

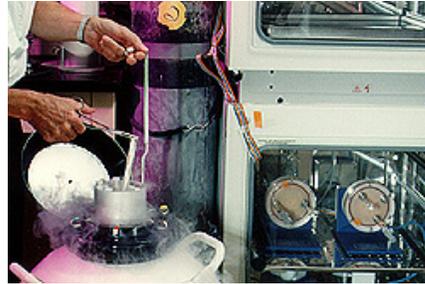
NASA using space incubator to understand breast cancer

**Bioreactor research could help women's health
on Earth and in space**

Oct. 1, 1998: A special incubator designed to grow tissue samples in space is being applied on Earth in a quest to understand how breast cancer works - and how it might be controlled.

Scientists are using NASA Bioreactors to culture breast cells on Earth to learn what controls the growth of both healthy and malignant breast tissues. Their findings could affect health care for women not only on Earth, but on missions to Mars.

Right: Dr. Robert Richmond of NASA/Marshall withdraws breast tissue specimens from cold storage in one of two liquid nitrogen Dewars that hold this unique collection. At right are two Bioreactors culturing breast tissue specimens. Credit: Dennis Olive, NASA/Marshall Space Flight Center.



"We know that many things - radiation, certain chemicals, genetic makeup - can contribute to the cause of breast cancer," said Dr. Robert Richmond, director of the recently created Radiation and Cell Biology Laboratory at NASA's Marshall Space Flight Center in Huntsville, Ala. Richmond is also a research associate professor of medicine at the Dartmouth Medical School in Hanover, N.H.

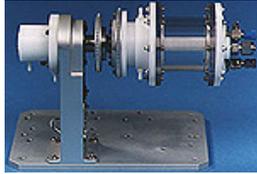
"We are culturing noncancerous mammary cells hoping to learn what guides their growth, and how we might use that knowledge to thwart malignancies before they are created. The type of mammary cells we are growing comes from an individual susceptible to breast cancer, and that susceptibility is likely driven by damage caused by ionizing radiation. Space exploration will involve slightly increased exposures of crew members to radiation, so what we learn from these cells could help help justify methods of female crew selection, and help manage breast cancer in the national population at the same time."

Cancer research is typically a collaborative and interdisciplinary effort. In this regard, Richmond was connected with a breast cancer susceptible donor of the mammary tissue now used in his laboratory by Dr. Mike Swift of the Medical College of New York, Hawthorne, N.Y. Drs. Olive Pettengill (Pathology Department of the Dartmouth Medical School) and Martha Stampfer (Lawrence-Berkeley Laboratory) helped him to select cells from this cancer susceptible breast tissue.

Turning a problem on its side

It has long been established that cells and tissue growing in microgravity - the weightless conditions obtained in space - can grow and mutate in ways different than on Earth. A perpetual challenge for the experimental study of these phenomena has been simulating the conditions of space so that complete laboratory studies can be done by numerous investigators on Earth. The simulated growth of mammalian cells in tissue culture needed to duplicate the quiet conditions of orbital free-fall in a way that allowed for maintaining fresh media and oxygenation.

To solve the problem, NASA in the 1980s developed the bioreactor (right), which is a can-like vessel equipped with a membrane for gas exchange and ports for media exchange and sampling. As the bioreactor turns, the cells continually fall through the medium yet never hit bottom. Under these quiet conditions, the cells "self assemble" to form clusters that sometimes grow and differentiate much as they would in the body. Eventually, on Earth, the clusters become too large to fall slowly and research has to be continued in the true weightlessness of space.



It has been well established that a number of cell types grow in the bioreactor on Earth for extended periods in ways that resemble tissue-like behavior. For this reason, the bioreactor also provides cell culture studies with a new tool for the study of 3-dimensional cell growth and differentiation.

Bioreactors have been used aboard the Mir space station to grow larger cultures than even terrestrial Bioreactors can support. Several cancer types, including breast and colon cancer cells, have been studied in this manner. Continued research using the NASA Bioreactor is planned aboard the International Space Station.

Within NASA, Richmond also interacts with Dr. Jeanne Becker, an associate professor at the University of South Florida College of Medicine in Tampa, and with investigators in the Biotechnology Cell Science Program at NASA's Johnson Space Center in Houston, home of the NASA Bioreactor.

For many people, culturing cells means putting some small number into nutrient media in a dish or a tube and letting them grow. However, this kind of approach does not provide the culture environment that supports tissue assemblies because in such an environment cells are "clueless." Without a proper 3-D assembly, epithelial cells (the basic cells that differentiate tissue into specific organ functions) lack the proper clues for growing into the variety of cells that make up breast tissue.

