Rheological characterization of dynamic re-engineering of the pericellular region by human mesenchymal stem cell-secreted enzymes in well-defined synthetic hydrogel scaffolds†

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Control experiments

Three control experiments are done. (1) Hydrogels are made without cells and incubated in media at the same environmental conditions, 5% CO_2 and $37^{\circ}C$, overnight. Changes in the state of the material are measured over $30-60$ *mins* in different areas in the hydrogel, Figure **??**. This experiment was repeated with three different hydrogel samples. (2) Human mesenchymal stem cells (hMSCs) are encapsulated in the hydrogel scaffold and their matrix metalloproteinase (MMP) activity is inhibited. Data are taken around $5-6$ different cells in each hydrogel and changes in the state of the material are measured around each encapsulated cell separately, Figure **??**. Three biological replicates are measured and at least two hydrogels are made for each replicate. (3) Hydrogels are made without cells and incubated in the serum-free and Phenol red-free media with TIMP antibodies. Changes in the state of the material are measured over $30-60$ *mins* for four different areas, Figure **??**. This experiment was only done once due to the high cost of the TIMP-1 and -2 antibodies. No degradation is observed in all experiments.

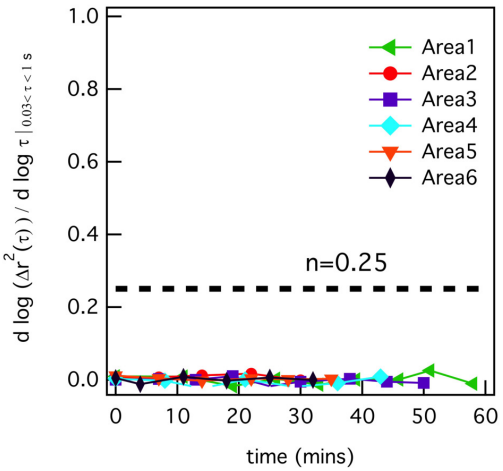

Fig. S1 Changes in the logarithmic slope of mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, over time for 6 different areas in a hydrogel without cells. For all measurements $\alpha \approx 0$ indicating no scaffold degradation.

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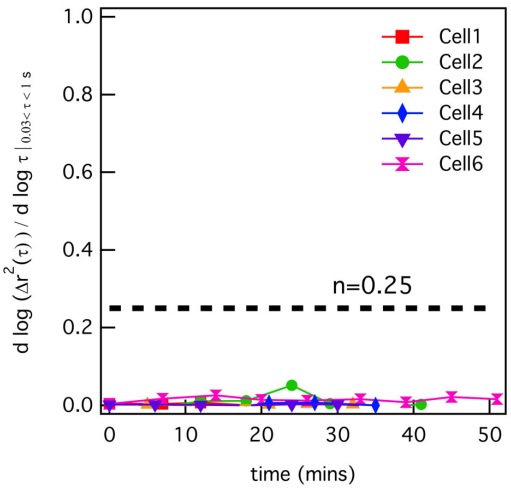

Fig. S2 Changes in the logarithmic slope of mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, around 6 different hMSCs over time after MMP inhibition. For all measurements $\alpha \approx$ indicating no scaffold degradation in the pericellular region around encapsulated hMSCs.

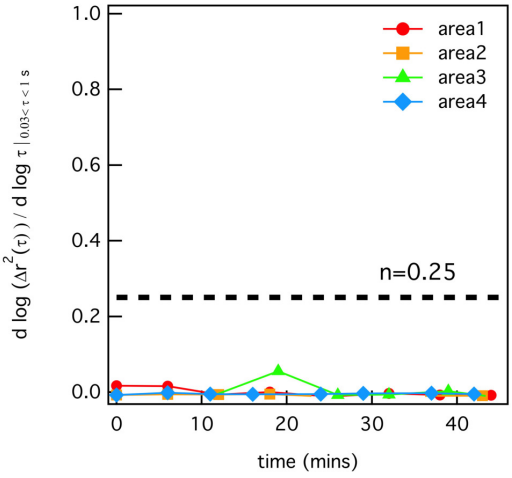

Fig. S3 Changes in the logarithmic slope of mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, over time for different areas in the hydrogel without cells incubated in serum-free and Phenol red-free media with TIMP antibodies. For all measurements $\alpha \approx$ indicating no scaffold degradation.

Western blots

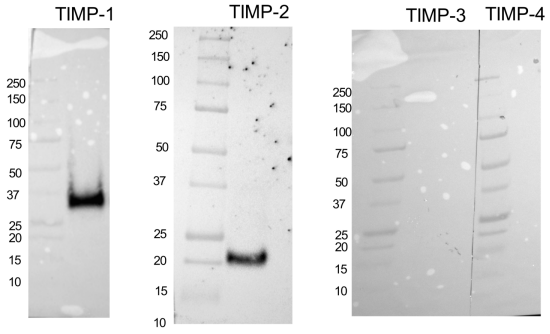

Fig. S4 Analysis of cell media samples using Western blotting. Cell media two days after incubation are analyzed with Western blotting for TIMP-1, -2, -3 and -4 expression. Appearance of bands in front of each molecular weight shows the existence of that specific protein in the sample. Molecular weight of TIMP-1, -2, -3, -4 are 29 kDa, 21 kDa, 26 kDa and 23 kDa, respectively.

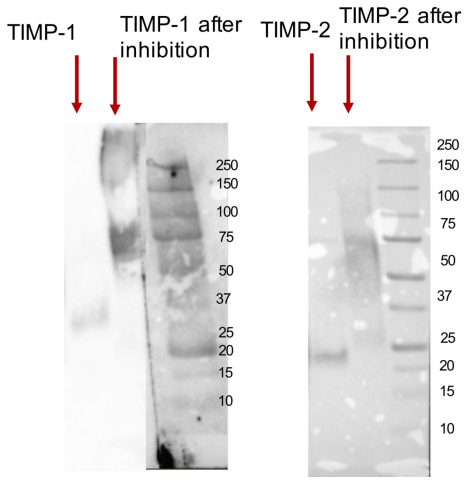

Fig. S5 Analysis of cell media samples using Western blotting. Cell media after two days on incubation is analyzed using Western blotting for TIMP-1 and -2 inhibition. The left column for each figure is cell media, which shows the presence of TIMP-1 and -2 by the bands in front of their molecular weight. The right column in each figure shows the disappearance of these bands.

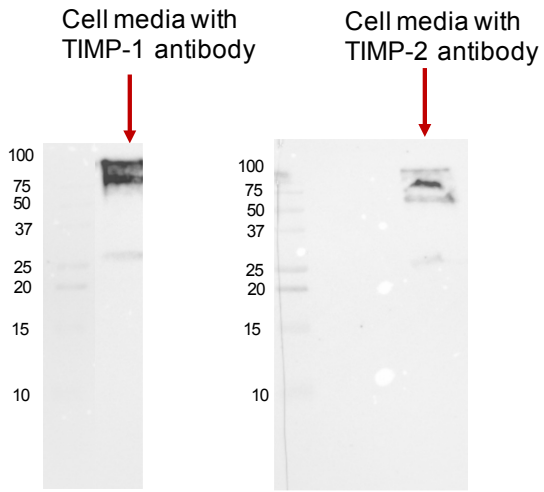

Fig. S6 Analysis of cell media samples using Western blots. Cell media samples are collected after two days of incubation and TIMP antibodies are added to the sample. Appearance of bands in front of $50-100\ kDa$ is due to existence of the antibodies in the samples.

Additional degradation profiles around encapsulated hMSCs

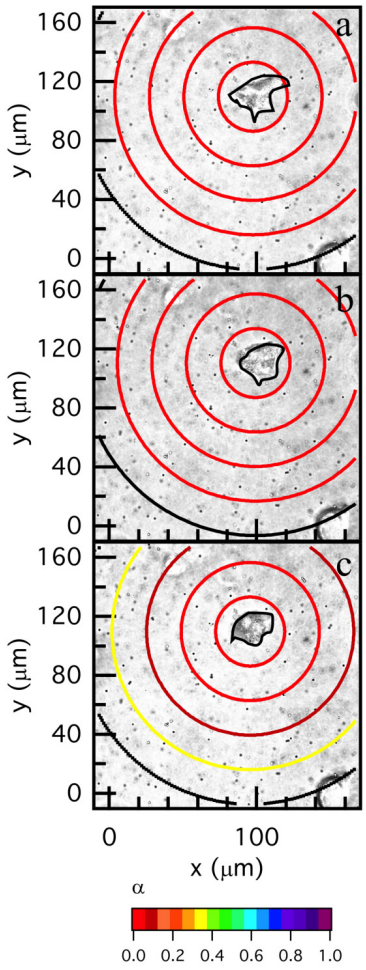

Fig. S7 Spatial degradation profiles around an encapsulated untreated hMSC. MPT data are collected over time after hMSC identification at (a) 0, (b) 18 and (c) 24 $mins$. The color of the rings indicates the logarithmic slope of the mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, in the hydrogel. Warm colors represent a gel and cool colors represent a sol. The transition between gel and sol occurs at $\alpha = n = 0.25$, which is represented by an orange color.

