Berkeley Researchers Lay Groundwork for Cell Version of DNA Chip

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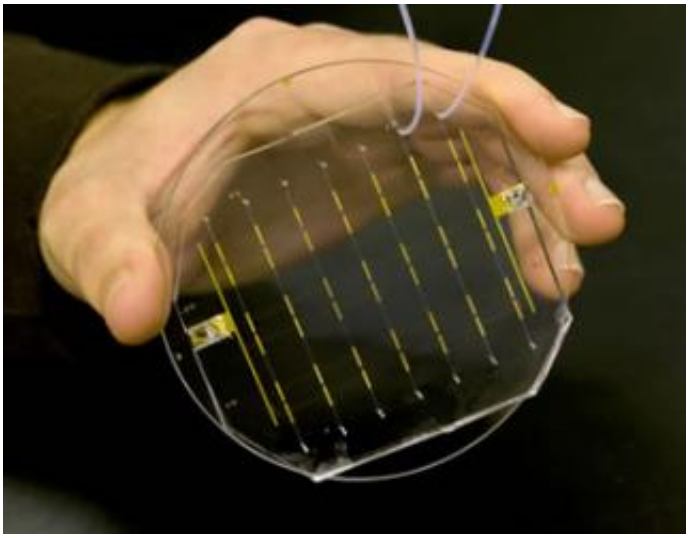

Using a DNA-based cell adhesion system, researchers can create cell chips, analagous to DNA chips, that could be used as biosensors for detecting the presence of pathogens, or for screening potential new therapeutic drugs.

A new technique in which single strands of synthetic DNA are used to firmly fasten biological cells to non-biological surfaces has been developed by researchers with the Lawrence Berkeley National Laboratory (Berkeley Lab) and the University of California at Berkeley. This technique holds promise for a wide variety of applications, including biosensors, drug-screening technologies, the growing of artificial tissues and the design of neural networks.

"Just as DNA chips revolutionized genome analysis, we hope to make cell chips (self-assembled arrays of cells on a thumbnail-sized chip) using our DNA-based cell adhesion strategy," said Ravi Chandra, a researcher affiliated with Berkeley Lab's Physical Biosciences Division and UC Berkeley's Chemistry Department. "Cell chips could be used as biosensors for detecting the presence of pathogens, or for drug screening, just to name of a few of the many possibilities."

Chandra is the lead author of a paper that appears in the latest issue of the international chemistry journal *Angewandte Chemie*. The other authors are Erik Douglas, Richard Mathies, Carolyn Bertozzi and Matthew Francis. The paper is entitled: *Programmable Cell Adhesion Encoded by DNA Hybridization*.

Many of the vast assortment of biological cells are naturally sticky, a property that enables individual cells to adhere to other cells and non-cellular components, which in turn enables them to assemble into different types of tissue, or carry out functions critical to an organism's health and well-being. Cell adhesion is now being used to incorporate biological cells into simple devices, but is expected to be important for the future production of complex nanotechnology devices.

To date, researchers have been attaching cells to surfaces using the array of cell adhesion proteins that Nature has provided, especially the proteins known as integrins. A surface will be laid out in a desired pattern with chemical handles called "ligands" to which the integrin-coated cells will bind. However, integrins are cell adhesion generalists, just about all of the different types of cells will stick to the same ligands. This makes integrin-coated cells ill-suited for applications that require precise patterns of multiple cell types.

The authors behind the Angewandte Chemie paper have solved the problem with the creation of a highly selective cell adhesion system that

uses single-strands of synthetic DNA to fasten the cells to a surface. This enables different types of cells to be selectively targeted and attached to specific locations on a surface based on the nucleotide sequences of the single-stranded DNA.

“We can pattern a surface with single-stranded DNA containing a specific nucleotide sequence, then coat cells with single-stranded DNA that contains a complementary sequence,” said Chandra, who is a member of both the Francis and Bertozzi research groups. Francis holds a joint appointment with Berkeley Lab’s Materials Sciences Division and the UC Berkeley Chemistry Department, and is an expert in linking organic molecules to nanoparticles to create hybrid structures. Bertozzi, who also holds a joint appointment with Berkeley Lab and UC Berkeley, is a leading authority on cell surface interactions. She is also a member of the Howard Hughes Medical Institute.

“Since the cells will adhere to the surface at locations where the complementary nucleotide sequences match, we can program cell adhesion events with a virtually unlimited number of possible coding options,” said Chandra. “The DNA effectively serves as a molecular barcode on the surface of living cells.”

Whereas the mounting of single-stranded DNA on the surface of a chip and using it as a probe to identify genetic matches is a well-established technology, this is the first time that single-stranded DNA has been attached to the surface of a biological cell. Chandra and his co-authors accomplished this feat through the adaptation of a technique, developed earlier by Bertozzi and her research group, called the “Staudinger ligation.” Bertozzi’s Staudinger ligation utilizes the sugar complexes called “oligosaccharides” that reside on the surface of every cell, and a reaction between two types of molecules, a nitrogen-containing azide, and a phosphorous-containing phosphine, as a means of attaching probes and reagents to cell surfaces, or to link different molecules inside of the

cells.

“The Staudinger ligation has been shown to be both cell friendly and precise, so we exploited the reaction to very specifically link a DNA molecule to the surface of a living cell,” said Chandra. “DNA is not normally found on the surfaces of cells, so the ability to deliver the DNA strands, keep them there, and use them for a productive purpose is a significant advance.”

Chandra, working under the direction of Francis and Bertozzi, used the Staudinger ligation to create a phosphine-DNA conjugate that makes it possible for the DNA to adhere to a cell’s surface. The phosphine-DNA conjugate
provides an outer layer of adhesion that is independent of the cell’s natural stickiness. As a bonus, the researchers are even able to bind their phosphine-DNA conjugates to cells that are not naturally sticky, providing the first known method for attaching non-adherent cell types to other surfaces. This could prove to be a real advantage.

“For example, it should be possible to array multiple types of non-adherent cells on a chip to create a mini-immune system for evaluating potential therapies,” said Chandra.

To test the ability of their phosphine-DNA conjugates to bind to cells, Chandra and his colleagues conducted a series of experiments with human embryonic kidney cells. From their results, they estimated that following treatment, each individual cell was coated with approximately 270,000 DNA molecules. Similar results were obtained using a line of human T-cells, called Jurkat cells, which are naturally non-adherent. Without the reactive phosphines, there was no detectable attachment of DNA to cell surfaces above background levels.

Once it was established that cell surfaces could be coated with single-

stranded DNA, Chandra worked with Douglas to demonstrate that this adhesion system could be used to attach cells to a non-biological surface. Douglas is a student under Mathies, director of UCB's Center for Analytical Biotechnology. Chandra and Douglas used a commercial chemical handle, the sulfur-based thiolate ion, to anchor single-stranded DNA onto gold pads, which were incorporated into microfluidic chips through standard photolithography. After the DNA-coated Jurkat cells were rinsed over the chips, fluorescence microscopy revealed that only those cells coated with single-stranded DNA complementary to the anchored DNA adhered to the gold pads. Cells that were otherwise identical but bearing mismatched DNA sequences were washed away.

"The adhesion of cells is very quick and far stronger than will be needed for most applications," said Chandra. "We just let them incubate for 35 minutes, rinse, and we're ready to go. We've kept our cells alive on the chip for up to 25 hours and the same proportion of cells survive using our method as cells cultured under identical conditions."

The next step, Chandra said, is to develop procedures for making complex arrays of cells quickly. The researchers also need to demonstrate that devices based on this cell-adhesion system will be useful for high-throughput screening applications.

Source: Berkeley Lab