Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

Culture Configurations Protocols

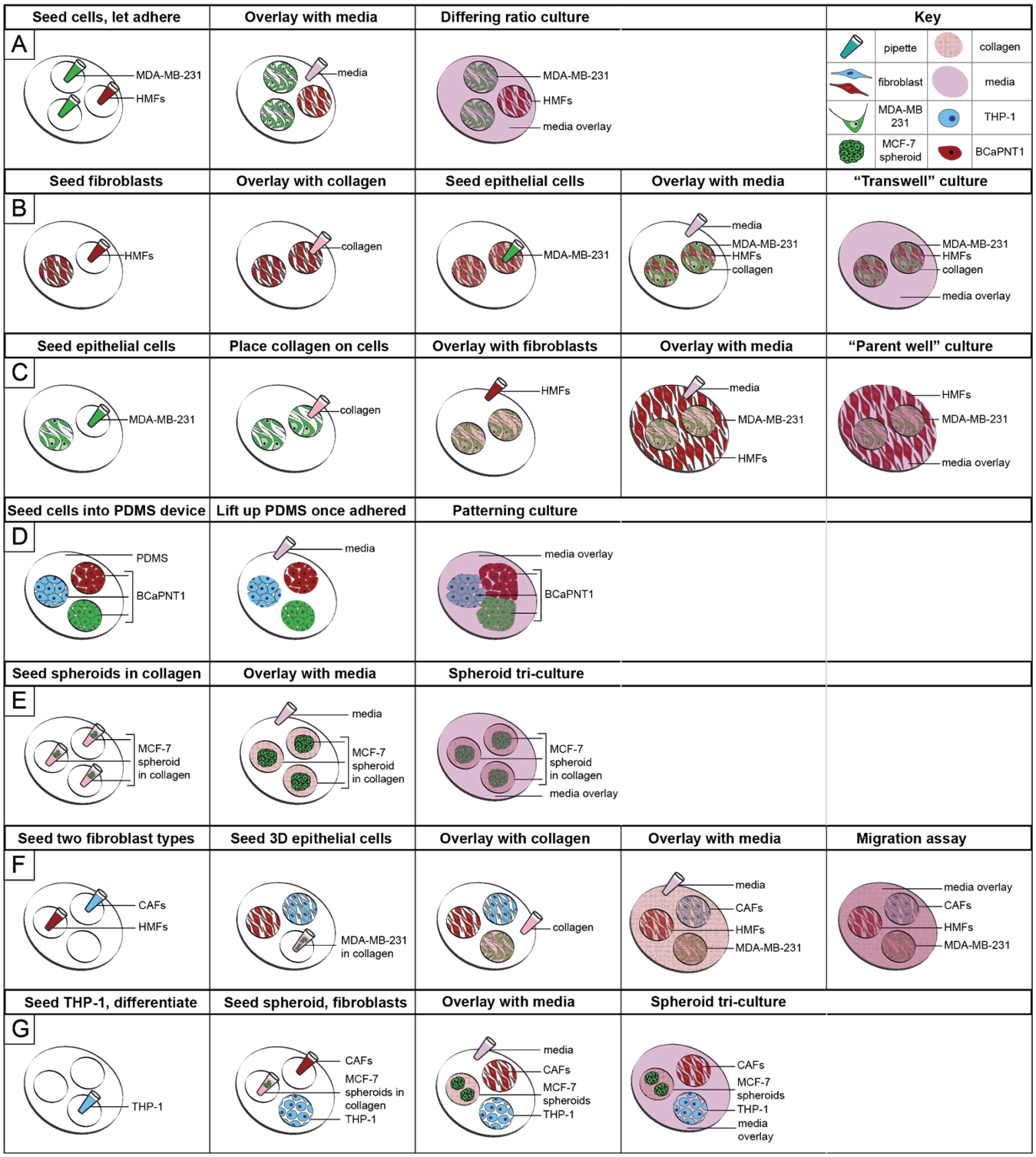

Figure S1 - Illustrated protocols for each culture configuration. Operation of the multi-culture device is performed with a standard micropipette. Steps (e.g., filling, seeding, and overlay) for preparing each culture configuration are illustrated. See **Movie S1 - Filing demonstration video caption**. Operation of the multi-culture device is performed with a standard micropipette. Steps for preparing each culture configuration is illustrated and listed below by subfigure.

