Cell and Protein Compatibility of Parylene-C Membranes

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Statement of Purpose: Parylene-C is an inert and non-degradable material, which can be used to fabricate mechanically robust microstructures. Parylene is used in biomedical research for a wide variety of applications, from 3-dimensional (3D) neurocages[1] to microfluidic channels. Recently micropatterned portable parylene stencils have been used for patterning proteins and cells, and for the generation of static and dynamic co-cultures. Given the increased applications of parylene-C in controlling the cell microenvironment, in this work we explored the cell and protein compatibility of the parylene-C substrates.

Methods: Parylene-C (di-chloro-di-para-xylylene) membranes were fabricated by depositing monomer (para-xylylene) using the PDS 2010 Labcoater 2 Deposition System (Specialty Coating Systems, Indianapolis). PDMS and parylene-C surfaces were modified by treating with air plasma for two minutes or incubating for one hour with a 5 μg/mL fibronectin solution. For protein adsorption studies, surfaces were saturated with FITC-BSA, FITC-Collagen type-1, and TRITC-IgG at 100 μg/mL, 500 μg/mL, 50 μg/mL respectively, incubated for one hour, rinsed and then imaged under a fluorescent microscope and analyzed using Image J software. For cell adhesion experiments, cells were seeded at various densities and the percentage of the cells attached was determined. To analyze cell spreading, the dimensionless shape factor computed as $S = 4\pi A/P^2$ was used, where A is the cell's area and P its perimeter, yielding a value of "1" for a perfect circle.

Results/Discussion: In this study, we evaluated and compared biocompatibility of parylene-C membranes with other commonly used substrates such as PDMS, glass and polystyrene. Surface hydrophobicity and roughness are important factors in the adhesion and morphology of attached cells. Though the plasma treatment process significantly increased the hydrophilicity of parylene-C and PDMS and lowered the contact angles of both materials, because of the viscoelastic properties of PDMS the contact angle increased to its original value over time but parylene-C surfaces remained hydrophilic over an extended period of time. Thus parylene can be used to generate hydrophilic surfaces for biological applications. Parylene-C surface was found to be rougher than PDMS and glass using AFM. The increased surface roughness of parylene-C may be due to the irregularities in the deposition process which may contribute to their enhanced cytocompatibility properties. Adsorption of IgG and BSA on unmodified parylene-C surfaces was comparable to the adsorption on glass substrates. In our studies, plasma treatment of parylene-C and PDMS or deposition of an initial layer of protein on the surface decreased the subsequent adsorption of BSA and IgG. NIH-3T3 fibroblasts and AML-12 hepatocytes were used to study the cell adhesion, spreading and proliferation. Our experiments show that while unmodified parylene-C did not promote cell adhesion and proliferation, parylene-C subjected to plasma treatment or coated with proteins like fibronectin enhanced cellular attachment and proliferation at rates comparable to materials like tissue culture treated polystyrene and glass. We also examined the degree of cell spreading on the various surfaces. The cells on surface modified parylene-C shows decreased shape factor, which indicates they are more spread.

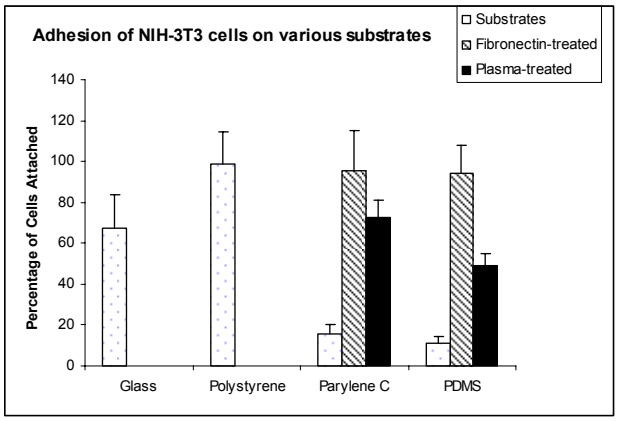

Figure 1. The adhesion characteristics of the NIH-3T3 cells on surface modified parylene-C are comparable to standard substrates like glass and polystyrene.

Conclusions: In this paper we have compared parylene-C to other commonly used cell culture substrates and demonstrate that surface modified parylene-C exhibits adhesion and cell spreading characteristics comparable to commercially available tissue culture-treated polystyrene. Parylene-C is a suitable substrate for culturing many types of mammalian cells and there are many advantages of fabricating devices made of this material than conventional materials such as PDMS, silicon and polystyrene.

References: 1.TookerA. IEEE Eng Med Biol Mag.2005:24:30-33