Supplementary Material (ESI) for Integrative Biology

Supplemental Material

Crosslinking of cell-derived 3D scaffolds up-regulates the stretching and unfolding of new extracellular matrix assembled by reseeded cells

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Figure S1: Chemical denaturation and strain curves to correlate FRET intensity ratios to fibronectin conformation and mechanical strain.

Chemical denaturation (A and C) and strain (B and D) calibration curves for the two batches of Fn-DA used in this study. Statistical variations in the number and placement of the donor molecules lead to slight differences in the correspondence between strain, conformation, and intensity ratio. Plots A and B correspond to data from all cell-derived ECM experiments (Figs. 2, 3, S2, and S3); plots C and D correspond to all manually deposited fiber experiments (Figs. 6 and 7). Chemical denaturation data were acquired by denaturing Fn-DA in different concentrations of guanidine HCl (black diamonds). The 1 M GndHCl point was also measured with Fn-DA that had been reduced to monomers (white squares) with DTT in order to eliminate intramolecular FRET due to cross-over of the dimer arms. Data points are the mean +/- s.e.m. from three independent experiments.

Strain data were acquired by straining manually deposited fibers containing Fn-DA on silicone sheets in a 1D strain device as described in the Materials and Methods. Percent strain is relative to the “fully relaxed” state of the fiber (point at which the fiber buckles; E.K. et al., submitted). Gray data points indicate measurements of individual fibers (mean intensity ratio +/- s.d.). Black points indicate the mean +/- s.e.m. of groups of fibers with similar length changes. The horizontal gray lines indicate the mean intensity ratio for the specified chemical denaturation point.

