Effect of Focal Adhesion Spatial Distribution on Cell-Substrate Adhesion Strength

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Statement of Purpose: Cell adhesion to extracellular matrix is a complex process involving integrin activation, mechanical coupling to extracellular ligands and subsequent interaction with the actin cytoskeleton via focal adhesion (FA) assembly [1]. Because the biochemical and biophysical processes in the FA complex are tightly coupled, mechanical analyses of the adhesion strength provide critical information on structure-function relationships that are critical to manipulating adhesive interactions in biomaterial and tissue engineering applications. Previously, a model for integrin mediated strengthening was developed by considering the contributions of adhesive area, integrin recruitment and focal adhesion assembly [2-3]. In this study, the adhesive areas of individual cells were engineered to provide further insight into the mechanisms regulating adhesion strength by elucidating the contribution of FA spatial distribution to adhesion strength.

Methods: Microcontact printing was employed to pattern substrates with adhesive domains surrounded by non-adhesive regions as described elsewhere [3]. To facilitate pattern transfer from PDMS stamps with low fill factors, a collapse barrier consisting of an annular column of lateral thickness of 250 microns was embedded at the stamp periphery. This ensured contact front propagation in the plane of the features and facilitated precise pattern transfer without roof collapse (Figure 1).

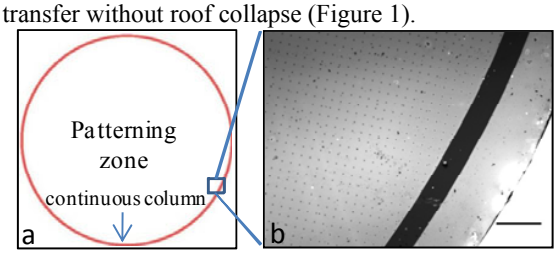

Figure 1: (a) Schematic diagram of PDMS stamp showing the position of annular column circumscribing the patterning region. (b) Bright field image of etched gold substrate after patterning.

Three geometries for cell adhesive area were chosen to dissect the contribution of adhesive area and FA position to adhesion strength (Figure 2a). Immuno-fluorescence microscopy showed that fibronectin (FN) localized to micropatterned areas (Figure 2b). NIH3T3 fibroblasts seeded on patterned substrates in the presence of serum individually adhered to the FN adhesive islands and maintained a nearly spherical shape, while the cell-substrate contact and FA assembly conformed to the pattern dimensions. A spinning disk device that applies a range of hydrodynamic forces to adherent cells was used to quantify steady-state cell adhesion strength on micropatterned surfaces [3].

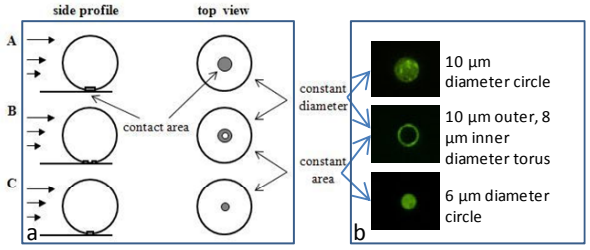

Figure 2: (a) Schematic diagram showing the adhesive area geometry and attached cell shape. (b) Images of immunostained FN localized to micropatterned islands.

Results: Adhesion strength measurements (τ_{50}) were taken at steady state (16 h) after seeding cells on micropatterned islands. Contrary to the expected result, $(\tau_{50})_{torus}$ was similar to $(\tau_{50})_{6\mu m}$. This value is 30% lower than $(\tau_{50})_{10\mu m}$. Interestingly, $(\tau_{50})_{torus}$ was also similar to the adhesion strength when the actin-myosin contractility of the cells on 10 µm circular islands was inhibited either due to serum starvation or by using pharmacological actin-myosin contractility inhibitors (Y-27632) (Figure 3). These results in concert with ongoing measurements of integrin binding and FA assembly will explain the role of FA position in adhesion strengthening.

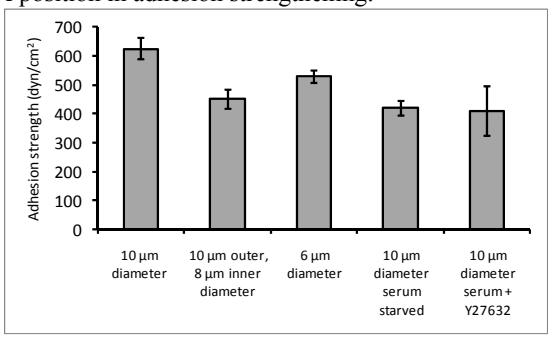

Figure 3: Mean adhesion strength for micropatterned cells and actin-myosin contractility regulators.

Conclusions: In experiments designed to independently determine the effects of FA position and total adhesive area, adhesion strength was observed to be strongly modulated by total adhesive area. However, it is important to note that by varying the geometry of adhesive area, cytoskeletal architecture is inherently modified. Hence current studies focused on dissecting the contribution of cytoskeletal architecture to adhesion strength will illuminate cell adhesion mechanisms that can be exploited in biomaterial and tissue engineering applications.

References: [1] Geiger, B. Nat. Rev. Mol. Cell Biol. 2001; 2: 793-805.
[2] Gallant, N. D. J. Biomech. 2007; 40: 1301-1309.
[3] Gallant, N. D. Mol. Biol. Cell 2005; 16: 4329-4340.