Fig S1A - MDA-MB-231s expressing GFP and HMFs stained with Hoechst were seeded into a 3-well device at a ratio of 2:1 MDA-MB-231 wells to HMF wells. After allowing the cells to adhere for 16 hrs, 50 μL of DMEM media was used to overlay the device.

Fig S1B - HMFs stained with Hoechst were seeded into the culture region of a 2-well device and adhered for 16 hrs. The media in the culture regions was aspirated and 5 μL of 3 mg/mL collagen was pipetted on top of each culture well. Once the collagen had polymerized after 5 min in a 37 °C incubator, 5 μL of MDA-MB-231s was placed onto each culture well. After 16 hrs incubation, the device was overlaid with 50 μL of warm DMEM media.

Fig S1C - MDA-MB-231s were seeded into the culture regions and adhered for 16 hrs. Next, the media in the culture wells was aspirated and 10 μL of 3 mg/mL collagen was placed on top of each culture well. Following polymerization of the collagen for 10 min in a 37°C incubator, the culture wells were overlaid with 50 μL of HMFs that were stained with Hoechst.

Fig S1D - Prior to cell culture, 3-well devices made by razor-printing a PDMS sheet, were soaked in 70% ethyl alcohol overnight, placed in in a 24-well plate and UV-sterilized. Before seeding, three populations of BCaPNT1 cells were stained independently with Hoechst, CellTracker TM Red (C34552, Thermo Fisher) or Calcein AM (C3100MP, Life Technologies) for 20 min and then 5 μL of each cell population was added to respective culture wells. After 12 hrs of culture at 37°C, the PDMS template was removed from the device and the cells were imaged. The device was imaged again following another 24 hrs of culture.

Fig S1E - The spheroids of MCF-7-eGFP cells presented were generated off-chip, resuspended in 3 mg/mL unpolymerized collagen, transferred to each culture well and subsequently allowed to polymerize in a 37°C incubator.

Fig S1F - The migration assay configuration was set up by seeding HMFs stained with Hoechst and CAFs stained with DRAQ5 into respective culture wells and allowing to adhere for 16 hrs. Next, MDA-MB-231s were resuspended in 6 mg/mL collagen and 10 μL of the collagen-cell mixture was seeded onto the third culture well of the device. The collagen was polymerized for 5 min at 23°C before overlaying the parent well with 30 μL of 6 mg/mL collagen that was polymerized for 30 min at 23°C before being placed inside the 37°C incubator for the duration of the migration assay. Devices were imaged after 48 hrs.

Fig S1G - In the spheroid tri-culture arrangement shown in Fig 2G, THP-1 cells stained with Hoechst were seeded onto the device and treated with RPMI 1640 containing 36 nM PMA (524400, Sigma-Aldrich) for 48 hrs. Spheroids of MCF-7-eGFP cells that were embedded in 3 mg/mL collagen were transferred from off-chip into one culture well. In the remaining culture well, HMFs stained with DRAQ5 were seeded and allowed to adhere for 16 hrs before overlaying the entire device with DMEM media.

Cell culture and maintenance

A monocyte cell line, THP-1 (ATCC® TIB-202™) was purchased from ATCC. MCF-7-eGFP, a mammary gland breast epithelial cells, were donated by the lab of Dr. Elaine T. Alarid (University of Wisconsin, Madison) and were created via stable transfection of MCF-7 cells with the pBABE-puro and pEGFP-N1 plasmids. They were selected for with 1 μg/mL puromycin (Sigma-Aldrich). The immortalized human mammary fibroblasts, RMF-621-tert cells, referred to as "HMFs" were derived from a reduction mammoplasty and donated by Dr. Lisa Arndt's lab (University of Wisconsin, Madison). BCaPNT1 were donated from Dr. William Ricke's lab (University of Wisconsin, Madison). Breast cancer associated fibroblasts, (CAFs) were gifted to us from Dr. Andreas Friedl's lab (University of Wisconsin, Madison).

