Supplemental Materials to: Substrate Viscosity Enhances Correlation in Epithelial Sheet Movement

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List of Figures

Supplemental Figure S1: *Along with the bulk rheology of the PDMS, the hydrophobicity of the surface of the PDMS changes. The hydrophobicity is important, as it effects how the adhesive protein - the fibronectin and collagen (ECM) binds to the PDMS. From our contact angle measurements, we see that there is essentially no difference in hydrophobicity between ζ=0.1 to 0.067. However, when, $\zeta = 0.0125$, the surface of the PDMS becomes wetting, as the contact angle drops from approximately 90° to 55°. We presume that much of the 'remodeling' of the ECM on PDMS with $\zeta \leq 0.0125$ is due to this effect. That is, that the remodeling of the protein on the surface is due to the ability for the cell to mechanically remove some of the protein from the surface, due to the decreased hydrophobicity. However, as can be seen in Fig 6, and Fig S2, there is still very tight coupling between adhesion and contraction of the surface, and deformation of the substrate.*

Droplets of deionized water (11 μl) on PDMS of ζ equal to: (A) 0.0167, (B) 0.0125, and (C) 0.0063. (D) Mean contact angle as a function of crosslinker content, ζ. Error bars are standard deviation.

Supplemental Figure S2: *Epithelial cells do not grow from low density to form confluent and stable sheets on viscous substrates. We therefore seed them at a very high initial cell density. The cells then adhere to the ECM and to each other simultaneously.*

(A) Brightfield image of cells during the initial stages of spreading and sheet formation. Scale bar is 100 μm. (B) Fluorescence image of the FITC collagen bound to fibronectin on the surface during the initial stages of spreading. Scale bar is 100 μm. (C) FITC collagen 50 minutes later shows significant remodeling. Scale bar is 100 μm. (D) PIV of the movement of the ECM boxed in red from (B) and (C). The vector field shows an inward flow of fluorescence intensity during the contraction of the collagen during the initial stages of spreading. (E) The vector field without the overlayed image of the ECM. (D) The MSD of the beads embeded within the region outlined in black in (B) and (C). The rise in the MSD is indicative of the buildup of stress that occurs during the formation of the epithelial sheet on a highly compliant substrate.

Supplemental Figure S3: *On highly viscoelastic substrates, there can be 'instabilities' that form in the epithelial sheet. These include what appear*

to be 'tension' induced holes in the sheet, which can grow or shrink during cell movement or aggregation. When cell-cell E-cadherin is blocked, these instabilities do not exist. Likewise, the correlation in movement is lessened, as is the overall contraction of the surface.

(A) Phase contrast image of epithelial cells on a substrate in Regime VE. (B) Phase Contrast Image of openings in the epithelial sheet that can occur when seeded on a substrate of this stiffness. (C) Fluorescence image of the E-cadherin at the cell-cell junctions. It shows that there is strong E-cadherin throughout the sheet, except at certain points along the periphery of the opening. (D) Fluorescence image of the nuclear stain of cells that are seeded with 100 μg/mL antibody against cell E-cadherin. (E) Vector Field of the displacement of cells in (D) that have had their E-cadherin blocked by antibody. Vector Fields are calculated over an interval of 25 min. (E) Vector Field of the displacement of the beads embedded in the PDMS within the same region as in (D) and (E). (G) Zoom of the region (a) outlined in (E) and (F). It shows the similarity between the displacement of cells (black) and the displacement of the embedded beads (red). In contrast, there are regions where the displacements are orthogonal with respect to each other, as they are in the outlined region (b). (H) The MSD for both cells (black) and beads (red) over all time.