Supplemental Figure S1

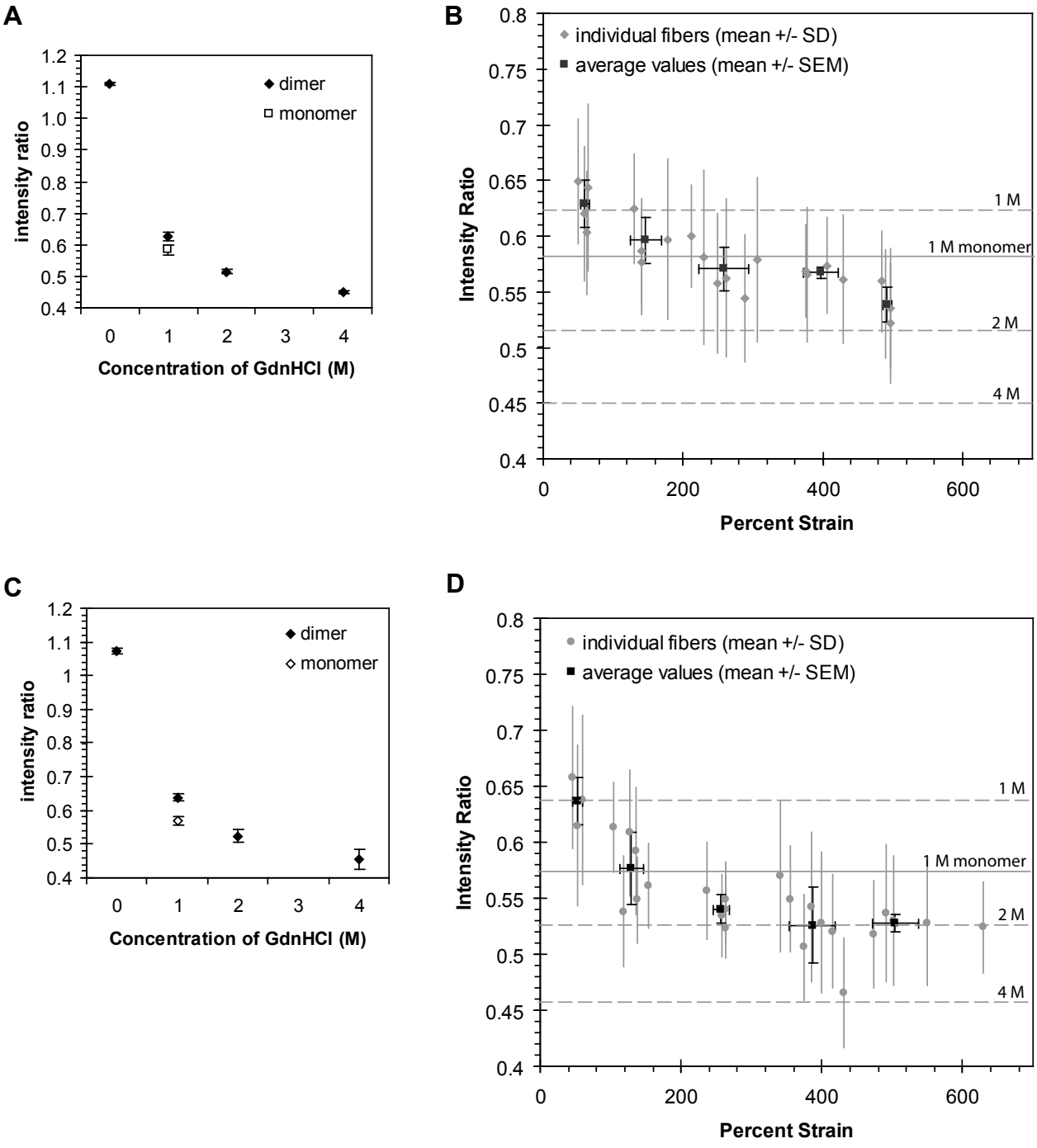


Figure S2: FRET intensity ratio distributions for scaffold fibers and new matrix fibers after reseeding.

Intensity ratio histograms and percent unfolding plots for scaffold fibers (A-C) and new matrix fibers (D-G) at 18 h (red) and 48 h (green) after reseeding. The histogram data and percent unfolding values are represented as described in Figure 2. The percent unfolding values are identical to those shown in Fig. 3I and are reproduced here in order to indicate the pertinent statistical differences. Stars and brackets indicate $p < 0.05$. Number of independent experiments in C are (from left to right) 5, 3, 3, 2, 3, 2; in G, 3, 3, 3, 4, 2, 3.

