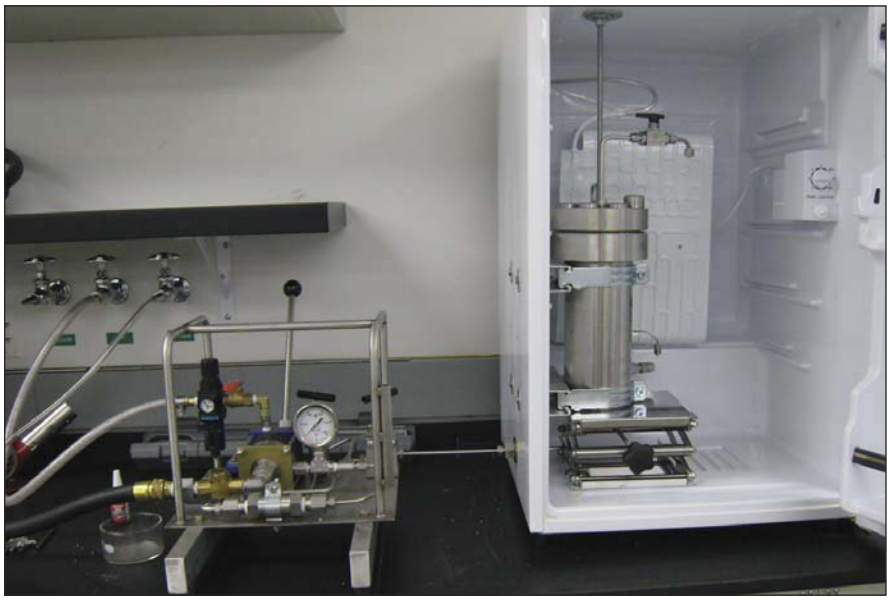
Apparatus for Cold, Pressurized Biogeochemical Experiments

Bacteria are grown under conditions imitating those at ocean depths.

NASA's Jet Propulsion Laboratory, Pasadena, California

A laboratory apparatus has been devised as a means of studying plausible biogeochemical reactions under high-pressure, low-temperature aqueous, anaerobic conditions like those conjectured to prevail in a liquid water ocean on Europa (the fourth largest moon of the planet Jupiter). The experiments to be performed by use of this apparatus are intended to enhance understanding of how life (if any) could originate and evolve in the Europa ocean environment. Inasmuch as terrestrial barophilic, psychrophilic organisms that thrive under anaerobic conditions are used in the experiments, the experiments may also contribute to terrestrial biogeochemistry.

The apparatus (see figure) includes a bolt-closure reaction vessel secured inside a refrigerator that maintains a temperature of 4 °C. Pressurized water is supplied to the interior of the vessel by a hydrostatic pump, which is attached to the vessel via high-pressure fittings.



This **Laboratory Apparatus** is used to study biogeochemical reactions in liquid water at high pressure and low temperature. Bacterial specimens are loaded from the top of the vessel into sample cells equipped with 0.2-μm filters. The vessel is filled with water, air is vented from the top through a valve, and then the water is pressurized to 5 kpsi (≈34 MPa).

The terrestrial organisms used in the experiments thus far have been several facultative barophilic, psychrophilic stains of *Shewanella* bacteria. In the experiments, these organisms have been tested for reduction of ferric ion by growing them in the presence of a ferric food source under optimized terrestrial conditions. The short-term goal of these experiments has been to select *Shewanella* strains that exhibit iron-reduction capability and test their ability to facilitate biogeochemical reduction of iron under temperature and pressure conditions imitating those in Europa's ocean. It is anticipated, that, once growth under Europa-like conditions has been achieved, the selected *Shewanella* strains will be used to facilitate biogeochemical reactions of sulfate and carbonate with hydrogen gas. Any disequilibrium of the products with the environment would be interpreted as signifying biogenic activity and the possibility of life in Europa's ocean.

This work was done by Xenia Amashukeli, Robert T. Pappalardo, and Stephanie A. Connon of Caltech and Damhnait F. Gleeson of the University of Colorado for NASA's Jet Propulsion Laboratory. For more information contact iaoffice@jpl.nasa.gov
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Growing B Lymphocytes in a Three-Dimensional Culture System

Cells grown in this system live long and closely resemble in vivo cells.

Lyndon B. Johnson Space Center, Houston, Texas

A three-dimensional (3D) culture system for growing long-lived B lymphocytes has been invented. The capabilities afforded by the system can be expected to expand the range of options for immunological research and related activities, including testing of immunogenicity of vaccine candidates *in vitro*, generation of human monoclonal antibodies, and immunotherapy.

Mature lymphocytes, which are the effectors of adaptive immune responses in vertebrates, are extremely susceptible to apoptotic death, and depend on continuous reception of survival-inducing stimulation (in the forms of cytokines, cell-to-cell contacts, and antigen receptor signaling) from the microenvironment. For this reason, efforts to develop systems for long-term culture of functional, non-transformed and non-activated mature lymphocytes have been unsuccessful until now.

