MICROMECHANICAL ELASTOMERIC DEVICES FOR INVESTIGATIONS OF MECHANOBIOLOGY IN HUMAN EMBRYONIC STEM CELLS

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ABSTRACT

Human embryonic stem cells (hESCs) provided a cell source for biotechnological and clinical applications. Yet, there is limited understanding of how mechanical signals in the microenvironment of hESCs regulate their fate decisions. Here, we applied a microfabricated micromechanical platform to investigate mechanoresponsive behaviors of hESCs. We demonstrated that hESCs are mechanosensitive, and they could increase their cytoskeleton contractility with matrix rigidity. Furthermore, rigid substrates supported maintenance of pluripotency of hESCs. Matrix mechanics-mediated cytoskeleton contractility of hESCs might be functionally correlated with E-cadherin expressions in cell-cell contacts and thus involved in fate decisions of hESCs. Our results provided a novel approach to characterize and understand mechanotransduction in hESC function regulation.

KEYWORDS

Human embryonic stem cells, pluripotency, mechanotransduction, cell mechanics, microfabrication

INTRODUCTION

Experimental evidence established in recent years has shown that mechanical signals experienced by mouse embryonic stem cells (mESCs) through their biophysical interactions with the extracellular matrix (ECM) can play critical roles in regulating survival, proliferation and differentiation [1]. However, there is still limited knowledge of how mechanical signals in the local cellular microenvironment regulate fate decisions of hESCs, and advancing in such knowledge will be critical for both fundamental biological understanding and clinical applications of hESCs.

Recently, our group and others have proposed the use of microfabricated elastomeric poly-dimethylsiloxane (PDMS) micropost arrays to regulate substrate rigidity, independently of effects on adhesive and other material surface properties [2, 3]. Our approach involves a library of replica-molded arrays of hexagonally spaced PDMS microposts from microfabricated silicon masters, which present the same surface geometry but different post heights, to control substrate rigidity. In this study, we proposed to apply the PDMS micropost array to study the mechanosensitivity of hESCs and how matrix mechanics could regulate pluripotency of hESCs.

EXPERIMENTS

The PDMS micropost arrays were fabricated using the protocol described previously [2]. Briefly, silicon micropost array masters were first fabricated by photolithography and deep reactive ion etching (DRIE). Negative PDMS mold was generated by peeling off the PDMS mold from the silicon master. To generate the final PDMS micropost array, 1:10 ratio PDMS prepolymer was poured over the negative PDMS mold and was cured at 110^{o}C for 40 hrs. The top surface of the PDMS micropost array was functionalized with human recombinant vitronectin to promote adhesion of hESCs. hESCs (H9 and H1, obtained from WiCell, Madison, WI) were maintained either on a feeder-free synthetic polymer coating (PMEDSAH) or mouse embryonic fibroblasts (mEFs). Single hESCs were obtained by treating cells with 0.25% Trypsin-EDTA and then seeded on the PDMS micropost array at a desired density.

RESULTS AND DISCUSSION

We first examined simultaneously expressions of Oct4 (a hallmark of stemness) in hESCs and their traction forces (Fig. 1). The evaluation of cytoskeleton contractility indicated a significant difference between Oct^+ and Oct^- cells, as Oct^+ hESCs showed significantly less total traction force per cell (Fig. 1B) and total traction force per cell area (Fig. 1C) as compared to Oct^- cells. Vinculin, a FA protein, was used to examine the functional role of FAs in regulating the mechanosensitivity of hESCs (Fig. 1D). Vinculin-expressing FAs appeared to be concentrated on the cell periphery of Oct^+ hESCs, while for Oct^- cells FAs were randomly distributed across the cell spreading area. Next we investigated whether single hESCs could sense and respond to changes in matrix mechanics and whether matrix rigidity could influence pluripotency of hESCs. Our data showed that matrix rigidity could indeed play a significant role in regulating pluripotency of hESCs, as 24 hrs after cell seeding, more single hESCs plated on the rigid PDMS micropost array remained as undifferentiated Oct^+ cells as compared to the cells on softer microposts (Fig. 2*A*) Oct^- cells also consistently showed greater cytoskeleton contractility than Oct^+ cells, regardless the micropost rigidity. Both Oct^+ and Oct^- cells increased their cytoskeleton contractility with micropost rigidity. We further examined the functional role of nonmuscle myosin II activity on rigidity-dependent self-renewal of hESCs, by using blebbistatin to inhibit myosin II activity. As shown in Fig. 2*D*&*E*, the effect of blebbistatin treatment on hESC self-renewal was time-dependent. Blebbistatin treatment for 24 hrs had no significant effect on pluripotency maintenance of hESCs on the rigid PDMS micropost array.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

