Bioreactor expands health research

NASA device gives new dimension to cell science

Good neighborhoods help you grow. It is true of humans, and of the cells of which we and all complex organisms are made. A challenge which has faced cell science throughout its short history is that most cell cultures produce flat, one-cell-thick specimens that offer limited insight into how cells work together. Such knowledge is crucial if we are to understand the chemistry and mechanics of healthy organs and of cancers, infectious diseases, immune system failures, and other public health problems.

The National Aeronautics and Space Administration, in concert with the biomedical community, has initiated work that offers significant advances in cell culturing technology on Earth that enables further unique research progress aboard Space Shuttle, *Mir*, and — soon — *International Space Station*. The NASA Biotechnology Cell Science Program, directed by Dr. Neal Pellis at NASA's Johnson Space Center, involves more than 100 scientists, engineers, and support personnel around the nation.

A new spin on cell growth

They are meeting the challenge with a unique new technology, the rotating wall vessel bioreactor. It spins a fluid medium filled with cells to neutralize most of gravity's effects and encourage cells to grow in a natural manner. The rotating bioreactor was invented by NASA as a model of microgravity effects on cells.

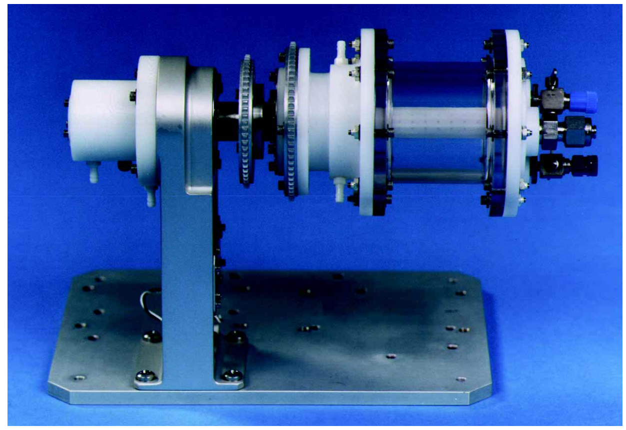
Ground tests of the bioreactor yielded three-dimensional tissue specimens approximating natural growth, a striking change from the pancake shapes of traditional cultures.

As cells replicate, they "self associate" to form a complex matrix of collagens, proteins, fibers, and other chemicals. This highly evolved microenvironment tells the cells who is next door or nearby, how they should grow and into what shapes, and how to respond to stimuli such as bacteria and wounds. Thus, we have the opportunity to study the complex order of tissue in a culture system that can be manipulated by drugs, hormones, and genetic engineering.

Staying in touch

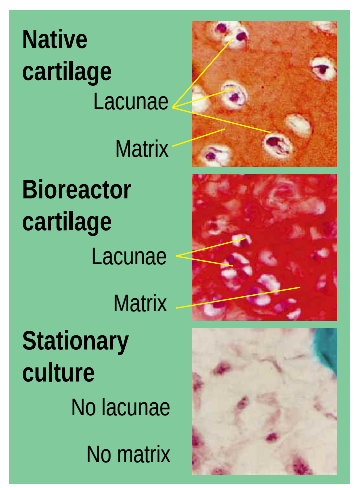
As with a city, the lives of a cell are governed by its neighborhood connections. Those connections that don't work have been implicated in muscular dystrophy, osteoporosis, glaucoma, and the spread (metastasis) of cancer cells throughout the body.

Studying these mechanisms outside the body on Earth is limited by gravity's effects because the cells do not easily self-associate to grow naturally. The bioreactor promotes self-association in a container about the size of a soup can.



The heart of the Bioreactor is the rotating wall vessel, shown here without its support equipment.

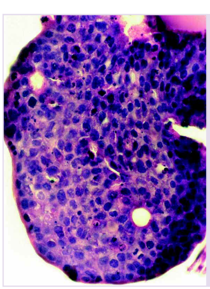
The clear shell allows scientists to check growth, and the center holds a cylindrical filter that passes oxygen and nutrients in and carbon dioxide and wastes out. This ensures that the fluid rotates without shear forces that would destroy the cells. The rotating vessel does not really cancel gravity, but ideally maintains cells in continual free-fall similar to that experienced by astronauts in the microgravity of space.