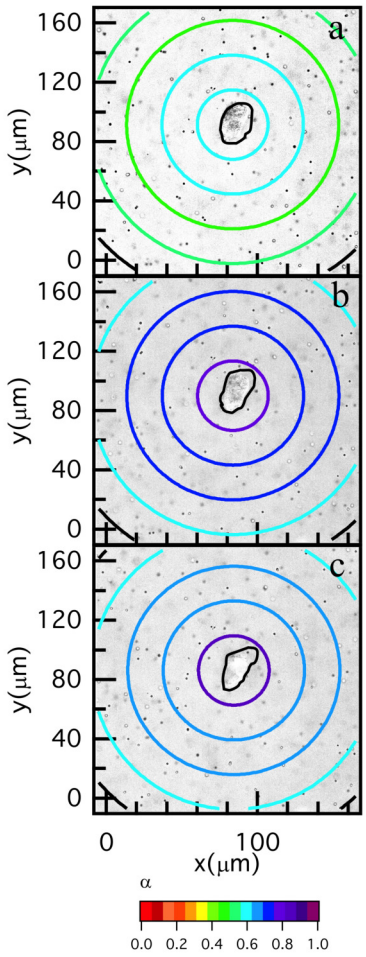

Fig. S8 Spatial degradation profiles around an encapsulated TIMP inhibited hMSC (treated with TIMP-1 and -2 antibodies). MPT data are collected over time after hMSC identification at (a) 0, (b) 8 and (c) 16 *mins*. The color of the rings indicates the logarithmic slope of the mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, in the hydrogel. Warm colors represent a gel and cool colors represent a sol. The transition between gel and sol occurs at $\alpha = n = 0.25$, which is represented by an orange color. This is the complete degradation profile for the data presented in Figure 6 in the main text.

