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14. ABSTRACT
The objective is to develop techniques for cell surface modification in order to interface cells with synthetic materials in the context of cell-based biosensors. Our approach uses the cell's metabolic machinery for oligosaccharide biosynthesis to deliver novel chemically reactive functional groups to the cell surface. We developed methods for cell adhesion using ketone-hydrazone condensation reactions and a new reaction we developed called the Staudinger ligation. We identified new metabolic pathways for delivery of reactive functional groups to cell surfaces. These tools were used to attach cells to synthetic surfaces in a robust manner with high resistance to shear stress.

15. SUBJECT TERMS
biosensor, cell adhesion, metabolic engineering, Staudinger ligation

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FINAL REPORT

GRANT #: N00014-98-1-0605

PRINCIPAL INVESTIGATOR: Professor Carolyn R. Bertozzi

GRANT TITLE: Metabolic Engineering of Reactive Cell Surfaces for Controlled Cell Adhesion

AWARD PERIOD: 01 June 1998-30 September 2001

OBJECTIVE:

The integration of biological components capable of performing complex functions into a synthetic device environment is a major frontier in the design of microscale and nanoscale devices. Proteins, for example, can serve as molecular motors or scaffolds; their natural ability to assemble into complex structures and transform chemical potential into mechanical force can be exploited in a device context. Living cells are capable of complex transformations such as multi-enzyme metabolic conversions that are useful for environmental bioremediation, energy harvesting and industrial fermentation. They can amplify and transduce signals in response to detection of soluble analytes, and thereby function in biosensing devices. All of these potential applications of biological components require their integration into a synthetic device environment. To achieve this, molecular control of the interface between the biological molecule or cell and the surrounding material is paramount. The objective of this project is to develop new strategies for engineering and characterizing biological/material interfaces.

APPROACH:

We developed a method of engineering chemical reactivity on living cell surfaces for specific covalent attachment to surfaces tailored with complementary chemical properties. We exploited metabolic pathways for oligosaccharide biosynthesis to introduce unique electrophiles such as ketones or azides into cell surface glycoconjugates. The cells were incubated with a simple sugar adorned with the electrophile of interest, and metabolic conversions within the cell incorporate the sugar into cell aurface components. The sialic acid biosynthetic pathway was found to be particularly amenable to this, as a wide range of N-acyl mannosamine analogs were found to be metabolized to sialic acids in mammalian cells. These electrophilic functional groups were then available for covalent reaction

with complementary nucleophiles anchored to surfaces. The resulting covalent adducts provided a means for specific attachment of cells to engineered surfaces.

ACCOMPLISHMENTS:

During the period of this grant we demonstrated that cells coated with ketones can be reacted with hydrazide compounds (1), and cells coated with azides can be reacted with phosphine reagents (2,3). We also expanded the repertoire of unnatural sialic acids we can introduce on cells (4). In addition, we discovered that the pathway for N-acetylgalactosamine metabolism can also be used for delivery of chemically reactive groups to cell surfaces (5). These discoveries significantly augment the types of cells that can be employed in artificial systems. Another major accomplishment is the development of surface coatings, based on supported bilayers that mimic cell membranes, that can support cell adhesion to an underlying substrate. As part of a collaborative effort with Prof. Jay Groves, we generated supported lipid bilayers on semiconducting material surfaces (i.e., silicon chips) that interfaced with engineered cell surfaces.

The hydrazone-mediated attachment of cells to the surface was found to be highly robust, as determined by the resistance of the interaction to increases in shear stress. ManLev-treated Jurkat cells were attached to bilayer-coated glass surfaces of various hydrazide density. The glass slides were placed in a parallel-plate flow chamber and buffer was passaged through the chamber at various flow rates. As shown in Fig. 1, cells on the high-percentage hydrazide surfaces were highly resistant to shear stress. At the highest shears (>6 dyne/sq. cm), cells detached via extraction of lipids from the bilayer; the hydrazone linkage remained intact even at these high shears (6).