The Bioreactor allows cells to reach a level of maturity rarely achieved by other methods.

Earth-based bioreactor cultures typically maintain cell growth for at least 60 days. On Earth, a sample then becomes so large (about 1 cm) that it is no longer suspended. In long space missions, large-sample growth in bioreactors can be studied in order to understand the extended growth and differentiation of cells, such as engineering complex tissue or modeling slow-growing tumors.

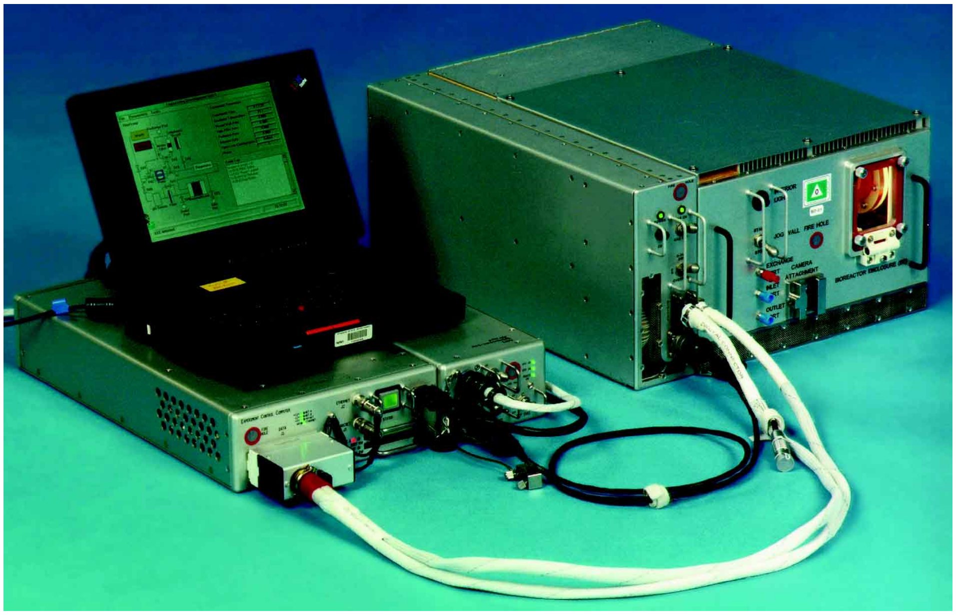
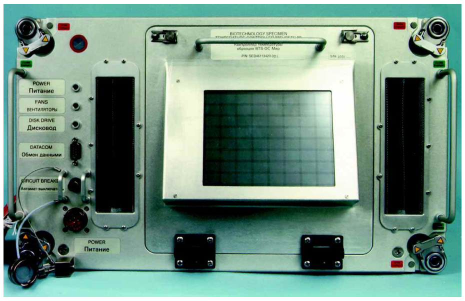
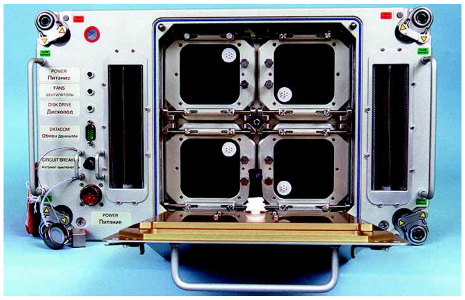
With long-term stays aboard the Mir space station and, soon, the International Space Station, NASA and the biomedical community have this opportunity to explore this new frontier in cell science and contribute to public health.



Targeted health issues

Infectious disease: Human immunodeficiency virus (HIV), Ebola virus, Lyme disease.
Cancer models: Prostate, breast, ovary, lung, and colon cancers.
Diabetes: Work on a pancreatic tissue for transplant.
Therapies: Musculoskeletal tissue disorders (in space and on earth).
Drug efficacy: Non-animal/nonclinical testing of drug effects and toxicity.

Tools

The Bioreactor rotating wall vessel sits at the front of the incubator module (window of large module in left photo) behind a window so the crew may monitor cell progress. The bioreactor is controlled by the Experiment Control Computer (ECC; flat module in the left photo), and the crew can reprogram the system through the payload general support computer atop the ECC. The BSTC (closed, top right, and open, bottom right) is an integral part of the bioreactor development program aboard Mir.