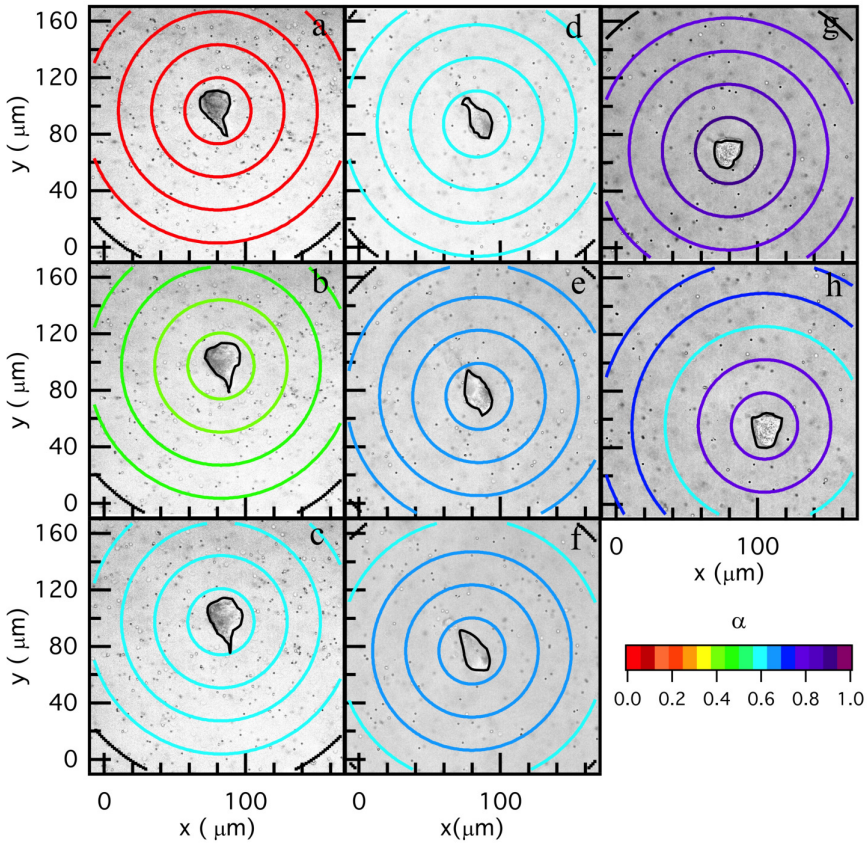

Fig. S9 Spatial degradation profiles around three different TIMP inhibited hMSCs. All motility groups are represented in this figure. The left column (a-c) is MPT data for an hMSC in the slow motility group, the middle column (d-f) shows the data around an hMSC in the fast motility group and the right column (g-h) is MPT data for an hMSC in the super fast motility group. MPT data are collected over time after hMSC identification at (a) 0, (b) 18, (c) 40, (d) 0, (e) 8, (f) 12, (g) 0 and (h) 6 *mins*. The color of the rings indicates the logarithmic slope of the mean-squared displacement, $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$, in the hydrogel. Warm colors represent a gel and cool colors represent a sol. The transition between gel and sol occurs at $\alpha = n = 0.25$, which is represented by an orange color.

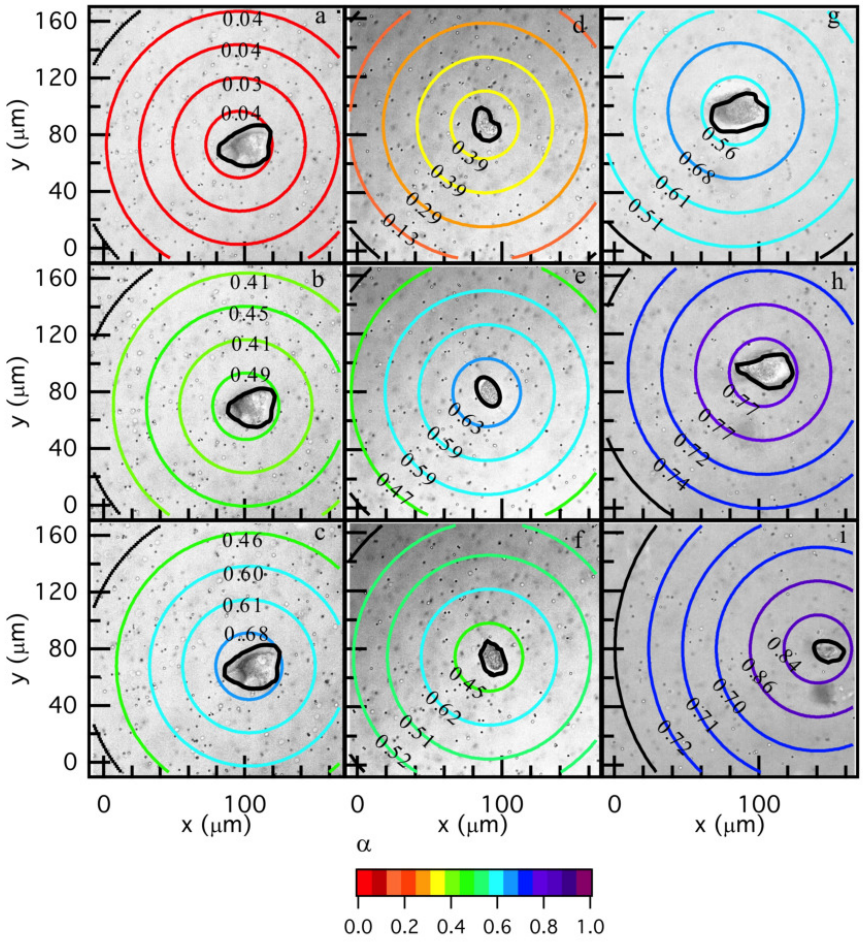

Fig. S10 Spatial degradation profiles around three different TIMP inhibited hMSC migration speed populations. The left column is MPT data for an hMSC in the slow motility group, the middle column shows the data around an hMSC in the fast motility group and the right column is MPT data for an hMSC in the super fast motility group. MPT data are collected through time for each hMSC after identification at (a) 0, (b) 32 and (c) 57, (d) 0, (e) 4 and (f) 8, (g) 0, (h) 8 and (i) 16 mins. The color of the each ring represents $\alpha = \frac{d\log\langle\Delta r^2(\tau)\rangle}{d\log\tau}$ that shows determines the state of the material in the hydrogel. The value of α has been added to each ring to illustrate the quantitative change as the distance from the cell center changes. This is a copy of Figure 5 in the manuscript with α values added.