SUPPLEMENTARY INFORMATION

Supplementary Figures

Supplementary Fig. 1
Supplementary Figure 1. Model formulation and characterization. (a) Schematic of lattice-spring network used to model the substrate. Simple springs are used as the linkages in a purely elastic case and the four-element Burger's model is used in the viscoelastic case. (b) Flowchart depicting the simulation algorithm. (c) Time-step dependence of algorithm. Simulations were run for 1 sec. in simulation time using various time steps and the resulting average spreading velocities for 20 simulation runs were recorded. Error bars represent S.D. (n=20). (d) Average spreading velocity as a function of substrate stiffness if lattice is removed and the number of clutches (n_c) is varied. Data is reported as the mean of five simulation runs. (e) Spreading velocity (blue) and number of molecular clutches (red) for a representative purely elastic substrate (k=1 pN/nm, 0.02 ligands/nm). Spikes are indicative of bond rupture. (f) Example force/extension curves from simulated tensile testing. Substrates were strained at 0.17 nm/ms and the resulting force in a given linkage was recorded. A representative purely elastic substrate is given in red and a representative viscoelastic substrate is given in green, both at the same initial elastic modulus.
Supplementary Fig. 2

Supplementary Figure 2. Simulations using Voigt and Maxwell element limits. (a) Dependence of cell spreading area as a function of ligand density and substrate stiffness when nodes connected by Voigt elements. (b) Difference between cell spreading area on elastic or Voigt substrates for the indicated substrate stiffnesses and ligand densities. (c) Dependence of cell
spreading area as a function of ligand density and substrate stiffness when nodes connected by Voigt elements. (d) Difference between cell spreading area on elastic and Maxwell substrates for the indicated substrate stiffnesses and ligand densities. (e) Cell spreading area as a function of stiffness and the Voigt dashpot value ($\eta_1$). (f) Cell spreading area as a function of stiffness and the Maxwell dashpot value ($\eta_2$).
**Supplementary Fig. 3**

**Supplementary Figure 3.** Stress relaxation and frequency dependent rheology data for all gel formulations used in manuscript. (a) Stress relaxation tests of the indicated gels. Indicated initial elastic modulus noted was determined using AFM. (b) Frequency dependent rheology of the indicated gels. Empty symbols indicate $G''$, and filled symbols indicate $G'$. All values are normalized by $G'(1 \text{ Hz})$ for the specific gel.
Supplementary Fig. 4

Supplementary Figure 4. 3T3 cell spreading on 2D substrates that are either elastic or exhibit stress relaxation. Cells were tested on substrates with an initial Young’s modulus of either 1.4 kPa or 3.4 kPa. Two RGD concentrations, 150 µM and 1500 µM, were compared at each initial modulus. Data are shown as mean +/- s.d. n = 19 – 135 cells analyzed for each condition. *** indicates p<0.001 (student’s t-test).
Supplementary Figure 5. Cell spreading area is not correlated with $G''$ or the ratio of $G'/G''$. (a) Cell spreading area from figure 3 is plotted as a function of $G''$ for each of the substrates. No correlation is observed. (b) Cell spreading area from figure 3 is plotted as a function of $G'/G''$. Again no correlation is observed.
Supplementary Fig. 6

Supplementary Figure 6. Greater clustering of RGD observed for cells on substrates that exhibit stress relaxation relative to purely elastic substrates. DIC and fluorescently coupled RGD for a representative cell plated on the indicated substrate. Thresholded images of the fluorescently coupled RGD are shown in the right panels. The initial elastic modulus for each substrate is 1.4 kPa, and the RGD ligand density is 1500 μM. Arrows indicate areas of local RGD clustering. The images are taken after 40 hours of culture.
Supplementary Tables

