

TAILORED SUBSTRATES FOR STUDYING AND CONTROLLING CELL ADHESION

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INTRODUCTION: Most cells are adherent and must attach to and spread on an extracellular protein matrix in order to survive, proliferate and carry out normal functions. The complex structure of this protein matrix often hinders mechanistic studies of cell adhesion, necessitating the development and use of model substrates. 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 mammalian cells with non-natural materials.

The approach emphasizes a molecular level design and preparation of monolayers that exhibit specific ligand-receptor interactions with cell-surface proteins. 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. 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. These model substrates—which permit complete control over the ligand-receptor interactions between cells and substrates—are valuable for mechanistic studies of cell adhesion and migration and for related applications in biotechnology.

METHODS:

RESULTS & DISCUSSION: *Self-Assembled Monolayers as Model Substrates for Cell Adhesion.*

The attachment of cells is mediated by the binding of integrin receptors to peptides contained in the extracellular matrix. The short tripeptide Arg-Gly-Asp is a common ligand for integrin receptors and has been used frequently to promote cell adhesion to materials. Monolayers that presented this peptide, at 1% density among tri(ethylene glycol)-terminated alkanethiolates supported the efficient attachment and spreading of 3T3 Swiss fibroblast cells. Immunostaining showed that cells had

mature focal adhesion complexes and actin stress filaments. Control experiments showed that cells did not attach to substrates presenting a scrambled peptide and that cell attachment could be inhibited by a soluble peptide, both demonstrating the specificity of cell-substrate interactions. This strategy has been applied to the design of monolayers used for studies of cell adhesion to several other peptide and carbohydrate ligands.

Dynamic Substrates for Studies of Cell Adhesion.

This strategy for engineering surfaces for selective interactions with cells has been extended to the design of dynamic substrates that can alter the presentation of ligands. These substrates, which can modulate ligand activity in real-time, offer a new opportunity for studying the cellular responses to changes in the composition and pattern of ligands on the underlying substrate. 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 ligands. This property stems from the Diels-Alder reaction of ligand-diene conjugates with a benzoquinone group of the monolayer. The dynamic property is based on the electrochemical reduction of quinone to a hydroquinone, which is not reactive with dienes. 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. It has also been used in a method to pattern the attachment of multiple cell types to a common substrate. A second example uses substrates that can selectively release immobilized ligands from the monolayer. Taken together, 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.