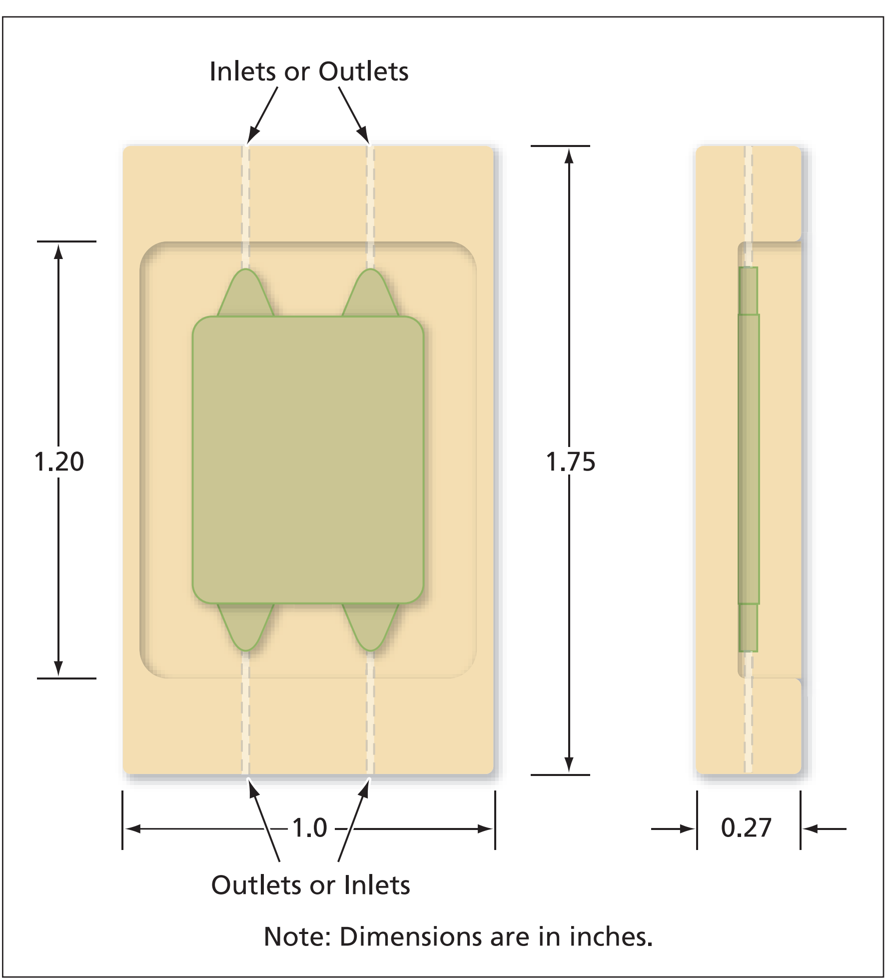
Miniature Bioreactor System for Long-Term Cell Culture

Cells can be cultured and sampled with minimal human intervention.

Lyndon B. Johnson Space Center, Houston, Texas

A prototype miniature bioreactor system is designed to serve as a laboratory benchtop cell-culturing system that minimizes the need for relatively expensive equipment and reagents and can be operated under computer control, thereby reducing the time and effort required of human investigators and reducing uncertainty in results. The system includes a bioreactor, a fluid-handling subsystem, a chamber wherein the bioreactor is maintained in a controlled atmosphere at a controlled temperature, and associated control subsystems. The system can be used to culture both anchorage-dependent and suspension cells, which can be either prokaryotic or eukaryotic. Cells can be cultured for extended periods of time in this system, and samples of cells can be extracted and analyzed at specified intervals. By integrating this system with one or more microanalytical instrument(s), one can construct a complete automated analytical system that can be tailored to perform one or more of a large variety of assays.

The bioreactor (see figure) is a thin culture chamber that has two or more inlets and two or more outlets for flows of liquids. The top face of the chamber is bounded by a membrane of porous respiratory active material that enables exchange of O_2 and CO_2 between the cell culture and the controlled atmosphere in which the bioreactor resides. The bottom face of the chamber can be either a second porous membrane or a microscope cover sheet, which enables microscopic imaging of cells in the chamber.



The **Bioreactor** is a thin culture chamber with inlets and outlets for liquids. The working volume of the bioreactor is 1 mL. Alternative designs can provide for more than the two inlets and/or for more than the two outlets shown here.

The fluid-handling subsystem includes an upstream and a downstream switching valve, flexible tubes that connect the upstream switching valve with three supply reservoirs and the bioreactor inlets, flexible tubes that connect the downstream switching valve with the bioreactor outlets and with waste and sampling reservoirs, and a peristaltic pump. The tubes on the downstream side are draped along the roller bearings of the peristaltic pump. There are three supply reservoirs: one containing the cell-culture nutrient medium, one containing a phosphate buffer solution (PBS), and one containing trypsin.