Supplemental Figure S4: *Illustration of the Radial Distribution Function, $g(r)$*

(A) An example of how the nuclei are identified by their peak fluorescent intensities. Shown is a fluorescent image of the stained nuclei of an epithelial sheet bound to a viscous substrate. Imaris (Bitplane) software was used to identify the peak intensities of the nuclei, which are shown as red spots. (B) The location of the peak intensities are used as the coordinates for the radial distribution function $g(r)$, which measures the density of cells as a function of r the distance from a reference cell. This reference cell is alternated to all cells in the field of view, and the ensemble average is taken. Shown is the subsection of cells that are outlined in a white square in (A). (C) Example of the $g(r)$ of the monte carlo simulation shown in (D). The red dots are meant to represent the cell nuclei. The system is crystalline, and thus shows very sharp peaks at all of the nearest neighbor distances. Scale bar is 100 μm. (E) Example of the $g(r)$ of the simulation shown in (F). The system is 'liquid-like', and thus there is correlation only for the nearest and next

nearest neighbors. Scale bar is 100 μm.

Supplemental Figure S5: *There are limitations to using PDMS as a cell substrate. In particular, the non-specific adsorption of fibronectin is not stable long-term. At long times, there is either degradation of the ECM, or is mechanically removed by the cells.*

(A) Brightfield image of epithelial cells attached to fibronectin bound non-specifically to PDMS in Regime E at T=0. This is the same sample as that used in Fig 6A. (B) Fluorescence image of FITC collagen at T=0. (C) Brightfield image of cells after approximately 12 hours. (D) Fluorescence image of the FITC collagen for the same region shown in (C). Scale bar is 100 μm. The ECM at the periphery has been separated from the surface of the PDMS, and the cell sheet is peeling off the dish.

Movie Captions

Movie S1: Cell Migration on PDMS of $\zeta = 0.1$. (left) SYTO nuclear stain of cells moving on $\zeta = 0.1$ PDMS (25x). (right) Substrate Displacement for PDMS of $\zeta = 0.1$ with 0.1μm beads embedded. Scale bar is 100 μm.

Movie S2: Cell Migration on PDMS of $\zeta = 0.0125$. (left) SYTO nuclear stain of cells moving on $\zeta = 0.0125$ PDMS (25x). (right) Substrate Displacement for PDMS of $\zeta = 0.0125$ with 0.1μm beads embedded. Scale bar is 100 μm.

Movie S3a: Cell "Migration" on PDMS of $\zeta = 0.0063$ (low magnification). (left) SYTO nuclear stain of cells moving on $\zeta = 0.0063$ PDMS (0.5x). (right) Substrate Displacement for PDMS of $\zeta = 0.0063$ (low magnification) with 0.1μm beads embedded. Scale bar is 500 μm.

Movie S3b: Cell "Migration" on PDMS of $\zeta = 0.0063$. (left) SYTO nuclear stain of cells moving on $\zeta = 0.0063$ PDMS (25x). (right) The same move on the left, after removing the drift. Scale bar is 100 μm.

Movie S4: Surface Traction on PDMS of $\zeta = 0.1$. (left) FITC collagen stain of surface fibronectin for cells moving on $\zeta = 0.1$ PDMS (25x). (right) Substrate Displacement for PDMS of $\zeta = 0.1$ with 0.1μm beads embedded. Scale bar is 100 μm.

Movie S5: Surface Traction on PDMS of $\zeta = 0.0125(+)$. (left) SYTO nuclear stain of cells moving on $\zeta = 0.0125(+)$PDMS (25x). (right) Substrate Displacement for PDMS of $\zeta = 0.0125(+)$ with 0.1μm beads embedded. Scale bar is 100 μm.

Movie S6: Surface Traction on PDMS of $\zeta = 0.0125(-)$. (left) SYTO nuclear stain of cells moving on $\zeta = 0.0125(-)$PDMS (25x). (right) Substrate Displacement for PDMS of $\zeta = 0.0125(-)$ with 0.1μm beads em-

bedded. Scale bar is 100 μm.

Movie S7: Cells Migrating with Blocked E-Cadherin. (left) SYTO nuclear stain of cells moving on $\zeta = 0.0125$ PDMS (25x). 100 μg/mL anti E-cadherin was added at seeding. (right) Substrate Displacement for PDMS of $\zeta = 0.0125$ with 0.1μm beads embedded. Scale bar is 100 μm.

