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Review

Patterning multiple cell types in co-cultures: A review

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ABSTRACT

With progress in materials, microelectronics, biological science, and some imagination we are in the position to manipulate and precisely control the local cellular environment to probe cell biology at the micron level. This precise control is important as cells interact strongly with their local environment including the extracellular matrix and neighboring cells. While various papers have reviewed the many techniques to pattern single-cell types, very few have focused on techniques to pattern multiple cell types in co-culture systems. Therefore, this review will explore various methods used to create patterned local cellular environments and the applications these techniques have found in probing cell–cell interactions. Emphasis will be placed on the creation of patterns incorporating multiple cell types in culture to study the interactions between different cell types in well controlled and biologically relevant environments. Such designs can be considered one of the greatest challenges bioengineers face to date.

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1. Introduction

With scientific progress in materials, microelectronics, and biological sciences, we are in the position to design a variety of substrates with pre-determined cell patterns which allow us to control the local cellular environment at the micron level. Studies focusing on the

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design of this local microenvironment are critical since it heavily influences cell behavior. In the body, this microenvironment consists of the extracellular matrix (ECM) – to which cells adhere – as well as numerous neighboring cells. These cells send out soluble signals to one another as a form of cellular communication. Neighboring cells can also form physical attachments to each other through adhesion proteins that interact with the cellular cytoskeleton, stimulate different signaling pathways, and thus influence numerous aspects of cell function. Cells also interact with the ECM, which serves to stimulate cell surface receptors, resulting in a cascade of signaling events which can eventually lead to cell proliferation, differentiation, and migration. The structure of the ECM can further impact the mobilization of soluble factors. It is the co-ordination of all of these influences in the microenvironment including soluble signaling, physical cell–cell interactions, and matrix interactions that act as positive or negative effectors which regulate cellular events [1–5]. Due to this strong cell dependence on their local environment, it becomes extremely important to develop technologies that can design and manipulate the cellular microenvironment. The patterning of cells on a surface allows us to control the degree of cell contact with supporting materials as well the degree of contact with neighbouring cells, including direct physical contact with both homotypic and heterotypic cell types. This enables us to conduct highly controlled *in vitro* experiments to gain insight into basic cell biology and will allow us a much greater flexibility in the control of cell behavior towards the development of new biotechnologies.

While patterning of single-cell types has been extensively reported and reviewed in the literature, there are relatively few studies tackling the additional challenge of patterning multiple cell types on the same substrate. This review will first briefly explore various methods used to design a patterned cellular environment. This will set the stage for techniques to pattern multiple cell types. Focus will be given to the methods used to create controlled co-culture systems and their applications found to date.

2. Overview of patterning techniques

The concept of single-cell-type patterning has been around for decades and over this period of time several major techniques have evolved. These techniques mark the first steps forward in designing more controlled local cellular environments. Some of the key developments will be briefly reviewed below in a historical manner.

2.1. In the beginning

Cell patterning first originated with Carter in the 1960s. These early works involved metal evaporation and subsequent deposition onto a cell non-adherent surface through a stencil-like mask. The deposited metal created patterned islands that supported cell growth. This technique brought about a way to create more controlled experiments; the pattern allowed one to track individual cells and to monitor their responses for several days *in vitro* over the course of a biological study [6–8].

Studies employing metal deposition patterning were continued by several other groups [9–12]. Through the 1970s and 1980s new patterning methods were developed with a trend towards using naturally occurring materials found in the cellular environment. Ivanova and Margolis patterned glass with a layer of non-adhesive phospholipids that could be subsequently scraped off in selected regions to allow cell adhesion [13]. Furshpan et al. applied droplets of collagen to a substrate in a pattern to create adhesive islands for the adhesion of cardiac

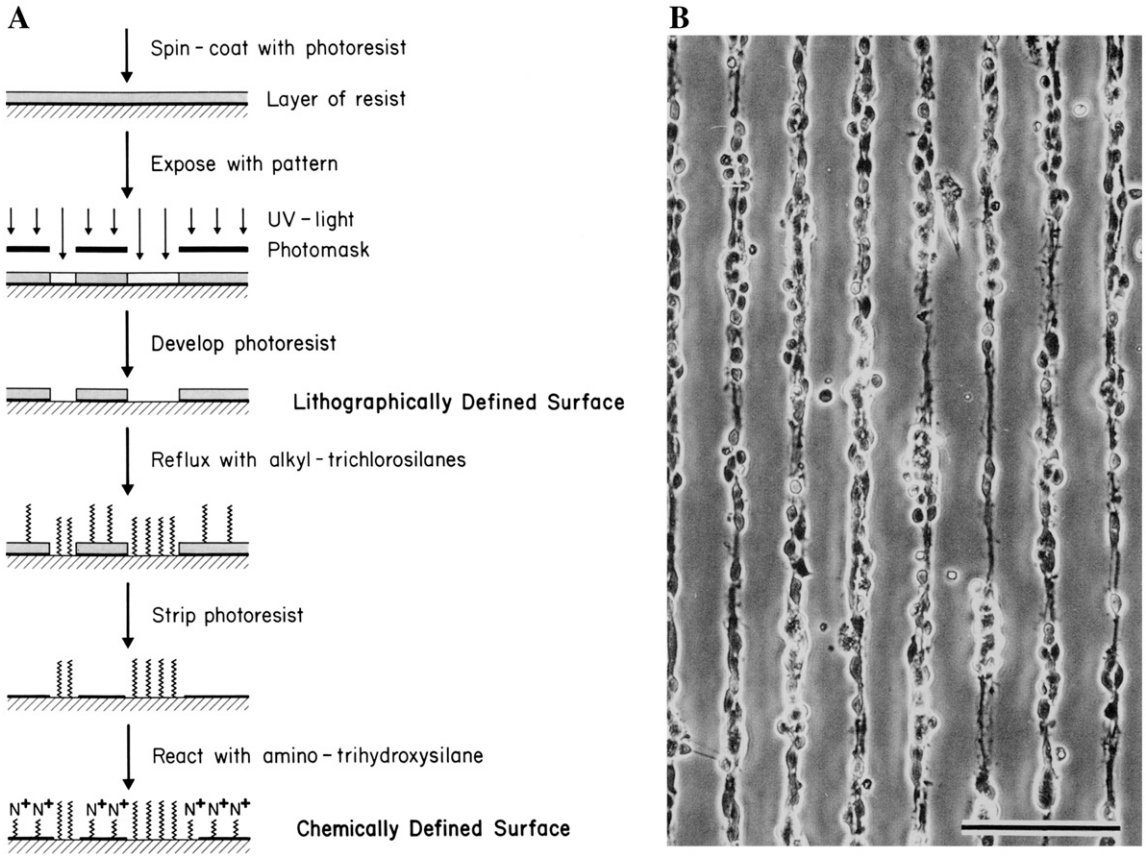

Fig. 1. (A) Schematic of photolithographic patterning method. The final pattern consists of alkyl-trichlorosilanes (cell non-adhesive) and amino-trihydroxysilanes (cell adhesive) chemically bound to the substrate. (B) Resulting patterned neurons, scale bar = 100 μm [18]. Reproduced from Kleinfeld, D. et al., Controlled outgrowth of dissociated neurons on patterned substrates. The Journal of Neuroscience 1988; 8(11): 4098–4120 by permission of The Society for Neuroscience.