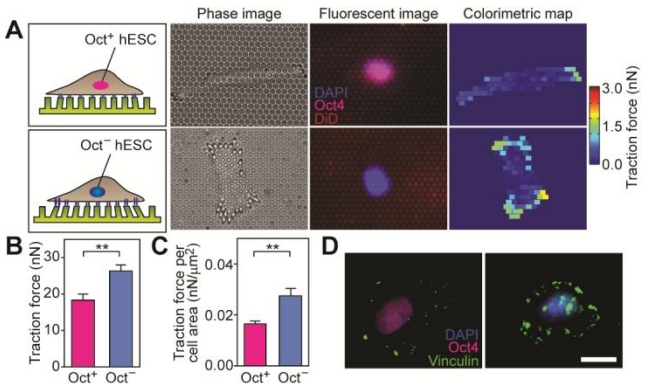

*Figure 1 Differential cytoskeleton contractility and vinculin distribution for single Oct4$^+$ and Oct4$^-$ hESCs. (A) Quantification of subcellular traction forces for single. (B&C) Bar plots of total traction forces per cell (B) and traction force per cell area (C). **, $p < 0.01$. (D) Immunofluorescence images showing vinculin distributions in single hESCs (left: Oct4$^+$; right: Oct4$^-$). Scale bar, 20 μm.*

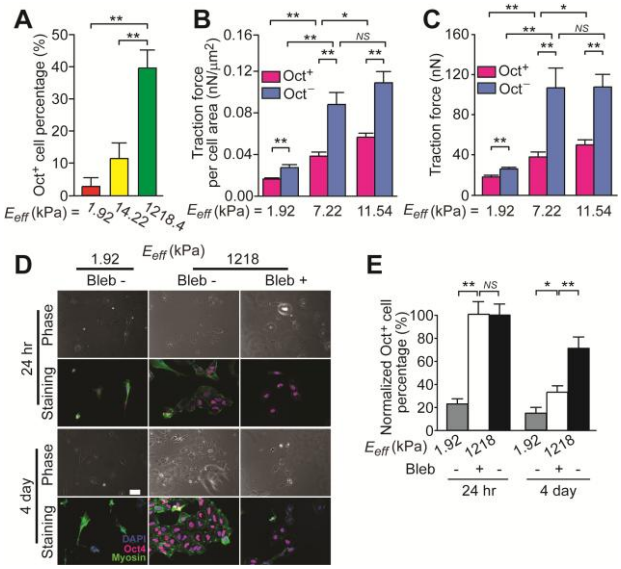

*Figure 2 Matrix mechanics-mediated behaviors of single hESCs on the PDMS micropost arrays of different rigidities. (A) Bar plot of percentage of Oct4$^+$ cells for single hESCs plated on the PDMS micropost arrays of different rigidities. (B&C) Traction force per cell area (B) and total traction forces per cell (C). (D) Phase contrast and immunofluorescence images of hESCs treated with or without blebbistatin on both soft (E_{eff} = 1.92 kPa) and rigid (E_{eff} = 1,218.4 kPa) PDMS micropost arrays. Scale bar, 50 μm. (E) Bar plot of percentage of Oct4$^+$ cells for blebbistatin treated hESCs and untreated controls as a function of the PDMS micropost array rigidity. Data in E was normalized to the value for untreated hESCs plated on the rigid micropost array under the 24-hr treatment condition. *: $p < 0.05$; **: $p < 0.01$; NS: $p > 0.05$.*

However, long-term exposure to blebbistatin resulted in loosely connected single hESCs and a significant decrease in cell density and the percentage of Oct$^+$ cells. Blebbistatin-treated single hESCs on the rigid PDMS micropost array appeared to behave differently from untreated cells on the soft micropost array, under both 24-hr and 4-day treatment conditions with blebbistatin (Fig. *2E*). This observation suggested that although it is known that actomyosin activity can be downregulated for mechanosensitive adherent cells plated on soft substrates, other molecular mechanisms might also exist and work in parallel to transduce the rigidity signal to regulate stem cell fate, such as integrin-mediated adhesion signaling.

We further investigated mechanosensitivity of small aggregates of hESCs and showed that larger aggregates of hESCs had a greater tendency to differentiate on softer micropost arrays while maintaining their Oct4 expressions on more rigid ones (Fig. 3*A*). Figure 3*B* also showed that the average total traction force per cell for hESCs plated on the same PDMS micropost array was not significantly different among single cells, doublets or triplets, for both Oct$^+$ and Oct$^-$ cells, suggesting that cell-cell contact might play an important role in regulating matrix mechanics-mediated hESC pluripotency. We performed E-cadherin inhibition assays and showed that blocking E-cadherin activity in hESCs on both rigid and soft micropost arrays resulted in loosely connected single cells as compared to compact colonies formed by untreated controls (Fig. 4*B*). Percentages of Oct$^+$ cells also decreased significantly for E-cadherin inhibited hESCs as compared to untreated controls, regardless micropost rigidity (Fig. 4*C*). Together, our findings in Fig. 4 supported a possible involvement of E-cadherin in rigidity-dependent self-renewal of hESCs.

