Reusable microfabricated parylene-C stencils for controlling the cell microenvironment

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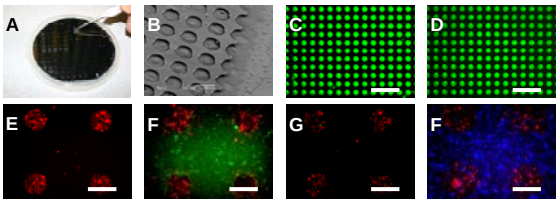
Statement of Purpose: Microscale technologies are potentially powerful tools for controlling the cellular microenvironment for a variety of applications. In general, methods that are easy to perform, versatile and inexpensive are believed to be important for spreading the use of microengineering techniques in biomedical applications. We have developed a method of using inexpensive, microfabricated parylene-C stencils to pattern proteins and cells and generate static and dynamic patterned co-cultures. Furthermore, we demonstrate the reusability of parylene-C stencils for at least 10 sequential patterning processes. The potential applications of this technology include high-throughput biological experiments, biosensors and tissue engineering.

Methods: Parylene-C [poly(chloro-*p*-xylylene)] stencils were fabricated on silicon wafers using a PDS 2010 Labcoater 2 chemical deposition system, and then etched in a Plasmaterm 790 using O_2. Individual parylene stencils were then peeled off from the wafer using tweezers. For protein co-patterning, FITC-BSA (50 ng/ml) was incubated on the stencil-substrate complex for 30 minutes, rinsed, following which the stencil was removed to reveal the patterned substrate. For patterning cells on various surfaces, fibronectin (FN) (5 µg/ml) was incubated either on top of the substrate or stencil for 45 minutes. Cells were then seeded on stencils and incubated for 6 hours. Subsequently, the stencil was peeled away to generate micropatterned cells. To fabricate patterned co-cultures, the primary cell type was patterned as described above and then incubated with the second cell type for 6 hours. For dynamic co-cultures, the stencil was incubated with hyaluronic acid (HA) (5 mg/ml) for 1 hour and the PDMS substrate was incubated with FN (5 µg/ml) for 45 minutes. The stencil was then sealed on a PDMS substrate. Mouse embryonic stem (mES) cells were seeded on the stencil-substrate complex, with cells adhering to the PDMS exposed through the holes in the stencil. After confirming cell adhesion, the surface was washed to remove non-adherent cells. The HA coating on the parylene surface inhibited the cell adhesion on its surface. Collagen (500 µg/ml) was then layered over the HA coating and incubated for 20 minutes. AML-12 cells were then seeded on the stencil coated with collagen to generate a co-culture of mES cells surrounded by AML-12 cells. Subsequently the stencil was peeled away, leaving the pattern of mES cells on the substrate. NIH-3T3 cells were then seeded on the pattern of the mES cells to create another co-culture of mES cells surrounded by NIH-3T3 cells. Stencils were recovered for reuse, by trypsinizing and/or plasma cleaning to remove proteins/cells.

Results/Discussion: Reversibly sealable parylene-C stencils can be used multiple times to generate precise high resolution patterns of cells and proteins as small as 1 micron. This technique can be applied to a variety of substrates with hydrophobic surfaces such as PDMS, acrylated glass and polystyrene, on planar and non-planar surfaces. Our cell adhesion and viability experiments suggest that surface modified parylene-C has a biocompatibility profile comparable to tissue culture-treated polystyrene. Furthermore, the mechanical properties of parylene-C are superior to the PDMS. By combining the reversibly sealable property of the parylene-C stencils with the layer-by-layer deposition of biomaterials[1] we demonstrate the ability to control the cell-cell interactions in a dynamic fashion. Our model can be used to regulate the spatial position, the type of cells, the temporal sequence of the interactions and the duration of such interactions. Since cues from surrounding cells influence stem cell behaviour, the technique may be used in directing stem cell differentiation. We also studied the stability of cell pattern over time and the effects of the cell migration on the patterned co-cultures.



(A) Individual stencils can be removed with tweezers. (B) SEM image of the stencil. (C)&(D) Protein patterns obtained with the same stencil 1st and 9th time. (E), (F), (G) & (H) Dynamic co culture process, mES cells (red) initially co-cultured with AML-12 cells (green) followed by NIH-3T3 cells (blue). Scale bar 250µm

Conclusions: Parylene-C stencils can be used to generate cell/protein patterns and static/dynamic patterned co-cultures. Parylene-C stencils can overcome several limitations associated with the use of PDMS in soft lithography. The reusability and low cost of parylene-C stencil can be used to make custom made stencils for applications in research labs or large scale fabrication of protein /cell patterns and co-cultures in biotech industry.

References: 1.KhademhosseiniA Biomaterials 2006:27:1479-1486