NASA's cell science program uses experimental hardware fitted to the requirements of the science community and the resources of the Space Shuttle orbiter middeck, and taking advantage of the endurance of the *Mir* space station. These are forerunners of systems for long-term investigations aboard the International Space Station.

Bioreactor Demonstration System (BDS) comprises an electronics module, a gas supply module, and the incubator module housing the rotating wall vessel and its support systems. Nutrient media are pumped through an oxygenator and the culture vessel. The shell rotates at 0.5 rpm while the inner filter typically rotates at 11.5 rpm to produce a gentle flow that ensures removal of waste products as fresh media are infused. Periodically, some spent media are pumped into a waste bag and replaced by fresh media. When the waste bag is filled, an astronaut drains the waste bag and refills the supply bag through ports on the face of the incubator. "Pinch" valves and a perfusion pump ensure that no media are exposed to moving parts. An Experiment Control Computer controls the Bioreactor, records conditions, and alerts the crew when problems occur. The crew operates the system through a laptop computer displaying graphics designed for easy crew training and operation.

Into the commercial sector

Bioreactors are entering the medical research community via the Technology Transfer Act. In 1990, NASA granted Synthecon, Inc., of Houston exclusive commercial license to NASA patents for the bioreactor system. This ensures that the research potential of the bioreactor will be available to a wide range of institutes and companies. More than $2,000,000 in Rotary Cell Culture Systems™ had been sold by mid-1998.

Synthecon has sponsored research agreements with the University of North Texas Science Center, Fort Worth, to commercialize replacement skin products, and Baylor College of Medicine, Houston, to develop a novel diabetic therapy. Synthecon is negotiating other agreements with major medical institutes to commercialize additional biological products. In addition, Synthecon develops new designs to advance their growth potential.

Biotechnology Specimen Temperature Controller (BSTC) will cultivate cells until their turn in the bioreactor; it can also be used in culturing experiments that do not require the bioreactor. The BSTC comprises four incubation/refrigeration chambers individually set at 4 to 50 ˚C (near-freezing to above body temperature). Each chamber holds three rugged tissue chamber modules (12 total), clear Teflon bags holding 30 ml of growth media, all positioned by a metal frame. Every 7 to 21 days (depending on growth rates), an astronaut uses a shrouded syringe and the bags' needleless injection ports to transfer a few cells to a fresh media bag, and to introduce a fixative so that the cells may be studied after flight. The design also lets the crew sample the media to measure glucose, gas, and pH levels, and to inspect cells with a microscope. The controller is monitored by the flight crew through a 23-cm (9-inch) color computer display on the face of the BSTC.

Biotechnology Refrigerator (BTR) holds fixed tissue culture bags at 4 ˚C to preserve them for return to Earth and post-flight analysis.

Gas Supply Module contains four rechargeable bottles of an air/carbon dioxide mixture to support optimal cell growth over long periods of time aboard a space station.

Ground-based research results

Since its invention in 1992, the bioreactor has been used in terrestrial laboratories for significant research and for work leading to clinical studies in a range of diseases. In May 1997, the Society for In Vitro Biology devoted an entire issue of *In Vitro Cellular & Developmental Biology—Animal* (**33**:5, 325-405) to the results of a special NASA workshop on bioreactor results. Many of the authors are investigators in NASA's bioreactor flight research program. Topics covered by *In Vitro* or reported through other media include:

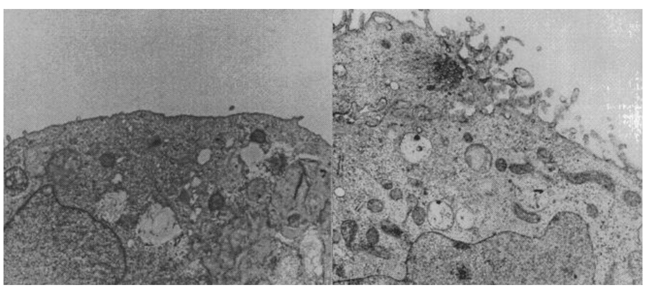
Cancer research. Bioreactors have cultured single cells of cancers of the skin (melanoma), prostate, ovary, breast, bone (osteosarcoma), and colon into viable cell cultures. Tissue structures can be grown for at least 60 days before they become too large to remain suspended in the bioreactor growth medium.

