Electronic Supplementary Material (ESI) for Soft Matter.

Measuring human mesenchymal stem cell remodeling in hydrogels with a step-change in elastic modulus†

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Additional Figures

S1 Elastic moduli, G', profiles of scaffolds with interfaces

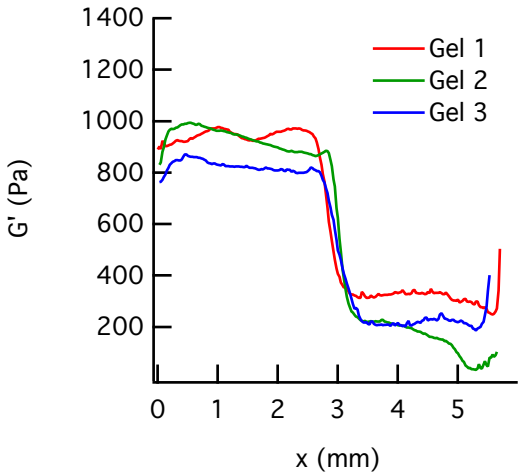

Figure S1: Three stiffness profiles for hydrogels with an interface in elastic moduli, G'. Reprinted (adapted) with permission from McGlynn and Schultz, "Characterizing Nonuniform Hydrogel Elastic Moduli Using Autofluorescence", *Macromolecules*, 2022, 55, 4469-4480.: DOI: 10.1021/acs.macromol.2c00241. Copyright 2022 American Chemical Society [1].

S2 Example of calculation of α values

α is calculated from the MSD and values of lag time, 0.033 $s \leq \tau \leq 1$ s and is the slope of the line of best fit on a log-log plot. The error in α is the error in fitting this slope.

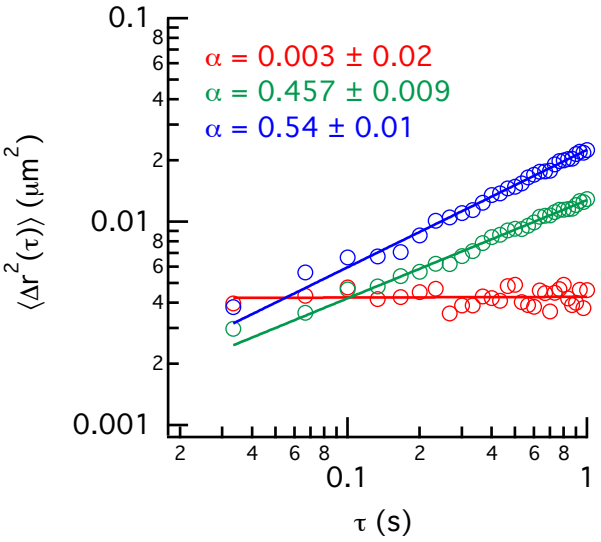

Figure S2: Example of how α is calculated from the MSD. A line is fit to the plot of MSD versus lag time, τ, which is α.

S3 Number of cells analyzed in each position relative to the interface on each day post-encapsulation

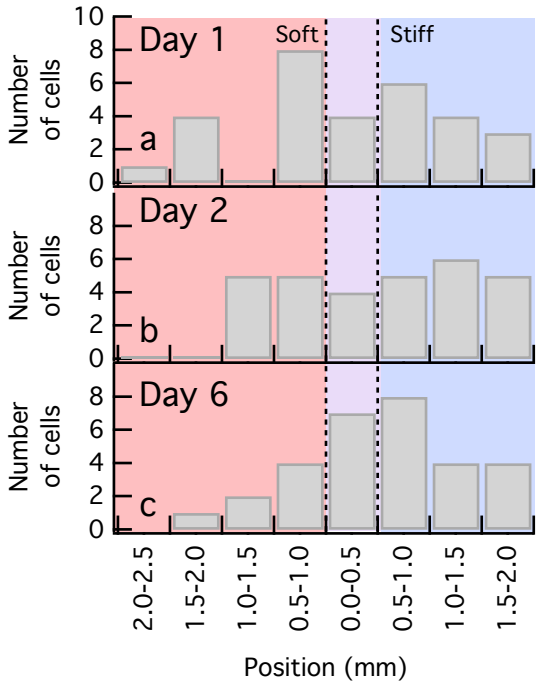

Figure S3: Number of cells whose pericellular regions are analyzed in each position relative to the interface on days (a) 1, (b) 2 and (c) 6 post-encapsulation. The background color represents the different regions of the hydrogel. Red and blue are the soft and stiff halves, respectively, and the region between the dashed lines is the region of rapid change in G' in the interface, shaded in purple.

