ORGANIZING COMPLEX MULTICELLULAR CONSTRUCTS USING STENCIL-DELINEATED ELECTROACTIVE PATTERNING (S-DEP)

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ABSTRACT

Attempts to regulate and monitor the roles of cell-cell interactions in engineered multicellular constructs have motivated numerous approaches to cell patterning. We present here a stencil-delineated electroactive patterning (S-DEP) that combines dielectrophoresis (DEP) and stencil patterning to create cell clusters with customizable shapes, positions, and internal cell organization. We demonstrate the use of S-DEP to investigate the motility of epidermoid carcinoma cells initially organized as striped cell clusters. In addition to cell tracking, S-DEP enables correlation of cell locations within the cluster to their phenotypes and provides avenues for creating mosaic tissue-like constructs of phenotypically or genetically distinct cells.

KEYWORDS: Stencil patterning, Dielectrophoresis, Cell patterning, BioMEMS

INTRODUCTION

Modulating the spatial organization of cell populations is critical to developing relevant *in vitro* platforms for probing the roles that cell-cell interactions play in tissue and organ function. Attempts to regulate and monitor such dynamics in engineered multicellular constructs have motivated numerous approaches to cell patterning. Protein stamping [1], physical confinement [2] and dielectrophoresis [3] have all been used to position individual or clustered groupings of cells in defined locations. Each of these approaches, used alone, can organize one cell type in isolated regions and surround that pattern with a bulk population of a second cell type. None, however, can structure the heterotypic patterns-within-patterns needed to form realistic tissue-like constructs (TLCs) (Figure 1). Stamping uses adhesive and non-adhesive surface chemistries and, like physical methods, lacks the

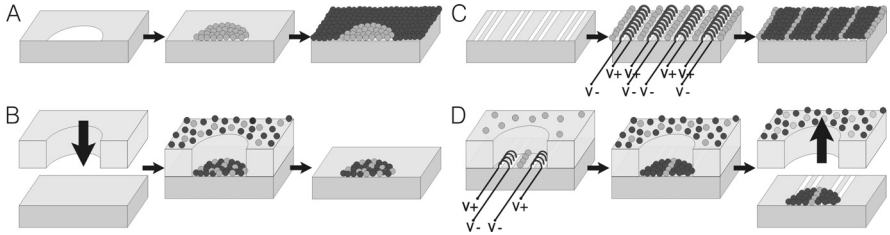

Figure 1: Comparison of patterning techniques. *(A) Protein patterning requires reversability of the initially non-adhesive region and prevents normal cell motion. (B) Stencil patterning cannot provide complex patterning of both cell types. (C) Dielectrophoresis requires complex electrode designs to give isolated cell constructs. (D) Pattern-in-a-pattern cell aggregate can be achieved using S-DEP.*

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"switchability" needed to organize structured assemblies of different cell types within larger isolated constructs. Dielectrophoresis (DEP) alone has never been used for creating patterns-within-patterns likely due to the complex electrode designs that would be required. We present here a stencil-delineated electroactive patterning (S-DEP) approach and demonstrate how this method can be used to create cell clusters with customizable shapes, positions, and internal cell organization.

METHOD

S-DEP utilizes the strength of regular DEP and stencil patterning. Stencils define overarching TLC geometries on the substrate. Negative-dielectrophoretic (n-DEP) forcing guides subgroupings of cells to desired positions within cell constructs. Our method uses a four-step procedure (Figure 2). We first place a PDMS stencil onto an electroactive substrate and subsequently seed the devices with an initial cell type. While loading, we turn ON the n-DEP electrodes, forcing settling cells into specified regions within the stencil openings. Next, we turn the electrodes OFF, await cell attachment, and then seed a second cell type. With the electrodes deactivated, this second cell type backfills exposed substrate areas within the stencil openings. We await attachment and remove the stencil, leaving in place isolated cell clusters with internally patterned subdomains. Because our patterning method does not depend on modification of surface chemistry, the patterned cell clusters will be able to migrate naturally on the substrate.

