Fluorescent Quantum Dots for Biological Labeling

Fluorescence is effectively turned on by enzymes specific to cells of interest.

NASA's Jet Propulsion Laboratory, Pasadena, California

Fluorescent semiconductor quantum dots that can serve as "on/off" labels for bacteria and other living cells are undergoing development. The "on/off" characterization of these quantum dots refers to the fact that, when properly designed and manufactured, they do not fluoresce until and unless they come into contact with viable cells of biological species that one seeks to detect. In comparison with prior fluorescence-based means of detecting biological species, fluorescent quantum dots show promise for greater speed, less complexity, greater sensitivity, and greater selectivity for species of interest. There are numerous potential applications in medicine, environmental monitoring, and detection of bioterrorism.

The established method of using fluorescent dyes to label live bacteria has several drawbacks:

- The high autofluorescence of many species renders many common chromophores invisible;
- The anaerobic conditions under which many bacteria live prevent proper folding of fluorescent proteins;
- Typical fluorescent dyes undergo rapid photobleaching and thereby rapidly cease to function as labels;
- Cells can be killed by the ultraviolet light needed to excite fluorescence in typical dyes used heretofore for labeling; and
- The addition of labeling dyes to cell cultures often leads to high background fluorescence, and bacteria are difficult to distinguish from debris, even when viewed through high-resolution microscopes.

When conjugated to suitable biological molecules that quench their fluorescence, fluorescent semiconductor quantum dots can be made to stick to the surfaces of, or to be taken up by, specific bacteria. To enable the on/off fluorescent detection of a specific bacterium, one chooses a fluorescence-quenching conjugate molecule that is removed by active enzymes on or in the bacterium.

Unlike conventional labeling dyes, fluorescent semiconductor quantum dots become photobleached very slowly and can be excited by blue light, which does not kill cells. Fluorescent semiconductor quantum dots can be manufactured to emit at wavelengths over a wide range — from blue through infrared. Spectral emission peaks of fluorescent semiconductor quantum dots are narrow — typically 10 nm or less in wavelength. The use of fluorescent semiconductor quantum dots entails the following disadvantages: (1) The dots are large and not always taken up by bacteria and (2) they contain heavy metals, which may prove toxic to organisms over long times.

Feasibility has been demonstrated in experiments on cadmium selenide quantum dots. First, the dots were conjugated to mercaptoacetic acid to render them soluble in water. The dots were then further conjugated to a variety of biological compounds. Conjugation was performed by use of a single-step carbodiimide reagent, which was then removed by dialysis versus pure water.

Conjugation to adenine, guanine, and tryptophan was found to quench all fluorescence from green-emitting quantum dots, and to quench >80 percent of the fluorescence from red-emitting quantum dots. Fluorescence did not return upon (1) exposure to ambient light for one week; (2) exposure to light from a 100-W, full-spectrum Hg lamp for 30 minutes; (3) incubation with a culture medium for 3 hours; or (4) incubation for 3 hours with metabolically inhibited bacterial cells [cells in a medium that contained ethylenediaminetetraacetic acid (EDTA), such that the cells remained intact but did not metabolize]. However, upon incubation for 3 hours in a culture medium with live bacterial cells, fluorescence returned and could be detected visually by color change, spectroscopically, and by fluorescence microscopy of individual cells.

This work was done by Gene McDonald, Jay Nadeau, Kenneth Nealson, Michael Storrie-Lombardi, and Rohit Bhartia of Caltech for **NASA's Jet Propulsion Laboratory**. *Further information is contained in a TSP (see page 1).*

NPO-30373

Growing Three-Dimensional Corneal Tissue in a Bioreactor

This method could help overcome the shortage of donated corneal tissue.

Lyndon B. Johnson Space Center, Houston, Texas

Spheroids of corneal tissue about 5 mm in diameter have been grown in a bioreactor from an in vitro culture of primary rabbit corneal cells to illustrate the production of optic cells from aggregates and tissue. In comparison with corneal tissues previously grown in vitro by other techniques, this tissue approximates intact corneal tissue more closely in both size and structure. This novel three-dimensional tissue can be used to model cell structures and functions in normal and abnormal corneas. Efforts continue to refine the present in vitro method into one for producing human corneal tissue to overcome the chronic shortage of donors for corneal transplants: The method would be used to prepare corneal tissues, either from in vitro cultures of a patient's own cells or from a well-defined culture from another human donor known to be healthy.

As explained in several articles in prior issues of *NASA Tech Briefs*, generally cylindrical horizontal rotating bioreactors have been developed to provide nutrient-solution environments conducive to the