Nano-Patterned Silicon Enhanced Fibronectin Binding and Promotes Organization of Glial Cells

Grace Tatagiri, Howard Matthew and Pamela VandeVord
Department of Biomedical Engineering, Wayne State University, Detroit, MI 48202

Statement of Purpose: Neural implants promise remarkable improvements in the treatment of a wide variety of disorders and diseases that affect the central and peripheral nervous systems. However, while impressive advances have been made in the technical design of neural prostheses and neuro-electronic interfaces, the tissue response to neural implants is still problematic. It is widely believed that binding of soluble proteins to the implant surface is the initial source of the inflammatory signal. By modifying the surface characteristics of the biomaterial on a nano-scale, it is intended to change and reduce the properties which elicit inflammation. In this study nano-patterned and non-patterned surfaces were compared in terms of fibronectin binding and cell growth.

Methods: Silicon wafers of 1.27cm^2 in size were used for this study. For our studies photolithography techniques were used to generate nanopatterns with a period of 350 nm (175 nm plateaus and 175 nm valleys) and a depth of 100 nm. Rectangular grating patterns were fabricated as 3 mm x 5 mm zones surrounded by non-patterned silicon. These silicon wafers were coated with fibronectin from human plasma with a concentration of 1µg/ml. The level of protein binding was qualitatively assessed using immunofluorescence. The images of these silicon samples were taken with a fluorescence microscope.

In order to analyze the rate of protein attachment, ellipsometry measurements were employed. Surfaces were placed in a Petri dish and immersed in a fibronectin solution with a concentration of 10µg/ml. The incident angle of the ellipsometer was set at 60°, which was the Brewster angle for this system. The ellipsometry readings proportional to protein layer thickness were then plotted against time.Glial cells (C6 cell line; ATCC) were grown on the fibronectin-coated silicon and incubated for 24 hours. Pictures were taken using Calcein AM fluorescence dye to assess cell attachment and morphology.

Results: Image analysis software (ImageJ) was used to analyze the fluorescent images. It showed a significant increase in fibronectin attachment as the adsorption was 40% more on the patterned surface as compared to the non-patterned surface. In Figure 1, the left picture depicts the border between the grid and the non-grid area. A sharp contrast is seen between the two surfaces. Glial cells aligned and spread in a particular direction of the grid surface. Outside the patterned area, the cells. were randomly scattered. Using SigmaScan Pro, shape factor of the cells was found to be significantly different with the cells on the patterned surfaces portraying a more elongated shape (p=0.001). The ellipsometric graph in figure 2 displays the ellipsometric y signal, which is proportional to the surface coverage with fibronectin. The adsorption of fibronectin on the nanopatterned surface increases with time whereas in the non-patterned area, it reaches a saturation point.

Figure1: Fluorescent microscope images. A: Image of patterned border at 4X. B: Cells outside the grid area at 10x. C: Cells on the grid at 10x

A. B. C.

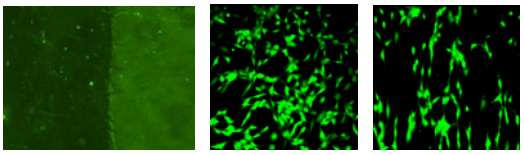

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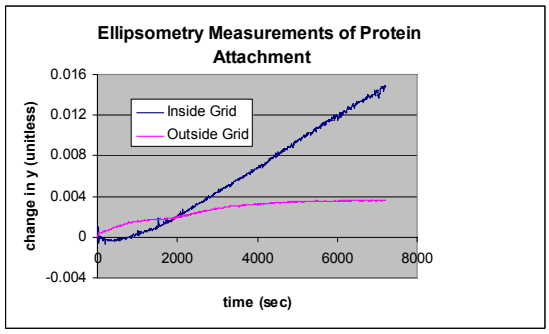
Figure 2: Ellipsometric y signal measured as a function of time.



Conclusions: The study was successful in detecting the presence of fibronectin attachment as the level of intensity of the nanopatterned area verses non-patterned surface was significantly different. The ellipsometric measurements further confirmed the increase in the thickness of protein adsorption in the nano-patterned surface. The results show that a one dimensional nanoscale pattern can substantially alter the protein adsorbtion kinetic on silicon, and that the alteration may strongly influence the organization of glial cells interacting with patterned surface.

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