Infectious disease. The U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick, Md., uses the bioreactor to grow cultures—rather than using live animals—in the study of how the Ebola virus is transmitted. At the NASA/NIH Center for Three Dimensional Tissue Culture (see box above), scientists use the bioreactor in a wide range of infectious disease studies.

Kidney failure. In addition to filtering waste from the bloodstream, the kidneys secrete crucial hormones, including erythropoietin and 1-25-diOH-D3. Replacing these hormones now costs $2.5 billion. Further, the technology for supplying them is (as with insulin for diabetics) inadequate to maintain a healthy body. Culturing of kidney cells offers the potential to produce these key hormones so that the health and quality of life of kidney patients can be improved.

Immune system repression. One of the marvels of the human immune system is how lymphocytes, like escape artists, squeeze through the tight spaces between cells in search of invading disease. Bioreactor studies using collagen reveal that lymphocytes exposed to simulated low-g for 72 hours do not move. This has been verified with experiments aboard Shuttle missions, and demonstrates that the bioreactor is a good simulation of low-g growth conditions.

Drug efficacy. Kidney and heart tissues cultured in the bioreactor show the appropriate drug receptor sites that allow testing of drugs to determine their safety without using animals. This also reduces the need to use human volunteers in final testing.



Cells from kidneys lose some of their special features in conventional culture (left) but form spheres replete with specialized cell microvilli ("hair") and synthesize hormones that may be clinically useful.

National Institutes of Health

In 1994, NASA and the National Institutes of Health signed an interagency agreement to provide NASA bioreactor technology to NIH and to establish a joint Center for Three Dimensional Tissue Culture at the National Institute of Child Health and Human Development. Since the original agreement, the bioreactor has been incorporated into more than a dozen laboratories within NIH.

The bioreactor is an excellent example of how the skills and resources of two distinctly different agencies can complement each other for the public good. Where NASA is chartered to explore and exploit space, NIH is chartered to develop tools to defeat disease.

This new center is conducting 16 bioreactor research projects. Foremost among these is the first *in vitro* tissue system permitting the study of the HIV pathway through the human lymphatic tissue. The other 15 projects address a range of human health issues. NASA bioreactor technology is used within several NIH institutes and other agencies covering virtually all of human health: allergies, dentistry, the human genome, digestive and kidney disease, neurology, and heart, lung, and circulatory health.

At the NASA/NIH Center for Three Dimensional Tissue Culture, scientists use tonsil tissue to grow live Human Immunodeficiency Virus (HIV-1) and thus observe more closely the transmission of the virus. The cultures have demonstrated the same progressive loss of CD4 T-cells as seen in AIDS patients. The NASA/NIH Center also is studying an intestinal parasite found on some imported fruit.

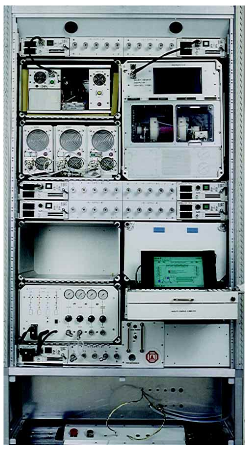
Attacking diabetes

Understanding that a key hormone is needed to move sugar from the bloodstream into cells allowed doctors to develop a treatment, daily insulin injections for life, for diabetes. While staving off death, this therapy is crude compared to the fine balancing act performed by the beta cells of a healthy pancreas. About half of the blindness, kidney failure, and limb loss in the United States are due to long-term complications of diabetes, for a public cost of $1 out of $7 spent in health care.

Bioreactor cell science opens new possibilities for more natural treatments. In 1997, NASA and the **Juvenile Diabetes Foundation** signed a Space Act Agreement for collaborative work including bioreactor research to understand the best route for cultivating and transplanting beta cells into Type I diabetics, and to develop a non-invasive blood sugar monitor which could enhance bioreactor operations.

Under a Technology Transfer Act Agreement, **VivoRx** of Santa Monica, Calif., is developing a method of encapsulating beta cells in treated seaweed membranes for implant in the abdomen. The seaweed allows insulin and glucose to diffuse back and forth so the transplanted cells work as an artificial pancreas. Microencapsulation results in human volunteers are highly promising. The bioreactor is being used to develop methods to expand the small numbers of cells available from donors to supply the large numbers of cells needed by diabetics.

