

MECHANICAL CONTROL OF STEM CELL DIFFERENTIATION USING MICRO-ENGINEERED MATRIX

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ABSTRACT

In this work, we explore the molecular mechanisms by which local mechanical properties (*e.g.*, rigidity) of the extracellular matrix (ECM) cooperates with soluble cues to regulate lineage commitment of human mesenchymal stem cells (hMSCs). We have established different micropost array substrates that can definitively decouple matrix rigidity from other properties including adhesiveness. We applied these substrates to investigate the influences of matrix rigidity on cell adhesion, cytoskeleton assembly/contractility, cell spreading, and proliferation. We further show that matrix rigidity regulates commitment of hMSCs to either adipogenic or osteogenic fate: soft matrix facilitates adipogenic differentiation while stiff matrix proves osteogenic.

KEYWORDS: Stem cell differentiation, Matrix rigidity, Mechanobiology, MEMS

INTRODUCTION

Cell function is classically thought to be regulated by extracellular stimuli primarily in the form of soluble factors or adhesive molecules that bind to cell surface receptors. Recently, there have been increasing lines of evidence suggesting that mechanical properties (*e.g.*, rigidity) of the ECM to which a cell adheres can also mediate cell function including proliferation, differentiation, and migration [1,2]. In the past, different methods had been used to modulate ECM rigidity, from varying concentrations of ECM to varying cross-linking density of synthetic gels such as polyacrylamide [1,2]. However, varying ECM concentrations changes not only mechanical rigidity but also ECM ligand density, leading to a varied adhesiveness. In synthetic gel substrates, varying rigidity also alters surface porosity and hydrophilicity, making it difficult to attribute a role for mechanical rigidity in regulating cell behaviour.

In this work, by using semiconductor microfabrication techniques, we have established a standard suite of micropost array substrates that can definitively decouple substrate rigidity from other properties including adhesiveness. Using this approach, spatially defined regions of desired rigidity can be patterned with unprecedented precision. Our strategy involves replica-molding of arrays of poly-(dimethylsiloxane) (PDMS) posts from microfabricated silicon post arrays (Fig. 1). Since the rigidity of PDMS microposts is solely determined by their geometry and by the Young's modulus of PDMS, the rigidity of the PDMS micropost substrate can be altered simply by varying post height L while keeping all other aspects of the substrate such as surface chemistry and ligand coating density unchanged. The mi-

croprost array substrate can therefore establish an efficient test bed to examine the rigidity-dependent cell function.

EXPERIMENTAL RESULTS

Figure 1 shows the design and fabrication of the micropost array substrates. Silicon post arrays with a post diameter d of 1-3 μm and a post height L of 1-14 μm were fabricated (Fig. 1B-F). PDMS micropost arrays replicated from these silicon substrates span a 40,000-fold range of rigidity from 0.12 to 5000 $\text{nN}/\mu\text{m}$ (Fig. 1G). These micropost substrates are utilized to investigate the influences of matrix rigidity on hMSC morphology, cytoskeletal (CSK) structure, focal adhesion, and traction force (Fig. 2). By immunofluorescence staining of the focal adhesion protein vinculin and the stress fiber protein actin, we show that both the actin stress fibers and the mature focal adhesions are primarily regulated by cell spread area, even though the hMSCs tend to increase their spread area with increasing micropost rigidity (Fig. 2B). We have also monitored simultaneously the effects of cell spread area and micropost rigidity on the CSK contractility of hMSCs [3]. Our experimental data show that the cell traction forces are closely correlated with the cell spread area, total fluorescence intensity and area of the focal adhesions, and matrix rigidity, indicating a strong functional role of mechanical forces and cell shape in the regulation of cell adhesion and cytoskeletal organization.

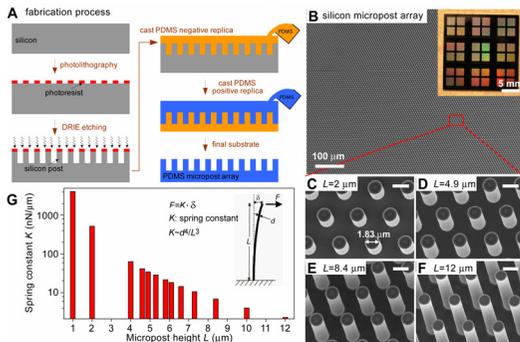


Figure 1. Microfabricated post array structures as ECM analogs. (A) Fabrication process of the micropost array. (B-F) Scanning electron microscopy (SEM) images of silicon micropost arrays of different heights. (G) Calculated spring constant K of the PDMS microposts as a function of post height L .

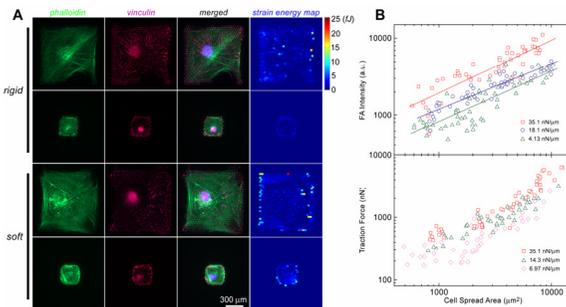


Figure 2. (A) Effects of micropost stiffness on stem cell morphology, cytoskeletal structure, adhesion, and traction force. (B) Correlations between total focal adhesion intensity (top), traction force (bottom) and cell spread area.

In Fig. 3, we show that matrix rigidity play an important role in the mechanical control of stem cell differentiation. The micropost arrays regulate commitment of hMSCs to either adipogenic or osteogenic fate in response to a bipotential differentiation medium that contains soluble inducers for both lineages. However, soft mi-

croprost matrix facilitates adipogenic differentiation while stiff matrix proves osteogenic. Furthermore, this osteogenic-adipogenic switch of hMSCs plated on stiff versus soft matrix is also regulated by cell plating density, indicating that both matrix rigidity and cell shape play important roles in the mechanical control of stem cell differentiation.

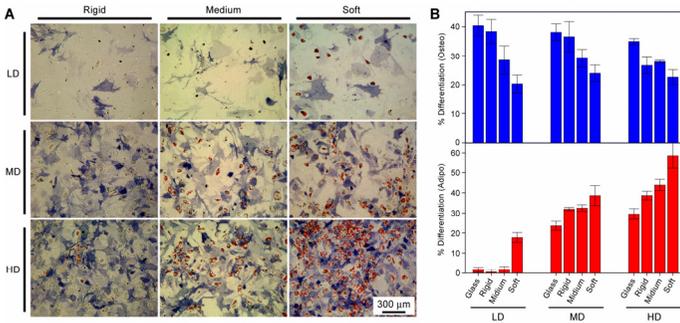


Figure 3. hMSCs plated at different densities on micropost array substrates of different rigidities selectively undergo adipogenic or osteogenic differentiation.

CONCLUSIONS

The molecular mechanisms relying signals from ECM rigidity to cell function is still largely unknown [4,5], which is partially due to a lack of engineering control in the rigidity sensing process by cells. Our novel microfabricated PDMS post array, by decoupling substrate rigidity and adhesiveness, can therefore provide a unique opportunity to study the molecular mechanisms by which local ECM rigidity regulates cell function, and more particularly how the CSK contractility is involved in transducing local ECM rigidity and growth factors collectively into a functional cellular response. Our results here suggest a strong functional interplay between stem cell differentiation, matrix rigidity and soluble factors, and will have great implications for understanding physical effects of the in vivo microenvironments in stem cell lineage specification and also for therapeutic applications of hMSCs.

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