Creating Multiple Organotypic Models on a Single 3D Cell Culture Platform

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Introduction

In vitro 3D cell culture models have emerged as a bridge between conventional 2D cell culture models and the complex & expensive in vivo animal models. By observing, analyzing and comparing the biological behavior of tissues embedded in 3 dimensional hydrogels, results are significantly different from the classic 2D cell culture. In particular, literature shows differences in terms of proliferation, morphology, drug response and gene expression [1-4]. These differences have been attributed to the topographically complex 3D environment surrounding the cells, where cell adhesion, structure, effector transport and mechanotransduction are substantially altered [5]. A carefully designed 3D model can therefore provide more physiologically relevant information using experimental designs unachievable by conventional 2D assays, at a fraction of the cost of in vivo models.

Current 3D cell culture assays like hanging drop culture often lack the capability to organize different co-cultured cell types in a meaningful way. The application of chemical gradients or flow is usually not possible.

We are now able to address this issue in a microfluidic platform (Figure 1a). The platform consists of a hydrogel channel and two flanking media channels, where cells can be meaningfully compartmentalized to study their interactions. The miniature posts that border the hydrogel channel maintain a vertical gel interface. Given the multi-channel design, a transient gradient of chemical factors can be set up across the hydrogel to yield a more physiologically relevant microenvironment.

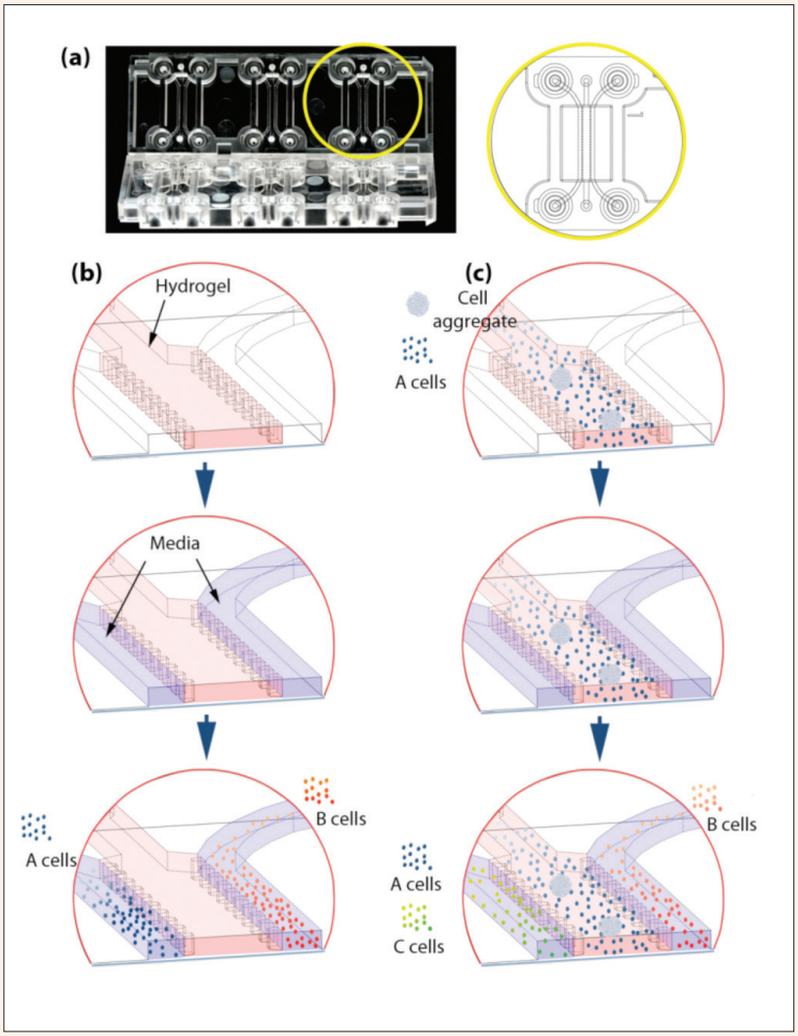

Figure 1. A schematic drawing to illustrate (a) the three-channel design of the chip and the workflow for (b) culturing cells in media channels; (c) culturing cells in hydrogel and media channels.