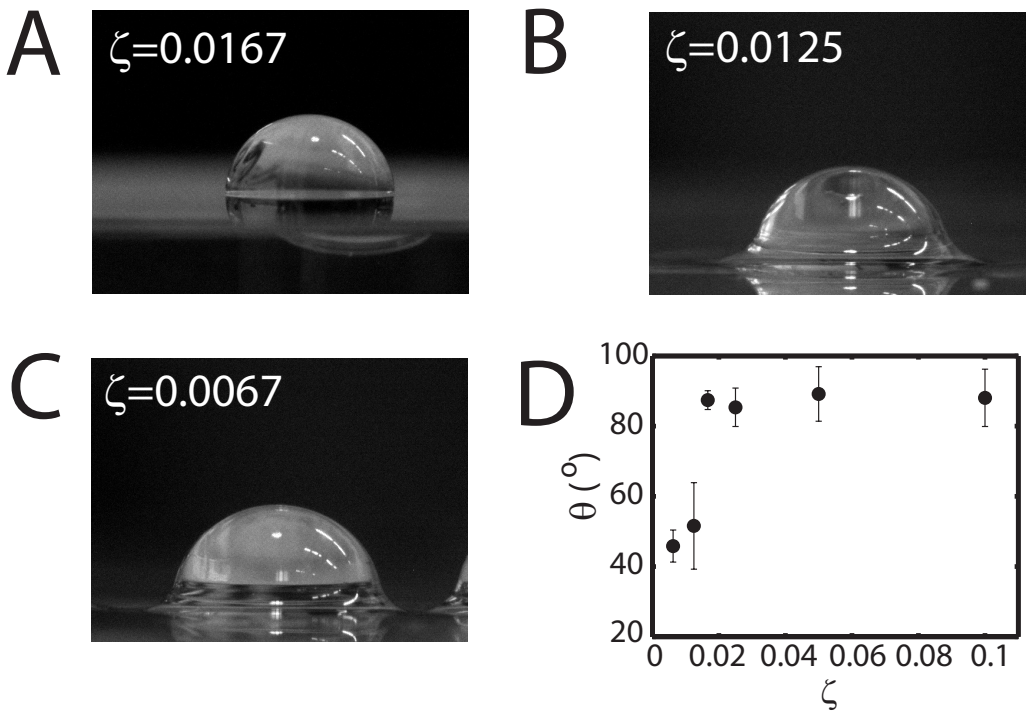

Figure S1: **Surface Wettability of PDMS is greater for low curing agent content**

