Biochemical and Biophysical Research Communications 461 (2015) 372–377

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Micropillar displacements by cell traction forces are mechanically correlated with nuclear dynamics

Qingsen Li [a, 1], Ekta Makhija [a, 1], F.M. Hameed [a], G.V. Shivashankar [a, b, *]

[a] Mechanobiology Institute, National University of Singapore, Singapore
[b] Department of Biological Sciences, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 27 March 2015
Available online 22 April 2015

Keywords:
Micropillars
Mechanotransducion
Correlation
Nuclear dynamics
Focal adhesion

ABSTRACT

Cells sense physical cues at the level of focal adhesions and transduce them to the nucleus by biochemical and mechanical pathways. While the molecular intermediates in the mechanical links have been well studied, their dynamic coupling is poorly understood. In this study, fibroblast cells were adhered to micropillar arrays to probe correlations in the physical coupling between focal adhesions and nucleus. For this, we used novel imaging setup to simultaneously visualize micropillar deflections and EGFP labeled chromatin structure at high spatial and temporal resolution. We observed that micropillar deflections, depending on their relative positions, were positively or negatively correlated to nuclear and heterochromatin movements. Our results measuring the time scales between micropillar deflections and nucleus centroid displacement are suggestive of a strong elastic coupling that mediates differential force transmission to the nucleus.

1. Introduction

Eukaryotic cells probe the stiffness of their local microenvironment to modulate gene expression and determine lineage specification [1]. Mechanical cues from the environment such as substrate rigidity [2], geometry [3,4] and force [5] impinge on cellular gene expression via biochemical and mechanical pathways. Towards this, forces are applied on the substrate by focal adhesions using acto-myosin contraction [6] and these substrate signals are then transmitted to the nucleus. While the biochemical pathways have been studied in detail over the past few years, physical links are only recently being explored [7–12].

The physical link from focal adhesions to the nucleus is mediated via cytoskeleton proteins, linkers of nucleoskeleton complex proteins and lamins [13–17]. To understand the viscoelastic nature of this mechanical coupling, various groups including ours [7,10–12] have measured the nuclear displacements and deformations upon perturbations of cytoskeletal and nuclear links or application of extracellular shear and compressive forces. However, the intrinsic time scales of focal adhesion to nucleus coupling, in unperturbed cells are yet unknown.

In this study, we probed the intrinsic coupling between micropillar array deflections induced by traction forces at focal adhesions and the resultant displacements of the nucleus and heterochromatin foci. Microfabricated pillar arrays, when used as substrate for cells, act as cantilever beams and their deflections can be used to calculate magnitude and direction of cell traction forces [18]. The tips of these micropillars have been shown to form focal adhesions [19]. Such micropillar deflections provide a measure for mapping force transduction from focal adhesions to the nucleus.

2. Materials and methods

2.1. Preparation of PDMS micropillars

PDMS micropillars were prepared from PDMS Elastomer Kit (SYLGARD 184, DOW Corning). The curing agent and precursor were mixed homogenously in the ratio 1:10 and then poured onto the micropillar array mould in a silicon wafer followed by curing at 80 °C for 2 h. The micropillars so formed were 2 μm in diameter, 5 μm in height with pillar centre to centre distance of 3 μm as confirmed by electron microscopy imaging.

* Corresponding author. Mechanobiology Institute, National University of Singapore, Singapore.
E-mail address: shiva.gvs@gmail.com (G.V. Shivashankar).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.04.041
0006-291X/

2.2. Stamping of micropillars with fibronectin

30 μl of 100 μg/ml fibronectin solution was deposited on a flat PDMS block substrate for 10 min. The solution was then removed and the block dried for 5 min. The stamp was then inverted over the UV treated micropillars for 5 min. The stamp was then removed and the PDMS micropillars washed with PBS and further treated with 1 ml of 2 mg/ml Pluronic F-127 (Sigma) for 2 h to passivate non-fibronectin coated regions

2.3. Cell culture, transfection and seeding

NIH3T3 cells were cultured in low glucose DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin. Transfections were carried out with fusion plasmid for core histone H2B tagged with EGFP and lifeact mRFP using Jetprime transfection reagent (Polyplus). All cell culture reagents were from GIBCO Invitrogen. Cells were seeded on fibronectin coated PDMS micropillar arrays and allowed to spread for 12 h before being inverted over coverslip dishes for imaging.

