

Heterogeneous Microchannel Assays Employing Primary Tumor Cells, Co-culture and 3D Matrices

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Introduction

There are several important differences between cellular drug discovery assays and the in vivo condition they hope to model. In vitro assays are typically monotypic, use immortalized cell lines, and are run on flat 2-dimensional plastic substrates. In contrast, cells in vivo are surrounded by other cell types, are primary cells by definition, and exist in a complex 3-dimensional extracellular matrix that provides physical support and signaling molecules. These differences are well documented to have profound effects on pharmacology.¹ We present here a microchannel array approach that enables highly parallel, primary cell, co-culture assays to be run in the presence of 3D extracellular matrices. Furthermore, these arrays are designed to allow fully automatable operation using standard liquid handling instrumentation.

Figure 1.

Microchannel Arrays used in this Study

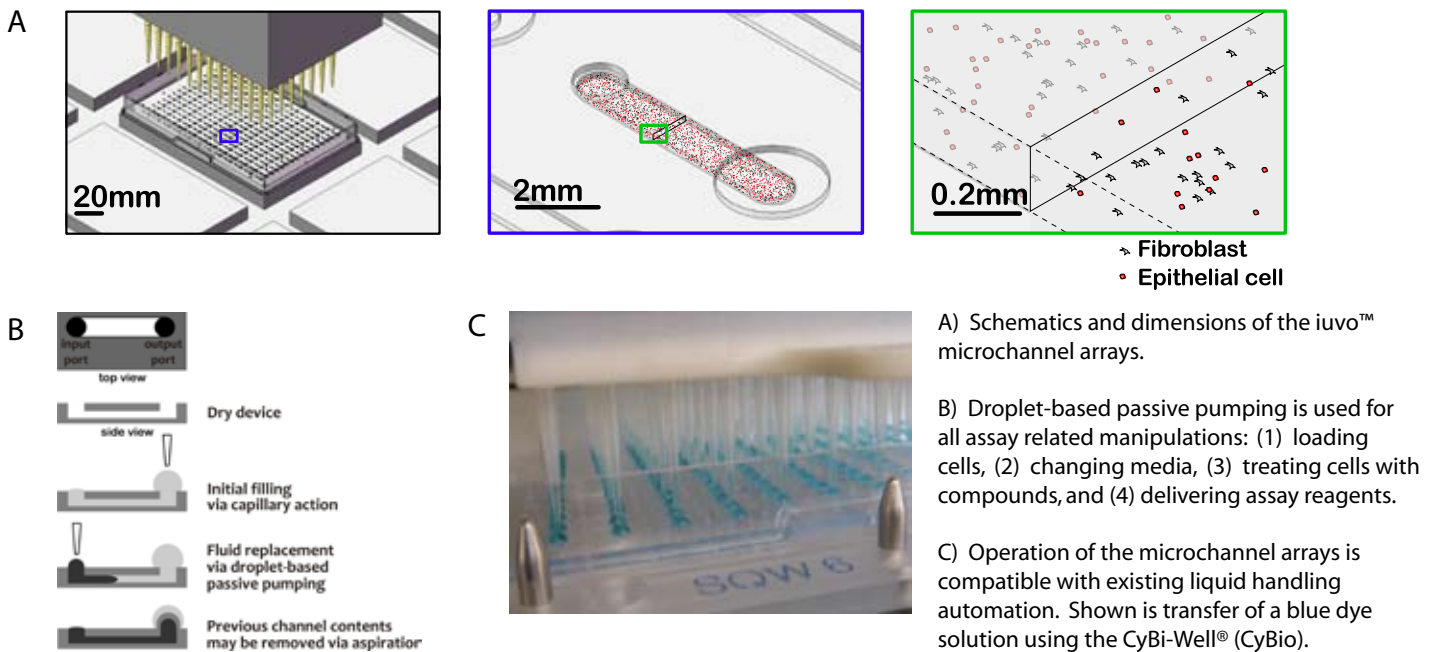
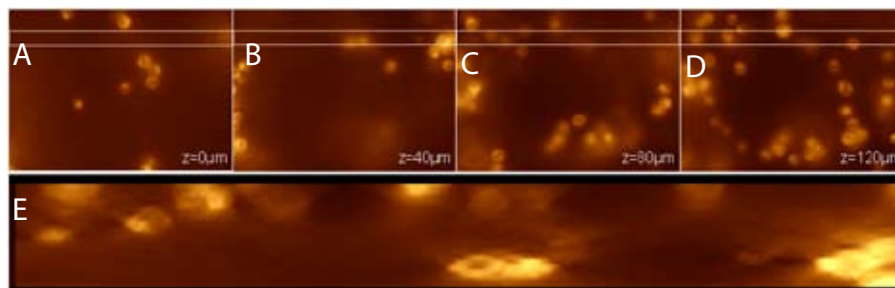