Supplementary Table 1

<table>
<thead>
<tr>
<th>Description</th>
<th>% alg.</th>
<th>cross-linker type</th>
<th>cross-linker conc. (mM)</th>
<th>Young’s Modulus (kPa)</th>
<th>Modulus measured by</th>
<th>Stress relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalently crosslinked, high modulus</td>
<td>2</td>
<td>AAD</td>
<td>3</td>
<td>9.7</td>
<td>AFM</td>
<td>None</td>
</tr>
<tr>
<td>Covalently crosslinked, inter. Modulus</td>
<td>2</td>
<td>AAD</td>
<td>0.6</td>
<td>3.4</td>
<td>AFM</td>
<td>None</td>
</tr>
<tr>
<td>Covalently crosslinked, low modulus</td>
<td>2</td>
<td>AAD</td>
<td>0.3</td>
<td>1.5</td>
<td>AFM</td>
<td>None</td>
</tr>
<tr>
<td>Ionically crosslinked, high modulus</td>
<td>3.6</td>
<td>Ca(^{2+})</td>
<td>72</td>
<td>8</td>
<td>AFM</td>
<td>Yes</td>
</tr>
<tr>
<td>Ionically crosslinked, inter. Modulus</td>
<td>2</td>
<td>Ca(^{2+})</td>
<td>48</td>
<td>3.3</td>
<td>AFM</td>
<td>Yes</td>
</tr>
<tr>
<td>Ionically crosslinked, low modulus</td>
<td>2</td>
<td>Ca(^{2+})</td>
<td>24</td>
<td>1.4</td>
<td>AFM</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Supplementary Table 1.** List of all hydrogel compositions used in study.
Supplementary Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{off}$</td>
<td>0.1 s$^{-1}$</td>
<td>Lele, et al.$^9$</td>
</tr>
<tr>
<td>$F_{rup}$</td>
<td>2 pN</td>
<td>Jiang, et al.$^{10}$</td>
</tr>
<tr>
<td>$v_u$</td>
<td>120 nm s$^{-1}$</td>
<td>Cuda, et al.,$^{11}$</td>
</tr>
<tr>
<td>$F_{stall}$</td>
<td>150 pN</td>
<td>Chan$^1$ and Molloy$^{12}$</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>1 s$^{-1}$</td>
<td>Chan and Odde$^1$</td>
</tr>
<tr>
<td>$\kappa_{clutch}$</td>
<td>5 pN nm$^{-1}$</td>
<td>Fisher, et al.$^{13}$</td>
</tr>
<tr>
<td>Initial adhered length</td>
<td>1 µm</td>
<td>Free parameter, adjusted to hasten simulation time required to observe differences in dynamics</td>
</tr>
<tr>
<td>$E_1$</td>
<td>0.01-100 pN nm$^{-1}$</td>
<td>Free parameter, ranges calibrated to experimental results on purely elastic sub., also (14))</td>
</tr>
<tr>
<td>$E_2$</td>
<td>0.01-100 pN nm$^{-1}$</td>
<td>Free parameter, ranges calibrated to experimental results on purely elastic sub., also (14)</td>
</tr>
<tr>
<td>$\eta_1$</td>
<td>0.01-100 pN s</td>
<td>Free parameter, ranges calibrated to experimental results on viscoelastic sub.</td>
</tr>
<tr>
<td>$\eta_2$</td>
<td>0.01-100 pN s</td>
<td>Free parameter, ranges calibrated to experimental results on viscoelastic sub.</td>
</tr>
<tr>
<td>Ligand Density</td>
<td>0.004-0.02 ligands nm$^{-1}$</td>
<td>Comisar, et al.$^7$</td>
</tr>
</tbody>
</table>

Supplementary Table 2. Parameter values used in simulations. Parameters were used from representative sources, although other values from other studies on the same order of magnitude could also have been used with free parameters adjusted accordingly.
Supplementary Notes

Supplemental Note 1 – Stochastic lattice spring model of cell spreading as a function of substrate mechanics and adhesion ligand density

Model Assumptions

This model tests the influence of substrate stress relaxation and adhesion ligand density in a dynamic simulation of cell spreading, building upon the framework of a previous model by Chan and Odde\(^1\). The goal of these simulations is to, even with a simple treatment of spreading mechanics, test for striking differences in cell mechanosensing as a function of material-side parameters. To that end, the model assumes no feedback into the number or applied force of myosin motors\(^2,3\) and does not distinguish between bundled and filamentous actin\(^3\), and the specific composition of linker proteins from the substrate to actin is not specified. Hence the linker proteins are treated as a simple spring and no adhesion strengthening is considered\(^3\). More complex features of cell spreading can be incorporated into future models, but are not expected to alter the essential effect of stress relaxation revealed by this model, as the initiation of and early stages of cell spreading are thought to be independent of adhesion strengthening (i.e. focal adhesion maturation)\(^4\).

The linkers are assumed, however, to undergo force-dependent dissociation as per the Bell model\(^5\) given by

\[ k_{off}^* = k_{off} e^{F_{clutch}/F_{rup}}, \]

where \(k_{off}^*\) is the force-dependent off-rate, \(k_{off}\) is the unloaded off-rate, \(F_{clutch}\) is the retarding force imposed by each clutch, and \(F_{rup}\) is the rupture force per bond. In addition, it is assumed that integrins bind adhesion ligands prior to incorporation into the adhesion complex.
Inhibition of actin retrograde flow velocity is assumed to be a function of the force sustained in the molecular clutches in the following manner:

\[
v_{\text{retrograde}} = v_u \left(1 - \frac{\sum_{\text{clutch}} F_{\text{clutch}}}{F_{\text{stall}}}\right)
\]  

(1)

where \(v_{\text{spread}}\) is the cell spreading velocity, \(v_u\) is the unloaded myosin motor velocity pulling the filament, \(v_{\text{retrograde}}\) is the actin retrograde flow velocity, \(\sum_{\text{clutch}} F_{\text{clutch}}\) is the sum of retarding forces sustained in all of the adhesion sites, and \(F_{\text{stall}}\) is the force required to stop actin retrograde flow velocity. It should be noted that the unloaded myosin motor pulling velocity is exactly balanced by the actin polymerization in the case of no substrate adhesions. Myosin motor pulling velocity treated as constant and the spreading velocity is thus given by \(v_{\text{spread}} = v_u - v_{\text{retrograde}}\). The cell spreading area is reported as the area that would be covered by the cell assuming isometric spreading, and thus is the area of the circle with a radius equal to the distance spread by the cell in 60,000 time steps.