Flight results



Bioreactor research aboard the Shuttle (above) is valuable but limited in duration. Completing work on Mir will let the bioreactor science team make full use of the Biotechnology Facility (right) proposed for International Space Station.

Photos: *Proceedings of the National Academy of Sciences* (page 4, top right). Synthecon (page 2, bottom). All others, NASA.

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Biotechnology
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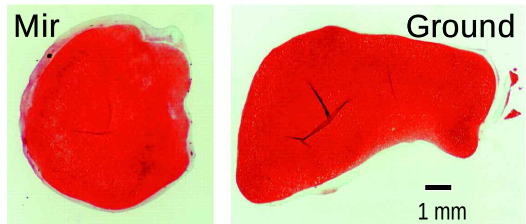
FS-1998-09-65-MSFC; 9/4/98

While bioreactor research in space is relatively new, it shows great promise. For 5 days on the STS-70 mission, a bioreactor cultivated human colon cancer cells, which grew to 30 times the volume of control specimens grown on Earth. This significant result was reproduced on STS-85 which grew mature structures that more closely match what are found in tumors in humans.

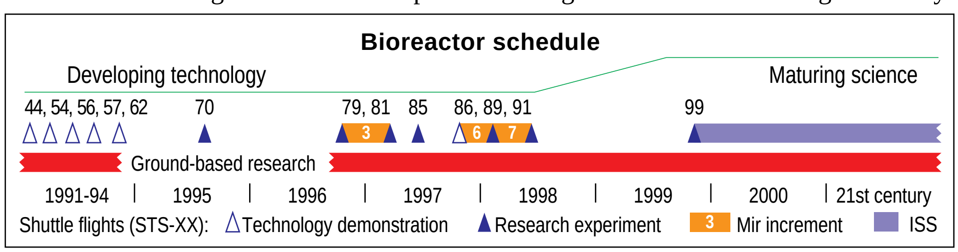


Engineered cartilage samples.

Mir Increment 3 (Sept. 16, 1996 - Jan. 22, 1997) grew specimens of tissue engineered cartilage that surprised investigators. In the December 1997 issue of the *Proceedings of the National Academy of Sciences* (**94**: 13885-90, 1997), Dr. Lisa E. Freed of the Massachusetts Institute of Technology and her colleagues reported that initially disclike specimens tend to become spherical in space, demonstrating that tissues can grow and differentiate into distinct structures in microgravity. The Mir samples were smaller, more spherical, and mechanically weaker than Earth-grown control samples.

These results demonstrate the feasibility of microgravity tissue engineering and may have implications for long human space voyages and for treating musculoskeletal disorders on earth. A separate commentary (*PNAS*, pp. 13380-2) notes that bioreactor research in space "offers an unprecedented opportunity for studying complex fluid assemblies...."

By mid-1998, the bioreactor investigators were deep into analysis of specimens from Mir Increments 6 and 7. Increment 6 (Sept. 25, 1997-Jan. 31-1998) will provide a stop-action movie of bone marrow, neuroendocrine, and kidney tubule cells as they are cultivated and then fixed every 7 to 10 days for up to 105 days in space (a portion of each batch is injected into new media to continue the cell line). Increment 7 (Jan. 22-June 12, 1998), was the first opportunity to grow human breast cancer cells in this most lifelike artificial setting as we work to understand how this killer grows and moves through the body.



International Space Station

Experience aboard Mir has turned microgravity bioreactor research into a mature science. The first long-duration Bioreactor experiment (Increment 3) saw the Bioreactor grow large cultures of bovine cartilage cells. A supporting technology mission (Bio-3D on Increment 6) demonstrated the BSTC as a repository for cells awaiting their turns in the bioreactor. The last NASA stay aboard Mir (Increment 7) was crucial as it brought everything together in an effort to culture human tissue in the Bioreactor. This was a full-scale rehearsal for operations aboard the International Space Station (center).

In addition to important science results, this work provided basic training on crew procedures and equipment design and operation. At the end of NASA activities aboard Mir in June 1998, the NASA bioreactor team will synthesize these lessons into an advanced program that will let them take maximum advantage of International Space Station at the earliest opportunity, and produce groundbreaking scientific results that will advance medical science.