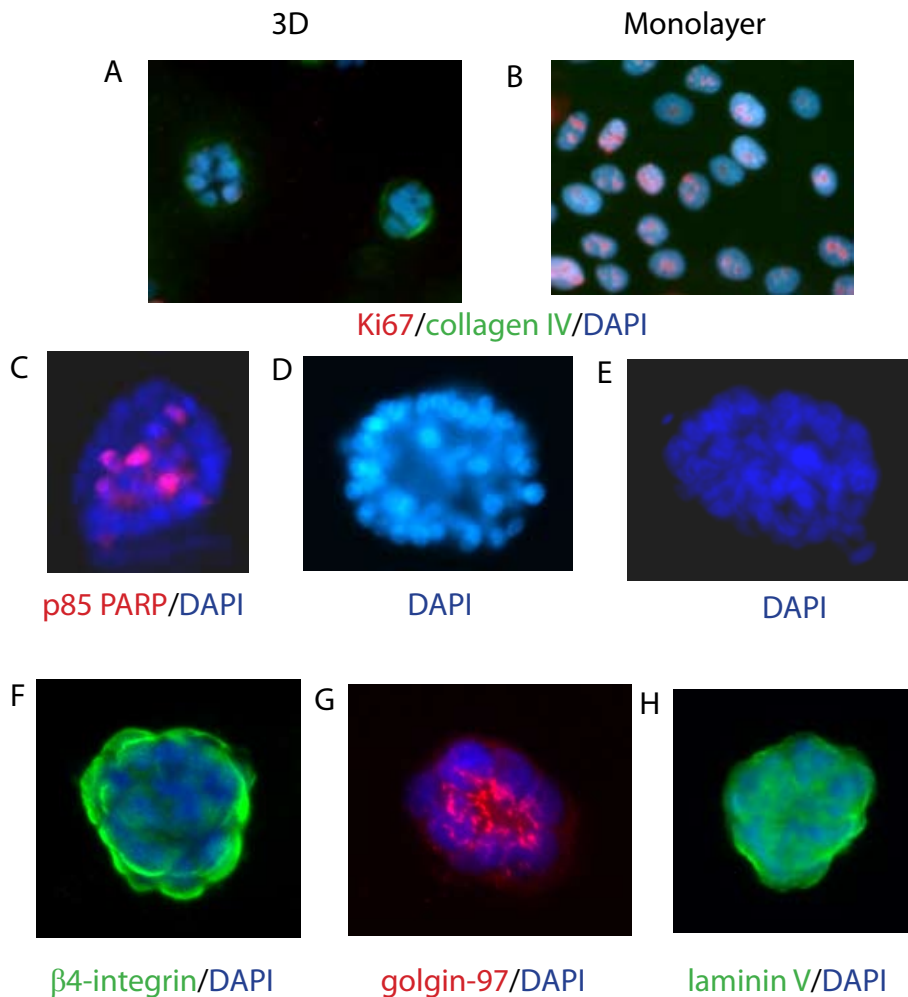
Figure 2.

