

Contract #: NAGW 4992 Year 1 (of 2 Years) Final Report
Title: "Altered Gravity & Early Heart Development in Culture"

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More than 200 precardiac explant pairs have been dissected and cultured as controls and bioreactor rotated (simulated microgravity) experiments in pairs. Each has been assessed for general morphology, and the ability to contract spontaneously. Many have been immunostained with antibodies to fibronectin and several images of these sections have been captured for computer-assisted image analysis. Electron microscopy has been carried out on several pairs of explants. All of these procedures are continuing as we conduct approximately weekly experiments.

All precardiac explants from the initial experiment were photographed using phase contrast microscopy. These and all others from subsequent experiments have been carefully observed. They possess the expected size and shape, and a vesicle is present. There have been no observable differences between control and bioreactor rotated explants.

Each explant has been observed for spontaneous contractions immediately following the 18 hour culture period. There is a clear inhibition of the development of contractile activity as a result of bioreactor rotation. The accumulated results from the first five experiments are displayed in the following table:

	Control	Bioreactor
Number of explants	87	75*
Number contracting	75	23
Percent contracting	86.2%	30.7%

*Some explants are lost during recovery from the bioreactor culture chamber when its medium is poured out.

Immunostaining of sections of the recovered explants has shown substantial differences between control and bioreactor rotated explants. Although positive staining is present in all, most of the rotated explants showed less intense staining in all areas. To date, 87 % of these are less stained than controls.

The control explants showed areas of intense, linear, extracellular

staining along epithelia. These are suggestive of basement membrane staining, but the areas were shorter and more discontinuous. Control sections also showed areas of intercellular staining in both mesenchymal tissue and myocardium. Areas of staining with this pattern were seen in sections from rotated explants as well, but they were more restricted. Usually confined to quite small areas of only a few cells, and they were less intense. We have our image analysis system assembled and have worked out the procedures for measuring intensity of red staining with background correction, and for measuring areas of red staining in square microns, both using Adobe Photoshop and NIH Image software. The analysis is proceeding.

Initial immunoassay results from two experiments have shown a possible correlation of tissue fibronectin accumulation with the immunostaining results. Five control and five bioreactor rotated explants were pooled, homogenized, diluted twofold to create a series of samples, and blotted onto nitrocellulose sheets. Fibronectin purified from adult chicken tissue by immunoaffinity column chromatography was diluted and applied as a standard. Immunostaining and quantitation of these by image analysis (NIH Image) have produced stained slots with a linear range of assay response. In these experiments we have observed an average 24% reduction in fibronectin. This would suggest a reduction of approximately 28 picograms of fibronectin per explant during bioreactor rotation. We are working to standardize the measured quantities of fibronectin per unit of DNA, in order to check whether the differences we have observed are authentic on a per cell basis.

Seven control and 9 bioreactor rotated explants have so far been analyzed by electron microscopy under the supervision of Dr. Spooner at Kansas State University. More will be analyzed to achieve reliable results. An improvement in fixation has been achieved with addition of 2% tannic acid to the glutaraldehyde/formaldehyde fixative, and change to cacodylate buffer. The cells that have been viewed exhibit the expected ultrastructure. Myofibrillar structures are apparent and are characteristic of the expected degree of development (Hamburger-Hamilton stage 6 or 7 plus 18 hours yields approximately stage 11). No differences between control and rotated explants have been apparent at this time.

Therefore, at this time, we have answered three questions. First, it is clear that rotation in the HARV bioreactor simulates microgravity effectively and we feel confident the instrument provides a good model system. Second, it is apparent that rotation is delaying or inhibiting the

development of spontaneous contractions in the tissues. Third, the amount and distribution of fibronectin is diminished from microgravity exposure. Our continuing experiments and particularly our image analysis measurements and electron microscopy will refine these conclusions further.

Our results may raise a new question concerning the production and assembly of myofibrils. Van Twest et al (1995) found delay or inhibition of myofibrils in spaceflight explants taken from stage 7 and especially stage 6 explants, yet so far, we have not found evidence for this effect in bioreactor experiments. Thus we now ask, does the microgravity environment of space shuttle flight differ from that of the bioreactor with regard to this dimension of myogenesis? Completion of our experiments may yield additional data that will help answer this question.

We have also encountered the possibility that certain vibrations may prevent the inhibitory effects of bioreactor rotation. The bioreactor we have been using was found to rotate unreliably at first because of its electric motor. Replacement of the original motor at Synthecon, Inc. (Houston, TX) solved the problem but resulted in considerably louder rotation. Subsequent experiments showed no differences between control and rotated explants in the development of contraction ability. The instrument was returned to Synthecon and dampened, and now the inhibition of development of contractions is apparent again. Future work may allow the investigation of the possible effect of vibrations on the cell surface mechanotransduction apparatus that is apparently sensitive to vibration as well as gravitation.

In the immediate future, we plan to determine fibronectin concentration in explant tissue more definitively, and also in the culture medium. If the tissues do have less fibronectin, we wish to know if it is being secreted into the medium in greater amounts, or if total production is less. Our results thus far with microgravity exposure also suggest that hypergravity exposure of precardiac explants will be important to investigate: if the gravity receptors are sensitive to microgravity, then interesting results might be seen in hypergravity. Experiments using centrifugation at the NASA-Ames Research Center are being planned

1996 Bibliography

Awards

Dean's Award for Superior Achievement in Research, College of Natural Sciences, University of Northern Iowa, 1996

Nomination for Donald N. McKay Faculty Research Award, 1997, University of Northern Iowa.

Presentations

Altered Gravity and Early Heart Development in Culture (1996). Peter Lwigale, Jill Denning, and Darrell Wiens. American Society for Gravitational and Space Biology Annual Meeting, Charlotte, N.C., October, 1996, poster.

M.S. Thesis

The Effect of HARV Bioreactor Microgravity on Early Development of the Heart in the Chick Embryo, Peter Lwigale, in preparation.

ALTERED GRAVITY AND EARLY HEART DEVELOPMENT IN CULTURE. P. Lwigale, J. Denning, and D. Wiens. Dept. Biology, Univ. N. Iowa, Cedar Falls, IA.

The macromolecules comprising the cytoskeleton and extracellular matrix of cells may be sensitive to gravitation. Since early development of organs depends on dynamic interactions across cell surfaces, altered gravity may disturb development. We investigated this possibility for heart development. Previous studies showed that the extracellular matrix glycoprotein fibronectin (Fn) is necessary for normal heart development. We cultured precardiac tissue explants in a high aspect ratio bioreactor vessel (HARV) to simulate microgravity. We observed tissue morphology, contraction, and Fn distribution by immunolocalization in HARV rotated and control (1xg) explants, cultured 18 hr. We also measured Fn amount by immunoassay. Explants in HARV were rotated at 6 rpm to achieve continuous freefall. Thirty-five of 37 control, but only 1 of 37 matched rotated explants exhibited contractions. Tissue architecture was identical. Immunolocalization of Fn showed remarkable differences which may be related to the development of contractions. The Fn staining in the HARV explants was less intense in all areas. Areas of linear staining along epithelia were present but shorter, and there was less intercellular staining in both mesenchymal tissue and myocardium. Initial immunoassay results of 5 matched pairs of explants showed a 22% reduction in total tissue Fn in the HARV rotated samples. Our results indicate that altered gravity in the HARV reduced the amount and distribution of Fn, as assessed by two independent criteria. This was correlated with a reduction in the development of contractile activity. This research is supported by NASA-NAGW-4992.