Engineered Hierarchical Surface Roughness Regulates Mesenchymal Stem Cell Spreading, Proliferation, and Differentiation on Titanium Implants

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Introduction

Titanium has been widely used for orthopedic implants given its excellent biocompatibility and high strength and durability[1]. Multiple surface modification techniques have been introduced to enhance cell interaction and differentiation, including promotion of osteogenesis and bone formation[2]. All cell types, including progenitor cells [e.g., mesenchymal stem cells (MSCs)], are responsive to biophysical cues they experience within their microenvironment. These cues can include material stiffness, organization, deformation, and surface topography[3]. Such cues elicit reorganization of the internal cyto-architecture and mechanical signaling of cells and can be leveraged to direct lineage specification. Our team recently developed a method to enhance the topology of titanium implant surfaces using an Electron Beam Melting (EBM), and here, we explored how this manufacturing process impacted initial MSC interactions, spreading, proliferation, expansion, and osteogenic differentiation.

Methods

Human mesenchymal stem cells were purchased (hMSC, Lonza, male 23 years), and passage two cells ($5 \times 10^{\wedge 4}$ cells) were seeded onto one of 3 surfaces: 1) Smooth Titanium (Smooth), and two proprietar 3D-printed (P3D) titanium surfaces [Electron Beam Melting (EBM)], which consist of a hierarchical surface roughness that spans from the macro to nano-scale having lesser 2) P3D1 or greater 3) P3D2 surface roughness. Surface characterization was performed using scanning electron microscopy and surface profiles were determined via image analysis (ImageJ) of brightfield micrographs, where average roughness values (R_a) were determined according to ASME B46.1. Cell seeded titanium surfaces (diameter 20 mm) were cultured in a serum containing basal growth media (BM) or in an osteogenic media (OM). Throughout a 21-day culture period, cell morphology was assessed by staining for filamentous actin (phalloidin), and cell area and aspect ratio (> 25 cells from n = 3 surfaces) was calculated using Image J. In addition, cell proliferation was assessed using the Alamar Blue assay (n = 6 samples/group) and osteogenic differentiation was evaluated by RT-PCR of Type-I Collagen (COL-I) and Osteocalcin (OCN) (n = 6 samples/group). Statistics were performed using ANOVA with Tukey's post hoc testing with 95% confidence interval.

Results

SEM images showed evidence of a hierarchical surface roughness spanning from the macro to the nanoscale [average roughness (R_a): P3D1: 50 ± 4 μm, P3D2: 65 ± 6 μm] (Figure 1A, B), and initial MSC adhesion showed marked differences between smooth and rough surfaces (Figure 1A). This was confirmed via Phalloidin staining and quantification shown in Figure 2. On smooth surfaces (Smooth), MSCs adopted a large spread area with abundant stress fibers on Day 1, while on the rougher surfaces (P3D1 and P3D2), MSCs had a smaller spread area (Figure 2A-C) with fewer and smaller focal adhesions (not shown). Both cell area and aspect ratio were significantly lower (by >25%) on the P3D1 and P3D2 surfaces, compared to cells on smooth titanium ($p<0.05$) (Figure 2A-C). With respect to proliferation, throughout the 21 days of culture, hMSCs expanded rapidly on both smooth and rough surfaces, with no significant differences in the either BM or OM culture condition (Figure 3A). RT-PCR analysis of BM conditions

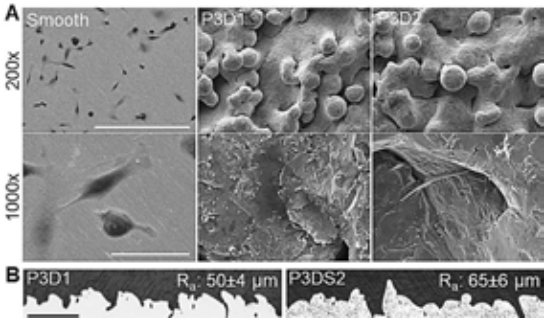

Figure 1. (A) Representative SEM images of MSC seeded titanium surfaces: Smooth, P3D1, or P3D2 [Scale bars = 300 μm (top) and 50 μm (bottom). **(B)** Representative micrographs of P3D1 and P3D2 and average roughness values (Ra) (Scale bar = 500 μm).

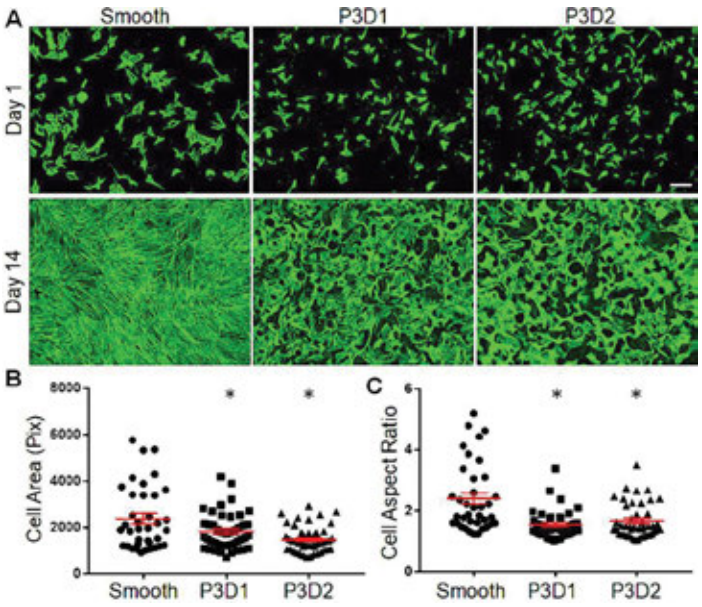

Figure 2. (A) Representative actin staining of MSC seeded titanium surfaces: Smooth, P3D1, or P3D2, Scale bar = 100 μm. **(B)** Quantification of cell area and aspect ratio on Day 1 (n > 30, mean ± SEM, *: p<0.05 vs. Smooth).

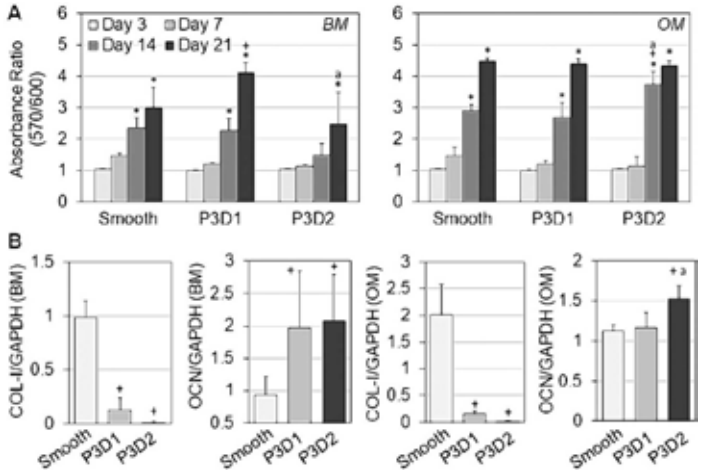

Figure 3. (A) Cell proliferation assessed by the Alamar blue assay. **(B)** Osteogenic gene expression determined by RT-PCR on Day 14 in hMSC cultured in basal growth media (BM) or osteogenic differentiation media (OM). (n = 6, *: p < 0.05 vs. Day 3, +: p < 0.05 vs. Smooth, a: p < 0.05 vs. P3D1).

showed that COL-I was more highly expressed on smooth surfaces than on rough surfaces, while OCN expression was higher on the rough surfaces (Figure 3A). In OM conditions, COL-I expression remained considerably higher on the smooth surface compared to the rough surfaces, while OCN expression was higher on the P3D2 surface (Figure 3B).These data indicate that the smooth surfaces may lead to fibrogenic differentiation of hMSCs, while surfaces with enhanced roughness may promote osteogenic differentiation.

Discussion

Our findings indicate that MSCs rapidly adopt a spread morphology on stiff, smooth titanium surfaces. Conversely, materials produced with a proprietary EBM build theme promoted less initial spreading but greater osteogenic differentiation over time when compared to smooth Ti. Taken together, these findings demonstrate that proprietary 3D printed titanium implants can be produced with surface features that regulate adhesion, spreading, and differentiation of bone marrow derived MSCs. Improvements engendered by rough surfaces may result in greater boney tissue formation when such surfaces are used for spinal fusion procedures. Future work will explore the mechanism by which this 3D printed surface roughness improves osteogenesis and its impact on mineral deposition and bone formation *in vivo*. Our data show that controlling the surface roughness on titanium implants regulates hMSC morphology and osteogenic differentiation propensity, which may enhance bone formation in clinical scenarios where rapid osseointegration and fusion are required.

References

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