3-Dimensional Distribution of Cells in ECM



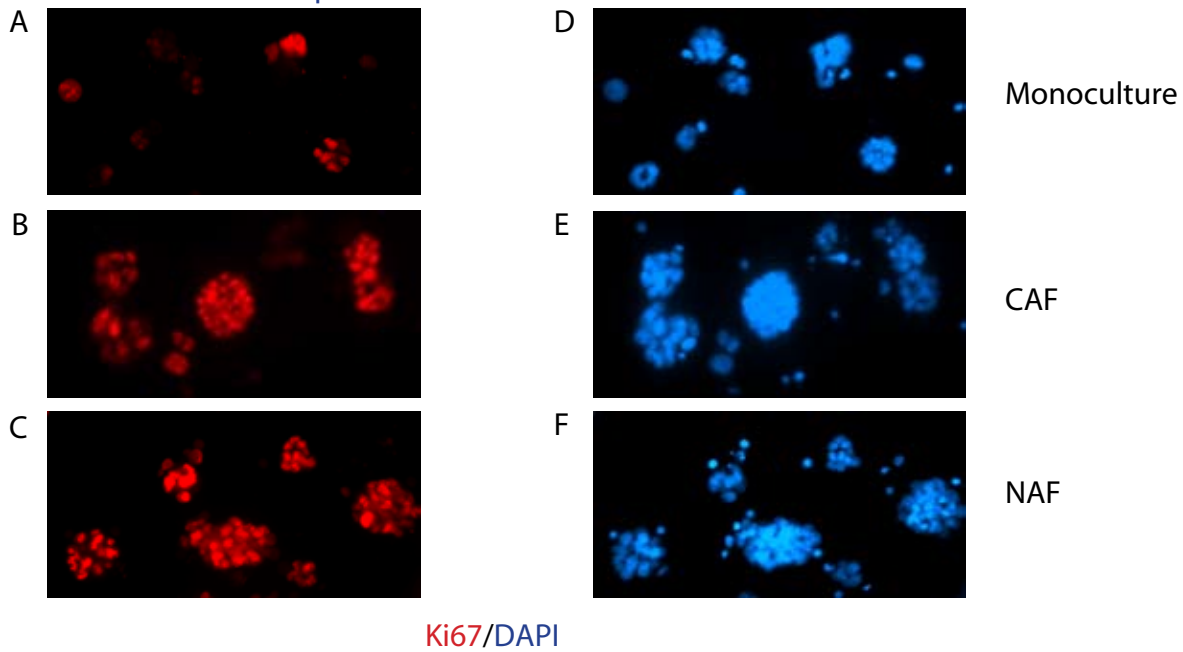
Passively pumping cells embedded in extracellular matrix results in 3D distribution of cells. Cells were labeled with an all cell stain, CellTracker Red (Molecular Probes), prior to loading into microchannels using 3D type I collagen. A-D) Fluorescence micrographs of cells embedded in collagen inside microchannels. Z axis height in microns for each image was 0 (A), 40 (B), 80 (C) and 120 (D). Different focal planes are shown from the same x,y stage coordinates. E) Side view of a channel loaded with cells embedded in 3D collagen: Images spanning the entire 137 µm channel height were cropped (white box in A-D) and subjected to 2D deconvolution using nearest neighbor analysis. The resulting images were processed for 3D reconstruction. All image analysis was performed using Metamorph software package (Molecular Devices).

