Tuesday Afternoon, October 3, 2000

Biomaterial Interfaces

Room 202 - Session BI+EL-TuA

Cell-Surface Interactions

Moderator: D.W. Grainger, Colorado State University

2:00pm **BI+EL-TuA1 Model Surfaces for Studying and Controlling the Adhesion of Cells**, ***M. Mrksich,*** The University of Chicago **INVITED**

This presentation will give an overview of the use of self-assembled monolayers of alkanethiolates on gold as model substrates for studying and controlling the interactions of cells with non-natural materials. This surface chemistry approach begins with monolayers terminated in short oligomers of the ethylene glycol group, because these films are inert to the non-specific adsorption of protein. Monolayers patterned into regions presenting glycol groups with the complementary regions presenting hydrophobic surfaces are excellent substrates for patterning the attachment of cells. The immobilization of ligands to these inert films gives substrates to which proteins can selectively bind, but which otherwise rule out non-specific interactions of proteins. This approach can be extended to give substrates that mediate the attachment of mammalian cells. Monolayers presenting the peptide Arg-Gly-Asp (a ligand for cell-surface integrin receptors) mediate the selective attachment and spreading of fibroblast cells. This presentation will also discuss the design of dynamic substrates that can alter, in real time, the presentation of ligands to an attached cell and hence influence the behaviors of adherent cells. These active substrates are based on electroactive monolayers that present redox-active groups which can be switched by applying electrical potentials to the underlying gold. A first example uses substrates that can be switched to turn on the immobilization of ligands. This strategy has been used to switch regions of the substrate from an inert state to a state that permits the adhesion and migration of cells. A second example uses substrates that can selectively release immobilized ligands from the monolayer. These examples establish that self-assembled monolayers of alkanethiolates on gold are an excellent model system for controlling the adhesion of cells and will find wide use both in fundamental studies for biology and in applied targets for biotechnology.

2:40pm **BI+EL-TuA3 Cell Respone to Chemically and Topographically Modified Surfaces**, ***D.S. Sutherland,*** *A.S. Andersson, K. Glasmastar, S. Petronis,* Chalmers University of Technology, Sweden*; F. Backhed, A. Richter-Dahlfors,* Karolinska Institute, Sweden*; U. Lidberg,* University of Gothenburg, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

The properties of surfaces have long been known to influence cellular behaviour. Both the chemistry and topography of surfaces have been shown to effect different aspects of cellular response. With the advent of micro and nanofabrication it is now possibl e to study these interaction in a more detailed fashion, isolating specific surface structures and systematically varying their size and shape. In a parallel multicentre project a range of micro and nanofabricated surfaces are used in cell culture experi ments with a range of cell types. The specific surface designs were selected to give chemical and topographic cues on a range of length scales from the micron and submicron to the nanometre and were used as a set, to screen for the influence of surface st ructure on cellular behaviour. Similar sets of well-characterised surfaces were used in a number of different cell culture systems, including epithelial, endothelial, mammary gland and pancreatic cells, to look for both cell-specific interactions and g ene ric correlations. The studies have taken advantage of recent advances in microbiological techniques, focussing on different aspects of gene expression, cell differentiation and cell-cell signalling as well as more traditional adhesion, proliferation and m orphologic analysis. Examples of preliminary results obtained so far include: 1. Non-adherence/proliferation of three cell types to lipid bilayers (so called supported membranes) 2. Expression of a specific cytokine by epithelial cells is influenced by the microtopography of the surface. Additional results from ongoing studies are expected within a few months.

3:00pm **BI+EL-TuA4 Directing Endothelial Cell Attachment and Growth Using a Novel Ozone Patterning Technique**, *S.R. Webb,* ***T. Boland,*** Clemson University

Being able to modify surfaces to control cellular behavior, i.e. adhesion, spreading, migration, and or proliferation is extremely important in the development of materials for tissue engineering applications. Of particular interest in the field of vascular research are surfaces that will direct cell attachment and growth in the presence of RGD containing serum proteins, which may adsorb to the material surface. In this study, cell response to patterned materials was examined by employing highly organized monolayers of self-assembled (SAM) octadecytrichlorosilane (OTS) on silicon oxide wafers. OTS surfaces were exposed to ozone for a varying amount of time ranging from 1-4 minutes. The remaining surfaces were exposed to ozone via a micron size mask, allowing only the exposed areas to be etched. The surfaces were analyzed by ellipsometry and electron spectroscopy for chemical analysis (ESCA). Bovine aortic endothelial cells (BAEC): were cultured in MEM + 10% Fetal Bovine Serum + 1% antibiotic solution. Cells were seeded and cultured in 96 well plates in the presence of pure and patterned OTS surfaces. Cell attachment and growth of endothelial cells on pure OTS monolayers was very poor, most likely because of the denaturing of serum proteins near the surfaces. The surfaces exposed to ozone showed varying film thickness depending on the dose, and a strong carbonyl peak in the ESCA spectra, indicating the presence of an oxidized thin organic film. Cell attachment to etched surfaces and growth exceeded the control tissue culture polystyrene. Cell density increased in regions of the pattern to a confluent layer. The cell spreading and attachment on the micro-patterned surfaces suggests that the cells may be able to attach more firmly to the extracellular proteins on the patterned surfaces. The result from this cell growth study will aid in designing micro-patterned surfaces varies areas, such as, cell-based biosensors, biocomputers, and new biomaterials.

