Parylene Peel-Off Arrays to Study Tumor Cell-Cell Interactions

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Abstract:
Microenvironmental conditions, such as cell-cell interactions, have been known to influence cell behavior and may be involved in cancer progression [1]. However the underlying biological mechanisms are poorly understood. We have developed a simple micropatterning approach utilizing a parylene peel-off template for controlling cell-cell contact in a reproducible manner. This has enabled the systematic investigation into the role of cell-cell interactions in tumorigenesis.

Summary of Research:
Cell behavior is regulated by the microenvironment, such as cell-cell interaction with neighboring cells, or cell interaction with the extracellular matrix. These microenvironmental conditions are important in maintaining homeostasis and deregulation of these conditions can lead to disease [2]. Angiogenesis, the formation of new blood vessels from existing ones, is important to supply a growing tumor with nutrients and provide a route for metastasis later. Previous studies indicate that cell-cell interaction may be correlated with the regulation of angiogenesis in cancer cell lines [3,4]. It is important to elucidate the underlying molecular pathways of angiogenesis leading to cancer, for the design of drugs towards more efficacious therapies.

Parylene has been used as a template to pattern arrays of biomolecules (e.g. cells, nucleic acids, antibodies, lipids) uniformly over a large area, in both wet or dry conditions [5-8]. Parylene is chemically inert and does not swell in aqueous solutions, thus overcoming the problems associated with other micropatterning technologies such as microcontact printing. The parylene template is easy to microfabricate using standard photolithography techniques (Figure 1). Briefly parylene is deposited on a substrate by chemical vapor deposition. A protective layer of photosresist is spun onto the parylene to serve as an etch mask. The parylene underlying the exposed openings on the resist is then etched away, while the parylene underneath the unexposed resist regions is protected. The resist is then removed to reveal the parylene template suitable for patterning cells.

We demonstrated that we can pattern single tumor cells and clusters of tumor cells using our parylene peel off arrays. We first incubated the parylene arrays with fibronectin, a component of the extracellular matrix to help cell adhesion. Next we peeled off the parylene template to reveal defined patterns of fibronectin squares with different sizes suitable for patterning single cells or clusters of cells, thus conferring control of cell-cell contact (Figure 1). Polyethylene glycol on
the substrate surrounding the fibronectin prevented nonspecific cell adhesion. We were able to culture and maintain the cells arrays for 24 hours as shown by phase contrast microscopy, for both the single cells and cell cluster arrays (Figure 2). Further staining of the cell arrays for cytoskeleton (phalloidin), nuclei (DAPI), and fibronectin (immunostaining using antibodies) confirmed that we have maintained the fidelity of our patterned biomolecules and cells (Figure 3).

We are currently using these micropatterned cell arrays to systematically profile protein secretions by the cells, in the absence or presence of cell-cell contact [9]. Our simple and adaptable technology is useful for other applications whereby a uniform deposition of biomaterials over a large area is critical, such as drug screening, and gene and protein expression microarrays [10-12].

References: