Supplementary Figure Captions

Figure S1: Fabrication of tumor-on-chip device. Generation of polyacrylamide (PAm) hydrogel tethered rectangular and circular glass coverslips (A), which is integrated with PDMS layer prepared on a silicon wafer with flow channels (B). UV Ozone treatment of B (C) followed by alignment and bonding (D).

Figure S2: Endothelial layer characterization. MCF7-laden bi-layer hydrogels with endothelial layer at the periphery stained for nuclei (blue), F-Actin (green), VE-Cadherin (Magenta), and CD31 (Red). The merged image shows the overlay of the four channels. Scale bar: 200 μm.

Figure S3: Mechanical characterization of GelMA hydrogels. Representative stress-strain curves of 7, 8.5, and 10% wt/v GelMA hydrogels under compressive loading (A) and the corresponding Young's Modulus (B).

Figure S4: Co-culture of cancer cells, monocytes, and endothelial cells within a single hydrogel layer. (A) XZ confocal sections of cylindrical green fluorescent beads-laden GelMA hydrogel sandwiched between two PAm hydrogels containing Magenta fluorescent beads. XY confocal sections at vertical positions Z1 and Z3 show the interface between the hydrogels and Z2 show the circular cross section of the GelMA structure. Horizontal and Vertical scale bars: 200 μm and 20 μm, respectively. Brightfield and fluorescent images of GelMA hydrogels containing a co-culture of endothelial cells and monocytes (B) or cancer spheroids, monocytes, and endothelial cells (C) immediately after encapsulation, Day 0, and after two days of culture, Day 2. Scale bar: 200 μm. (D) Monocyte density within the GelMA hydrogels as a function of culture

time. (E) Distribution of monocytes within the GelMA hydrogels after two days of culture in the absence (white) and presence (shaded) of a cancer spheroid. The number of cells within each zone, Z1 through Z4, is normalized to the total number of monocytes within the entire hydrogel. Cancer spheroids, monocytes, and endothelial cells are denoted by CS, Mo, and E, respectively. * and ** indicate statistically significant differences with $P < 0.05$ and 0.01 between samples designated by • and ➔.

Figure S5: MCF7 cultures involving dispersed cells. Bright field image of the cell-laden hydrogels after 2 days of culture. Magnified images show cell aggregates of 3-5 cells. Scale bar: 50 μm.

Figure S6: TALL-104 cell distribution within the bi-layer GelMA hydrogels loaded with MCF-7 cancer cells and monocytes. Fraction of TALL-104 cells residing within each annular region, shown in the inset, for hydrogels containing CS + Mo, CS, DisC + Mo, DisC, and Mo at (A) Day$_T$ 0, (B) Day$_T$ 1, and (C) Day$_T$ 2.

Figure S7: TALL-104 cell infiltration into bi-layer GelMA hydrogels laden with cancer cells (MDA-MB-231), monocytes (THP-1), and endothelial cells. (A) Brightfield and fluorescent images of bi-layer hydrogels containing CS, DisC, CS+Mo, or DisC+Mo two days post TALL cell infiltration (Day$_T$ 2). The fluorescent images contain monocytes and TALL cells labeled with green and red fluorescent dye, respectively. Scale bar: 200 μm.

Supplementary Movie 1: 3D Rendering of cancer spheroids and monocytes encapsulated within GelMA hydrogel. 3D rendering of a confocal Z-stack image of a cancer spheroid and monocytes within the interior of the bi-layer cylindrical hydrogel after 4 days of culture. The image is rotated about the y-axis and the cancer spheroid and monocytes are fluorescently labeled as green and red, respectively.

Supplementary Movie 2: 3D Rendering of dispersed cancer cells and monocytes encapsulated within GelMA hydrogel. 3D rendering of a confocal Z-stack image of dispersed cancer cells and monocytes within the interior of the bi-layer cylindrical hydrogel immediately after encapsulation. The image is rotated about the y-axis and the cancer cells and monocytes are fluorescently labeled as green and red, respectively.