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A Redundant, Orthogonal Yeast-cell-based Biosensor

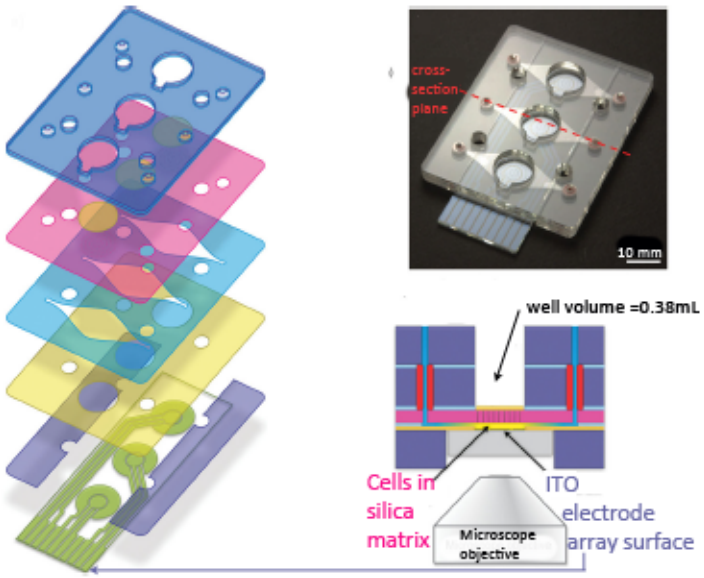
Using Sandia's /UNM's silica living-cell encapsulation method, with microfluidics and laser-machining produces a device in which living cells detect an analyte by three complementary, noninterfering assays

Challenge

Canary in a coal mine? Not quite—more like a yeast cell in a silica micro-chamber. But the principle is quite similar. The canary served as a toxic gas detector in mines—a biosensor. In like manner, the silica-encapsulated cells on this technology can serve as sensors of a variety of environmental agents. The difference, perhaps, is that the canary had to collapse, often die, whereas the cells need only send and amplify a signal telling a human observer that something of interest has been detected. There are several reasons to look at living cells as potential biosensors, not the least of which is that cells possess intrinsic amplification mechanisms to transduce detected analytes into more robust responses. Additionally, the diversity of cellular proteins and other macromolecules provides a huge battery of molecular specificities for accurate recognition events. On the other hand, unlike strictly electromechanical devices, cells require an environment that sustains their metabolism and protects them from dehydration.

Research

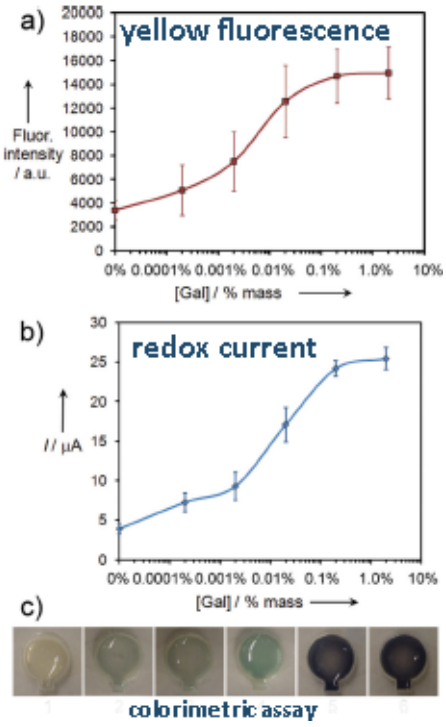
The last of these requirements has benefitted greatly from a solution that has been developed in the laboratory of Sandia Distinguished Staff member, Jeff Brinker, namely an organic-inorganic system of lipids and silica-related compounds to create micro-enclosures for living cells that allow the free exchange of nutrients, wastes, and signaling (hormone-like) molecules. In the current research, these encapsulated cells have been incorporated into a microfabricated device to provide the ability for three parallel methods of cell signaling upon detection of a target analyte.



Views of the chip utilized in this research. Left: disassembled to show multi-laminate structure; top: photograph; bottom: drawing of cross-section to show location of encapsulated cells and electrode array for electrochemistry.

Fabricated from 9 layers of flat materials ranging from Mylar and polymethylmethacrylate to indium tin oxide (ITO), this biosensor chip provides a microfluidic system for the exchange of aqueous solutions, a chamber for micro-encapsulated cells and a system of electrodes for detection of charge-transfer-related events.

The encapsulated cells are those of the bakers' yeast, *Saccharomyces, cerevisiae*, and three separate engineered populations are placed into the cell chamber. One type of yeast cell is engineered with a gene encoding yellow fluorescent protein, a fairly routine method in molecular biology, and this cell type responds to the presence of the simple sugar galactose by synthesizing this fluorescent marker. A second yeast cell type has been engineered to express and secrete the enzyme glucose oxidase (GOx) upon exposure to the simple sugar galactose. This enzymatic reaction is accompanied by the reduction of hydrogen peroxide and, hence, an electrical current from the transfer of electrons.



Data from exposure of the engineered yeast cell populations to the target analyte, galactose. Top: fluorescence obtained from the galactose induction of gene expression for yellow fluorescent protein; center: electrical current due to the galactose-induced expression of glucose oxidase and the reduction of H_2O_2; bottom: colorimetric assay for the presence of galactose at various concentrations.

Results of exposure of the chip to galactose showed both fluorescence and electrical current measurement with similar kinetics and similar dependency on galactose concentration. And as if this redundancy of detection wasn't sufficient, the presence of galactose, the target analyte, was also detectable by a colorimetric assay that produces a deep bluish green color upon the introduction of galactose. All in all, this set of experiments illustrated how redundant detector assays can be set up for biosensors, thereby greatly reducing both the possibility of detection failure and the possibility of false positives, increasing confidence in the validity of detection and diminishing interference from other substances in solution.

As a consequence of the clever encapsulation system of the cells, the biosensor also demonstrated reasonable longevity, with 40% of the yeast cells still viable after 60 days.

A final portion of this research was the co-encapsulation of both eukaryotic (the yeast cells) and prokaryotic cells (cells of the bacterium *E. coli*). With the yeast expressing yellow fluorescent protein in response to galactose, and the bacteria expressing green fluorescent protein upon exposure to theophylline, the sensor was capable of detecting which

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substance was present: yellow fluorescence for galactose, green fluorescence for theophylline, both yellow and green if both analytes were present.

Significance

Although still somewhat preliminary work, the results of this study point the way to possible routes to biosensors, with the silica-encapsulation methodology forming the basis of a technology that is much closer to realization than most other biosensor mechanisms. Engineering simpler eukaryotic cells such as yeast is well studied and it is conceivable that cells can be engineered to detect many different substances, from chemical and bio toxins to radionuclides and to express that recognition through a variety of colorimetric, electrochemical, and fluorescent mechanisms. The system engineered with micro-encapsulation thus opens the doors to a diversity of biosensor types.

For More Information: J .C. Harper et al., *Small online*: www.smalljournal.com, DOI:10.1002/smll.201200343 (2012).

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