Supplemental Figure 2

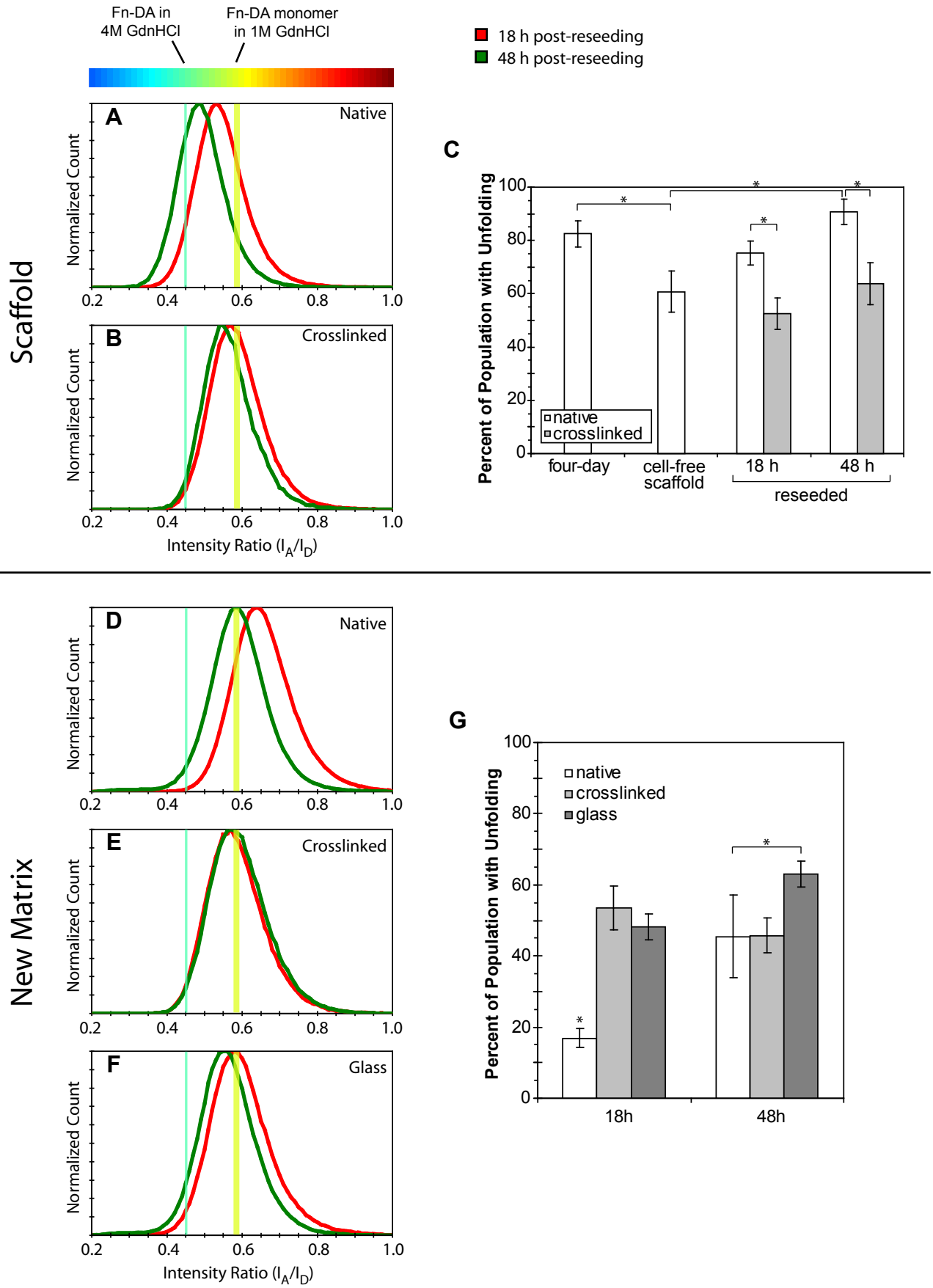
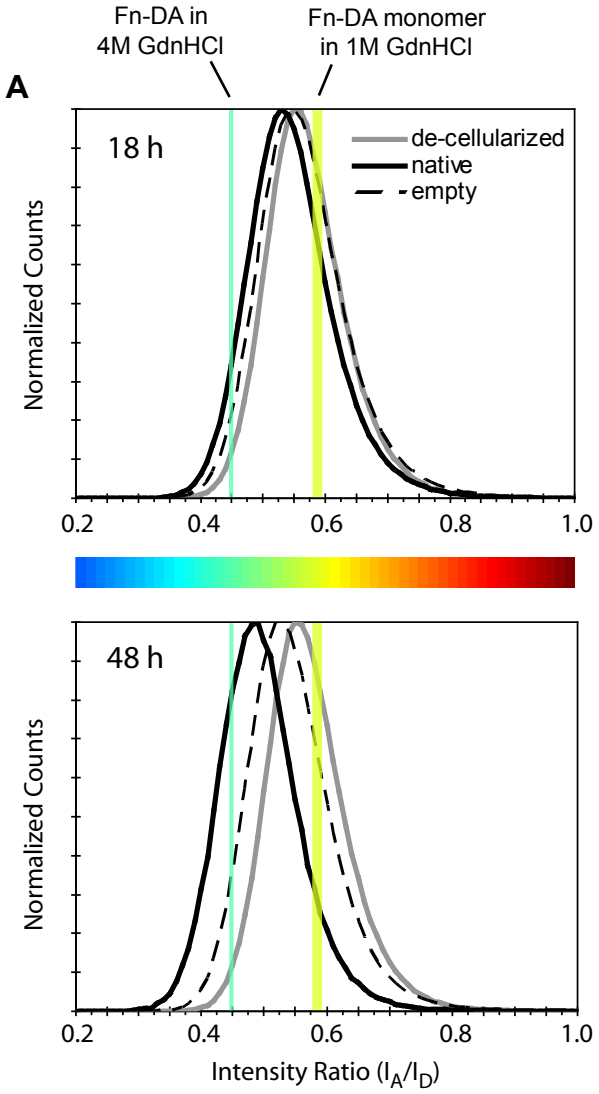


Figure S3: Intensity ratios of un-seeded scaffolds decrease slightly over time.