Materials and Methods

Culture Cells in Media Channels

Fill polymerizable hydrogel (e.g. type I collagen, fibrin gel, Matrigel) into the gel channel (the middle channel shaded in red in Figure 1b). Once the gel has polymerized, fill cell culture medium into the media channels (the flanking channels shaded in blue in Figure 1b). Seed cells into the media channels. Set up chemical gradient across the gel region or apply flow in the channels, depending on your applications. Allow the cells to grow and interact with the microenvironment in an incubator. Observe the biological processes with phase contrast, fluorescent or confocal microscopy. Perform downstream analysis (e.g. ELISA, RT-PCR) on media or extracts collected from the chips to further understand the biological processes.

Culture Cells in Hydrogel Channels

Mix cell suspension or cell aggregates with the hydrogel of choice. Fill the hydrogel with cells into the gel channel. Once it has polymerized, fill cell culture medium into the media channels. Seed cells, of the same cell type or of a second cell type, into the media channels. Observe and study the biological processes as described above.

Results

Different biological models can be devised depending on the cell types and the locations of the cells being cultured. Cells (e.g. cancer cells) can be seeded in the media channels and be positioned at the gel interface for cell migration assays. With appropriate chemical gradients, the cells can invade into the 3D hydrogel and this process can be monitored in real time. Endothelial cells tend to form a monolayer in the media channel which itself is useful for permeability tests. In addition, with the appropriate angiogenic signals and interstitial flow, an endothelial monolayer can be induced to form sprouts in the 3D hydrogel and serve as an angiogenesis model. The same monolayer can also be co-cultured with tumor cells and be used to study transendothelial migration, i.e. extravasation or intravasation, depending on the location of the target cells.

Endothelial cells mixed with fibrin gel will undergo morphogenesis to form vascular networks in the gel through vasculogenesis. The vascular networks can then be used for more advanced assays including flowing cells through the networks.

Solid tumor dissemination can be modelled by seeding tumor cell aggregates in the hydrogel. Under the right culture conditions (with co-culture or stimuli), tumor cells will disseminate from the aggregates and invade the surrounding matrix, marking the first step of metastasis.

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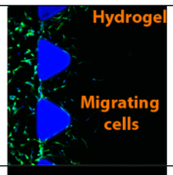
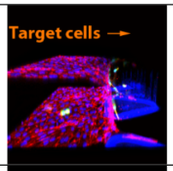
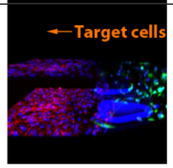
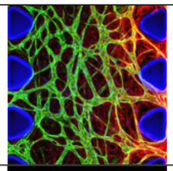
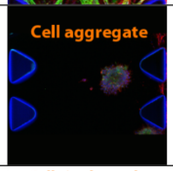
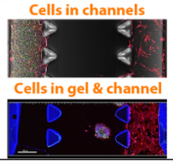
Cell Seeding		Purpose of Experiment
Individual cells in the media channels		Cell migration/ invasion of various cell types
Endothelial cells forming monolayer in the media channels		Permeability test, basis for angiogenesis and transendothelial migration assays
Endothelial monolayer in the media channels, stimulated with angiogenic factors		Angiogenesis or screening of anti-angiogenic compounds
Endothelial monolayer and target cells in the media channels		Extravasation : Transendothelial migration of cells from luminal to abluminal side
Endothelial monolayer in the media channels and target cells in the hydrogel channel		Intravasation: Transendothelial migration of cells from abluminal to luminal side
Endothelial cells and stromal cells in the hydrogel channels		Formation of perfusable vascular networks; and studies of tumor or immune cell extravasation
Tumor cell aggregates in the hydrogel channels		Dissemination of tumor cells from the primary tumors
Co-culture of cells in different channels		Cell-cell communication and interactions

Table 1. Biological models that have been successfully created in the platform. Additional references to related publications can be found at http://www.aimbiotech.com/publications.html

References

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