Effect of Changing Surface Hydrophobicity Alters Protein Expression and Cellular Dynamics

Ian S. Miller [1], Stephen C. Penney [1], Darran P. O'Connor [1], Iseult Lynch [2], Kenneth A. Dawson [2], William M. Gallagher[1].
[1]School of Biomolecular and Biomedical Sciences, Conway Institute of Biomolecular and Biomedical Research University College Dublin, Ireland
[2]Centre for Colloid Science and Biomaterials, School of Chemistry and Chemical Biology, University College Dublin, Ireland

Statement of Purpose: The success or failure of a biomaterial is dependant on how the cells regulate their adhesion, morphology and biochemical response to their environment. In the past, research focused on the actual presented surface of the implant, however in recent times, the emphasis of the research has switched focus to protein expression elicited from the surface. Using our novel model system of N-isopropylacrylamide (NiPAAm) and N-tertbutylacrylamide (NtBAAm) co-polymers of increasing hydrophobicity (50:50 65:35 85:15 NiPAAm to NtBAAm), we have shown that protein adsorbed to surfaces of differing hydrophobicity effects various cellular activities, including cell adhesion, cytoskeletal re-arrangement, cell growth and protein expression.

Methods: NipAAm (99%; Acros Oragnics, Fairlawn, NJ USA) and NtBAAm (Fluka, Switzerland) were recrystallised from hexane and dried at room temperature in a vacuum. The NiPAAm: NtBAAm co-polymer was provided by Dr. Iseult Lynch (Dept of Chemistry University of Lund, Sweden) and was synthesized as outlined in Rochev *et al*[1]. Co-polymer films were cast from a 5% (w/w) solution of each co-polymer in 100% ethanol. Appropriate volumes of co-polymer solutions were added to dish surfaces of varying size (0.69 1.7 9.6 and 57cm^2) to create films 5µm thick. For immunofluorescence, HeLa cells (Human cervcix epithelial carcinoma cell line) were grown for 24hrs on the co-polymers. Cells were fixed with 3.7% formalin solution (Sigma USA), permeabilised in a 0.1% Triton X100 solution, blocked in 1% FBS. Cells were subsequently immunostained with mouse monoclonal anti-vinculin (1:100) and chicken anti-mouse FITC 2^o (1:1000) (Sigma), DAPI (1:50000) and stained with Rhodamine Phalloidin for F-actin (1:100) (Fluka). Images were taken using a Zeiss Axiovert. For SDS PAGE cellular extracts were run on 10% bis tris acrylamide gels and immunoblotted with rabbit anti-p-FAK y861 (1:1000) (Santa Cruz, CA, USA) and detected with a horseradish peroxidase conjugated 2^o antibody (1:7500) (Sigma). For 2D analysis IPG gels strips (pH 4-7 Amersham, Sweden) were loaded with 100µg of protein and isoelectrically focused then run in the 2nd dimension on 10% bis tris acrylamide gels. Gels were fixed and subsequently silver stained.

Motility: Cells were seeded onto polymer or tissue culture plastic (TCP) and grown for 24 hrs. Phase contrast images were then taken (Zeiss Axiovert, Germany) every 10 mins to monitor cell activity. Movement, distance and velocity were quantified using Cellbuster software

Results / Discussion: Using our novel model system of N-isopropylacrylamide (NiPAAm) and N-tertbutylacrylamide (NtBAAm) co-polymers, we have previously shown that protein adsorbed to surfaces of increasing hydrophobicity effects various cellular activities, including cell adhesion, cytoskeletal re-arrangement and cell growth. Here we show that cells display a distinct phenotypic shift to a stellate morphology as surface hydrophobicity increases. Moreover, we show that adsorbed serum proteins are responsible for distinct morphological and biochemical changes in the cell. It was found that that p-FAK is down regulated in response to the polymer and by a combination of gel electrophoresis and mass spectrometry, we show that changing the surface hydrophobicity of our co-polymer series influences further changes in cellular protein expression. Additionally, results of time-lapse experiments reveal a distinct increase in velocity of HeLa cells on the most hydrophobic polymer surface compared to TCP. (19.50µm/hr compared to 9.05µm/hr respectively)

Conclusions: It has been shown that changing the hydrophobicity of a surface alters various biochemical and morphological processes in the cell.

References: [1]Rochev *et al*. Prog Colloid Polym. Sci (2001;118: 153-56)