

NASA microgravity research highlights Advancing heart research

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The Microgravity Research Program supports microgravity-based research in mammalian cell science and tissue engineering. Many of the scientists in the program culture cells and tissue using the NASA-designed rotating wall bioreactor, which models some aspects of a microgravity environment. Two scientists involved in cardiac research are finding promising results using the NASA bioreactor for heart muscle research, drug testing, and possibly the eventual growth of transplantable heart tissue.

Cardiovascular disease is responsible for a preponderance of health problems in the United States. In the vast array of research conducted under the auspices of private and federal funding, there is a compelling need for isolated, bench top, tissue-based research. Standard cell culture of cardiac muscle cells seldom yields tissue modeling consistent with the development of new strategies that offer novel platforms for research and the eventual development of transplantable heart muscle.

The NASA bioreactor affords such a strategy by promoting the assembly and maturation of small heart muscle constructs that bear remarkable resemblance and coordinated performance similarities with native heart muscle. The engineered constructs respond to cardioregulatory drugs and perform electrophysiologically such that their activity can be monitored using electrocardiography. Thus with this new platform, research will target near-term basic research and include as a long-term goal the production of transplantable tissue.

Cardiac Tissue Engineering at A.I. duPont Hospital for Children

The underdevelopment or absence of tissue structures in the heart can be life threatening. Each year, approximately 25,000 children in the United States undergo surgical procedures to correct structural defects of the heart. In addition, each year thousands more children and adults are treated for functional problems arising from injury, infection, or maladaptation of the heart.

Treatments for both congenital-heart-defects (missing structures) and cardiomyopathies (poor function) have become highly advanced, but when they fail, organ replacement remains the only other treatment option. At present, heart transplantation is limited by both the number of donors and by the limited lifespan of implanted organs. New approaches



Small heart muscle constructs cultured in the NASA-developed rotating wall bioreactor (on the left) bear remarkable resemblance to native heart muscle, offering new research potential.

that expand the current treatment options of medical management, corrective surgery, and transplantation are needed. One possibility is tissue engineering of cardiac implants.

The A.I. duPont Hospital for Children (AIDHC) in Wilmington, Delaware, is one of the world's premier facilities for the treatment of congenital heart defects. With the support of the NASA Cellular Biotechnology program and the Nemours Foundation, Dr. Charles Hartzell established one of the first cardiac tissue-engineering programs in the country at AIDHC in 1992. By 1993, the team produced its first tissue-engineered cardiac constructs and has been a leader in the field of cardiac tissue engineering ever since.

Researchers in the cardiac tissue engineering program at AIDHC, now under the direction of Dr. Robert Akins, use NASA-designed bioreactors to study how the component cells of the heart interact to form cardiac structures outside the body. "AIDHC has a long history of culturing and characterizing animal cardiac cells, and we are applying all that experience to develop constructs for use in humans," says Dr. Akins, "and our approaches span cell biological, biochemical, and molecular biological methods." The group focused its early efforts on studying the conditions needed to grow bioreactor-derived tissue constructs on a variety of surfaces like plastics, synthetic polymers, and naturally occurring proteins. These early efforts were very fruitful and demonstrated that the NASA bioreactors were desirable vessels for cardiac tissue engineering.

The team's initial results growing constructs that had the outward appearance and function of small pieces of tissues were very encouraging; however, they were not altogether surprising. The beating of isolated cardiac cells had been described many years earlier, and the culture of cardiac cells in three dimensions was well known long before the term tissue-engineering was coined. "Spontaneous contraction

is characteristic of cells isolated from very young animals. Groups of cultured rat heart cells beat in unison within 36 hours of seeding, but the outward signs of 3-D shape and contractile effort don't tell you much about the underlying organization of the cells" explains Dr. Akins. There were surprises to be found in the architecture of what the team was growing, and when they looked at the micro-organization of their constructs, they found structure that was indistinguishable from the intact tissue. Dr. Akins continues, "Cells isolated from rat hearts were able to regenerate aspects of the very thing that is disrupted in congenital heart disease: tissue structure. They did this without any external cues from us, which was very surprising and very exciting ... the cells themselves showed an intrinsic ability to re-establish structure." The research group believes that it may be possible to harness this organizational ability to grow individually designed heart implants or even an entire transplantable organ.

