PARTITION OF MICROCHANNELS WITH COLLAGEN FOR FABRICATING TUNABLE 3D CELLULAR MICROENVIRONMENTS

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ABSTRACT

The ability to model accurately the cellular microenvironment is required to improve studies of cell biology in vitro. Here, we describe a method based on the use of laminar flow that makes it possible to pattern biologically-derived hydrogel (collagen). This technique allows the replication of the cellular microenvironment with control over the distribution of (i) various types of cells and of (ii) gradients of soluble factors, such as cytokines. This technique does not require the use of elements that might be toxic (UV light or photoinitiators) for biological cells. We demonstrate this method by investigating the intercellular communication between two types of macrophage-like cell lines (BAC and LADMAC cells).

KEYWORDS: microenvironment, 3D cell culture, collagen, microchannels

INTRODUCTION

Cells \textit{in vivo} receive, integrate, and respond to numerous signals that originate from direct contact with other local cells (such as gap junctions in myocytes), from soluble factors secreted by neighboring or distant cells (such as growth factors and hormones), and from the surrounding extracellular matrix (ECM), which contributes both chemical and mechanical signals. \cite{1} Methods to investigate the independent contributions of each signal to cells in 3D matrices require to (i) control the position of and distance between cells on a length scale similar to that observed in tissue (i.e., a few to hundreds of microns), and to (ii) expose the cells in culture to steady gradients of soluble factors that are not disturbed by convective flow. \cite{2}

Several techniques have demonstrated that microfabrication makes it possible to reduce the dimensions of the structures of hydrogel but they can generate only planar patterns (e.g., \cite{3}) or require the use of photochemical initiators and UV light, which are often toxic to cells, and are limited to synthetic hydrogels (e.g., \cite{4}). Other methods to pattern hydrogels containing embedded cells (e.g., \cite{5}) require a new design of the microchannel for each new configuration of the structures of hydrogel. Here we describe a technique that uses laminar flow to partition microchannels with one or more microslabs of hydrogel (Figure 1) into various subchannels separated by walls of collagen in which we cultured cells \cite{6}. Our patterning process relies on thermal curing of the gel—from a liquid state at 4 °C to a gelled state at 25-37 °C—and allows one design of the channel to yield multiple experimental configurations.
EXPERIMENTAL

NIH/3T3 fibroblasts were cultured at 10% CO₂ in DMEM with 10% fetal bovine serum. Monocyte-derived LADMAC cells (ATCC; CRL-2420) were cultured at 5% CO₂ in Eagle’s MEM with 1.5 g/l sodium bicarbonate and 10% fetal bovine serum. BAC1.2F5 macrophages (generously donated by Dr. E. R. Stanley; Albert Einstein College of Medicine, New York) were cultured at 5% CO₂ in αMEM supplemented with 10% newborn calf serum and 36 ng/ml recombinant human CSF-1. To assess the viability of cells, we stained them with a 100 μg/ml solution of propidium iodide in culturing medium.

To fabricate walls of gel inside microchannels (Fig. 1), we delivered a stream of uncured hydrogel (Growth Factor Reduced Matrigel®, mainly composed of laminin and collagen IV [7]) flanked with two streams of buffer (220 mg/ml PEG 8000 in PBS), followed by rapid thermal gelation (37 ºC); microchannels were fabricated by soft lithography using standard protocols.

After gelation, the walls of gel extended from top to bottom of the channel, creating independent subchannels (Fig. 2).

RESULTS AND DISCUSSION

The technique described here allowed the culture of mammalian cells either in the subchannels (Fig. 2b) or within the walls of hydrogel (Fig. 3) for up to a week (renewing the culturing medium every 1-2 days). To form walls of gel with embedded cells, we delivered a suspension of cells in uncured gel flanked with streams of buffer (220 mg/ml PEG) into the microchannel, and increased the temperature to 37°C.

We applied this technique to co-culture BAC1.2F5 and LADMAC cell lines within spatially separated regions of 3D matrix of Matrigel. We formed an ensemble of gel (Fig. 3) consisting of one subchannel and three contiguous slabs of gel: (i) BAC cells encapsulated in gel, (ii) pure gel, and (iii) LADMAC cells in gel.
LADMAC and BAC cells represent a simple model system for intercellular communication in which the LADMAC cells secrete a cytokine (CSF-1) that is required by the BAC cells for survival. Impaired communication between the two types of cells (i.e., impaired delivery of CSF-1 to BAC cells) will result in death of the BAC cells. In the presence of LADMAC cells (Fig. 3), the BAC cells remained viable for a week. In an ensemble similar to that in Fig. 3 but lacking LADMAC cells (slab at far right comprised pure Matrigel), BAC cells died within two days.

**CONCLUSIONS**

We demonstrated that this method makes it possible to (i) culture cells either within the walls of gel, or on the surface of the subchannels created by the walls, (ii) pattern different types of cells in 3D matrices of gel, and (iii) achieve effective diffusion of soluble factors through gel. These characteristics make this technique a powerful and versatile tool for modeling of *in-vivo* cellular microenvironments and suitable for studying intercellular communication mediated by soluble factors.

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**REFERENCES**