

MiCA-M32P APPLICATION NOTE

3D-ECM Perfusion Culture of MCF-10A Mammary Epithelial Cells

INTRODUCTION

Cells cultured in three-dimensional extracellular matrix (3D ECM) display many biologically relevant functions not found in 2D monolayers.¹ A well-studied example of the benefits of 3D ECM culture is acinus formation of human mammary epithelial cells when cultured in laminin rich 3D ECM, such as BD Matrigel.² This condition has been found to better reflect the *in vivo* cancer phenotype when compared to 2D monolayers.³ In addition, the anti-cancer drug sensitivity of cells cultured in 3D ECM has been found to be more representative of the *in vivo* response.^{4,5} The widespread use of 3D ECM culture for screening applications is limited by the tedious manual manipulations of current *in vitro* 3D ECM culture methods.

The M32P microfluidic plate enables long term, continuous perfusion 3D ECM culture in a user-friendly format. The M32P is designed according to the CellASIC MiCA specification (www.cellasic.com/MiCA), allowing multiplexed perfusion culture in a standardized 96 well format. The plate operation requires only a pipette and an incuba-

tor, allowing easy integration with existing workflows. The cells/gels are dispensed directly into the 2 mm diameter culture chamber, which is fed with continuous medium perfusion through micro-capillaries located adjacent to the cell/gel culture chamber. The plate design allows long term live cell analysis by microscopy and is fully compatible with standard optical, biochemical, and automation assays.

The operation of the M32P plate was demonstrated by culture of MCF-10A mammary epithelial cells in Matrigel. The cells exhibited 3D acinus formation in the microfluidic environment over 12 days. In contrast, MCF-7 cells developed into a mass-like structure. This is consistent with previous findings of how breast cancer cell lines respond to 3D culture. Exposure of the 3D cultures to the anti-cancer drug Paclitaxel for 72 hours indicated a differential response when the 2D and 3D cases were compared, suggesting that screening on the 3D format may provide information not available in standard 2D experiments. The flexibility of the M32P plate design allows multiplexed perfusion culture in 3D or 2D of virtually any cell type, including primary cells, isolated tissues, stem cells, and single cells.

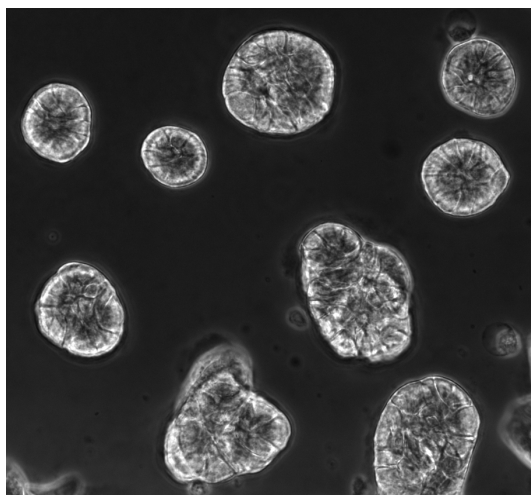


Figure 1. MCF-10A cells cultured in Matrigel within the microfluidic chamber adopt a 3D spheroidal morphology. Image taken with a 20X objective lens.

PLATE DESIGN

The layout of the M32P microfluidic array is depicted in Figure 2. There are 32 independent units tiled on a standard 96 well plate, with each flow unit occupying 3 wells. The 3 wells correspond to a flow inlet, the open-top cell culture chamber, and a flow outlet. Cells/gels are dispensed directly into the open culture chamber with a standard pipette. The 2 mm diameter open chamber is separated from the flow channels by a microfluidic perfusion barrier (Figure 3). The barrier consists of a 75x4 micron (LxH) microfluidic ring surrounding the entire culture region. This design localizes the cells and gels to the culture chamber, and prevents blocking of the flow channels. The chamber height is 1 mm, with a volume of 4 μ l. The bottom surface of the cell culture chamber is a 170 μ m thick glass slide, allowing for high quality cell imaging on an inverted microscope.