Figure 3.
Acinar Morphogenesis of Mammary Epithelial Cells



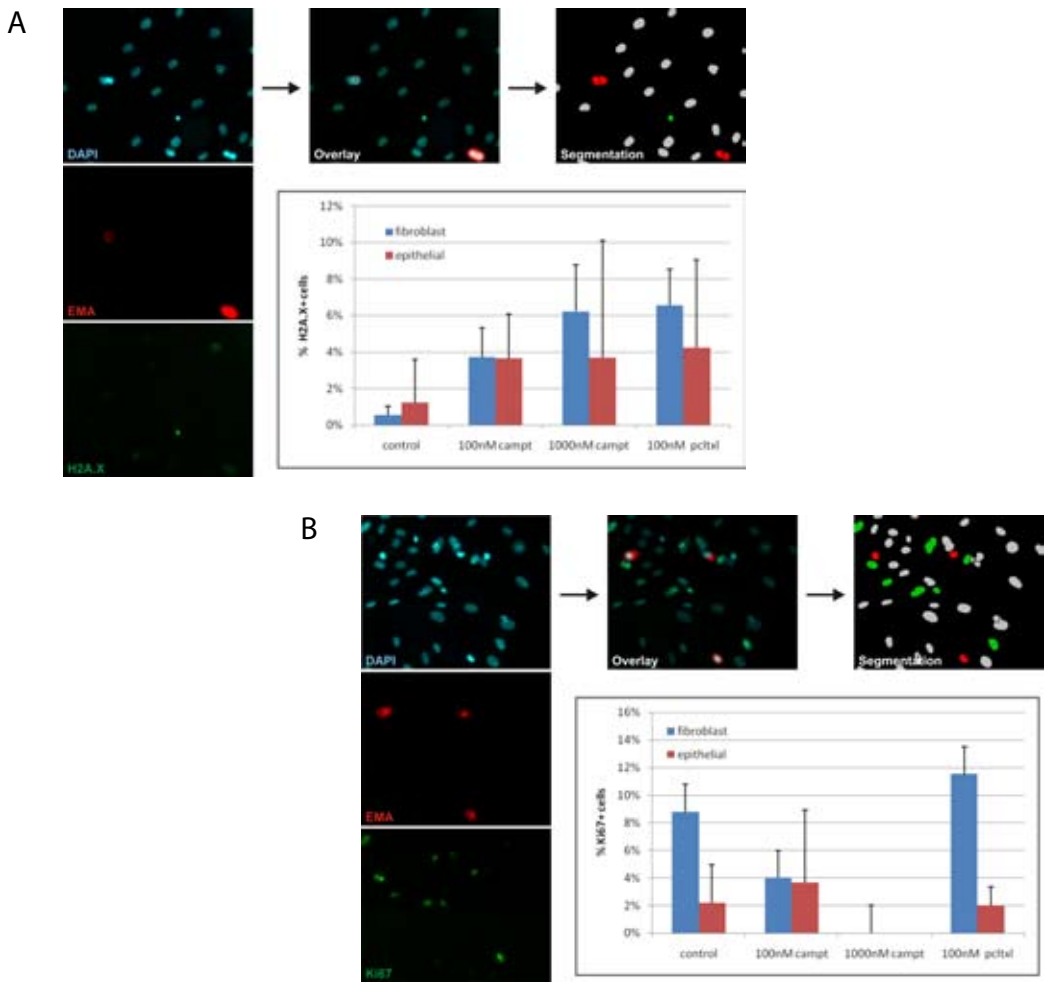
Acinar morphogenesis of mammary epithelial cells occurs normally in microchannel arrays. Mammary epithelial cells (MCF10A and HMT3522 S1) were embedded in Matrigel and loaded into microchannel arrays using droplet based passive pumping. Cells were cultured for up to 14 days. Key events associated with acinar morphogenesis were detected by immunocytochemistry. When embedded in 3D IrECM (A) cells undergo growth arrest and begin to deposit basement membrane proteins by day 6, unlike when plated in monolayer (B). MCF10A cells in 3D IrECM were loaded into microchannels. After 7 days, cells were immunostained for the proliferation associated marker Ki67; collagen IV, a component of the basement membrane; and the nuclear stain DAPI. As a control, cells were allowed to adhere to the bottom prior to addition of 3D matrix. These cells grew as a monolayer instead of the ordered clusters observed in 3D IrECM. C) Apoptosis of cells in the cluster interior occurs during morphogenesis. MCF10A cells grown as above were stained for the apoptotic marker p85 PARP (red) and DAPI (blue). D) MCF10A cells at day 18 show hollow center as viewed by DAPI staining. E) Tumorigenic T4-2 cells do not form polarized, hollow structures, and instead grow into a disordered solid mass of cells. Shown are cells at day 14 stained with DAPI. F-H) Polarity and basement membrane deposition can be detected during acinar morphogenesis. Mammary epithelial cell line HMT3522 S1 cells were cultured for 8 days in microchannels. Immunocytochemistry revealed basal polarization of β 4-integrin (F), apical localization of prosecretory granules evidenced by the trans-Golgi marker golgin97 (G), and secretion of laminin V, a basement membrane component (H). Laminin V antibody is human-specific, and does not recognize the mouse laminin ortholog present in IrECM.

Figure 4.
Co-culture of Fibroblast and Epithelial Cells



Coculture of Fibroblast and Epithelial Cells. Effect of fibroblast coculture on epithelial cells embedded in 3D matrix in microchannel devices. HMT3522 S1 cells embedded in matrigel were plated either alone (A, D); with human primary carcinoma-associated fibroblasts (CAF) (B, E); or with normal breast-associated fibroblasts (NAF) (C, F) in microchannels using passive pumping. 7 days after plating, cells were immunostained for the proliferation marker Ki67 (A-C) and counterstained with the nuclear stain DAPI (D-F).

