(68g) VE-Cadherin Signals and Substrate Stiffness Regulate Force Transduction through Endothelial Monolayers

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The vascular endothelium forms a semi-permeable barrier, restricting the transfer of proinflammatory cytokines and endothelial hormones within body fluids to underlying tissues. Vascular endothelial cadherin (VE-cad) complexes are the main cohesion and force transducing proteins at endothelial adherens junctions, connecting cytoskeletons to form an

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integrated dynamic mechanical network, regulating endothelial function and permeability. Past studies showed that localized forces on VE-cad receptors trigger changes in global endothelial mechanics when cells were cultured on glass [Barry, A.K. et al., J. Cell Sci. 2015, 128, e0705]. The signals further propagated through the monolayer to affect distant cells. In this study, we used traction force and monolayer stress microscopy to investigate the impact of matrix stiffness cadherin-activated cell responses and the long-range propagation of mechanically-activated signals.

Polyacrylamide gels (8.8 kPa and 40 kPa) with embedded fluorescent fiducial markers were prepared as described [Muhamed, I.,Â et al.,Â J. Cell Sci.Â 2016]. Cured gels were dried overnight and micro-spotted with fibronectin, to create circular 500 Âµm diameter patterns. Gels were then re-hydrated, UV-sterilized, and seeded with human pulmonary artery endothelial cells (HPAECs) for 24 hr, to yield a confined, confluent monolayer with 200-280 cells/array of 10 Âµm thickness. Next, carboxyl ferromagnetic particles functionalized with VE-cadherin were placed on the apical cell surface, and a twisting torque was applied to VE-cad receptors with a magnetic twisting cytometer (MTC) [Wang, N.Â et al.,Â ScienceÂ 1993,Â 260, 1124â??1127]. Time lapse images of the fiducial markers in the gels recorded during MTC experiments were post-processed to

calculate cell-matrix (traction) and cell-cell (monolayer) stresses. Tractions were calculated using a custom-written software and a Fourier transform algorithm. Monolayer stresses were computed by finite element analysis [Tambe, D. T.,Â et al.,Â Nat. Mater.Â 2011,Â 10, 469â??475].

We first assessed monolayer tractions (Fig. 1a). On soft substrates (8.8 kPa), the population averaged tractions of unperturbed (-) and perturbed (+) monolayers were constant over the course of the experiment, as opposed to behavior on stiff substrates (40 kPa). The same trend was observed for monolayer stresses. Interestingly, computed stresses on perturbed monolayers on both substrate stiffness were initially higher than on unperturbed monolayers (Fig. 1b, c, and d). Additionally, on stiff substrates and perturbed cells, the monolayer stress distribution exhibited a rapid reduction in stress magnitudes with respect to unperturbed monolayers, due to the formation of endothelial gaps and loss of interendothelial connectivity. We consider how the substrate stiffness affects long-ranged monolayer disruption activated by VE-cadherin force transduction.

Our study indicates that basal (initial) traction and monolayer stresses anti-

correlate and that external mechanical stimuli via VE-caderin shifts traction stresses from focal adhesions to adherens junctions. Collectively our data demonstrate how matrix stiffness and interendothelial stresses coordinate to regulate the collective integrity of endothelial monolayers.

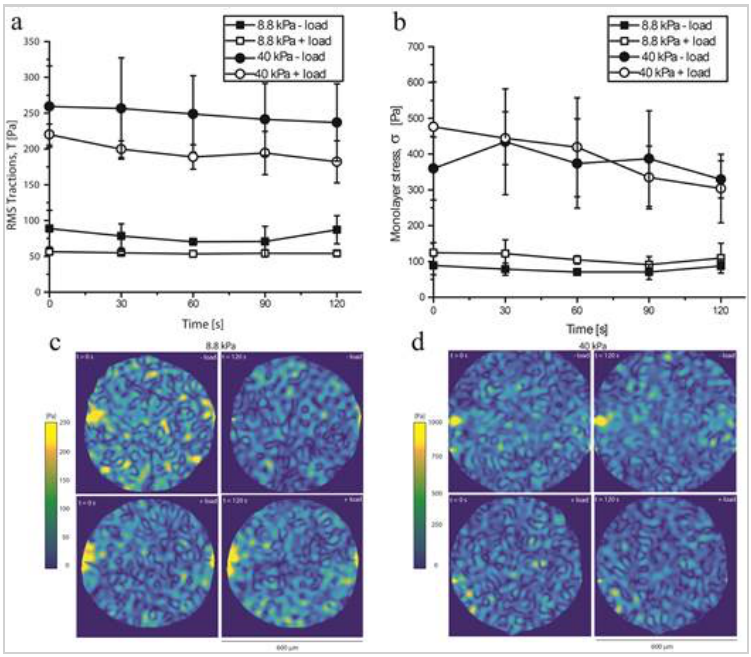

Fig. 1.a) time resolved (TR) RMS traction, b) TR magnitude stress distribution, and c) and d) initial and final heat maps displaying stress distribution at time points 0s and 120s for 8.8 kPa and 40 kPa -/+ load.