Cell loading can be performed in a number of configu-

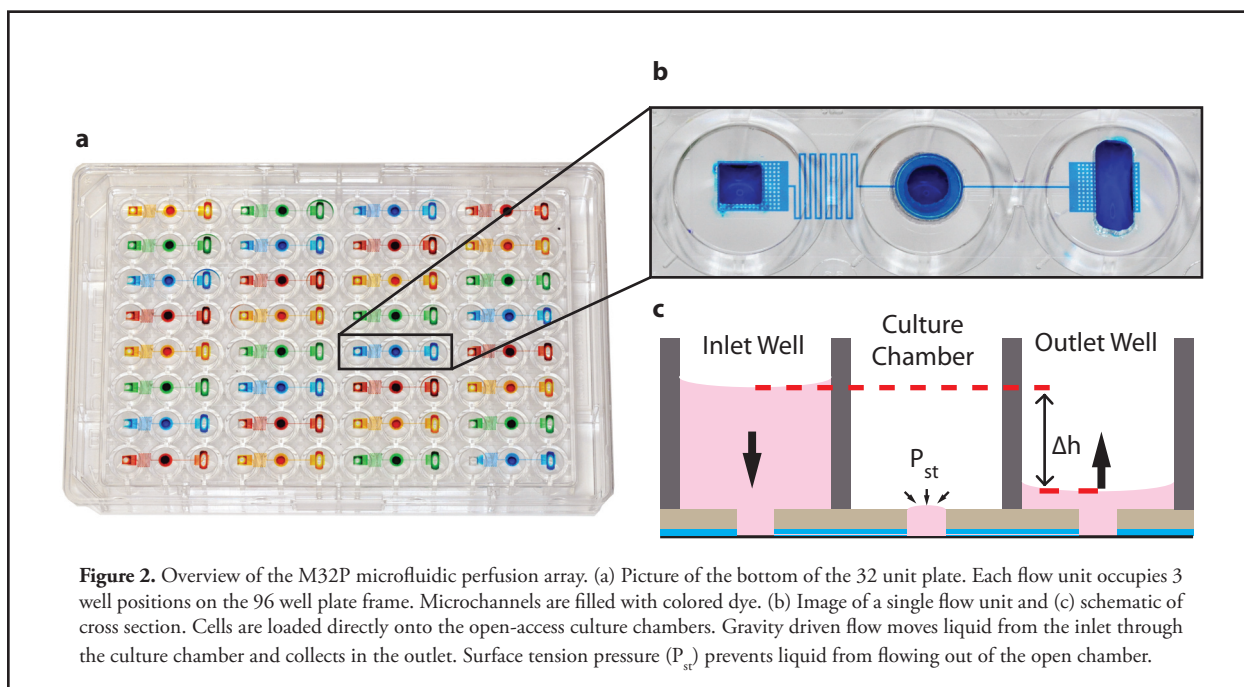


Figure 2. Overview of the M32P microfluidic perfusion array. (a) Picture of the bottom of the 32 unit plate. Each flow unit occupies 3 well positions on the 96 well plate frame. Microchannels are filled with colored dye. (b) Image of a single flow unit and (c) schematic of cross section. Cells are loaded directly onto the open-access culture chambers. Gravity driven flow moves liquid from the inlet through the culture chamber and collects in the outlet. Surface tension pressure (P_{st}) prevents liquid from flowing out of the open chamber.

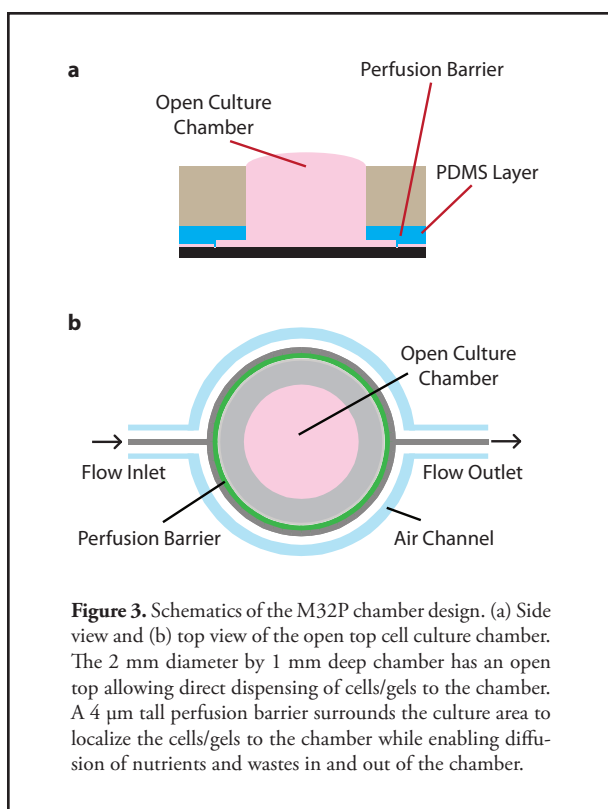


Figure 3. Schematics of the M32P chamber design. (a) Side view and (b) top view of the open top cell culture chamber. The 2 mm diameter by 1 mm deep chamber has an open top allowing direct dispensing of cells/gels to the chamber. A 4 μm tall perfusion barrier surrounds the culture area to localize the cells/gels to the chamber while enabling diffusion of nutrients and wastes in and out of the chamber.

rations, depending on the experiment. For 3D embedded culture, cells are mixed with the gel and dispensed into the chamber to be polymerized. For 2D culture, cell solution is dispensed into the chamber and allowed to attach to the glass bottom. For 2D overlay, gel is added to cells that have attached to the bottom. For sandwich culture, the bottom surface can be coated with a 2D or thin 3D layer prior to overlay.