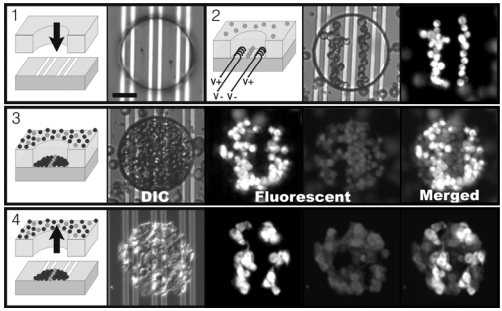

Figure 2: S-DEP procedure*. (1) Place a PDMS stencil over an electroactive substrate. (2) Seed the 1st cell type with electrodes ON. (3) With electrodes OFF, load the 2nd cell type after cell attachment. (4) Remove the stencil after all cells attach, rendering TLCs with internally patterned subdomains. Scale bar = 50 µm.*

RESULTS

By programming a microprocessor and electrical switches to supply the electrical current to different sections of the substrate in cycle, we have successfully patterned large arrays of TLCs on the substrate using reduced power requirement (Figure 3). We then used S-DEP to study how cell motility alters initially-prescribed patterns within growing cell clusters (Figure 4A). Such analysis enables us to uncover the

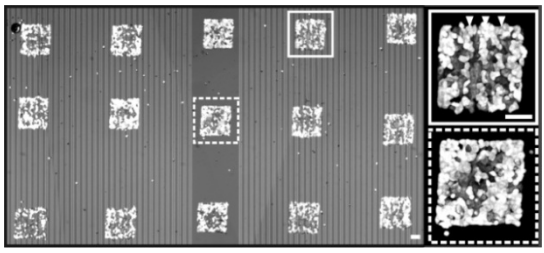

Figure 3: Large-scale TLC patterning. *S-DEP enables parallelized TLC formation on a shared substrate. The insets show closer views of cell organization within example TLCs. White arrows show cell banding. Scale bars = 100 µm.*

impact that cell spatial organization plays in governing cell-decision processes. We first patterned A431 epidermoid carcinoma cells in stencil-defined square footprints. Internal to these structures, we patterned single-cell-wide strips of red-stained cells within backfilled regions of green-stained populations. Figure 4B illustrates the growth and motility dynamics of one such TLC monitored over two days. The assay demonstrates the mixing of the two subpopulations and the disappearance of the initial striations, indicating dynamic cell migration within the growing clusters.

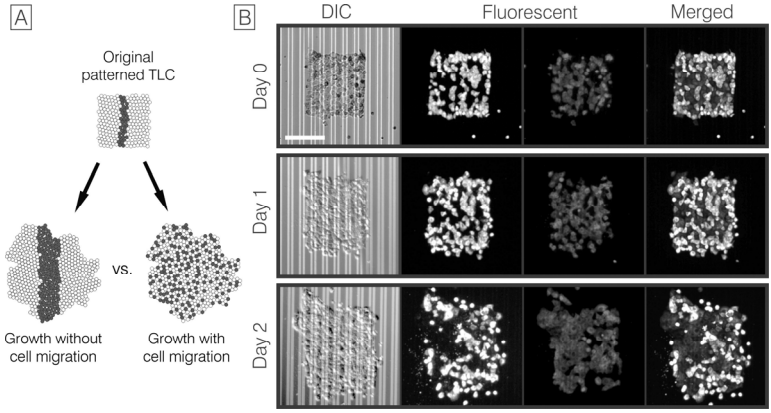

Figure 4: Cell tracking within TLCs made available with S-DEP. *(A) Cell motility within an individual TLC is readily monitored by forming TLCs with regularly defined, labeled subdomains. As the cell constructs proliferate, the positions of the originally patterned cells provide information on dynamics within cell aggregates. (B) Images of a square-patterned TLC monitored over the course of two days. The red patterned A431 cell strips disappear after day 2 as cells migrate within the cell agglomerate. Scale bar = 200 μm.*

CONCLUSION

We provide a straightforward approach for generating complex TLCs and show their relevance for studying motility in evolving cell clusters. Beyond cell growth and motility tracking, S-DEP enables correlation of cells' cluster location to phenotype and provides avenues for creating mosaic TLCs of phenotypically or genetically distinct cells. Such diversified chimeras could allow the study of, for instance, stem cell differentiation in complex cellular environments.

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