Dr. Akins is quick to point out that there is an enormous amount of research to be carried out before cardiac tissue engineering can be applied in a surgical setting. "The amount of work to be done is daunting," Dr. Akins says. Just a short list of some crucial questions illustrates his point: Where will the cells come from? How will these cells, and the constructs prepared from them, be maintained and stored? How will the simultaneous formation of both macro- and micro-structures be controlled so that a construct of the desired size and shape has the specific cellular and sub-cellular organization needed for long-term function? How will blood be supplied to the constructs when they're put into a heart? Clearly, bringing cardiac tissue engineering to the clinical setting will take a large amount of work in a large number of labs, but Dr. Akins is optimistic. "There is a growing number of labs working in the field, and things can progress very dramatically as more people work together," he says.

Thanks to NASA support, the AIDHC research program is moving ahead with studies into the interactions between cardiac cells and their surroundings to see how these interactions affect the structure and function of tissue-engineered constructs. The group is beginning to dissect the steps by which cells form tissue architectures outside the body. While they do this, they are also analyzing how the establishment of tissue structure affects cell function.

“NASA support made the cardiac tissue engineering program at AIDHC a reality, and NASA biotechnologies have given us tools for looking at the complex interactions that occur when cells freely associate into three dimensional tissue structures,” says Dr. Akins, “the prospects are very exciting.”

Working to Make Cardiac Patches a Reality

Lisa Freed and Gordana Vunjak-Novakovic, both of the Massachusetts Institute of Technology (MIT), have become used to taking on tremendous challenges in their more than eight years of partnership in cell tissue culture research. Their efforts, funded in part by the microgravity biotechnology cell science program, have paid off enormously with the first-ever laboratory observation of engineered cardiac tissue beating in unison.

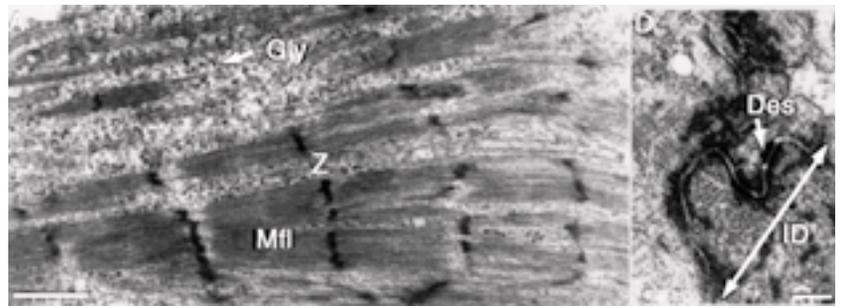
Using techniques that have also allowed the successful culturing of functional cartilage tissues, Freed and Vunjak-Novakovic have taken the first steps toward engineering heart muscle tissue that could one day be used to patch damaged human hearts. “We start from cells that are isolated from very young animals,” explains Freed. The cells are then attached onto a three-dimensional polymer scaffold. “Usually our scaffold is a fibrous mesh of a polymer that is biodegradable and synthetic,” she continues. Cells are attached on all surfaces of the scaffold and between its pores, unlike standard cell culture, where cells are spread flat, almost two-dimensionally, in a petri dish. This technique allows the cells to behave in ways that more closely mimic activity in a living organism. The attachment process takes only a few hours, after which the scaffold containing the cells is placed in a NASA bioreactor containing a solution of nutrients to feed the cells and a membrane for gas exchange. The bioreactor rotates, keeping the scaffold freely suspended in the liquid.

It takes about a week of just the right conditions for the more than 5 million (on average) cells to begin to form connections among themselves. The process is exacting, explains Freed, because cardiac cells are very sensitive to their growth environment and require lots of oxygen. The researchers start with such a large number of cells because cardiac cells essentially do not divide in culture. “If you start with 5 million cells,” says Vunjak-Novakovic, “you end up with 5 million cells, more or less.” The object of culturing individual cardiac cells is not to produce more cells, but to encourage the cells to develop connections and to begin to contract in unison, as a piece of functional tissue. Vunjak-Novakovic explains the significance of this behavior: “Transmission or conduction of electrical signals is possible only if the cells are

functionally connected. This is the goal, actually — to make and reorganize or reconstruct the cells’ native tissue.”

