Supplemental Figure 1. Steps for quantitative assessment of cell clustering. Images of cell nuclei labeled with propidium iodide from corneal fibroblasts plated on top of fibrillar collagen matrices were acquired. Using the “Find Maxima” process in image J, point selections were automatically made at the center of each cell nucleus and were subsequently converted to a binary mask. Then distances between points were computed to produce a map of connected components, an adjacency list, and a distance matrix. Distribution of the nearest nearest-neighbor distances were calculated from distance matrices, while cluster sizes were extracted from adjacency lists.

Supplemental Movie 1. Fibroblast motility on top of 3-D collagen matrix. Time lapse DIC imaging (20 minute intervals) of cells cultured in PDGF. Cells moved randomly and did not form stable clusters Total time is 20 hours.

Supplemental Movie 2. Fibroblast motility on top of 3-D collagen matrix. Time lapse DIC imaging (20 minute intervals) of cells in PDGF, immediately following the addition of thrombin.
Following addition of thrombin, cells gradually moved toward each other to form clusters. Total time is 14 hours.

**Supplemental Movie 3. Fibroblast motility on top of 3-D collagen matrix.** Time lapse DIC imaging (20 minute intervals) of cells in PDGF+thrombin after the addition of Y-27632. Following addition of Y-27632, cells that were grouped began to separate and move apart, suggesting that Rho kinase-dependent contractile forces are necessary to maintain corneal fibroblast clusters in response to thrombin. Total time is 10 hours.

**Supplemental Movie 4. Fibroblast migration through collagen ECM.** Time lapse phase contrast imaging (10 minute intervals) of corneal fibroblast migration in a nested collagen matrix, in media containing PDGF+thrombin. Once cells escaped from the inner matrix, they moved in a random walk pattern and neither stable interactions nor grouping of cells were observed. Total time is 29 hours.

**Supplemental Movie 5. Fibroblast migration through fibrin ECM.** Time lapse phase contrast imaging (10 minute intervals) of corneal fibroblast migration in media containing PDGF+Y-27632. Corneal fibroblasts migrating into 3-D fibrin matrices moved into the outer fibrin ECM while maintaining connection to cells in the inner matrix at the rear. Other cells followed behind these cells along the same paths, producing lines of interconnected cells. As migration into fibrin proceeded, adjacent cells having lateral protrusions became interconnected, resulting in the formation of a mesh-like structure Total time is 26 hours.