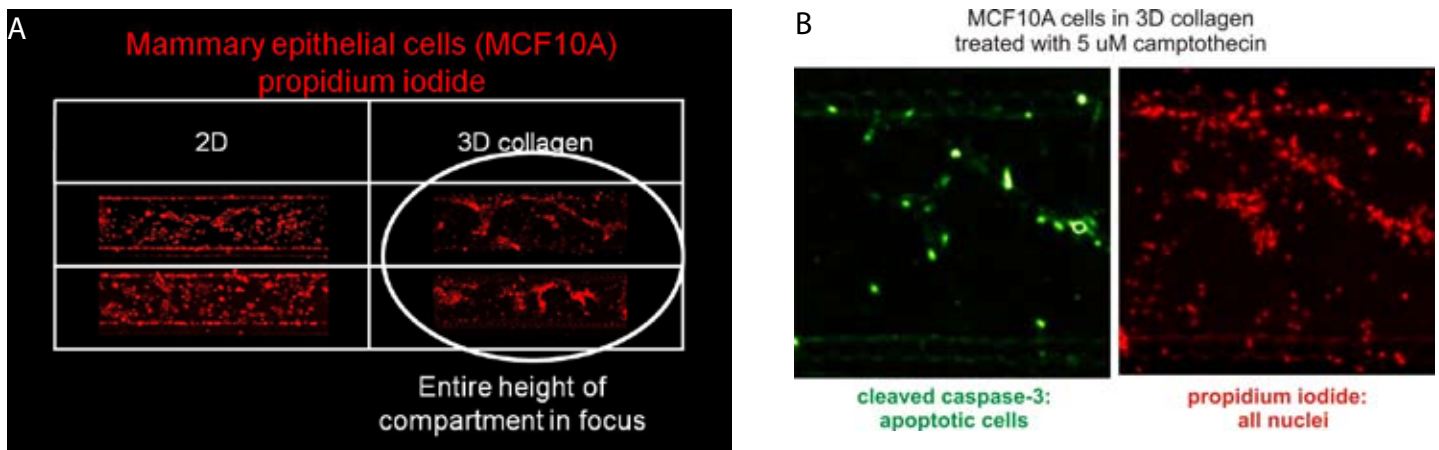
Figure 5.
Assessing DNA Damage and Proliferation using Primary Cells Cultured from Resected Tumors



Mixtures of epithelial cells and fibroblasts were seeded into microchannel arrays, and treated with various concentrations of apoptotic agents camptothecin or paclitaxel for 36 hours. Immunocytochemistry was carried out using either A) anti-H2A.X for DNA damage or B) Ki67 for proliferative status. In addition, anti-EMA antibody was included to identify epithelial cells, and DAPI as a nuclear counterstain for all nuclei. Each panel contains three images on the left edge representing each fluorescence channel. The top middle image is an overlay of all three channels, and the top right image is a segmentation based on algorithms designed to score cells based on nuclear staining, EMA, and either Ki67 or H2A.X. The chart at the bottom right shows graphically the results of triplicate analysis of various conditions. 1000 nM paclitaxel was excluded from analysis due to the extreme cell destruction observed.

Figure 6.

Imaging the Microchannel Array using the Isocyte Microplate Scanner



Imaging the microchannel array using the Isocyte Microplate Scanner (Blueshift Biotechnologies) allows rapid scanning and analysis of the entire plate, and importantly, the entire channel height is in focus. MCF10A cells were either suspended in growth media or embedded in 3D collagen and seeded into microchannel arrays using droplet-based passive pumping. Cells were subjected to various concentrations of drug treatments for 36 hours. Following treatment, immunocytochemistry was performed using Alexa488 labeled secondary antibody to detect cleaved caspase-3 antibody. Nuclei were counterstained with propidium iodide in the presence of RNAase. A) Cells in both 2D (left panels) and 3D collagen (right panels) were visualized in the propidium iodide channel using the Isocyte instrument. B) Cells in 3D collagen were visualized for cleaved caspase-3 to detect apoptosis, and also propidium iodide.

Conclusions

- Passive pumping can support cellular assays in micro-conduit arrays, operated by pipettors or standard liquid handling instrumentation.
- By keeping the height of the channel small, the entire 3D cellular compartment can be accessed by microscopy without the need for cryosectioning.
- Highly differentiated multicellular structures, such as those of mammary acinar morphogenesis can be formed in microchannels. Key features of this complex biological process were preserved, including growth arrest, apoptosis, polarization and deposition of basement membrane proteins.
- Paracrine signaling can be observed in microchannel assays. Fibroblasts were shown to delay growth arrest of epithelial cells when co-cultured in a three dimensional matrix.
- Analysis of primary fibroblast and epithelial cell cultures from breast cancer resections can be carried out in 2D and 3D collagen culture, including analysis using antibody-based staining.
- The Isocyte plate scanner is capable of rapidly scanning and analyzing the entire array of microchannels. The properties of the optics allow the entire channel height to be imaged in focus, thus avoiding the need to scan multiple heights across the Z-axis.

References

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