S4 Visualization of EFF calculation

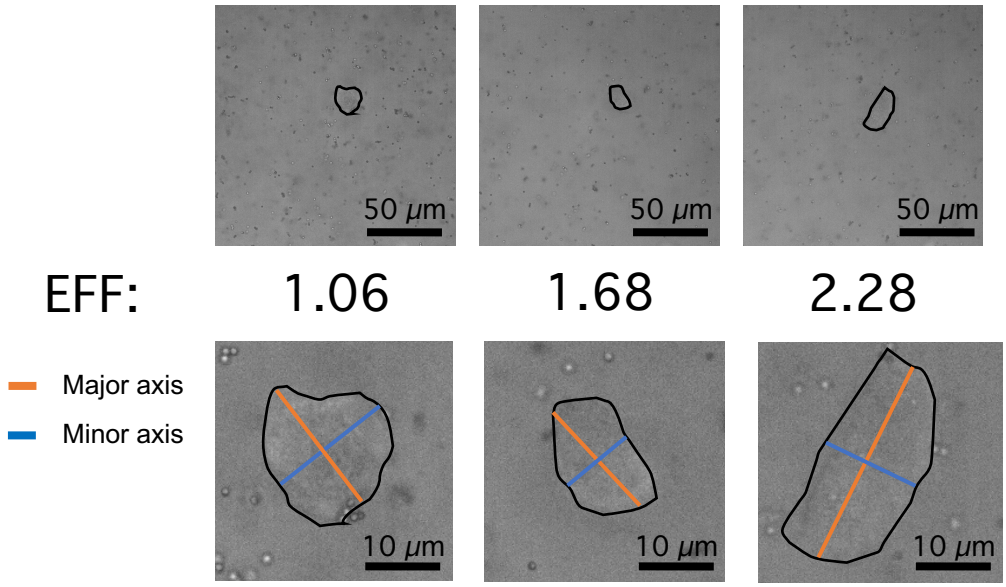

Figure S4: Illustration of how EFF is calculated for representative cell images. The approximate cell trace is shown in black, which is used to determine the major and minor axes, shown in orange and blue respectively. The ratio of the lengths of the major and minor axis is the EFF.

S5 Cell speed in each region of the hydrogel

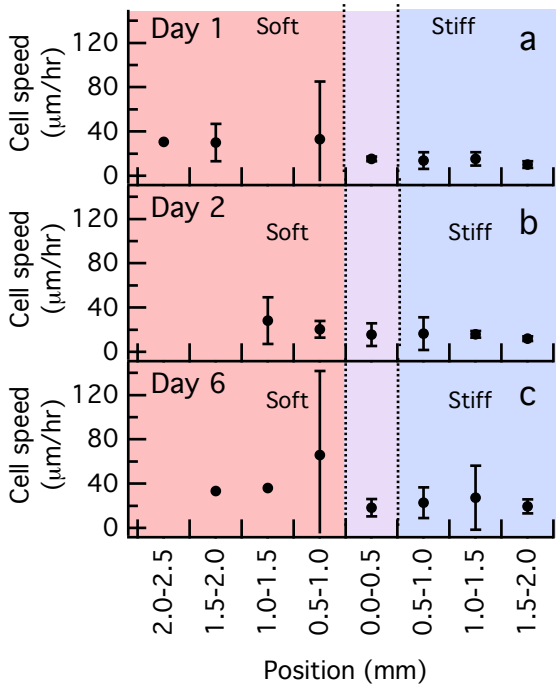

Figure S5: Cell speed in each region of the hydrogel relative to the interface on days (a) 1, (b) 2 and (c) 6 post-encapsulation. Cell speed is represented as an average of all speeds measured in that region with an error bar that is the standard deviation. Red and blue represent the soft and stiff halves, respectively, and the region between the dashed lines is the region of rapid change in G' in the interface, represented as purple.

S6 Additional analysis of α values in the interface region

In this section we describe further analysis of pericellular regions in the interface. We don't draw major conclusions from this analysis because not enough cells significantly remodel the interface until day 6 post-encapsulation.

To investigate the relationship between the rapid stiffness change in the interface region and hMSC-mediated remodeling, we analyze 40 μm wide sections of the pericellular region around each cell in the interface. We begin by fitting a line to the α values of each horizontal section in the interface region. This procedure is outlined for a single sample in Figure S6.

