Supplemental Material

Spatial organization and mechanical properties of the pericellular matrix on chondrocytes

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I. Probing the fluorescently-labeled pericellular matrix shows beads penetrate into PCM

Movie S1 shows a three micron passivated microsphere carried towards a living cell whose pericellular matrix has been labeled with neurocan-GFP (which binds to hyaluronan). The bead visibly enters into the cell coat, as seen by the fluorescent material that surrounds the bead as it moves closer to the cell surface. In principle, a cross-linked matrix might be impenetrable or at least partially deform as a particle moves into it.

II. Probing the cell coat with two holographic traps shows no force coupling between the beads

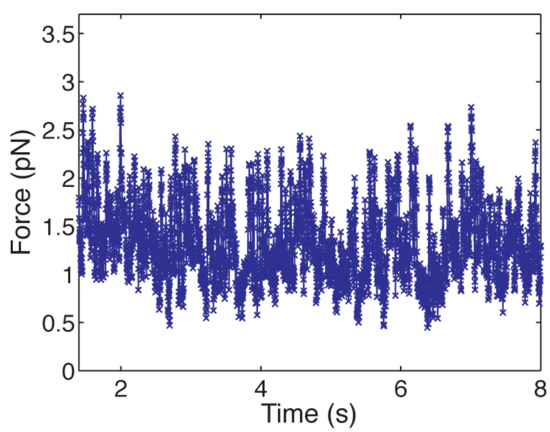

Fig. S1 The monitored force on the inner bead during the two bead HOT experiments. The force remains constant as the second probe is carried into the matrix. This supports the observation that forces are not easily transmitted through the matrix from one bead to the other, providing evidence that minimal crosslinking is present.

III. Estimating the pressure profile from the equilibrium force curve

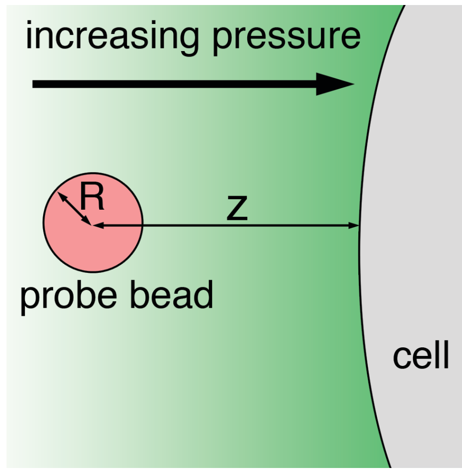

Fig. S2 Schematic of osmotic pressure gradient on a bead as a result of a varying concentration (green) in the cell coat.

The relationship between the equilibrium force and the local pressure on the bead is

$$\vec{F}_{osm}(z) = \int P(z')d\vec{A} \qquad \text{Eq. S1}$$

where P is the pressure, z is the distance from the cell membrane to the center of the bead, and z' is the distance to the outside of the bead, as illustrated in Fig. S1.

Since the pressure acts in the inward radial direction on the bead, we rewrite Eq. S1 as

$$\vec{F}_{osm}(z) = -\int P(z')dA\hat{R} \qquad \text{Eq. S2.}$$

Considering that the experiment is symmetric in every direction but z, we rewrite the above as

$$\vec{F}_{osm}(z) = -\int P(z')\cos\theta dA\hat{z} \qquad \text{Eq. S3}$$

Replacing z' with z'= z - R$\cos\theta$, where θ is the standard spherical coordinate, gives

$$\vec{F}_{osm}(z) = -\int P(z - R\cos\theta)\cos\theta dA\hat{z} \qquad \text{Eq. S4}$$

where R is the bead radius. Integrating the surface element dA over r and φ gives $dA=2\pi R^2\sin\theta d\theta$, since their contributions are constant and independent of θ. This results in

$$\vec{F}_{osm}(z) = -\int_0^\pi P(z - R\cos\theta)2\pi R^2\sin\theta\cos\theta d\theta\hat{z} \qquad \text{Eq. S5}$$