The intensity ratio of de-cellularized scaffolds was found to decrease slightly over time if incubated under conditions identical to those of reseeded scaffolds, but without cells. The intensity ratios of native cell-derived scaffolds with (native) and without (empty) cells were measured at 18 and 48 h. (A) Histograms compiled from three random regions in three separate experiments. The compiled histogram from de-cellularized scaffold is also shown for comparison. The normally reseeded scaffold (native; solid black line) and de-cellularized (solid gray line) histograms are the same as in Fig. S2. (B) Percent unfolding is plotted as in Fig. 2. The bracket and stars indicate $p < 0.05$ ($N = 3$ for all samples). Although percent unfolding of the empty scaffold increases over time, it is not significantly different from the freshly de-cellularized scaffold. The percent unfolding of the reseeded scaffold is significantly higher than the de-cellularized scaffold and the empty scaffold at 48 h.

Supplemental Figure S3



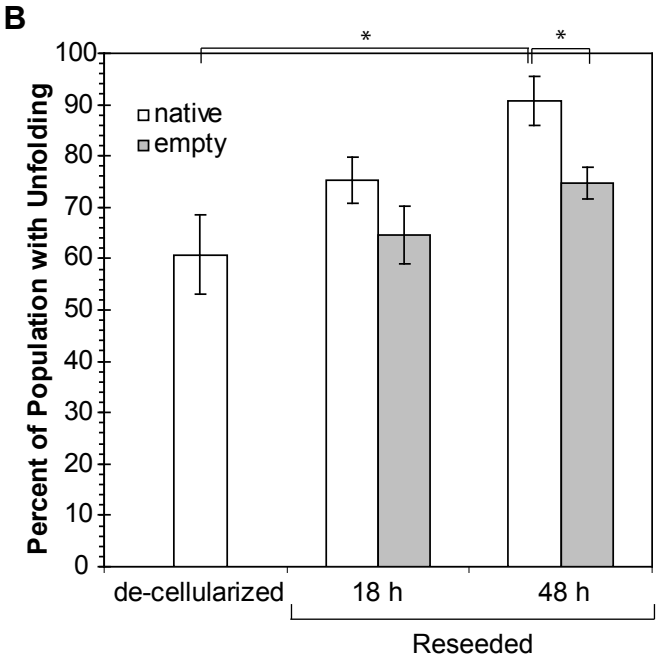
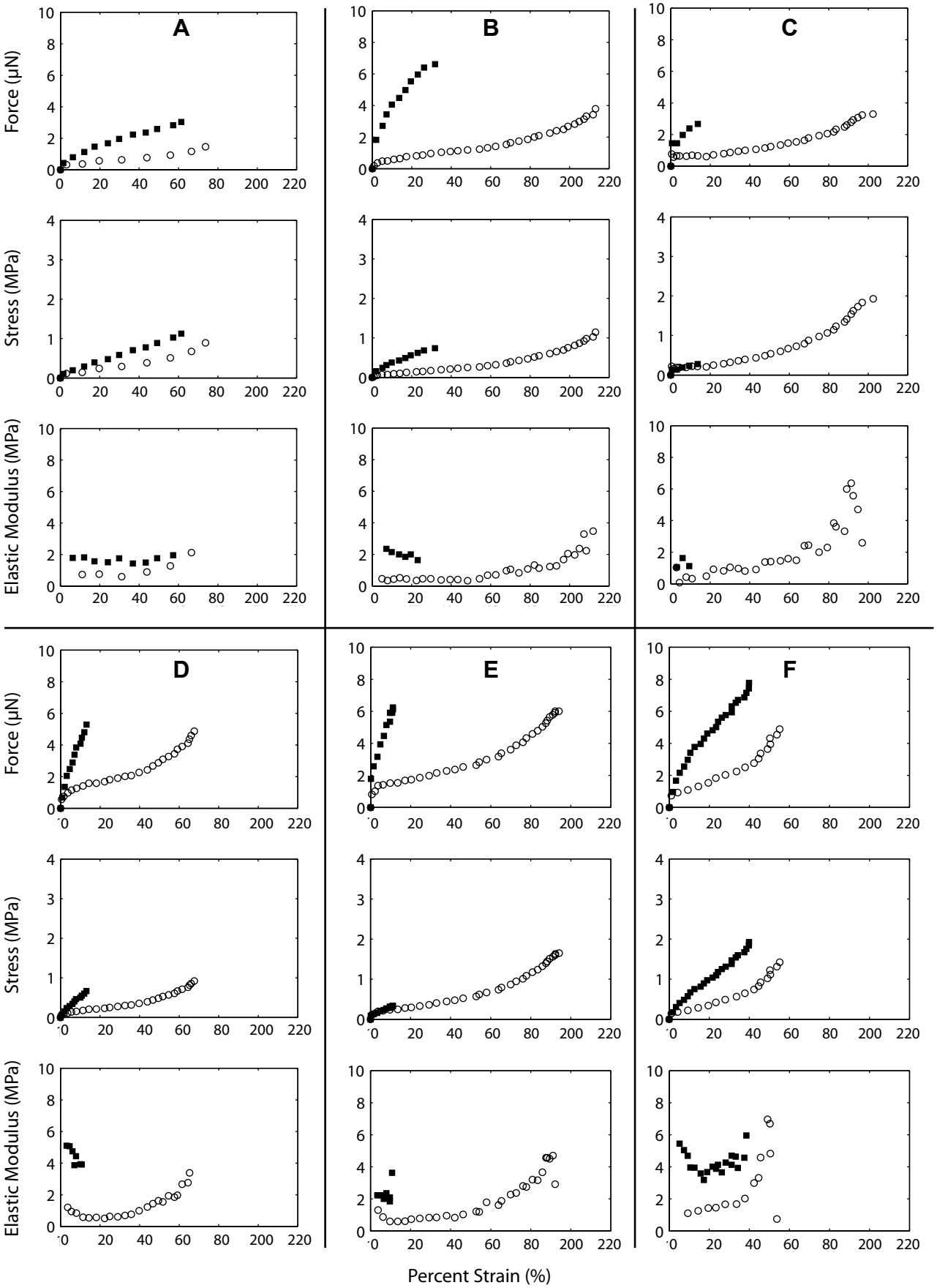