The bone-marrow microenvironment supports the growth and differentiation of many hematopoietic lineages, in addition to B-lymphocytes. Primary bone-marrow cell cultures designed to promote the development of specific cell types *in vitro* are highly desirable experimental systems, amenable to manipulation under controlled conditions. However, the dynamic and complex network of stromal cells and insoluble matrix proteins is disrupted in prior plate- and flask-based culture systems, wherein the microenvironments have a predominantly two-dimensional (2D) character. In 2D bone-marrow cultures, normal B-lymphoid cells become progressively skewed toward precursor B-cell populations that do not retain a normal immunophenotype, and such mature B-lymphocytes as those harvested from the spleen or lymph nodes do not survive beyond several days *ex vivo* in the absence of mitogenic stimulation.

The present 3D culture system is a bioreactor that contains highly porous artificial scaffolding that supports the long-term culture of bone marrow, spleen, and lymph-node samples. In this system, unlike in 2D culture systems, B-cell subpopulations developing within 3D cultures that have been modified to foster lymphopoiesis retain an immunophenotype that closely recapitulates cells in fresh bone marrow harvests. The 3D culture system has been found to be capable of supporting long-lived (8 weeks) populations of B and T lymphocytes from peripheral lymphoid organs, in the absence of activation signals, to an extent not achievable by conventional culture techniques. Interestingly, it has been found that 3D-culture B cells display a phenotype that has characteristics of both B1a and B2 cells. These promising preliminary observations suggest that the 3D culture system could be used with success in the study of peripheral-B-lymphocyte biology and in the development of biotechnological techniques and processes.

This work was done by J. H. David Wu and Andrea Bottaro of the University of Rochester for Johnson Space Center. For further information, contact the Johnson Commercial Technology Office at (281) 483-3809.

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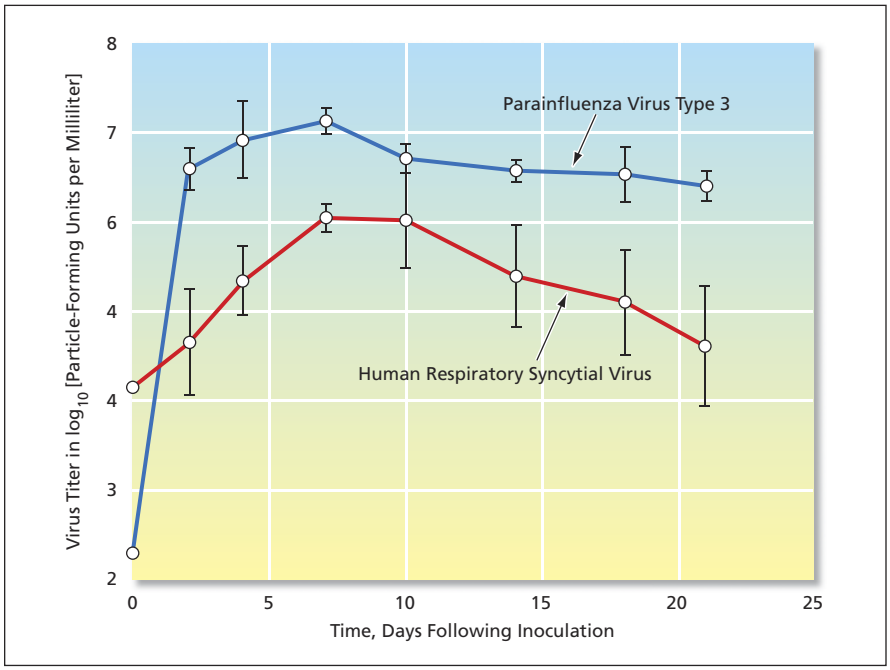
Tissuelike 3D Assemblies of Human Broncho-Epithelial Cells

Experimental conditions are more realistic than those of 2D monolayer cell cultures.

Lyndon B. Johnson Space Center, Houston, Texas

Three-dimensional (3D) tissuelike assemblies (TLAs) of human broncho-epithelial (HBE) cells have been developed for use in *in vitro* research on infection of humans by respiratory viruses. The 2D monolayer HBE cell cultures heretofore used in such research lack the complex cell structures and interactions characteristic of *in vivo* tissues and, consequently, do not adequately emulate the infection dynamics of *in-vivo* microbial adhesion and invasion. In contrast, the 3D HBE TLAs are characterized by more-realistic reproductions of the geometrical and functional complexity, differentiation of cells, cell-to-cell interactions, and cell-to-matrix interactions characteristic of human respiratory epithelia. Hence, the 3D HBE TLAs are expected to make it possible to perform at least some of the research *in vitro* under more-realistic conditions, without need to infect human subjects.

The TLAs are grown on collagen-coated cyclodextran microbeads under controlled conditions in a nutrient liquid in the simulated microgravitational environment of a bioreactor of the rotating-wall-vessel type. Primary human mesenchymal bronchial-tracheal cells are used as a foundation matrix, while adult human bronchial epithelial immortalized cells are used as the overlying component. The beads become coated with cells, and cells on adjacent beads coalesce into 3D masses. The resulting TLAs have been found to share significant characteristics with *in vivo* human respiratory epithelia including polarization, tight junctions, desmosomes, and microvilli. The differentiation of the cells in these TLAs into tissues functionally similar to *in vivo* tissues is confirmed by the presence of



These **Virus Titers** indicate rapid growth of virus populations during the first few days.