MDA-MB-231-GFP, MCF-7-eGFP, HMFs and CAFs were maintained in DMEM high glucose media with L-Glutamine (D5796, Sigma-Aldrich) supplemented with 10% heat inactivated fetal bovine serum (F6765, Sigma-Aldrich) and 1% penicillin/streptomycin (P4333, Sigma-Aldrich). THP-1 and BCaPNT1 cells were cultured up to passage 10 in RPMI 1640 Medium (Gibco, 22400089) supplemented with 10% heat inactivated fetal bovine serum, 2% glutamax (35050-061, Gibco) and 1% penicillin/streptomycin P/S (15140-122, Gibco).

Seeding different device configurations

The open device design used is flexible, making it possible to adapt the culture layout in terms of types of cells, matrices and endpoints (Fig 3). Throughout configurational experiments, cell seeding densities were kept at 400,000 cells/mL. Culture areas were seeded consistently with 5 μL of cell volume resulting in 2,000-4,000 cells/culture region. Cells were

left to adhere in the culture area for a minimum of 16 hrs in a cell culture incubator. After which, the media from the culture regions was manually aspirated by micropipette in order to remove any non-adherent cells while avoiding inadvertent joining of droplets or disruption of cellular monolayers.

Collagen preparation

High concentration (8-11 mg/ml) rat tail collagen I (Corning Life Sciences, 354249) was neutralized and corrected to a pH of 7.4 using 10× PBS and 1 N sodium hydroxide (S318, Fisher Scientific) on ice. Final gel concentrations were either 6 or 3 mg/mL and were achieved by diluting with DMEM high glucose media.

Spheroid Preparation

The hanging drop method with methylcellulose was used for mass generating spheroids off-chip for later multi-cell-type culture in devices. Briefly, 6 grams of high viscosity methyl cellulose (4000 cP, Sigma m-0512) was dissolved into 500 mL of warmed RPMI 1640 basal media (Gibco, 22400089) and centrifuged at 5000g for 2 hrs, after which the clear supernatant was removed for use. MCF-7-eGFP cells at 1e5 cells/mL were mixed 1:4 (v/v) with the methyl cellulose solution. Then, 25 µL droplets of this mixture were placed on the lid of a petri dish, sterile water was put within the dish and the entire petri dish incubated in a 37 °C incubator. After two days of culture in hanging drop, spheroids were transferred to the open culture device.

Staining and Imaging

All cells were either stained off-chip with nuclear dyes before seeding or expressed GFP. Pre-staining was done to pictorially demonstrate the individual nature of the separate culture areas and to visualize the placement of the cells. HMFs, CAFs and THP-1s were either stained with Hoechst at 10 mg/mL (H3570, Thermo Fisher) or DRAQ5 (62251, Thermo Fisher) at 1:10000 and were washed once with DMEM media before seeding. The other cell types, MDA-MB-231s and MCF-7s expressed GFP. Following culture, devices were imaged on a Nikon Ti microscope with NIS Elements software. Image processing was done using JEX. Images were contrast enhanced, in order to see cellular patterns clearly.

COMSOL modeling of closed multi-culture device

Modeling results for this device are obtained from a previous publication.[27] The device geometry and potential configurations are illustrated here to aid visualization.

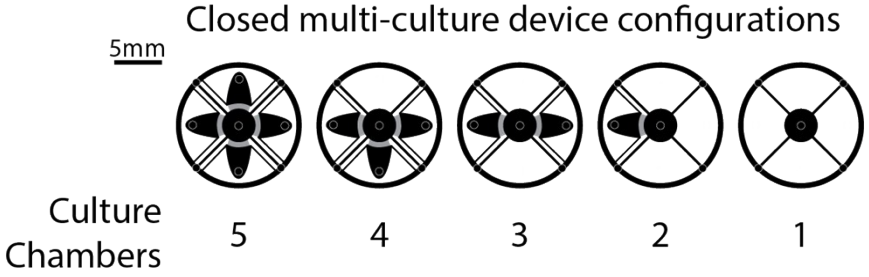

Figure S2 - Closed multi-culture device. The center black region and each black lobe represent a culture region. Between the lobes and the center region is a diffusion port (gray) that allows diffusion directly from one region to another. The number of lobes are varied according the the number or ratio of cell types to be studied. The straight lines and out ring represent microchannels used for seeding the device and replacing culture media. Each culture region has a small port for seeding and treatments (white circles).