Figure S4: Chemical cross-linking increases Fn fiber rigidity – additional supporting data.

Each set of plots is from a different fiber that was measured as in Figure 5, before (open circles) and after (black squares) chemical cross-linking. See Figure 5 caption for a description of the plots. Some of the variation between the stress-strain characteristics of the fibers comes from small differences in the initial strain of each fiber. The small offsets in the stress vs. strain and force vs. strain curves are due to a residual force on the MEMS device (e.g. D and E).

Supplmenental Figure S4



Movie Descriptions

Movie 1: Cells migrating through a native scaffold: cell encountering obstructing fibers.

The left panel shows a z-projection of the fluorescently labeled scaffold; the right panel shows a z-projection of the fluorescently labeled fibroblasts. The movie corresponds to the images in Figure 1C. The cell in the center invades the scaffold, polarizes and migrates toward the bottom of the frame while pushing obstructing fibers out of the way. See the caption for Figure 1C for a longer description. This movie plays at six frames-per-second; 1 s of movie time equals 2 h of real time.

Movie 2: Cells migrating through a native scaffold: cell migrating along aligned fibers.

The cell just outside of the top of the frame, a little left of center, migrates into the frame along a set of parallel fibers without causing much fiber rearrangement. This movie plays at six frames-per-second; 1 s of movie time equals 2 h of real time.

Movie 3: Cells migrating through a crosslinked scaffold.

This movie shows cells migrating through a crosslinked scaffold. Note that the extent of the fiber movements is greatly reduced relative to those seen in Movies 1 and 2. Note also that the cells generally move slower, although one very fast cell is also observed (enters and leaves image on right side). Images in this experiment were taken every 30 min. instead of every 20 min. as in Movies 1 and 2. Therefore, to enable comparison between the movies, the frame rate of this movie (four frames-per-second) was adjusted so that 1 s of movie time equaled 2 h of real time.