGF- β [9,11]. A balance between these macrophage activations is required for the proper healing of an injury and biomaterial integration [1,14]. The aim of our study was to examine the effect of surface microstructure and wettability on macrophage activation, polarization, and cytokine production in response to Ti substrates.

2. Materials and methods

2.1. Disk preparation

Ti disks were provided by Institut Straumann AG (Basel, Switzerland), with SLA and modSLA corresponding to the commercially available SLA[®] and SLActive[®] respectively. Each disk was created by a 15 mm punch from 1 mm thick sheets of grade 2 unalloyed Ti. The disks were sized to fit securely in a 24 well plate. The sample disks were prepared as previously described [4]. Disks were cleaned and degreased by acetone bath, and then processed in a 2% ammonium fluoride/2% hydrofluoric acid/10% nitric acid solution at 55 °C for 30 s to produce the pretreatment (PT) surfaces. SLA surfaces were created by coarse-grit blasting PT disks with 0.25–0.50 mm corundum followed by acid etching in a mixture of HCl and H₂SO₄ in order to create surface structures and roughness at the macro and micro scale. PT and SLA disks were rinsed in deionized water and dried after processing. modSLA disks were created using the same procedure as SLA but were rinsed under nitrogen protection to prevent air exposure and stored in an isotonic NaCl solution in sealed glass tubes until use. This process results in a hydrophilic surface with roughness at the micron-, submicron-, and nano-scale. Disks were sterilized by γ -irradiation.

A set of hydrophilic, PT and SLA disks were created by oxygen plasma cleaning (plasmaPT, plasmaSLA) as established in prior experiments [15,16]. Disks were treated in an oxygen plasma cleaner (PDC-32G, Harrick Plasma, NY) at medium radio frequency for 2 min per side. The hydrophobic aged modSLA surface was created by sonicating modSLA surfaces in ultrapure water for 10 min two times to remove the residual saline solution and aged by exposure to air for two weeks under sterile conditions.

2.2. Surface characterization

The roughness generated under each surface condition was determined by laser scanning confocal microscopy (LSCM, Zeiss LSM 710, Carl Zeiss). Measurements were taken with a scan size of 600 μm \times 600 μm with a 20 \times lens. Roughness values (average roughness over area: S_a , skewness: S_{sk} , kurtosis: S_{ku} , and developed interfacial area ratio: S_{dr}) were calculated with a 100 μm threshold. Measurements were taken at three different points on each disk.

Qualitative assessments of macro-, micro-, and nanostructure of each surface were acquired by scanning electron microscopy (SEM, Zeiss Auriga, Carl Zeiss, Jena, Germany) at 1k \times and 100k \times magnifications. Disks were analyzed without the addition of a conductive coating with a secondary electron detector at 5 kV, under vacuum and a distance of approximately 2 mm.

The wettability of each surface type was indirectly measured by sessile drop contact angle (ramé-hart contact angle goniometer 250, model 100-25a, ramé-hart instrument co., Succasunna, NJ). Measurements using 1 μL drops of deionized water were taken at three locations of six disks per surface condition. A contact angle of 0° considered hydrophilic and greater than 80° considered hydrophobic.

The chemical composition of the surface was determined by X-ray photoelectron spectroscopy (XPS, ESCALAB 250, Thermo Scientific, Waltham MA) under ultra-high vacuum (10⁻⁹ Torr or below) with a microfocused monochromatic AlK α X-ray source. The focus of the XPS was to assess differences in carbon levels; therefore, disks were secured to the mount with stainless steel clips in order to remove potential carbon readings from the adhesive tape. Prior to analysis clips and mount were sonicated in acetone. Survey scans were completed at each region, followed by high-resolution scans for C1s, Ti2p, O1s, Na1s, and Cl2p. Scans were aligned to the binding energy of the C1s peak at 284.8 eV. Thermo Advantage software was used to evaluate spectrum results. Each surface characterization procedure was performed on three regions of six disks per surface condition. modSLA surfaces were rinsed in ultrapure water prior to each surface characterization procedure.

2.3. Cell culture

Primary murine macrophages were isolated from femurs of 6–8 week-old male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) under VCU IACUC approval using previously described methods [17]. Briefly, bone marrow cells were flushed from the femurs using Dulbecco's phosphate-buffered saline (Life Technologies, Carlsbad, CA). Red blood cells were lysed from the bone marrow extract with ACK Lysing Buffer (Quality Biological, Inc., Gaithersburg, MD). Cells were counted (TC20™ Automated Cell Counter, Bio-Rad Laboratories, Hercules, CA) and plated in a 75 cm² flask at a density of 500,000 cells/mL in 10 mL RPMI 1640 media (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 50 U/mL penicillin-50 $\mu\text{g}/\text{mL}$ streptomycin (Life Technologies), and 30 ng/mL macrophage colony-stimulating factor (M-CSF, PeproTech, Rocky Hill, NJ). Cells were cultured at 37 °C, 5% CO₂, and 100% humidity. Fresh media supplemented with M-CSF was added after four days. Seven days after plating, macrophages were passaged and seeded onto Ti surfaces for experiments. modSLA surfaces were removed from saline solution and rinsed with ultrapure water prior to cell seeding.

2.4. Cell staining

Differentiated macrophages were plated on surfaces at a density of 20,000 cells/cm² in RPMI with 10% fetal bovine serum (Life Technologies), 50 U/mL penicillin-50 $\mu\text{g}/\text{mL}$ streptomycin (Life Technologies) without M-CSF and cultured at 37 °C, 100%

ID	Title	Pages
164	Titanium surface characteristics, including topography and wettability, alter macrophage activation	10

Download Full-Text Now



<http://fulltext.study/article/164>



-  **Categorized Journals**
Thousands of scientific journals broken down into different categories to simplify your search
-  **Full-Text Access**
The full-text version of all the articles are available for you to purchase at the lowest price
-  **Free Downloadable Articles**
In each journal some of the articles are available to download for free
-  **Free PDF Preview**
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>