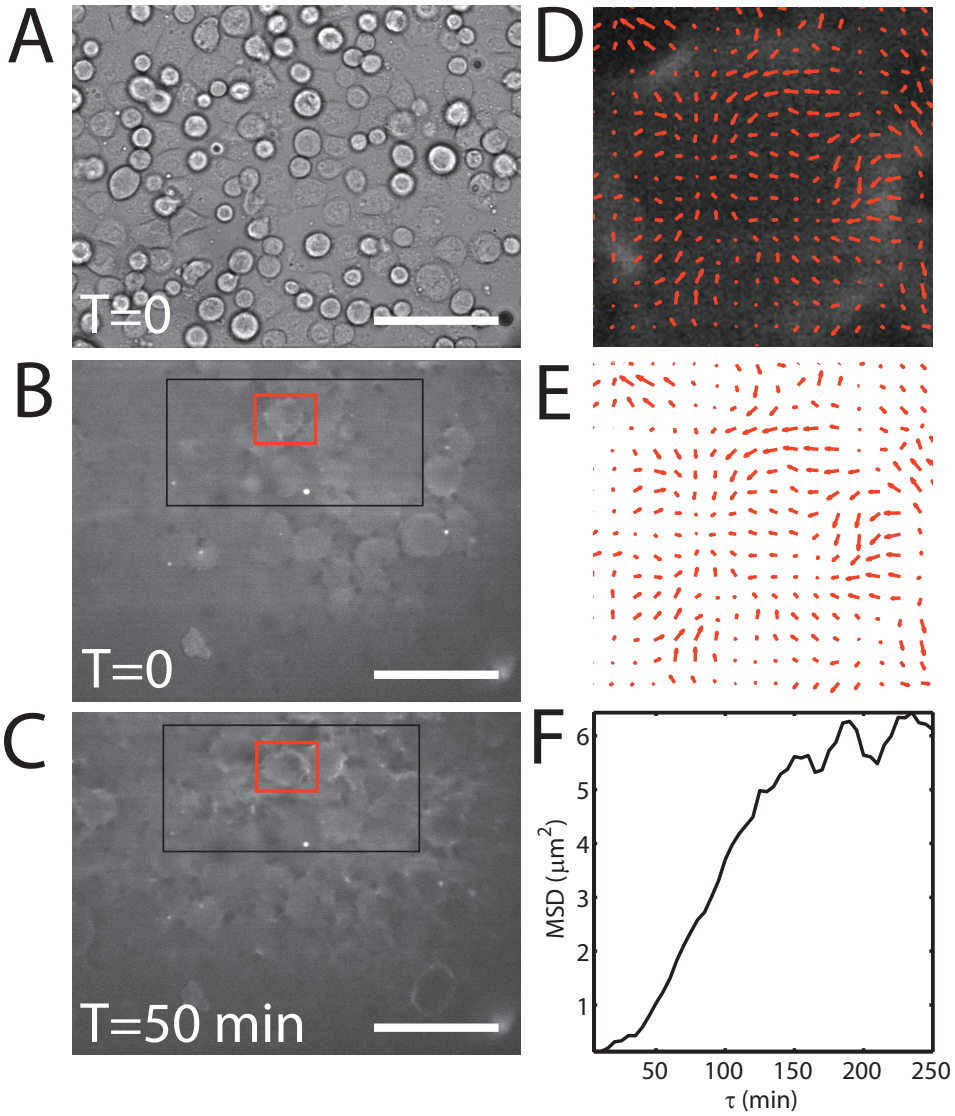

Figure S2: **Epithelial Sheets Can Form on Highly Viscoelastic Substrates**