Setting x = Rcosθ, the force becomes

$$\vec{F}_{osm}(z) = -2\pi\int_{-R}^{R} P(z-x)xdx\hat{z} \qquad \text{Eq. S6}$$

Now, Taylor expanding P(z-x) around z

$$P(z-x) = P(z) - \frac{\partial P(z)}{\partial(z-x)}x + \frac{1}{2}\frac{\partial^2 P(z)}{\partial(z-x)^2}x^2 - \frac{1}{6}\frac{\partial^3 P(z)}{\partial(z-x)^3}x^3 + \frac{1}{24}\frac{\partial^4 P(z)}{\partial(z-x)^4}x^4 + \ldots \qquad \text{Eq. S7}$$

and using $z' = z - x$ and the chain rule which gives $\frac{\partial P(z)}{\partial z'} = \frac{\partial P(z)}{\partial z}\frac{\partial z}{\partial z'}$ where $\frac{\partial z}{\partial z'} = 1$, the integral for the force becomes:

$$\vec{F}_{osm}(z) = -2\pi\int_{-R}^{R}\left(P(z) - \frac{\partial P(z)}{\partial z}x + \frac{1}{2}\frac{\partial^2 P(z)}{\partial z^2}x^2 - \frac{1}{6}\frac{\partial^3 P(z)}{\partial z^3}x^3 + \frac{1}{24}\frac{\partial^4 P(z)}{\partial z^4}x^4\right)xdx\hat{z} \qquad \text{Eq. S8}$$

Due to the limits of integration, the terms odd in x above drop out yielding

$$\vec{F}_{osm}(z) = \frac{4}{3}\pi R^2\left(\frac{\partial P(z)}{\partial z}R + \frac{\partial^3 P(z)}{\partial z^3}\frac{1}{10}R^3 + \ldots\right)\hat{z} \qquad \text{Eq. S9}$$

Assuming that the solution for the pressure has an exponential dependence

$$P(z) = he^{-dz} \qquad \text{Eq. S10}$$

and using the experimental observation that the equilibrium force has an exponential dependence with known parameters a and c,

$$F_{osm}(z) = ae^{-cz} \qquad \text{Eq. S11}$$

we can solve Eq. S9 to find an approximate solution for the pressure. It is apparent that d=c, while the relation between a and c is found to be

$$a = \frac{4}{3}\pi R^2 h\left(cR + \frac{1}{10}(cR)^3 + \ldots\right) \qquad \text{Eq. S12}$$

Since we know that c=0.5 and that R=1.5, we have cR = 0.75. This gives $0.1(cR)^3$=.04 and therefore we can neglect the 3rd and higher order terms. The final approximate expression for the pressure profile is

$$P(z) \approx \frac{3a}{4\pi R^3 c} e^{-cz} \qquad \text{Eq. S13}$$

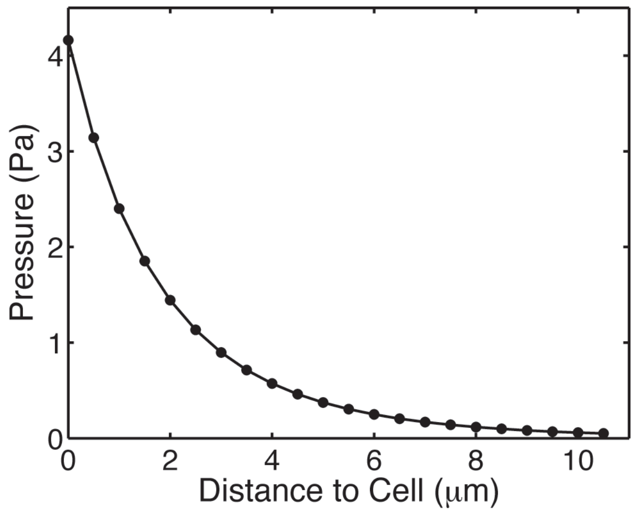

Fig. S3 Pressure profile of the coat using the approximate solution in Eq. S13 and the parameters (a,c) acquired from optical force probe assays.