3:20pm **BI+EL-TuA5 Cellular Interactions with Self-assembled Monolayers**, ***G.J. Leggett,*** University of Manchester Institute of Science and Technology, UK **INVITED**

The development of a detailed understanding of the influence of surface chemical structure on mammalian cell attachment has been confronted with difficulties. Not only are the biological problems inherently complex, but until recently there have not been adequately well defined model surfaces for fundamental studies. The advent of self-assembled monolayers (SAMs) has promised to transform this situation, by providing well-defined surfaces with structures and chemistries that may readily be controlled, and the past five years have seen growing interest in the use of SAMs to model cellular interactions with artificial substrata. In the present work, SAMs with a range of alkyl chain lengths and terminal groups have been used in studies of the attachment of murine 3T3 fibroblasts and primary human osteoblast-like cells. The sensitivity of cellular attachment to subtle changes in adsorbate molecular structure and order has been explored. The responses of cells to micropatterned substrata formed using photopatterning methods have been explored. The organisation of structural elements, including filamentous actin organisation and focal contact formation, within the cell cytoskeleton has been explored using immunochemical methods. The effect of protein adsorption has been probed by comparing attachment from serum-free and full media, and by pre-exposing surfaces to protein solutions. Valuable insights have been gathered into the relationship between surface chemical structure and cellular behaviour.

4:00pm **BI+EL-TuA7 Artificial Networks of Rat Hippocampal Neurons on Microelectrode Arrays**, ***C.D. James,*** *A.J. Spence,* Cornell University*; N. Dowell,* Wadsworth Center/Department of Health*; H.G. Craighead, M.S. Isaacson,* Cornell University*; J. Turner, W. Shain,* Wadsworth Center/Department of Health

The construction of artificial neuronal networks from dissociated primary neurons will permit study of synaptogenesis, synaptic plasticity, and neuronal processing. However, a thorough investigation of these processes requires two important components: a flexible method of producing patterned cell networks, and long-term (weeks) studies of such cell networks. To address these issues, microelectrode arrays have been fabricated to conduct long-term, non-invasive extracellular measurements of spontaneous and induced action potentials. In addition, we have used two methods, microcontact printing and conventional photolithography, to align patterns of molecules, such as poly-L-lysine and laminin, to the microelectrode arrays. Surface analysis of the patterned molecules was completed to assess the relevant factors for successfully promoting cell attachment and neurite guidance. Issues dealing with the reliability and stability of the microfabricated electrode arrays, specifically for primary neuron cell cultures, will also be addressed.

4:20pm **BI+EL-TuA8 Living Neural Cells as Components in Sensors and Computational Devices**, ***J.J. Hickman,*** Clemson University **INVITED**

We are developing the methodology to build hybrid biological/non-biological systems to create new information technology devices. This presentation will focus, from a bioengineering standpoint, the steps necessary to build such a device and some of the possible functions of

these devices. We are using self-assembled monolayers (SAMs) to control the intrinsic and geometric properties of surfaces in contact with biological systems. The use of surface modification techniques allows us to tailor the interface between biological/nonbiological materials independent of the bulk composition of the nonbiological material. The ability to control the surface composition of the in vitro system as well as controlling other variables, such as growth media and cell preparation, all play important roles in creating a defined system for devise operation. This defined system has been used as a test-bed to evaluate surface coatings for neuronal interactions with electronic materials. We have used the geometric control of the surface composition afforded us by SAMs to create in vitro circuits of mammalian neurons. We have also recorded the electrophysiological signals produced by neurons on the patterned SAMs in response to stimuli. The surfaces have been characterized by X-ray photoelectron spectroscopy (XPS), imaging XPS and contact angle measurements and we have related the intrinsic properties of the surface and the proteins deposited by the cells to cellular development. We are using what we learn for a more fundamental understanding of cellular development and also to create sensors using living neurons as the sensor element. The continuing development of this technology will be discussed, our latest results, as well as the implications and applications for (a) biosensor fabrication, (b) neuronal circuit design, and (c) biological computation.

5:00pm **BI+EL-TuA10 Tissue Formation of Hepatocytes on Micro-Porous Films of Polylactide***, **T. Nishikawa,*** RIKEN, Japan*; K. Nishikawa, R. Ookura, J. Nishida, S.-I. Nishimura, H. Ookubo, H. Kamachi, M. Matsushita, S. Todo,* Hokkaido University, Japan*; M. Shimomura,* RIKEN, Japan

Control of interaction between cells and material surfaces has been considered as a fundamental issue in designing and developing bio-materials for various purposes such as cell culture, implantation, and tissue regeneration. Surface morphology is one of the factors which can control the interaction. We previously reported that two-dimensional regular honeycomb pattern appear as a surface morphology of polymer films which were fabricated by casting dilute solution of amphiphilic polymers on solid substrates in a humid atmosphere. Recently we found that the honeycomb morphology can be applied to micro-patterning of cell culture substrates and that rat hepatocytes recognize the micro-patterned surfaces from chemical and morphological aspects and change their morphology and functions. Here we show that self-supported honeycomb films can be fabricated by casting a dilute solution containing polylactide (PLLA) as major component of the films and amphiphilic polymer as component for induction of honeycomb morphology. The honeycomb films worked as cell culture substrates for rat hepatocytes. Hepatocytes on the honeycomb films formed a colony, which exhibited tissue-like structure and express high level of albumin secretion, which was comparable to that of spheroids of hepatocytes. The tissue formation of hepatocytes specifically occurred on the honeycomb films of PLLA, but not on flat films of PLLA. The colony of hepatocytes kept the morphological features and liver specific function at day 14. This indicates that micro-porous films of PLLA would be appropriate for long term culturing of hepatocytes. Recently we succeeded in culturing hepatocytes on both sides of the self-supported honeycomb films of PLLA. In this sense, we believe that our materials possessing regular micro-pores are applicable to artificial extra-cellular matrices for tissue engineering.

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