Transmigration of cancer cells across blood/lymph vessel endothelium requires coordinated tumor-endothelial crosstalk. In doing so, mechanochemical signaling is utilized to direct cellular cytoskeletal rearrangement. Due to a lack of combined experimental and theoretical models, the mechanisms underlying physical interactions are difficult to observe.

We demonstrate that an engineered 3D in vitro endothelial-epithelial co-culture system can be used to isolate molecular and physical tumor-endothelial interactions in a platform that is easily modeled, quantified, and probed for experimental investigation. Using this platform along with a mathematical model, we show that metastatic cells display unique behavior with the endothelium, exhibiting a marked increase in association with the endothelium in addition to elongation of the metastatic cell in comparison to normal breast epithelium. We establish energetic favorability for deformation of the epithelial cells prior to breeching endothelial junctions, expending less energy as compared to the undeformed state. Finally, we demonstrate pharmacological inhibition of the cytoskeleton, leading to disruption of the metastatic cells’ ability to elongate/interact with endothelium, resulting in a less invasive phenotype.

**Figure 1.** (A) Range of invasive potential: normal epithelial cells (left); tumorigenic epithelial cells (center), maintaining rounded phenotype, forming characteristic mammospheres near the vessel; metastatic epithelial cells (right) elongating and aligning with the vessel. (B-D) Fluorescent microscopy and (E-F) SEM images corresponding to the range of invasive potential above. Normal cells (B) maintain characteristic round shape in the presence of the endothelium and do not have a high affinity for directly contacting vessels, nor do they aggregate as much as (C,E) tumorigenic cells, or elongate like (D,F) metastatic cells. Endothelial tubes are outlined with dotted lines in SEM images. (G) Association and Elongation parameters for primary (pink), tumorigenic (green), and metastatic (blue) cells quantify the degree of epithelial-endothelial interaction. Metastatic cells exhibited increased association with and elongation along the endothelial tubes compared to other cell types.