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.

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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Refer to MSC-23571-1, volume and number of this Medical Design Briefs issue, and the page number.

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...
tissue-like differentiation marker compounds, including villin, keratins, and specific lung epithelium marker compounds, and by the production of tissue mucin.

In a series of initial infection tests, TLA cultures were inoculated with human respiratory syncytial viruses and paramyxovirus type 3 viruses. Infection was confirmed by photomicrographs that showed signs of damage by viruses and virus titers (see figure) that indicated large increases in the populations of viruses during the days following inoculation.

This work was done by Thomas J. Goodwin of Johnson Space Center. Further information is contained in a TSP (see page 1).

This invention is owned by NASA, and a patent application has been filed. Inquiries concerning nonexclusive or exclusive license for its commercial development should be addressed to the Patent Counsel, Johnson Space Center, (281) 483-0837. Refer to MSC-24164-1.

Isolation of Resistance-Bearing Microorganisms

NASA’s Jet Propulsion Laboratory, Pasadena, California

Strategies were explored for inactivating resistance-bearing microorganisms, focusing on a new species (*Bacillus horneckii* sp. nov.) discovered on the surfaces of the Kennedy Space Center cleanroom facility in which the Phoenix lander was assembled. Two strains that belong to this novel species were isolated and subjected to a comprehensive, polyphasic analysis to characterize their taxonomic position.

Both phenotypic and genotypic analyses clearly indicate that these isolates belong to the genus *Bacillus*, and represent a novel species. In addition to the phylogenetic affiliation, structurally the spores of this novel bacterium possess an extraneous layer, which might be responsible for increased resistance to space radiation conditions. The chemical characterization of this novel, extraneous layer of spores will reveal the mechanisms behind radiation resistance.

This work was done by Kasthuri J. Venkateswaran, Alexander Probst, Parag A. Vaishampayan, and Sudeshna Ghosh of Caltech; and Sharrif Osman of Lawrence Berkeley National Laboratory for NASA’s Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov. NPO-46337

Oscillating Cell Culture Bioreactor

This bioreactor is well suited to work with different biological specimens.

Lyndon B. Johnson Space Center, Houston, Texas

To better exploit the principles of gas transport and mass transport during the processes of cell seeding of 3D scaffolds and *in vitro* culture of 3D tissue engineered constructs, the oscillatory cell culture bioreactor provides a flow of cell suspensions and culture media directly through a porous 3D scaffold (during cell seeding) and a 3D construct (during subsequent cultivation) within a highly gas-permeable closed-loop tube. This design is simple, modular, and flexible, and its component parts are easy to assemble and operate, and are inexpensive. Chamber volume can be very low, but can be easily scaled up. This innovation is well suited to work with different biological specimens, particularly with cells having high oxygen requirements and/or shear sensitivity, and different scaffold structures and dimensions.

The closed-loop changer is highly gas permeable to allow efficient gas exchange during the cell seeding/culturing process. A porous scaffold, which may be seeded with cells, is fixed by means of a scaffold holder to the chamber wall with scaffold/construct orientation with respect to the chamber determined by the geometry of the scaffold holder. A fluid, with/without biological specimens, is added to the chamber such that all, or most, of the air is displaced (i.e., with or without an enclosed air bubble). Motion is applied to the chamber within a controlled environment (e.g., oscillatory motion within a humidified 37 °C incubator). Movement of the chamber induces relative motion of the scaffold/construct with respect to the fluid. In case the fluid is a cell suspension, cells will come into contact with the scaffold and eventually adhere to it. Alternatively, cells can be seeded on scaffolds by gel entrapment prior to bioreactor cultivation.

Subsequently, the oscillatory cell culture bioreactor will provide efficient gas exchange (i.e., of oxygen and carbon dioxide, as required for viability of metabolically active cells) and controlled levels of fluid dynamic shear (i.e., as required for viability of shear-sensitive cells) to the developing engineered tissue construct.

This bioreactor was recently utilized to show independent and interactive effects of a growth factor (IGF-I) and slow bidirectional perfusion on the survival, differentiation, and contractile performance of 3D tissue engineering cardiac constructs.

The main application of this system is within the tissue engineering industry. The ideal final application is within the automated mass production of tissue-engineered constructs. Target industries could be both life sciences companies as well as bioreactor device producing companies.

This work was done by Lisa E. Freed, Mingyu Cheng, and Matteo G. Moretti of Massachusetts Institute of Technology for Johnson Space Center. For further information, contact the Johnson Technology Transfer Office at (281) 483-3809.

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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Refer to MSC-24270-1, volume and number of this Medical Design Briefs issue, and the page number.