

Supplementary

Fig S1 - Co-culture with HMF cells increased the invasion of MCF-DCIS cells. MCF-DCIS cells were pre-labeled with green cell tracker dye (C) and both MCF-DCIS cells and HMF cells were stained with Hoechst 33342 (H) after 36hr incubation in invasion chambers. C represents the number of MCF-DCIS cells and H represents both MCF-DCIS and HMF cells.

Fig S2 - Collagen I or Matrigel alone did not support both MCF-DCIS growth and fibroblast survival. MCF-DCIS cells cultured in collagen I (1.3mg/ml) did not grow in 3D cluster for 7 days, and HMF cells cultured in 50% matrigel did not express stretched fibers during 7 days. Scale bar, 60 μ m.

Fig S3 - Variation in distance between MCF-DCIS cells and HMF cells in microfluidic channels. Three input/one output ports channels were used to provide 0 and 0.5 mm gap distance, and four input/one output ports channels were used to provide 0.75 and 1.5 mm gap distance. Two different configurations of 0.75 mm distance were prepared to examine cell density influence. The numbers in each figure represent gap distance. Scale bar is 0.6 mm.

Fig S4 - Variation in distance between MCF-DCIS cells and HMF cells in conventional transwells and 48 wells. (a) A liquid spacer (culture media, \sim 0.8mm) separates gels containing MCF-DCIS (bottom) and HMF (top). Scale bar is 200 μ m. Color codes describe the condition of each layer. (b) Multiple layers of gels containing cells and media (regular, conditioned) in 48 wells. Colored bars in the left bottom corner of each image represent a specific arrangement of gels and media. Dotted line indicates cell

clusters showing protrusive activity. Conditioned media was collected from HMF culture. Scale bar is 200 μm .

Fig S5 - SHG image of mixed co-culture. HMFs change collagen architecture greatly, making it challenging to separate collagen structure associated to MCF-DCIS cells only.

Fig S6 - Inhibited migration of HMFs into MCF-DCIS compartment. HMFs loaded next to blank (BK) gel compartment show trans-migration after 4 days culture, while fibroblasts loaded next to MCF-DCIS compartment do not show migration. HMFs and MCF-DCIS cells are stained with phalloidin for F-actin.

Fig S7 - Different characteristics of collagen rearrangement associated with MCF-DCIS transition and with cell-to-cell contractile force. Dotted circles indicate the area influenced by the two events. Collagen rearrangement by cell-to-cell contractile force is relatively continuous and homogeneous between two cells/clusters while rearrangement at the invasive front is discontinuous and concentrated around MCF-DCIS clusters.

Fig. S8 - Collagen fiber arrangement around MCF-DCIS clusters. (a,b) Collagen fibers and cells at 24hrs after loading showed random collagen arrangement around single cells. Collagen fibers (c) after 12 days culture at interface showed aligned arrangement along the invasive cell cluster (d), while the fibers at control (e) showed radial arrangement around non-invasive cell clusters (f). Scale bar, 30 μm .

Fig. S9 - SHG intensity calibration curve for both pure collagen I (1-4 mg/ml) and mixed matrix (final collagen I concentration 0.8-2 mg/ml).