The flow passages in the valves are arranged so as to allow only the one correct liquid to flow through a given tube at a given time. The upstream valve enables the selection of flow of either fresh nutrient medium or PBS to the inlets. Alternatively, for the purpose of effective disconnection of part of the bioreactor, the upstream valve can be set to infuse trypsin through one inlet and the nutrient medium through the other inlet. The downstream valve can be set to connect all outlets to the waste reservoir or to connect a specified outlet or all outlets to a sampling reservoir.

Because the rates of flow required to sustain cell cultures are small and the system is operated accordingly, the flow velocity in the thin culture chamber is so small that the flow can be considered to be essentially laminar and two-dimensional, so that a given infinitesimal volume of liquid can be considered to travel smoothly along a simple, well-defined path. This flow characteristic can be exploited in harvesting cells from a specific region of a culture of anchorage-dependent cells, without disturbing

cells from other regions. In the case of suspension cells, harvesting is performed upon the infusion of fresh nutrient medium. Incorporated into the miniature culture system is a temperature-control system and gas-control loop. The inclusion of these two systems will enable the miniature culture system to be autonomous.

This work was done by Steve R. Gonda of Johnson Space Center and Stanley J. Kleis and Sandra K. Geffert of the University of Houston.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should addressed to:

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Refer to MSC-24210-1, volume and number of this NASA Tech Briefs *issue, and the page number.*

Electrochemical Detection of Multiple Bioprocess Analytes

Key analytes can be detected using sample volumes of only 100 mL.

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An apparatus that includes highly miniaturized thin-film electrochemical sensor array has been demonstrated as a prototype of instruments for simultaneous detection of multiple substances of interest (analytes) and measurement of acidity or alkalinity in bioprocess streams. Measurements of pH and of concentrations of nutrients and wastes in cell-culture media, made by use of these instruments, are to be used as feedback for optimizing the growth of cells or the production of desired substances by the cultured cells. The apparatus is designed to utilize samples of minimal volume so as to minimize any perturbation of monitored processes.

The apparatus can function in a potentiometric mode (for measuring pH), an amperometric mode (detecting analytes via oxidation/reduction reactions), or both. The sensor array is planar and includes multiple thin-film microelectrodes covered with hydrous iridium oxide. The oxide layer on each electrode serves as both a protective and electrochemical transducing layer. In its transducing role, the oxide provides electrical conductivity for amperometric measurement or pH response for potentiometric measurement. The oxide on an electrode can also serve as a matrix for one or more enzymes that render the electrode sensitive to a specific analyte. In addition to transducing electrodes, the array includes electrodes for potential control. The array can be fabricated by techniques familiar to the microelectronics industry.

The sensor array is housed in a thin-film liquid-flow cell that has a total volume of about 100 mL. The flow cell is connected to a computer-controlled subsystem that periodically draws samples from the bioprocess stream to be monitored. Before entering the cell, each 100-mL sample is subjected to tangential-flow filtration to remove particles. In the present version of the apparatus, the electrodes are operated under control by a potentiostat and are used to simultaneously measure the pH and the concentration of glucose. It is anticipated that development of procedures for trapping more enzymes into hydrous iridium oxide (and possibly into other electroactive metal oxides) and of means for imparting long-term stability to the transducer layers should make it possible to monitor concentrations of products of many enzyme reactions — for example, such key bioprocess analytes as amino acids, vitamins, lactose, and acetate.

This work was done by R. David Rauh of EIC Laboratories, Inc., for Johnson Space Center. Further information is contained in a TSP (see page 1).

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

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Fabrication and Modification of Nanoporous Silicon Particles

Biodegradable drug carriers allow sustained drug release for days or even weeks.

Lyndon B. Johnson Space Center, Houston, Texas

Silicon-based nanoporous particles as biodegradable drug carriers are advantageous in permeation, controlled release, and targeting. The use of biodegradable nanoporous silicon and silicon dioxide, with proper surface treatments, allows sustained drug release within the target site over a period of days, or even weeks, due to selective surface coating. A variety of surface treatment protocols are available for silicon-based particles to be stabilized, functionalized, or modified as required. Coated polyethylene glycol (PEG) chains showed the effective depression of both plasma protein adsorption and cell attachment to the modified surfaces, as well as the advantage of long circulating.

Porous silicon particles are micromachined by lithography. Compared to the synthesis route of the nanomaterials, the advantages include: (1) the capability to make different shapes, not only spherical particles but also square, rectangular, or ellipse cross sections, etc.; (2) the capability for very precise dimension control; (3) the capacity for porosity and pore profile control; and (4) allowance of complex surface modification. The particle patterns as small as 60 nm can