α values in the pericellular region are plotted against their normalized y-position relative to the interface in Figure S6a. These values match the values of α which color the graphs in Figure S6b. The slope of the line (m) is the rate of change in α across the interface region. m also quantifies the orientation of cellular degradation. Negative values of m indicate that degradation increases towards the soft half of the hydrogel. Positive values of m indicate that degradation increases towards the stiff half of the hydrogel. y-position is normalized by dividing the y-coordinate of each measurement by 160 μm, which is the maximum y-value in the field of view. Normalizing the position between $0-1$ ensures that both α values and position are varying with the same order of magnitude, which makes interpretation of the slope simpler. We determine m for each time point measured for cells in the interface region on each day post-encapsulation.

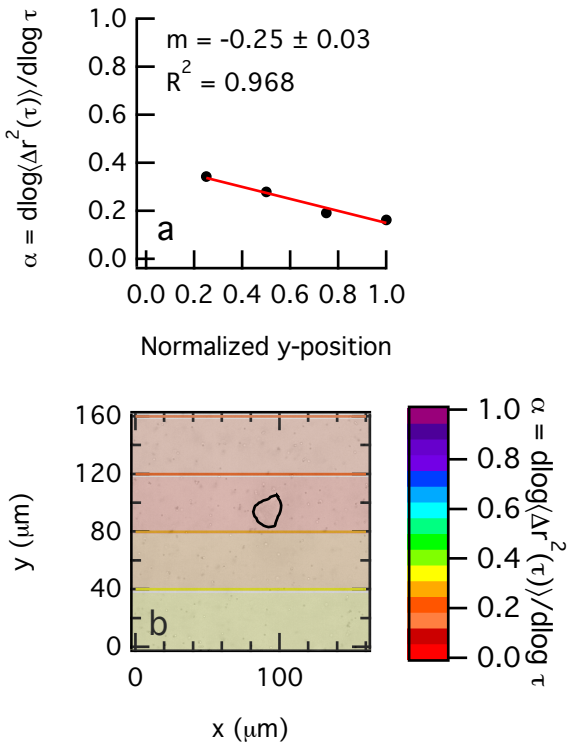

Figure S6: This figure illustrates how m and R^2 are calculated for a single sample. (a) α values from each section are plotted against normalized y-position and the value of the slope, m, and the coefficient of determination, R^2, are calculated. These α values match the colors plotted as the 40 μm sections in (b).

This analysis method determines how cellular remodeling changes in the pericellular region relative to the location of the interface. However, not all calculated lines of best fit accurately represent the change in the value of α along the interface. To identify lines which do not fit well, we multiply the calculated value of m by the coefficient of determination (R^2), which quantifies how well a fitted line represents the data. $R^2 = 1$ is when all points fall perfectly on the fitted line. Lower values of R^2 indicate the fitted line does not represent the data well. When $m \times R^2 \approx 0$, the relationship between α and position in the field of view is not linear, α does not change significantly with position or both.

To determine how m varies on each day post-encapsulation, we assign each sample measured around

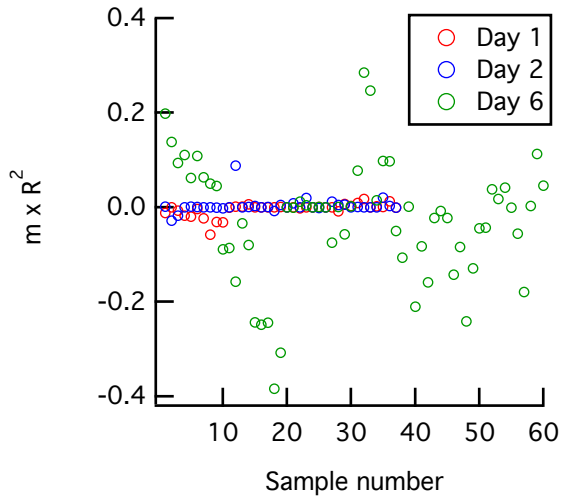

Figure S7: $m \times R^2$ versus arbitrary sample number on each day post-encapsulation. All significant variation in $m \times R^2$ is on day 6 post-encapsulation.

a cell in the interface region on a given day an arbitrary sample number and plot $m \times R^2$. This is shown in Figure S7. For days 1 and 2 post-encapsulation, we measure no significant variation in $m \times R^2$ with all values $m \times R^2 \approx 0$. This is likely because little degradation occurs in the interface region on days 1 and 2 post-encapsulation preventing any measurable gradient in α. On day 6 post-encapsulation, we measure significant variation in $m \times R^2$ indicating degradation is towards the soft and stiff halves of the hydrogel. Because the only significant variation in α occurs on day 6 we will only discuss data from this day. The mixed values of $m \times R^2$ suggest that cells encapsulated in the interface region behave differently based on the structure of their surroundings. This is an example of the feedback between hMSCs and their environment changing hMSC remodeling.