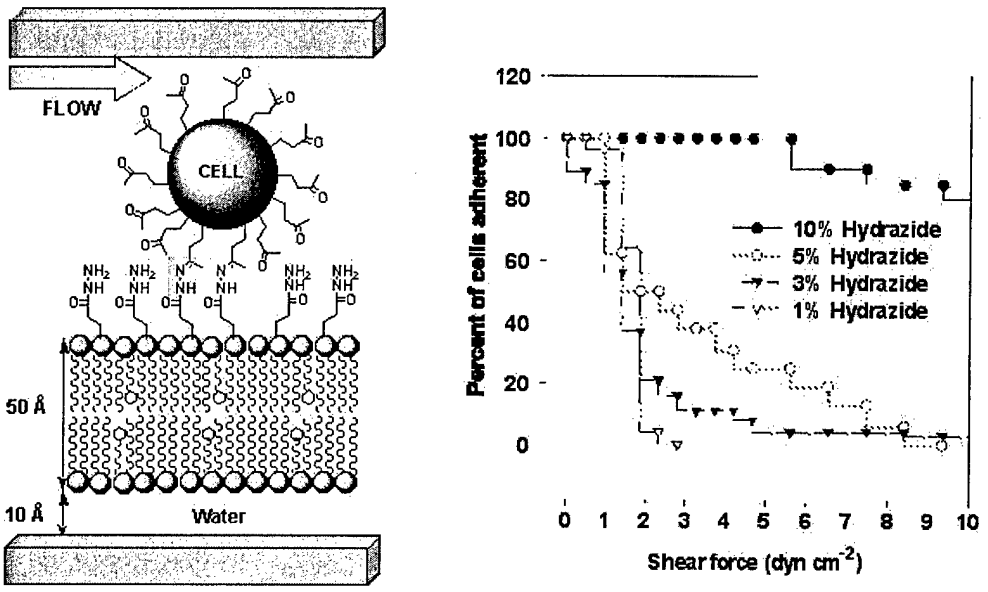

Figure 1. *Ketone-coated Jurkat cells attached to bilayer-coated surfaces via hydrazones resist detachment under shear stress. Cells were attached to surfaces as above, and the glass slide was placed in a parallel plate flow chamber. Increases in flow rate correlate with increased shear stress. The number of cells remaining attached to the surface after 15 minutes was determined.*

Finally, we have extended our metabolic engineering efforts from oliogsaccharides into the realm of proteins. In collaboration with Prof. David Tirrell at Caltech, we demonstrated that azides can also be incorporated into proteins by feeding cells unnatural amino acids bearing the azido group. The azide serves as a unique chemical handle for attachment of proteins to surfaces or other materials applications via a reaction we developed called the Staudinger ligation (7).

CONCLUSION:

We developed a method for engineering novel chemical properties onto cell surfaces and for attaching cells to synthetic surfaces in a chemically controlled fashion.

SIGNIFIGANCE:

This work provides new technologies for development of cell-based biosensors and bioreactors.

AWARD INFORMATION:

Donald Sterling Noyce Prize for Excellence in Undergraduate Teaching (2001); UC Berkeley Distinguished Teaching Award (2001); ACS Award in Pure Chemistry (2001); Merck Academic Development Program Award (2000); UC Berkeley Department of Chemistry Teaching Award (2000); Presidential Early Career Award in Science and Engineering (PECASE) (2000); MacArthur Foundation Award (1999); Camille Dreyfus Teacher-Scholar Award (1999); Arthur C. Cope Scholar Award (ACS) (1999); Joel H. Hildebrand Chair in Chemistry (1998-2000); Beckman Young Investigator Award (1998); Prytanean Faculty Award (1998); Glaxo Wellcome Scholar (1998); Research Corporation Research Innovation Award (1998); Office of Naval Research Young Investigator Award (1998)

PUBLICATIONS AND ABSTRACTS: (for total period of grant)

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