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The synergistic effect of micro-topography and biochemical culture environment to promote angiogenesis and osteogenic differentiation of human mesenchymal stem cells

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ABSTRACT

Critical failures associated with current engineered bone grafts involve insufficient induction of osteogenesis of the implanted cells and lack of vascular integration between graft scaffold and host tissue. This study investigated the combined effects of surface microtextures and biochemical supplements to achieve osteogenic differentiation of human mesenchymal stem cells (hMSCs) and revascularization of the implants in vivo. Cells were cultured on 10 μm micropost-textured polydimethylsiloxane (PDMS) substrates in either proliferative basal medium (BM) or osteogenic medium (OM). In vitro data revealed that cells on microtextured substrates in OM had dense coverage of extracellular matrix, whereas cells in BM displayed more cell spreading and branching. Cells on microtextured substrates in OM demonstrated a higher gene expression of osteoblast-specific markers, namely collagen I, alkaline phosphatase, bone Sialoprotein, and Osteocalcin, accompanied by substantial amount of bone matrix formation and mineralization. To further investigate the osteogenic capacity, hMSCs on microtextured substrates under different biochemical stimuli were implanted into subcutaneous pockets on the dorsal aspect of immunocompromised mice to study capacity for ectopic bone formation. In vivo data revealed greater expression of osteoblast-specific markers coupled with increased vascular invasion on microtextured substrates with hMSCs cultured in OM. Together, these data represent a novel regenerative strategy that incorporates defined surface microtextures and biochemical stimuli to direct combined osteogenesis and re-vascularization of engineered bone scaffolds for musculoskeletal repair and relevant bone tissue engineering applications.

1. Introduction

Bone is the second most common transplanted tissue [1,2] with an estimated 2.2 million grafting procedures performed annually worldwide [3]. The gold standard for bone regeneration is to use autologous bone grafts for traumatic injuries, fracture non-unions, spinal fusion, and hip joint replacements, and other bone-related diseases [4]. However, autologous grafting is associated with limited availability of donor tissue for clinical applications [5–7] and significant patient morbidity and complication rates of 8.6–20.6% [1,8,9]. Allograft technologies have been developed to address the limited availability of autograft bone. However, the process of decellularization, sterilization, and allograft storage disrupts the osteoinductive nature of the tissue and results in clinical failure rates of 16–35% predominantly due to the inability of these grafts to adequately re-vascularize [10,11]. As such, there is a significant and growing need for strategies to promote vascularized bone graft substitutes for this unmet clinical need. Several strategies have been used to stimulate musculoskeletal healing, such as controlled release of growth factors to promote vascularization [12–15] or regulation of osteogenesis with mechanical properties [16–21]. Nonetheless, the lack of host vasculature penetration and integration between engineered bone scaffold constructs and host tissue still remains a fundamental challenge during bone fracture healing [22–24]. An understanding of the scaffold's underlying physical properties and its biochemical environment is crucial to elicit the desired biological responses for bone regeneration [25,26].

Surface topographical characteristics are important aspects in designing biological implants, as they have great implications in cell guidance and behavior [27–35]. The development of microfabrication and related microelectromechanical systems (MEMS)

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technologies such as soft lithography offers unprecedented reproducibility and precision to create surface topography that can interact with cells and tissues in a systematic manner [36–39]. For instance, Dalby et al. demonstrated that different arrangements of topographical disorder could modulate osteogenic differentiation of human mesenchymal stem cells (hMSCs) [40]. The Chen group demonstrated that the inherent rigidity of different sized micropatterns could shift the balance of hMSC fates, alternating between osteogenesis and adipogenesis [41,42]. Our laboratory also reported that 10 μm diameter post microtextures on polydimethylsiloxane (PDMS) significantly increased proliferation and osteogenesis of human bone marrow-derived connective tissue progenitor cells (CTPs) compared with those cultured on smooth surface [21,43]. These results suggest that varying geometry and arrangement of the surface topography can result in the modification of cell proliferation and morphology as well as the potential to enhance lineage specificity. However, there has been little investigation on the synergistic effects of microscale surface topography and biochemical attributes for tuning hMSC fate in vivo. Particularly, in vivo studies concerning microtextured materials have demonstrated mixed results in which microgrooved surfaces produced the same cellular and tissue response as smooth surfaces [44,45]. It has been suggested that an amorphous layer was formed between the surface microtexture and the connective tissue due to the influence of inflammatory cells present at the site of implant surface. Hence, cells from the connective tissue were unable to sense and respond to the micro-topography [44,46].

The goal of this study is to evaluate the long-term combined effect of microtextured topography and biochemical cues on hMSC proliferation and osteogenic differentiation, and re-vascularization in mice for potential bone tissue engineering applications. Specifically, PDMS substrates with 10 μm cylindrical post (diameter, height, and interspace) were created to culture hMSCs for 6 weeks under two different conditions: (1) one group in the proliferative basal medium (BM) during the entire study; (2) another group in the BM for the first five weeks and in the differentiative osteogenic medium (OM) for the last week. The in vitro pre-culture maximized material-cell interaction for lineage specificity on microtextured scaffolds, ensuring adequate hMSC sensing and response to topographical signals for in vivo development. The in vivo study investigated the potential osteogenic differentiation and vascular integration of hMSCs with the host environment by subcutaneous implantation of hMSCs on microtextured substrates in BM and OM for 6 weeks.