To examine whether substrate rigidity could regulate differentiation of hESCs toward specific lineages, we investigated the neural induction of hESCs plated on micropost arrays (Fig. 5). Single hESCs were seeded on PDMS micropost arrays of different rigidities. The cells were cultured in media containing knockout serum replacer, bFGF, and retinoic acid for 7 days before fixed and stained with antibodies against Sox1 (a transcription factor found in neuroepithelial cells) and Islet1/2 (transcription factor found in developing motor neurons). Our results suggested that soft micropost arrays were conductive to and could promote and accelerate neural differentiation of hESCs.

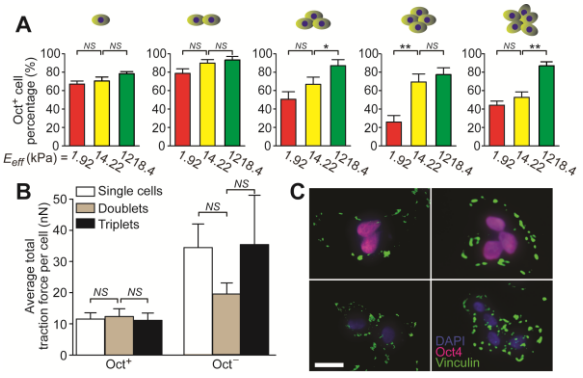

*Figure 3 Matrix mechanics-mediated cellular functions of small aggregates of hESCs on PDMS micropost arrays with different rigidities. (A) Bar plots of percentage of Oct$^+$ cells for clustered hESCs of different sizes as a function of the PDMS micropost rigidity. (B) Plot of average total traction force per cell for both Oct$^+$ and Oct$^-$ cells contained in different sized hESC aggregates. Data in A & B represents the means ± s.e.m from 3 independent experiments. *: $p<0.05$; **: $p<0.01$; NS: $p>0.05$.*
(C) Immunofluorescence images showing FA distributions in Oct$^+$ (top) and Oct$^-$ (bottom) hESC aggregates, as indicated by vinculin staining. Scale bar, 25 μm.

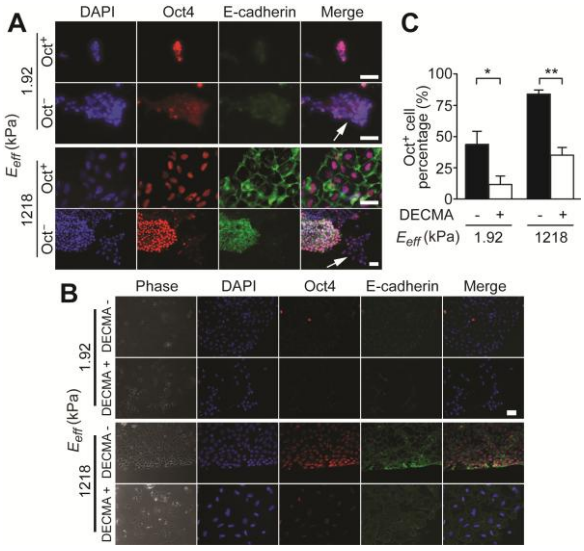

Figure 4 E-cadherin expression of hESCs modulated by substrate rigidity. (A) Immunofluorescence images taken for Oct$^+$ and Oct$^-$ hESC colonies on soft (E_{eff} = 1.92 kPa) and rigid (E_{eff} = 1,218.4 kPa) PDMS micropost arrays, as indicated. Differentiated hESC colonies were marked with an arrow. Scale bars, 50 μm. (B) Phase contrast and immunofluorescence images of hESCs treated with or without DECMA-1 on both soft (E_{eff} = 1.92 kPa) and rigid (E_{eff} = 1,218.4 kPa) PDMS micropost arrays. Scale bar, 50 μm. (C) Bar plot of percentage of Oct$^+$ cells for DECMA-1 treated hESCs and untreated controls as a function of the PDMS micropost rigidity.

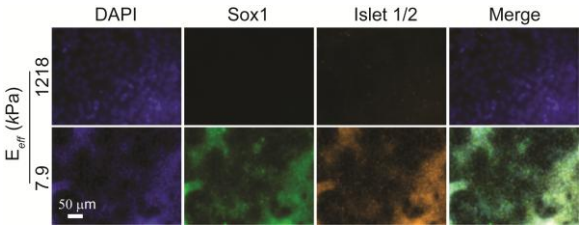

Figure 5 Soft substrates could accelerate neural differentiation of hESCs. Immuno -fluorescence images showing hESC colonies after 7-d neural induction on soft (E_{eff} = 7.9 kPa) and rigid (E_{eff} = 1,218.4 kPa) PDMS micropost arrays.

CONCLUSIONS

Together, our results highlighted the important functional link between matrix rigidity, cellular mechanics, and pluripotency and differentiation of hESCs and provided a novel approach to characterize and understand mechanotransduction and its involvement in hESC function regulation.

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