So, Richmond and Becker are using NASA Bioreactors to fool mammary cells into thinking they are in a normal environment, and thus culture them into larger assemblies whose natural growth can be studied.

At NASA/Marshall, Richmond has established a research program using a unique collection of healthy breast cells that contain a significant genetic weakness towards cancer.

Right: Two High-Aspect Ratio Vessels turn at about 12 rpm to keep breast tissue constructs suspended inside the culture media. Syringes allow scientists to pull samples for analysis during growth sequences. The tube in the center is a water bubbler that dehumidifies the air to prevent evaporation of the media and thus the appearance of destructive bubbles in the bioreactor.



Becker, in collaboration with coworkers at NASA/Johnson, has grown primary breast cancer cells (obtained directly from different surgical specimens) into masses that resemble the original tumor. She hopes to further our understanding of the factors important in the growth and the spread of tumors.

"We have grown noncancerous human breast cells in the NASA Bioreactor," Richmond said. "Our observations suggest there is much to learn and value to be gained from the study of their tissue-equivalent growth."

Culturing of primary breast cancer cells for long periods is rarely achieved in standard tissue culture dishes. With tumor cells from 27 different breast cancer patients, Becker could get only 5 specimens to grow enough to fill the dish. None of the five could then be expanded further when passed to new dishes.

In contrast, however, tumor samples from another five breast cancer patients grew successfully for long periods of time as 3-dimensional cocultures in the NASA Bioreactor.

These primary breast tumor cell constructs were grown successfully for up to 3 months, and the cancerous fraction increased. These constructs grew up to 3 mm in diameter, at which point they were removed for analysis and thus prevented from additional growth.

The information relating to the patient-derived breast cancer constructs grown in the bioreactor by Becker and coworkers at NASA/Johnson suggests that this model simulates events that occur as breast tumors progress within the body. This line of research therefore offers potential for increasing knowledge on the basic biology of human breast cancer. For more immediate application, this research also provides for the first time an opportunity to test breast cancer therapies on a patient's cancer cells in culture before extending that therapy to the patient herself.

With the healthy cells, Richmond is developing a normal breast tissue-equivalent model, a scientific description of how healthy breast tissue grows. A routine capability to model patient-specific breast cancer then could allow for testing and developing of realistic therapies.



Left: Dr. Harry Mahtani analyzes the nutrient media sampled from the bioreactors.

For example, hormonal therapy is an important treatment option for approximately a third of previously untreated breast cancer patients. It is well known that

breast tissue responds to estrogens. However, normal human mammary epithelial cells (HMEC) in a standard 2-dimensional culture dish do not demonstrate any estrogenic response.

Richmond plans experiments that will determine if 3-dimensional constructs of normal breast tissue in the bioreactor will respond to estrogen. If so, then Bioreactors could be used to tailor hormonal therapies that more closely match what will stop growth of cancer cells with minimal side effects for the patient.

To begin this research, Richmond established a cell repository from noncancerous breast tissue donated by a young woman carrying a single defective ATM gene. The debilitating syndrome ataxia-telangiectasia (A-T) results when both of the two ATM genes normally present in cells of the body become defective. These A-T individuals have about a 100-fold increased risk of all cancers plus other serious problems. Women carrying only one defective ATM gene are clinically normal, but have about a 5-fold increased risk, or susceptibility, to breast cancer. To reduce her breast cancer risk to near-zero, the donor elected to have a double mastectomy.

Her breast tissues now reside in a cell bank as perfectly matched cell types - preserved in liquid nitrogen - that will allow experimental results of today to be compared with experimental results obtained for many years to come.

In the bioreactor, these cells will grow in normal fashion because they are normal except for the single defective ATM gene. Once the normal tissue-equivalent model is defined, then these same cells can be manipulated to mimic the stages of breast cancer formation, and the model-related differences evaluated.

A normal tissue-equivalent model would thus hopefully promote the understanding of the creation of breast cancer and, eventually, allow development of therapies tailored to the individual patient.

Dodging a bullet

In addition to bringing the space bioreactor to bear on terrestrial health issues, NASA is also concerned about ionizing radiation, an issue for the human exploration and development of space environment.

Ionizing radiation actually has two components, photons - X-rays, and gamma rays - and particles - naked atomic nuclei blasted out from stars and supernovas. "Ionizing" means the radiation can energize electrons to break away from atoms. Such ionization in the nucleus of a cell can cause genetic damage that promotes the formation of cancer.

Space radiation is of little risk to us on the ground. Earth's atmosphere protects us on the surface from the great majority of space radiation, and the Earth's magnetic field shields space crews in low orbits from all but the most energetic particles.