To determine how the structure of the pericellular region impacts the direction of hMSC remodeling in the interface region, we plot the average α value for the pericellular region versus m in Figure S8. We also use color to indicate the direction of cell migration, quantified by $\sin\theta$. This shows the relationship between the gradients in α and whether the cell is undergoing durotaxis. We restrict the data in Figure S8 to only include values of m with $R^2 > 0.7$ to prevent poorly fitting lines from obscuring the data. 26 individual measurements of the pericellular region on day 6 post-encapsulation have $R^2 > 0.7$. 34 samples do not meet this criteria and are not analyzed further.

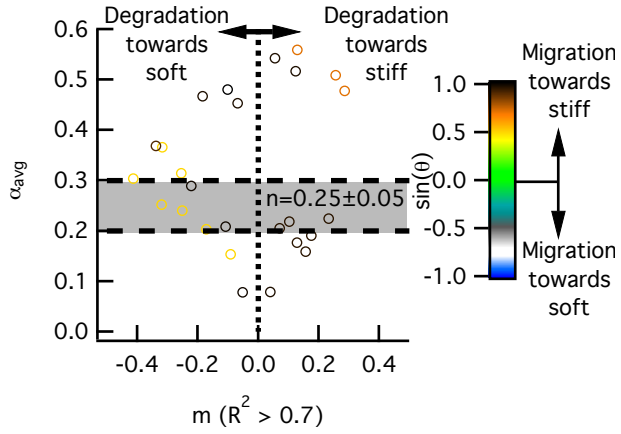

Figure S8: Average α values plotted against the value of m defined as the slope of α versus normalized y-coordinate for sections in the interface. The color of each marker represents the migration direction of the cell, quantified as $\sin\theta$. The gel-sol transition region is the shaded region.

The data plotted in Figure S8 shows that two populations exist: one with positive values of m and one with negative values of m. Most of the samples with negative values of m have moderate α values

$(0.25 < \alpha < 0.45)$. This indicates that these cells have more degradation towards the soft half of the hydrogel than towards the stiff half. We hypothesize that these cells with negative m values are secreting MMPs which degrade the lower cross-link density regions more rapidly than the higher cross-link density regions near the stiff half.

Samples with positive m values have degradation which is oriented towards the stiff half of the hydrogel. These samples separate into two sub-populations with $\alpha < n$ and $\alpha > n$. We use the cellular persistence data from Figure 4 to determine if the direction of migration of the cell, quantified by $\sin\theta$, is related to the m value. The samples with high α and positive m values have $\sin\theta = 0.81 \pm 0.14$ while those with low α and positive m values have $\sin\theta = 0.94 \pm 0.01$. This means that cells in highly degraded regions (high α values) do not migrate as directly to the stiff half of the hydrogel (indicated by lower $\sin\theta$) as cells in regions which are still a gel ($\alpha < n$). We hypothesize that this is because cells surrounded by material that is a sol ($\alpha > n$) are not able to sense the gradient in stiffness of the interface because there is no longer a sample-spanning network. The cells surrounded by gel ($\alpha < n$) with positive m have higher $\sin\theta$ because they can better sense the gradient in stiffness at the interface and migrate in response to the durotactic cue.

The value of m may also be impacted by the change in the gradient in G' in the interface as the hydrogel degrades. As cells secrete MMPs in the interface region, the interface will have its G' reduced as cross-links are broken. We measure that α values in the interface region increase over the course of the experiment indicating that G' is decreasing. Because material in the stiff half of the hydrogel degrades more rapidly due to the increase in MMPs, the difference in moduli between the two halves decreases. This will lower the magnitude of the gradient in G' and the rheological properties will become more uniform. Cells which were originally in a region of rapid change in G' may be in a more uniform area of the hydrogel. We hypothesize that this effect will become more prominent at later days post-encapsulation when cells have had more time to secrete MMPs and remodel the interface.

References

[1] John A McGlynn and Kelly M Schultz. Characterizing nonuniform hydrogel elastic moduli using autofluorescence. *Macromolecules*, 2022.