2.4. Live imaging

All images were captured using inverted confocal microscope (Perkin Elmer Spinning Disk, 60X, 1.2 NA objective) at 1 frame every 3–7 s for at least 3 min. The PDMS micropillars were placed upside down on glass bottom petridish (Ibidi) with a 60 μm thick PDMS membrane placed at the edges of PDMS pillar block as spacer.

2.5. Pillar deflection and nuclear movement measurements

The deflections of the pillar tips and the nucleus and the heterochromatin foci were calculated using custom code written in MATLAB. The tips of the pillars show up as bright spots in the bright field images. To calculate the position of the pillar tips, bright field pillar images were thresholded and centroid was calculated for each time point. The images were corrected for XY drift using the mean displacement of pillars not in contact with the cell. A square lattice was generated by calculating the distance between adjacent pillars from the control region. The lattice was then best fit to the cell region so as to minimize the deflection of pillars outside the cell. The centroid of the nuclei was obtained by thresholding for H2B-EGFP and calculating the centroid in ImageJ. The centroids of the heterochromatin were obtained by utilizing the same method after cropping out everything but the heterochromatin foci.

2.6. Correlation analysis

The time series were analyzed for autocorrelation and cross correlation using custom written code in MATLAB. The autocorrelations of the displacement (magnitude) of the pillar, the nucleus, and heterochromatin foci were calculated and plotted to arrive at their typical timescales. Further, the cross correlations between displacements (component along direction of nucleus movement) of individual micropillars at different regions under the cell were plotted with respect to the displacements (component along direction of nucleus movement) of nucleus and heterochromatin foci.

3. Results

3.1. Micropillar deflections map spatial distribution of cell matrix interactions

NIH3T3 fibroblast cells stably expressing H2B-EGFP were allowed to spread for 12 h on force sensitive micropillar arrays.

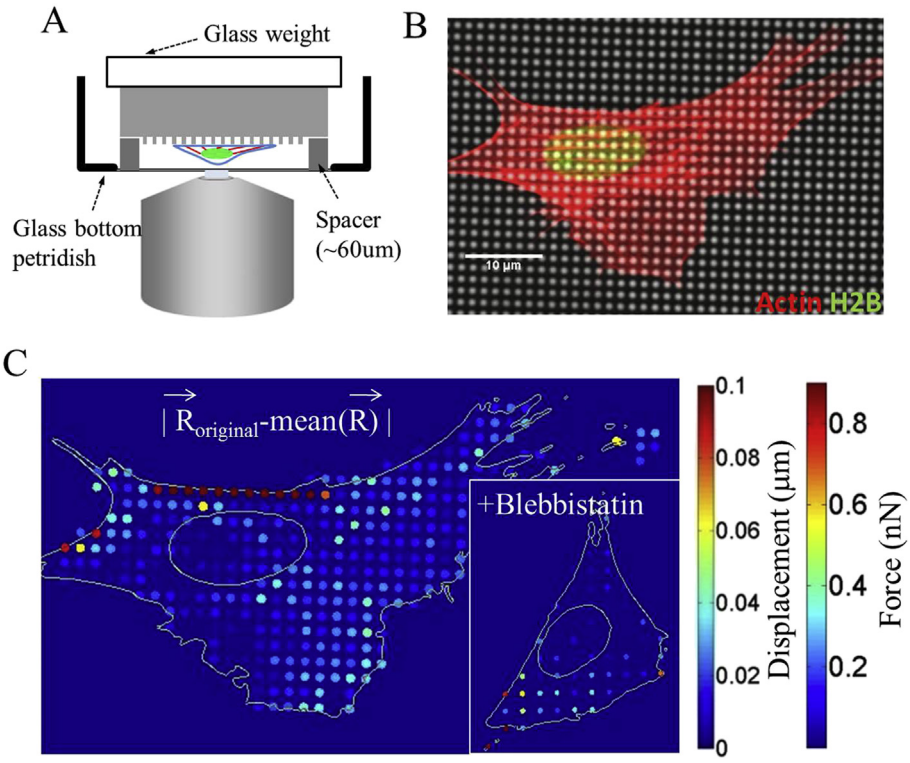

Fig. 1. Experimental setup. A) Inverted imaging configuration B) Image of pillars, H2B-EGFP nucleus and lifeact-RFP actin C) Color coded pillar deflections and corresponding force magnitude. Inset shows similar results for cell treated with blebbistatin.