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MECHANICAL REGULATION OF STEM CELL DIFFERENTIATION ON GEOMETRICALLY MODULATED ELASTOMERIC SUBSTRATES

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ABSTRACT

We report the use of micromolded elastomeric micropost arrays to modulate substrate rigidity independently of effects on adhesive and other material surface properties. We demonstrate that micropost rigidity impacts cell morphology, focal adhesions, cytoskeletal contractility, and stem cell differentiation. Furthermore, these micropost arrays reveal that changes in cytoskeletal contractility can precede stem cell differentiation and be utilized as a non-destructive predictor for fate decisions at the single cell level.

INTRODUCTION

Cell function is regulated primarily by extracellular stimuli, including soluble and adhesive factors that bind to cell surface receptors. Recent evidence suggests that mechanical properties of the extracellular matrix (ECM), particularly rigidity, can also mediate cell signaling, proliferation, differentiation, and migration (1, 2). Culturing cells on hydrogels derived from natural ECM proteins, such as fibrin and collagen type I, at different densities has dramatic effects on cell function. However, changing protein concentrations of the gels impacts not only mechanical rigidity, but also the amount of ligand, leaving uncertainty as to the relevant contribution of these two matrix properties on cell function. Synthetic ECM analogs such as polyacrylamide or polyethylene glycol gels, which vary rigidity by modulating the hydrogel cross-linker density, has revealed that substrate rigidity alone can modulate many cellular functions including stem cell differentiation (3, 4). However, altered cross-linker density may affect non-mechanical material properties including porosity, surface chemistry, as well as conformational flexibility and binding of immobilized adhesive ligands. Consequently, whether cells sense a macroscopic change in substrate rigidity or a concomitant change in substrate surface properties still remains an open question. While hydrogels will continue to play a major role in characterizing and controlling cell-material interactions, alternative approaches are necessarily needed to explore the physical basis by which cells sense changes in substrate rigidity.

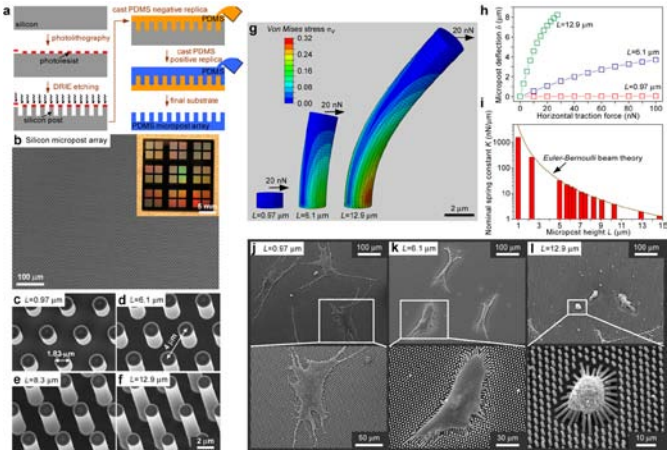

FIGURE 1. MICROMOLDED ELASTOMETIC PDMA MICROPOST ARRAYS TO ENGINEER SUBSTRATE RIGIDITY

Here we have established a suite of elastomeric micropost array substrates to decouple substrate rigidity from adhesive and surface properties. Our strategy involves replica-molded arrays of hexagonally spaced poly-(dimethylsiloxane) (PDMS) microposts from microfabricated silicon micropost array masters, which present the same surface geometry but different post heights to control substrate rigidity (Fig. 1). Conventional high-resolution photolithography and deep reactive ion-etching techniques were used to generate the silicon micropost arrays with a post diameter d of 1.83 μm and post heights L ranging from 0.97 to 14.7 μm. After replica-molding, the PDMS micropost arrays were rendered adhesive by microcontact printing of fibronectin across the top of the microposts. Micropost rigidity was characterized by computing the nominal spring constant, K, using the finite element method (FEM), where K is obtained by calculating the micropost deflection δ in response to a horizontal force F applied uniformly on the top of the micropost ($K=dF/d\delta$ ($\delta \rightarrow 0$)) (Fig. 1). The PDMS micropost arrays used here span a more than 1,000-fold range of rigidity from 1.31 nN/μm (L=14.7 μm) up to 1,556 nN/μm

(L=0.97 μm), and can therefore establish an efficient test bed to examine the rigidity-dependent cell function.