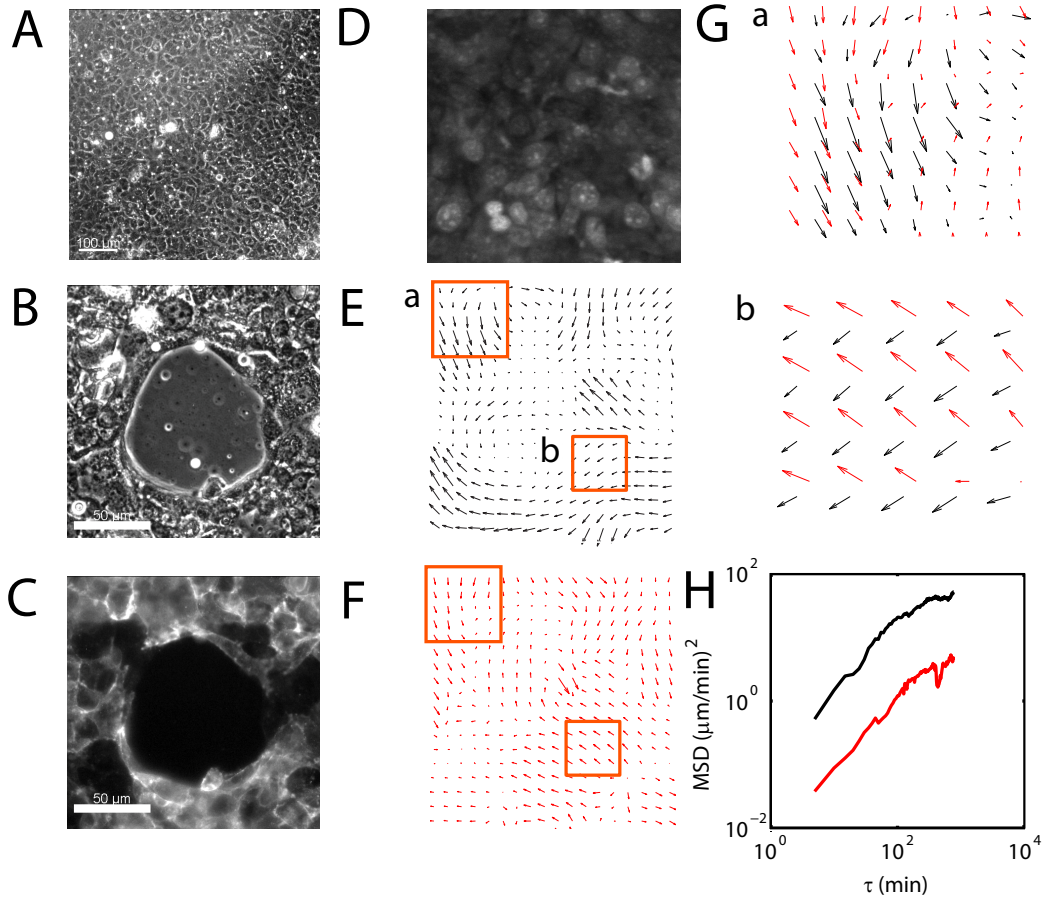

Figure S3: **The Migration of an Epithelial Sheet with Blocked Antibody in Regime VE**