COMSOL modeling of 2-region nested co-culture device

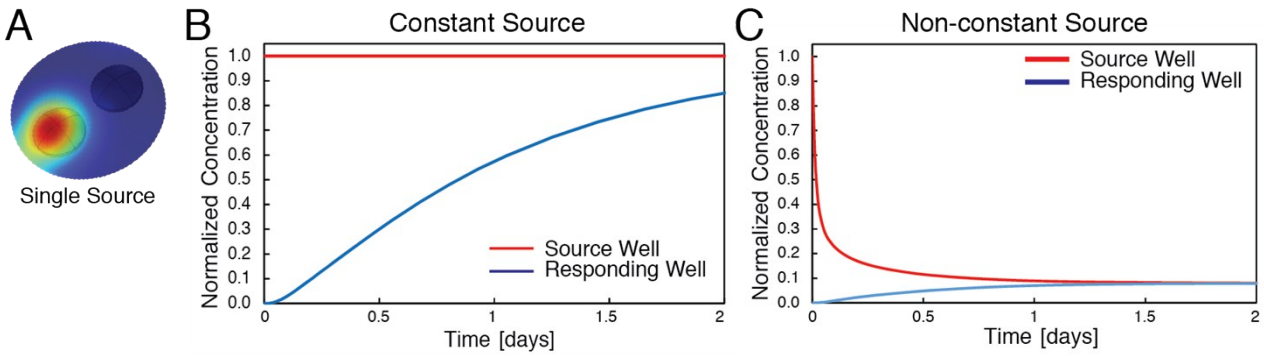

Figure S3 - Comsol simulation results for the 2-region co-culture device.

Movie S2.mov - Animation of simulation results for a constant source over the course of 48 hrs.

Movie S3.mov - Animation of simulation results for a non-constant source over the course of 48 hrs.

Confirmation of Shh Activity

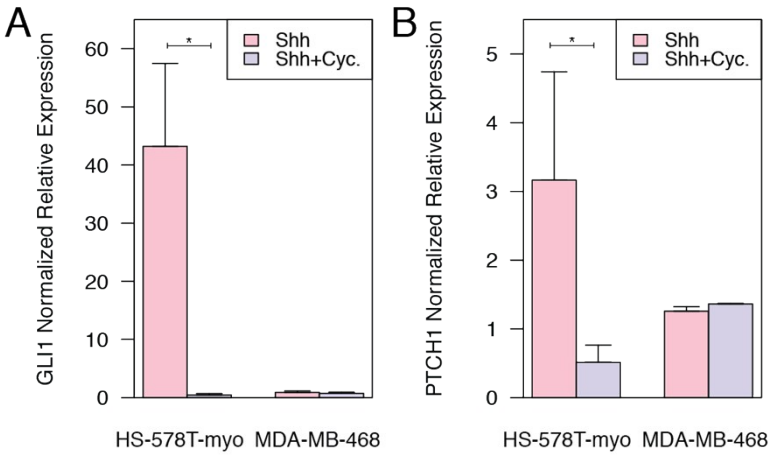

Figure S4. (A-B) Up-regulation and pharmacological inhibition (5 μM cyclopamine) of Hedgehog target genes GLI1 and PTCH1 confirms active Hedgehog signaling in HS-578T-myo during co-culture while levels of GLI1 and PTCH1 were not significantly affected by Shh or cyclopamine in MDA-MB-468. Student t-test was done to determine statistical significance based on p-value<0.05.

Separability of endpoints

To further support results of Fig 5 and specifically demonstrate that cells can be separately extracted from adjacent culture wells after co-culture with minimal cross-over of cell material, we quantified human GLI1 (hGLI1) and mouse GLI1 (mGLI1) using species-specific primers from adjacent culture wells containing human and mouse cell lines, MCF-7 and NIH 3T3. Tab. 4 illustrates the expected pattern where gene expression was not detected in samples taken from culture wells where the species of the primer does not match the species of the cell line.

Table S1 - Separable PCR endpoints from adjacent culture wells. Expression (mean ± se, 2 independent experiments) of human and mouse GLI1 is expressed as relative to human and mouse GAPDH, respectively. PCR is done by pooling 8-10 small culture wells before PCR. (-) Indicates undetectable signal for all technical replicates at 40 cycles and demonstrates the ability to effectively analyze each cell type separately.

Gene	MCF7	NIH 3T3
mGLI1	(-)	5e-6 ± 4e-6
hGLI1	9e-6 ± 3e-6	(-)