2. Materials and methods

2.1. Experimental design

Bone marrow derived hMSCs were cultured for 6 weeks on 10 μm micropost PDMS substrates under two conditions: (1) in the proliferative basal medium (BM) during the entire study, and (2) in the BM for the first five weeks and in the differentiative osteogenic medium (OM) for the last week. Cell seeded scaffolds were analyzed in vitro using fluorescent microscopy, scanning electron microscopy (SEM), histological stains, and real time polymerase chain reaction (RT-PCR). Cell seeded scaffolds were also subcutaneously implanted into mice for 6 weeks.

2.2. In vitro evaluation of hMSCs cultured on microtextured substrates

2.2.1. Fabrication of microtextured substrates

The microfabricated-patterned mold was prepared using soft lithography techniques as previously described (Fig. 1) [43]. To prepare the PDMS substrate, liquid PDMS base and curing agent (Sylgard 184; Dow Corning) were mixed at a ratio of 10:1, degassed, and poured uniformly on top of the patterned mold. After curing the patterned mold at 85 °C for 2 h, solidified PDMS casts with 10 μm cylindrical posts were released from the mold (Fig. 3a). Patterned PDMS substrates were cut into 4 mm diameter disks, sterilized for 30 min with 70% ethanol, and washed 3 times with phosphate buffered saline (PBS) for subsequent cell culture.

2.2.2. Cell culture

Human MSCs (PT-2501, Lonza, Allendale, NJ) were cultured on microtextured 4 mm disks under standard culture conditions. For the BM condition, hMSCs seeded on microtextured PDMS scaffolds were grown in Dulbecco's modified Eagle's medium (DMEM) low glucose (CCFAA001, UCSF Cell Culture Facility) supplemented with 10% fetal bovine serum (FBS) (Life Technologies), 1% penicillin–streptomycin (P/S) (CCFGK003, UCSF Cell Culture Facility), and 10 ng/mL recombinant human FGF-2 (R&D Systems, Minneapolis, MN) for 6 weeks. For the OM condition, hMSCs seeded on PDMS substrates were first grown under BM for 5 weeks before being exposed to osteogenic media (OM) in DMEM high glucose (CCFAA005, UCSF Cell Culture Facility) supplemented with dexamethasone (10^{-7} M) (D4902, Sigma), L-ascorbic acid 2-phosphate (100 μM) (A8960, Sigma), β-glycerol phosphate (10 mM) (50020, Sigma), sodium pyruvate (100×) (CCFGE001, UCSF Cell Culture Facility), ITS + (BD Biosciences, NJ), and 1% P/S for another week. Seeding density for both conditions was 2×10^6 cells/cm^2.

Mouse preosteoblast cell lines MC3T3 (obtained from R. Franceschi, University of Michigan, Ann Arbor, MI) and calB 2T3 (obtained from S.E. Harris, University of Texas, San Antonio, TX) were cultured on microtextured substrates as positive controls.

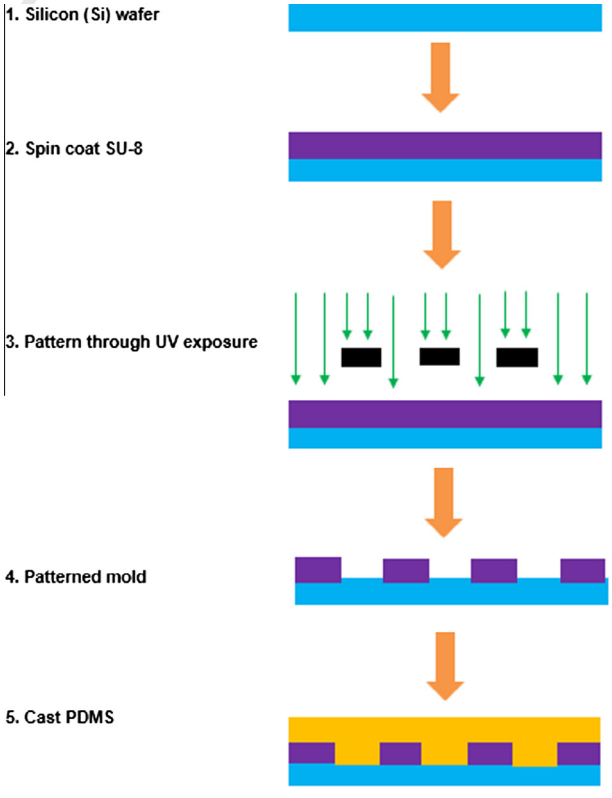

Fig. 1. Fabrication of PDMS post microtextures by soft lithography. Briefly, 10 μm thick SU-8 2010 photoresist was spin coated on top of silicon wafers. The post microtexture pattern with 10 μm inter-space was transferred from a photomask onto the photoresist under UV exposure. The liquid PDMS and curing photocatalyst were mixed at a ratio of 10:1, degassed for 20 min, and then poured uniformly on top of the patterned mold. The PDMS substrates were cured at 85 °C for 2 h.

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