RESULTS AND DISCUSSION

Human mesenchymal stem cells (hMSCs) have previously been shown to respond to mechanical cues both *in vivo* and *in vitro* (4). We tested whether hMSCs would respond to rigidity changes in our micromolded PDMS micropost arrays. hMSCs sparsely plated on these micropost arrays exhibited marked differences in their degrees of attachment and spreading (Fig. 1). On the rigid microposts, hMSCs were well-spread with prominent and highly organized actin stress fibers and large focal adhesions (FAs). In contrast, cells on the soft microposts displayed a rounded morphology, poorly organized actin filaments, and small, punctate adhesion complexes. Quantitative morphometric analysis of cell populations on these substrates revealed strong correlations between FAs and cell spreading, regardless of micropost rigidity (data not shown). We also observed a strong correlation between traction force and cell spreading, as well as a small independent effect of micropost rigidity on traction force, regardless of cell spreading (data not shown). Overall, these observations suggest that cell shape, FA structures, and cytoskeletal (CSK) tension are tightly coupled systems involved in rigidity sensing, independently of nanoscale changes in adhesive and surface properties.

To investigate whether micropost rigidity could also regulate the lineage commitment and differentiation of these stem cells, hMSCs were plated on micropost arrays with three different post heights L, and were exposed to either a basal growth medium (GM) or a bipotential differentiation medium (MM) supportive of both osteogenic and adipogenic fates (Fig. 2). hMSCs cultured in GM failed to express differentiation markers at any micropost rigidity (data not shown). In contrast, following a two week induction in MM, we observed substantial osteogenic and adipogenic differentiation on the micropost arrays, as indicated by alkaline phosphatase activity (ALP, blue) and formation of lipid droplets (Lip, red), respectively (Fig. 2). Importantly, micropost rigidity shifted the balance of hMSC fates: osteogenic lineage was favored on rigid micropost arrays whereas adipogenic differentiation was enhanced on soft ones.

To further confirm these histological studies, we assayed rigidity-driven hMSC differentiation using quantitative real-time PCR (qRT-PCR) to detect changes in gene expression of osteogenic (ALP, bone sialoprotein (BSP), and frizzled B (FrzB)) and adipogenic (CCAAT-enhancer-binding protein alpha (CEBPα), lipoprotein lipase (LPL), and peroxisome proliferator-activated receptor gamma (PPARγ)) markers. Consistent with the histological data, ALP, BSP, and FrzB were all highly induced on the rigid micropost arrays, while CEBPα, LPL, and PPARγ were all upregulated on the soft ones (Fig. 3). Thus, rigidity of the micropost arrays can serve to switch hMSCs between osteogenic and adipogenic lineages.

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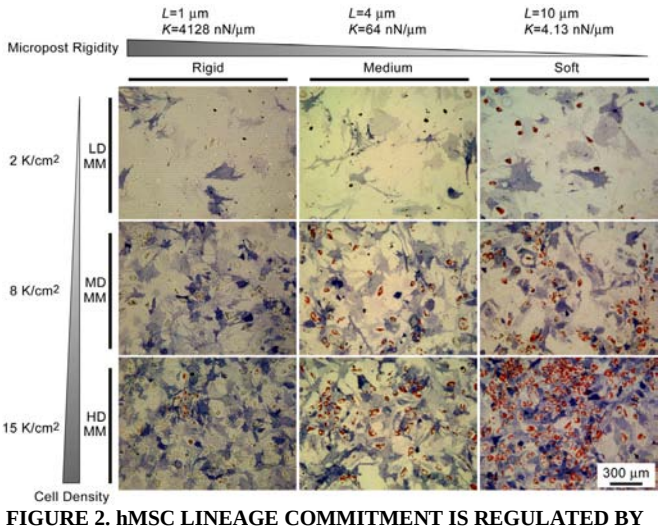

FIGURE 2. hMSC LINEAGE COMMITMENT IS REGULATED BY MATRIX RIGIDITY

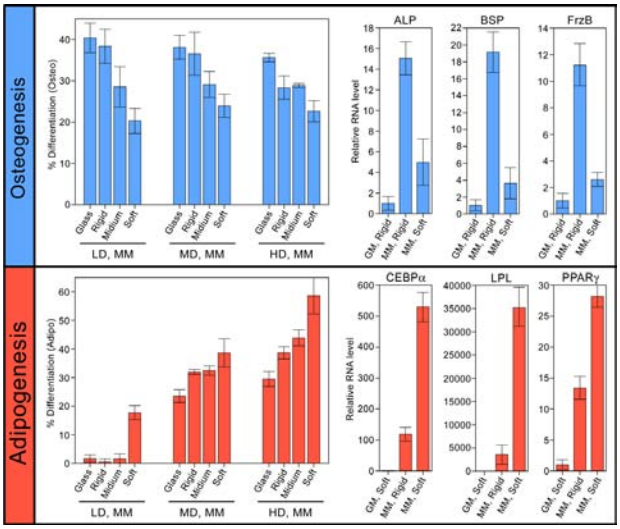

FIGURE 3. hMSC GENE EXPRESSION IS REGULATED BY MATRIX RIGIDITY