The second goal of the research begins where culturing in the bioreactor leaves off — studying the engineered tissue. Freed, Vunjak-Novakovic, and a number of researchers and students at MIT and Boston University characterize the tissues in order to learn about their electrophysiological, histological, and molecular properties. Using an electrode array, researchers can study the propagation of electrical waves through the tissue, command the tissue to beat at a prescribed rate, and study the range of frequencies that can be used to pace the tissue, much like the function of a pacemaker implanted in a human heart patient. “We can use antibodies to look at the connecting proteins that are joining the cells together and study those channel proteins,” says Freed. “We can also use biochemical methods to look at construct metabolism and enzyme levels.”

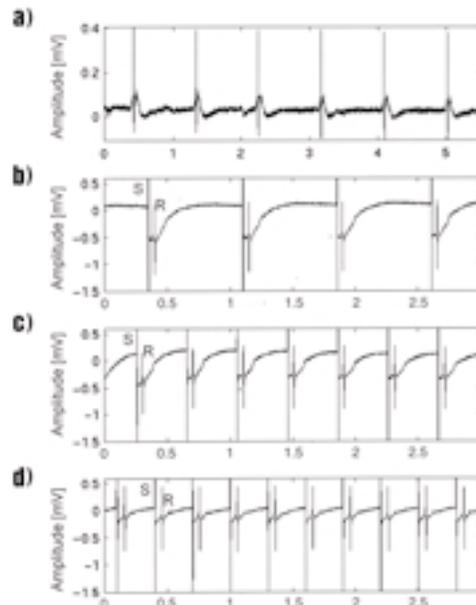
Though excited about their successes thus far, Freed and Vunjak-Novakovic know that



Success in heart tissue engineering comes one step at a time. To date, researchers are capable of engineering only very thin patches of heart muscle, but they are working toward the even greater challenges of creating in-vitro, thick, vascularized tissue. Here, a transmission electron micrograph of engineered tissue shows a number of important landmarks present in functional heart tissue: (A) well-organized myofilaments (Mfl), z-lines (Z), and abundant glycogen granules (Gly); and (D) intercalated disc (ID) and desmosomes (DES).

there is a long road ahead of them before engineered heart muscle patches are ready to help patients in need. “Right now we’ve just made the muscle component, and even that muscle is very thin. To get a real piece of implantable cardiac tissue, that cardiac muscle that we now have has to be integrally fed by a vascular system. It’s sort of the \$64 million question for tissue engineering,” says Freed. A further challenge will be learning to solve the same engineering challenges with human cells. Current experiments on rat cells offer the advantage of a mammalian analog to human cardiac function, but only human cells can give researchers the ability to move from laboratory observation to an actual clinical application. One possibility for resolving the gap between research on rat cells and studies of human cardiac cells lies in the use of stem cells, or primitive cells, which are still capable of cell division and differentiation. “Amplification, vascularization, and immunological compatibility could all be solved with stem cells,” says Freed. “It’s just that we are at the very, very beginning of that research.”

Freed, Vunjak-Novakovic, and their team of researchers have published their findings in two articles: “Cardiac Muscle Tissue Engineering: Toward an In-Vitro Model for Electrophysiological Studies,” *American Journal of Physiology*, **277** (*Heart Circ. Physiol.* 46) H433–H444, 1999; and “Cardiac Tissue Engineering: Cell Seeding, Cultivation Parameters, and Tissue Construct Characterization,” *Biotechnology and Bioengineering*, **64**: 580–589, 1999.



Functionally connected heart cells that are capable of transmitting electrical signals are the goal for tissue engineering researchers Freed and Vunjak-Novakovic. Electrophysiological recordings of engineered tissue show spontaneous contractions at a rate of 70 beats per minute (a), and paced contractions at rates of 80, 150, and 200 beats per minute respectively (b, c, and d).

Additional information

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