The substrate is modeled as a 2D lattice of mass nodes, with each node connected to its neighbors via a simple spring in the purely elastic case, or a Burger's model, in the viscoelastic case (Supplementary Fig. 1a). In the Burgers model, stress and strain are related by:

\[
\sigma + \left(\frac{\eta_1}{E_1} + \frac{\eta_2}{E_2}\right) \frac{d\sigma}{dt} + \left(\frac{\eta_2}{E_2}\right) \frac{d^2\sigma}{dt^2} = \left(\frac{\eta_2}{E_1 E_2}\right) \frac{d\varepsilon}{dt} \frac{d^2\varepsilon}{dt^2}
\]  

(2)

where \(\sigma\) is the stress sustained in the linkage, \(\varepsilon\) is the linkage strain, \(E_1\) is the Maxwell element stiffness, \(E_2\) is the Voigt element stiffness, \(\eta_1\) is the Maxwell element damping coefficient, and \(\eta_2\) is the Voigt element damping coefficient. Alginate, the material used experimentally in this study, has previously been modeled using a Burger's model\(^6\). Material parameter ranges were calibrated based on canonical spreading responses to different parameters in the purely elastic case, with the low end for stiffness being those purely elastic substrates on which cells do not
spread and the high end being those on which cells do spread (Supplementary Table 2).
However, simulations were also run in the limiting cases that the Burger’s model becomes a
Maxwell element or a Voigt element (Supplementary Fig. S2), and the effect of varying the
damping coefficient for each of these limiting cases is tested. For the Maxwell limit simulations,
the Voigt elements were made essentially infinitely stiff with no damping, and visa versa for the
Voigt limit simulations. For example, in the Maxwell limit, the Voigt spring constant was held at
10e30, while the damping coefficient was held at 1.

On the top level of nodes, only certain nodes are made available for binding by the cell as
determined by the ligand density. Ligand densities were approximated, based on previous
approaches to quantify spacing of RGD ligands on alginate hydrogels for varying degrees of
coupling that are consistent with those used in this study.\textsuperscript{7}

At each time step in the simulations, the leading edge spacing was calculated as the
distance between the leading node to which there is an adhesion, and the adjacent node in the
direction of spreading. For the comparison of this spacing, this value was averaged over 60,000
time steps.

\textit{Model Implementation}

The simulations are carried out in MATLAB (Supplementary Fig. 1b). The model was
tested for a range of time steps and a time step within the stable range was chosen (0.2 ms,
Supplementary Fig. 1c). At each time step it is determined whether the cell spreading front has
passed a new available adhesion ligand on the substrate. If so, a new bond may be formed, as
determined by \( k_{on} \) and the number of adhered clutches is incremented. Given the current
filament velocity, a strain of \( dt \times v_{fil} \) is imposed on the substrate via the clutches. Supp. Eq. 2 is
discretized using a Backward Euler method and the force between each node and its neighbors is calculated based on the new strain. For each node in the lattice, the resultant horizontal and vertical forces are found by summing the horizontal and vertical components of the force between the node and each of its neighbors; the equations of motion are then solved using an implicit Beeman scheme to find the new position of each node in the lattice for that time step.

At this point, based on the new strain profile of the lattice, the new force sustained in each molecular clutch is calculated using the clutch spring constant, $k_{\text{clutch}}$, and each molecular clutch is tested for dissociation per the Bell model described above. The new forces sustained in each clutch are used to update the actin retrograde flow velocity for the next time step.

**Supplemental Model Characterization**

By directly prescribing the number of molecular clutches and treating the substrate as a collection of independent simple springs, each connected to a clutch, instead of a lattice, the presence of a “stiffness optimum” that is mediated by clutch number as reported in Bangasser and Odde\(^8\) is recreated, as is the “load and fail” and “frictional slippage” regimes (Supplementary Fig. 1d-e). Additionally, the increase in velocity and optimum shift with increasing number of clutches, as well as the stalled state, or high spreading velocity that is independent of substrate stiffness, at a higher number of molecular clutches previously shown by Bangasser and Odde\(^8\) is captured. Upon incorporating the material lattice and ligand density, dissociation events as well as clutch addition due to spreading are noted (Supplementary Fig. 1e). In order to validate the behavior of the lattice, simulated tensile tests were performed as to confirm purely elastic or viscoelastic behaviors. At a constant strain rate, purely elastic substrates demonstrate a linear force/extension relationship, while viscoelastic substrates demonstrate stress relaxation,
confirming the capability of the substrate lattice to capture both purely elastic and viscoelastic behaviors (Supplementary Fig. 1f).
Supplementary references: