

Programmed Surface Topology and Roughness in 3D-printed Titanium Implants can Improve Osteoblastic Genes Expression in Bone marrow Mesenchymal Stem Cells

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INTRODUCTION: Interbody fusion cages are frequently used to stabilize diseased spinal segments and treat painful degenerative spine conditions. Achieving a successful spinal fusion requires the proper biomechanical environment. Attaining optimal spinal fusion in interbody fixation procedures requires a rapid and stable fixation at the bone-implant interface, which may reduce implant-related complications such as subsidence, expulsion and nonunion. Commonly utilized interbody fusion cages are comprised of Polyetheretherketone (PEEK) or Titanium (Ti). Although PEEK interbody spacers have favorable attributes such as biocompatibility and an elastic modulus that is similar to bone [1], PEEK implants generally have a smooth, hydrophobic surface that does not directly bond to bone and promotes fibrous tissue formation [2,3]. Similarly, Ti cages with a smooth surface (SmTi), such as commonly utilized in traditional interbody cages manufactured through a machining process, have demonstrated inferior osteogenesis and bony integration [4] as compared to those with roughened surfaces. Programmed porosity and surface roughness properties of Ti interbody implants have been shown to promote stability and fusion by facilitating improved bone repair and interlocking between the implant and bone interface zone. Specifically, multi-scale Ti surface topography (e.g. those that contain macro-, micro- and nano-scale features), can shorten the bone ingrowth and integration phase (**Figure 1**). The purpose of this study was to compare P3D with SmTi and PEEK surfaces for their bone differentiation capability by utilizing transgenic bone marrow stem cells that fluoresce when osteogenesis is initiated. In addition, to evaluate the rate and magnitude of osteogenesis, gene expression was evaluated longitudinally with a comprehensive panel of bone-related genes that include a master transcriptional regulator of bone formation, and early- and late-stage markers for bone differentiation.

METHODS: In this study we evaluated mesenchymal stem cells obtained from the bone marrow of a transgenic mouse in which the expression of two genes that are critical for the proper mineralization of bone could be monitored fluorescently (**Figure 2**).

Bone marrow was harvested from the femur and tibia bones of the Bone Sialoprotein (BSP)-Green Fluorescent Protein (GFP) tpz/Dentin Matrix Protein (DMP)-Red Fluorescent Protein (RFP)chry Bacterial Artificial Chromosome (BAC) transgenic reporter mouse and cultured under non-osteogenic conditions to expand the attached stem cells. The BSP and DMP proteins are located deposited in bone tissues and are involved in hydroxyapatite nucleation and collagen mineralization. The BSP gene is activated in newly formed osteoblasts and DMP expression is limited to mineral embedded osteocytes (**Figure 2**). Low density, first passaged cells from the primary bone marrow stromal culture (BMSCs) were seeded at 1x10⁶ cells/ mL onto three replicate plates: P3D, Ti with smooth surface (SmTi) or PEEK (ultra-low cell attachment plates, Corning Fisher Scientific). From day 7 through 22, cell-constructs were placed under osteogenic conditions (alpha MEM (ThermoFisher Cat# 12571063) supplemented with 10% FBS, 50 µg/ml ascorbic acid and 4 mM β-glycerophosphate). Media was replenished every 2-3 days. The plates were harvested at days 3, 7, 14 and 22 and evaluated for fluorescence and RNA expression. Cultures were rinsed with PBS and treated with Accutase for 30 minutes at 37°C. The remaining cells were scraped, resuspended and centrifuged. The cell pellets were resuspended in PBS containing 2% FBS, loaded into a disposable cell counting chamber slide (SD100, Nexcelom Bioscience, USA), counted in the Cellometer with phase image using filters for GFPtopaz (P/N: VB-535-402) and mCherry (custom made: VB-625-502) and analyzed with FCS4 Express Flow Cytometry software (De Novo Software, USA). Fluorescence intensity below 1000 was counted as fluorescence negative. Cells that have entered osteogenesis were identified as being GFPtopaz positive, mCherry positive, and/or GFPtopaz/mCherry double positive at day 22. Cells that did not express fluorescence were defined as not having initiated osteogenesis.

RESULTS: The data showed that a significantly greater percentage of BMSC-derived progenitor cells were osteogenic on the P3D Ti surface at day 22 (Fig 2, p<0.05) as compared to smooth Ti and PEEK. Osteogenic gene expression was evaluated over time on each surface (**Figure 3**). On each surface, almost all osteogenic markers increased over time with maximum osteogenic gene expression generally occurring at approximately day 14. The magnitude of gene expression was substantially higher for most markers on the P3D surface as compared to PEEK or SmTi (compare y-axis of all three).

At each time point, the fold-increase for osteogenic expression between P3D and PEEK or SmTi was evaluated (Fig 3). BMSC-derived progenitor cells cultured on P3D resulted in significantly (P<0.005) greater increases in the late osteogenic markers Bglap and Phex (indicative of osteocyte formation) by more than 1,000- and 100-fold, respectively, at day 14. At day 22, stem cells cultured on P3D resulted in significantly (P<0.005) greater increases in the early osteogenic markers Alp and Col1A1 by more than 15- and 35-fold, respectively, at day 22.

DISCUSSION: The 3D-printed rough Ti surface enabled a significantly greater proportion of the stem cells to enter the osteogenic lineage relative to PEEK and Smooth Titanium surfaces. Stem cells on the P3D surface resulted in significantly greater gene expression compared to PEEK or to SmTi for a broad spectrum of bone related molecules that includes a master regulator of bone differentiation, early bone markers and markers associated with bony mineralization. Faster and more robust late-stage osteogenic differentiation was demonstrated by over a 100-fold increase in osteocalcin and Phex for P3D relative to PEEK and SmTi. These attributes may facilitate a more rapid and stable fixation of bone-implant interface during spinal fusion.

SIGNIFICANCE: Hierarchical roughness of 3D printed titanium surface may facilitate a more rapid and stable fixation of bone-implant interface during spinal fusion.

REFERENCES: [1] Kurtz, S.M.+ Biomaterials, 2007. [2] Cho, D.Y.+, Neurosurgery, 2002. [3] Olivares-Navarrete, R.+ Spine, 2015. [4] Klein, M.O.+, Clin Implant Dent Relat Res, 2013.

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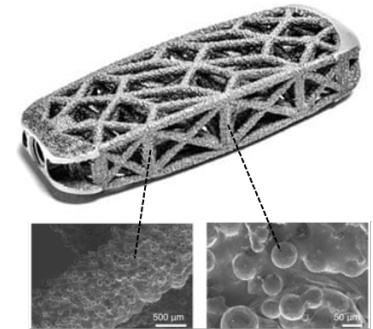


Figure 1. Scanning Electron Microscopy of the 3D surface at two different magnifications shows hierarchical surface roughness spanning from the macro to nanometer scale. Left: 500 micrometer scale (macro). Right: 50 micrometer scale.

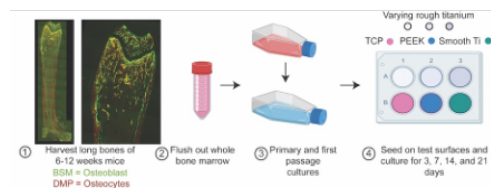


Figure 2. Experimental setup of mesenchymal stem cells extraction.

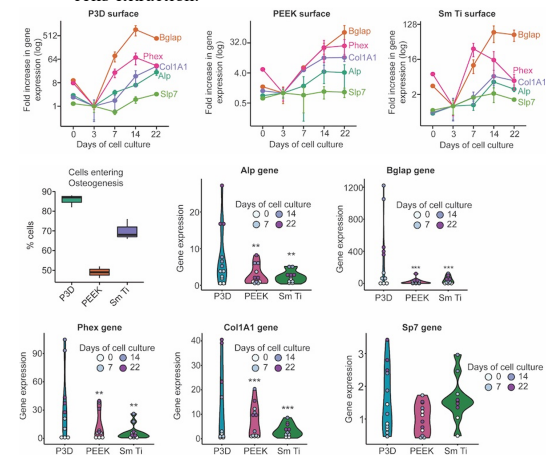


Figure 3. Osteogenic gene expression over time (day 3, 7, 14 and 22) for the BMSCs cultured on P3D, PEEK and smooth Ti.