GEL PERFUSION

Perfusion flow to the chamber is driven by gravity when there is more liquid in the inlet well than the outlet well. Liquid from the inlet well enters the microfluidic channels, through the culture chamber, and collects in the outlet well. The microfluidic channel resistance is tuned such that in normal operating conditions, approximately 80 μl of medium flows through the chamber every 24 hours. Liquid is prevented from flowing out of the open chamber by surface tension. This feature allows the M32P to be operated for 2D perfusion culture.

The microfluidic channels were designed so the flow rate was not affected by the absence or presence of gel in the culture chamber (Figure 4). When the inlet was filled with 300 μl of solution and the outlet has 30 μl , the flow volume in the first 24 hours was $\sim 80 \mu\text{l}$, and $\sim 35 \mu\text{l}$ in the second 24 hours. As long as the inlet was refilled and the outlet emptied every two days, the cells received continuous flow. The advantage of the gravity flow configuration is that no external pump is necessary to drive perfusion to all 32 flow units on the plate. In addition, multiple plates can be run in parallel inside a standard cell culture incubator for increased throughput.

The 4 μm opening of the perfusion barrier allowed free diffusion of solutes into and out of the gel (Figure 4 b-d). The diffusion of a 3 kDa fluorescent molecule (fluorescein conjugated dextran) into the center of the gel during continuous perfusion took about 20 minutes, consistent with calculations from the diffusion equation. This time scale was also desirable from a cell culture standpoint based on previous observations. The diameter of the open chamber was set at 2 mm to ensure adequate nutrient transport for cell culture.

3D CELL CULTURE

Cell suspensions were mixed with Matrigel at a 1:8 ratio and loaded into the microfluidic chambers, followed with incubation at 37°C for 15 minutes to polymerize the Matrigel. Culture medium was added to the inlet wells for gravity perfusion culture, with replacement every 2 days. During 9 days of perfusion culture, the MCF-10A cells underwent cell division and adopted an acinar morphology (Figure 5). This morphology was not seen on 2D perfusion culture in the microfluidic chamber. This is consistent with previous work⁶ that describes MCF-10A cells forming a well-polarized outer layer of cells that is in direct contact with the matrix.

The acinar structure was visualized by fixing the cells and staining the actin cytoskeleton (Figure 6). In 3D cultured MCF-10A cells, the cell clusters showed characteristic polarization. In MCF-7, a malignant breast cancer cell line, the

cell clusters were unorganized. Similarly, when MCF-10A cells were cultured in 2D (in the M32P plate), no acinar morphology was observed. These data are fully consistent with prior literature, and indicate that the M32P microfluidic plate is suitable for performing long term 3D culture experiments of breast cancer cell lines.

ANTI-CANCER DRUG SCREENING

To demonstrate the M32P microfluidic plate for anti-cancer drug sensitivity screening, we treated the MCF-10A mammary epithelial cells with Paclitaxel, and assayed with the CytoTox-One cytotoxicity kit (Promega). The cells were grown in standard media for 4 days with perfusion in 2D and 3D, and then exposed to drug for 3 days. As shown in Figure 7, the sensitivity of the cells cultured in 3D and in 2D is significantly different ($P < 0.05$), and consistent with the previously reported studies on altered drug sensitivity of 3D cultured cells.^{4,5}