But outside the magnetic field, the exposure and risk are greater. The exact amount of damage caused by space radiation varies with the length of the trip, the type of shielding used, and the makeup of solar and galactic radiations.

At this time the radiation damage for a trip to Mars is predicted to provide approximately lifetime cancer risk for 30 year-old males of about 28% as compared to 20% on Earth. This is unacceptably high, and scientists are trying to reduce it to about 23%. Because the radiation cancer risk to women is projected to be substantially greater - largely as breast and ovarian cancer - mission planners lean towards all-male crews.

It is important to note that scientists talk of risk, not of absolute predictions. Risk factors are applied to groups of people, and vary greatly from one individual to another because several steps are required for the final development of cancer. It is not possible to know exactly where an individual might be in this chain. Only the average outcome of any normal population can be used to predict risk factors.

As the genetic controls of cancer development become better understood, however, the "normal population" used for predicting cancer risk factors will also become better defined.

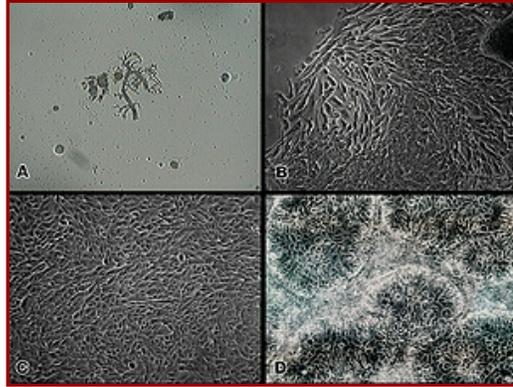
"Normal" now means "apparently healthy." However, the many genetic steps leading to cancer can be invisible in a "normal" person.

The phrase "cancer susceptibility" frequently mentioned these days indicates a genetic predisposition to cancer. For example, breast cancer is associated in part with defects in the BRCA1, BRCA2, and ATM genes.

Damage in both of the ATM genes, for example, sets a course for expression of a devastating clinical syndrome called ataxia-telangiectasia, or A-T, which includes an approximately 100-fold increased risk of cancer. On the other hand, studies by Dr. Mike Swift and coworkers have shown that when only one ATM gene is damaged (called A-T heterozygous), then a woman has about a 5-fold increased risk of cancer. Despite the fact she appears clinically normal.

Furthermore, scientists suspect that radiation damage is the principal initiator of increased breast cancer susceptibility in women with one defective ATM gene. It would seem prudent, therefore, to consider identifying A-T heterozygous women who might otherwise be selected for extended living within the space environment and thus not expose them to conditions that would further increase their risk of breast cancer.

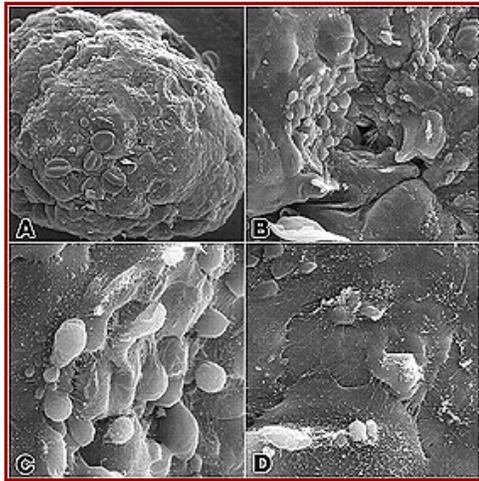
Isolation of human mammary epithelial cells (HMEC) from breast cancer susceptible tissue.



A: Duct element recovered from breast tissue digest.
B: Outgrowth of cells from duct element in upper right corner cultured in a standard dish; most cells spontaneously die during early cell divisions, but a few will establish long-term growth.
C: Isolate of long-term growth HMEC from outgrowth of duct element; cells shown soon after isolation and in early full-cell contact growth in culture in a dish.
D: Same long-term growth HMEC, but after 3 weeks in late full-cell contact growth in a continuous culture in a dish. Note attempts to reform duct elements, but this time in two dimensions in a dish rather than in three dimensions in tissue.