To check the approximations made above, we can take a slightly different approach starting from

$$\vec{F}_{osm}(z) = -2\pi \int_{-R}^{R} P(z-x)\,x\,dx\,\hat{z} \qquad \text{Eq. S14}$$

If we assume that the pressure has an exponential form (Eq. S10), we can solve for the exact expression for the pressure:

$$F_{osm}(z) = \frac{2\pi h}{d^2}\left(e^{dR}(dR-1) + e^{-dR}(dR+1)\right)e^{-dz} \qquad \text{Eq. S15}$$

Considering that from the data the equilibrium force has an exponential dependence with known parameters a and c, we can solve for the parameters d and h to find the pressure:

$$ae^{-cz} = \frac{2\pi h}{d^2}\left(e^{dR}(dR-1) + e^{-dR}(dR+1)\right)e^{-dz} \qquad \text{Eq. S16.}$$

Again we find that d=c. Solving for h yields the an exact expression for the pressure profile throughout the pericellular matrix,

$$P(z) = \frac{ac^2}{2\pi\left(e^{cR}(cR-1)+e^{-cR}(cR+1)\right)}e^{-cz}. \qquad \text{Eq. S17}$$

This exact solution can be compared with the first order solution (Fig. S4), verifying that it is sufficient to use the first order approximation (Eq. S13) for the pressure profile.

This expression can then be used to relate the pressure profile to the correlation length versus distance to the cell surface throughout the pericellular matrix:

$$P(z) \approx \frac{k_B T}{\xi^3(z)} \qquad \text{Eq. S18}$$

giving a correlation length profile with an exponential variation in space,

$$\xi(z) \propto e^{\frac{cz}{3}} \qquad \text{Eq. S19.}$$

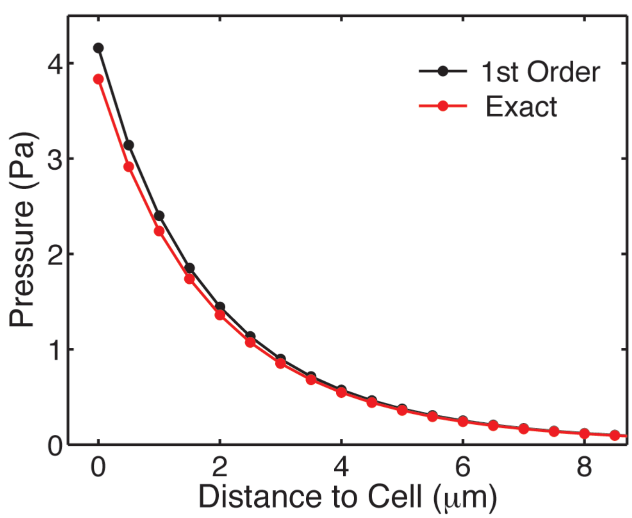

Fig. S4 Comparison of the first order solution (Eq. S13) to the exact solution (Eq. S17) for the pressure profile in the pericellular matrix, where to find Eq. S17 it was assumed that the pressure has an exponential profile.

IV. Quantitative Particle Exclusion Assays – A summary of the data

Bead diameter (nm)	**Effective thickness, d_{eff} (μm)**	**N**
40	1.4 ± 0.3	31
100	3.3 ± 0.4	43
200	5.2 ± 0.6	40
300	6.7 ± 0.5	65
400	7.1 ± 0.7	38
500	8.5 ± 0.8	59
2000	8.1 ± 0.9	58
3000	8.6 ± 1.4	19

Table S1 Full results from the quantitative particle exclusion assay (qPEA) including data not shown in the text. The effective thickness is the distance where the bead concentration becomes constant, indicating beads of that size can easily access that portion of the pericellular matrix. d_{eff} is an average number extracted from the analysis of intensity profiles of the bead distributions from N cells for each bead size. Error reported is twice the standard error.

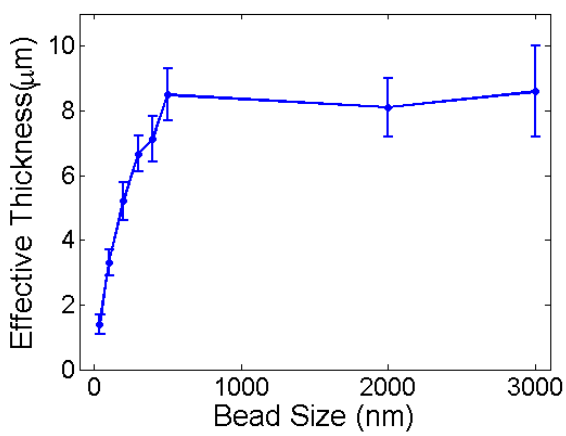

Fig. S5 The effective thickness increases with probe size until it plateaus at ~8.5 μm where probes 500 nm in diameter and larger are excluded from the PCM completely. The change in d_{eff} indicates that the cell coat acts like a sieve.