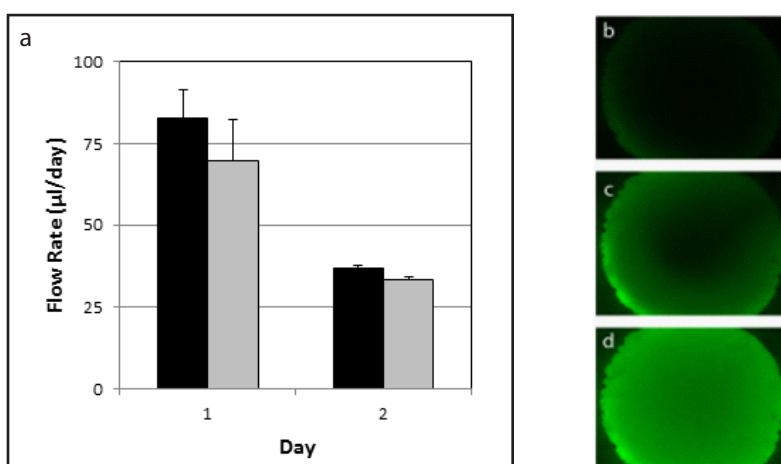


Figure 4. Perfusion exchange in the M32P culture chamber. (a) The gravity driven flow rate is equivalent with no gel (black) or Matrigel (gray) in the culture chambers. The flow rate slows over time as the liquid level difference equalizes, typically over 3 days. Diffusion of FITC-Dextran (3 kDa) from the flow channel into the culture chamber at 0 min (b), 10 min (c), 20 min (d) minutes. The circular chamber is 2 mm in diameter.

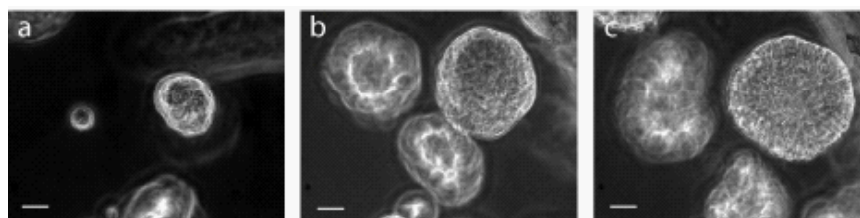


Figure 5. 3D ECM culture of MCF-10A cells in the M32P. MCF-10A cells were cultured embedded in Matrigel with continuous medium perfusion. Images show cells at (a) 3 days, (b) 6 days, and (c) 9 days. Scale bar = 20 µm.

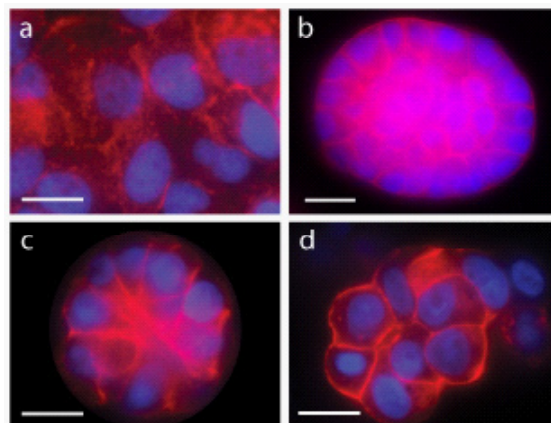


Figure 6. Actin staining of cell morphology. MCF-10A cells cultured in 2D (a) show no polarization, while in 3D Matrigel (b), the cells adopt an acinar structure (day 9). The morphological difference is also evident when comparing MCF-10A (c) to MCF-7 (d) cells cultured in Matrigel (day 4). Scale bar = 20 μ m.

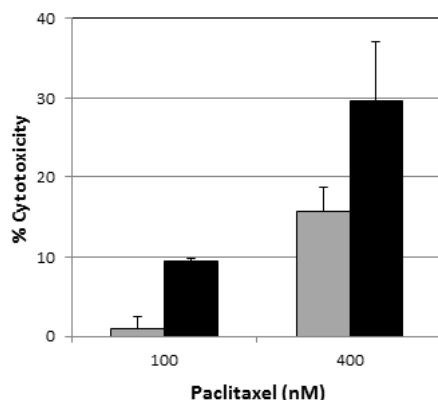


Figure 7. Paclitaxel toxicity of MCF-10A cells in 3D vs. 2D culture. Cells were cultured in the M32P plates for 4 days, followed by treatment with Paclitaxel for 3 days. Note the significant difference between cells grown in 3D (gray) vs. 2D (black).

SUMMARY

3D cell culture has many advantages for in vitro screening, yet current methods are not amenable to high throughput application. The M32P microfluidic plate offers a platform for long term 3D cell culture and assay in a 96 well format. The continuous perfusion culture environment was demonstrated to support acinar morphology in MCF-10A cells, as well as feasibility for anti-cancer drug screening in 3D ECM culture. Each microchamber uses only 4 μ l of gel, offering a substantial gel savings compared to static culture methods. The plates require no external connections, and is compatible with existing cell culture incubators and automated platforms. Additional applications of the M32P plate include use with primary cells, isolated tissues, and stem cells.

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