Credit: Dr. Robert Richmond,
 NASA/Marshall Space Flight Center

Epithelial cell monoculture

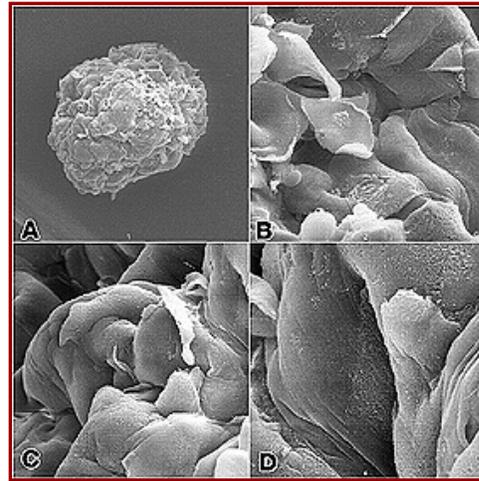


Long-term growth of human mammary epithelial cells (HMEC) grown in monoculture as 3-dimensional constructions in the presence of attachment beads in the NASA Bioreactor.

A: A typical construct about 3.5 mm (less than 1/8th in) in diameter with slightly dehydrated, crinkled beads contained on the surface as well as within the 3-dimensional structure. The center of these constructs is hollow. Crinkling of the beads causes a few to fall out, leaving crater-like impressions in the construct. The central impression shows a small hole that accesses the hollow center of the construct.
B : A close up view of the cells and the hole the central impression.
C, D: Closer views of cells in the construct showing cell-to-cell interactions.

Credit: Dr. Robert Richmond,
 NASA/Marshall Space Flight Center

Epithelial and fibroblast coculture

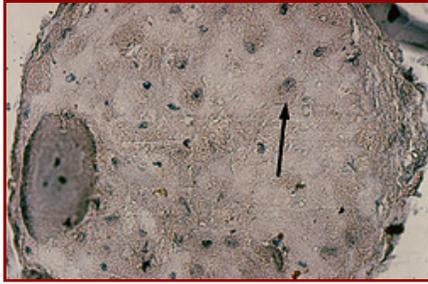


Long-term growth HMEC admixed in coculture with fibroblasts from the same initial breast tissue grown as 3-dimensional constructions in the presence of attachment beads in the NASA Bioreactor.

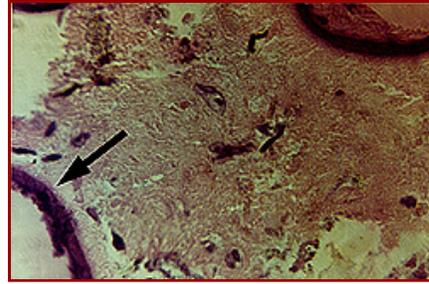
A: A typical construct about 2.0 mm in diameter without beads on the surface. The center of these constructs is hollow, and beads are organized about the inner surface. Although the coculture provides smaller constructs than the monoculture, the metabolic rate of the organized cells is about the same.
B, C, D: Closer views of cells showing that the shape of cells and cell-to-cell interactions appear different in the coculture than in the monoculture constructs.

Credit: Dr. Robert Richmond,
 NASA/Marshall Space Flight Center

Human primary breast tumor cells after 56 days of culture in a NASA Bioreactor.

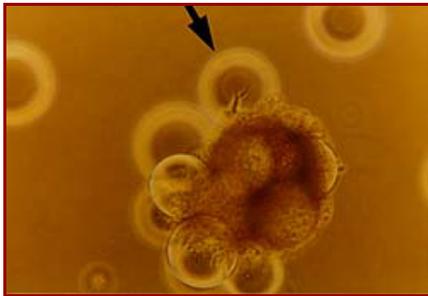


A cross-section of a construct, grown from surgical specimens of breast cancer, stained for microscopic examination, reveals areas of tumor cells dispersed throughout the non-epithelial cell background. The arrow denotes the foci of breast cancer cells.
Credit: Dr. Jeanne Becker, University of South Florida.

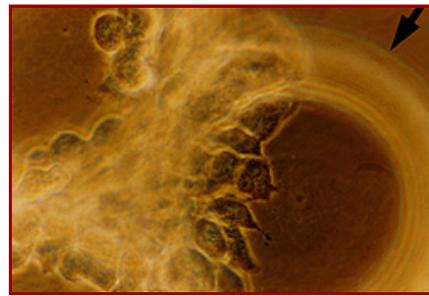


Higher magnification of view at left. The arrow points to bead surface indicating breast cancer cells (as noted by the staining of tumor cell intermediate filaments).
Credit: Dr. Jeanne Becker, University of South Florida.

Human primary breast tumor cells after 49 days of growth in a NASA Bioreactor.



Tumor cells aggregate on microcarrier beads (indicated by arrow).
Credit: Dr. Jeanne Becker, University of South Florida.



Higher magnification of view at left, illustrating breast cancer cells with intercellular boundaries on bead surface and aggregates of cells achieving 3-dimensional growth outward from bead.
Credit: Dr. Jeanne Becker, University of South Florida.