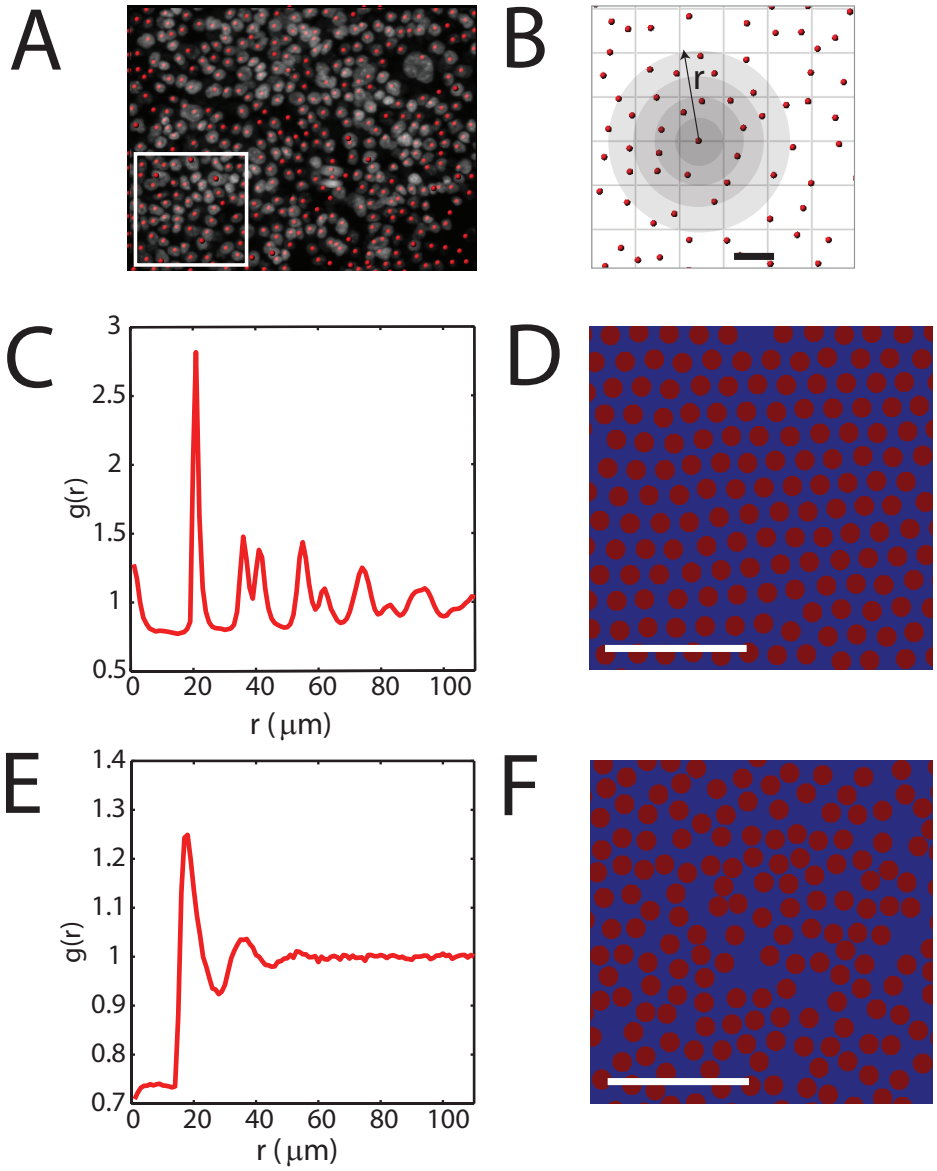

Figure S4: **Radial Distribution Function and Cell Positional Order**

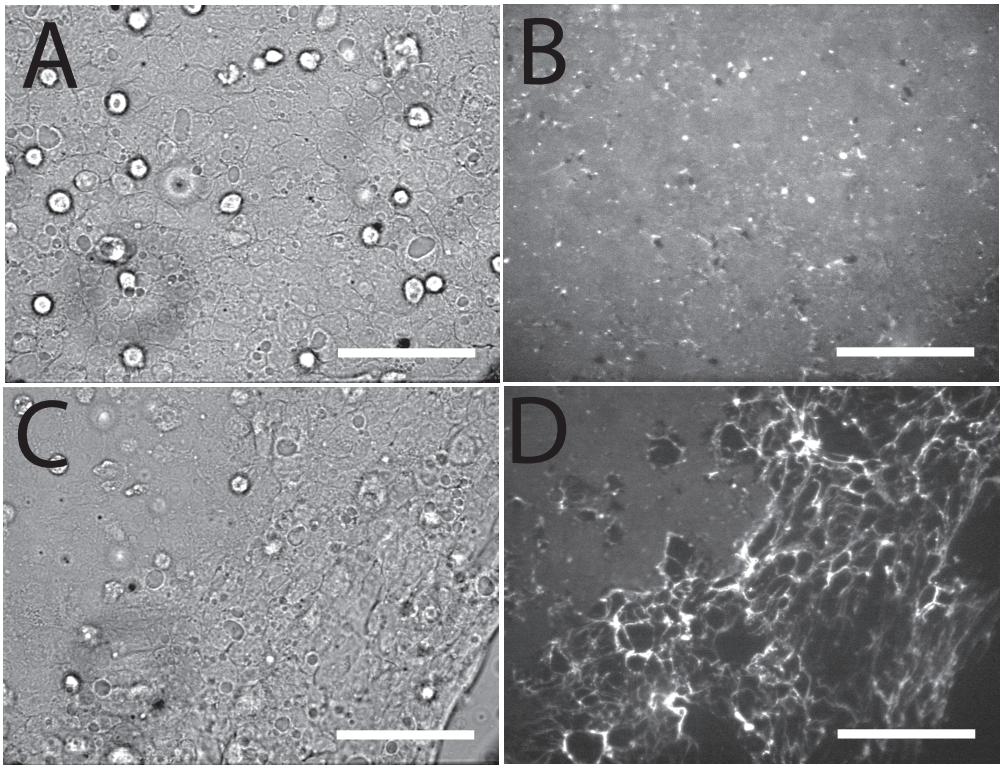

Figure S5: **Limitations to using PDMS**