Tissue Engineering of Cartilage in Space

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I. INTRODUCTION:

Exposure to microgravity can affect cells and tissues at a variety of levels. Musculoskeletal changes (e.g. significant bone and muscle loss) occur even when astronauts exercise regularly, but the mechanisms are not yet understood. Tissue engineering, a new field that enables tissue equivalents to be created from isolated cells in combination with biomaterials and bioreactors, potentially can provide a basis for systematic, controlled *in vitro* studies of tissue growth and function. We previously developed methods to create three dimensional cartilaginous tissue based on cartilage cells (chondrocytes), synthetic polyglycolic acid (PGA) scaffolds, and bioreactors. The scaffold induces cell differentiation and biodegrades at a defined rate, whereas the bioreactor maintains controlled *in vitro* culture conditions that permit tissue growth and development. In the present experiment, tissue engineering was studied in space using cartilage as a model musculoskeletal tissue. Cartilage was selected because of its resilience and low metabolic requirements. Previous microgravity experiments involving mammalian cells focused on the cells themselves in monolayers and lasted for six to twenty eight days. In the present experiment, engineered cartilage grown for three months on Earth followed by four months on the Mir Space Station was compared with that grown for seven months on Earth. The objectives were to: (1) examine cell viability and differentiated function in a long-term flight study, and (2) to assess the effects of the space environment on tissue growth and function.

II. METHODS:

<u>Pre-flight (M.I.T.):</u> Cell-polymer tissue “constructs” based on bovine calf articular chondrocytes and polymer scaffolds (5 million cells per 5 mm diameter, 2 mm thick PGA disc) were prepared according to previously established methods and cultured on Earth in NASA developed rotating bioreactors (Synthecon, Houston, TX). In particular, constructs were cultured in a 110 mL Slow Turning Lateral Vessel (STLV), configured as the annular space between two cylinders, the inner of which was covered with a silicone membrane and served as an internal gas exchanger. The vessel, filled with culture medium and eleven constructs was rotated as a solid body around its central axis at 15 – 28 rpm. The constructs within maintained freely suspended by adjusting vessel rotation speed to establish a dynamic equilibrium between the acting forces, such that culture on Earth simulated some aspects of actual microgravity. Fluid was exchanged batch-wise, at a rate of 50 % every 3-4 days.

<u>Flight and ground control studies (astronaut John Blaha, JSC team):</u> After three months of culture on Earth, constructs were divided into two groups and transferred into two NASA-provided rotating, perfused bioreactors (Biotechnology system, BTS) for an additional four months of cultivation on either Mir or on Earth. In particular, one BTS containing ten constructs was transferred to the Priroda module of Mir via the US space shuttle STS-79 (9/16/96 launch), operated in microgravity, and brought back to Earth via STS-81 (1/22/97 landing). A second BTS with ten constructs was operated on Earth (at NASA-JSC) served as a ground control. The BTS used for the present study was a prototype of the rotating, perfused bioreactor system under development for the Biotechnology Facility aboard the International Space Station. **Figure 1** shows a schematic of the BTS, which had dimensions of approximately 17 x 20

x 25 inches (2 1/2 middeck shuttle lockers) and weighed 137 lbs. Constructs were cultured in an 125 mL bioreactor configured as the annular space between two cylinders, that could be differentially rotated. Fluid entered the bioreactor at one end of the vessel and exited through a mesh on the inner cylinder. The inner cylinder also had a disc attached to one end to enhance secondary fluid flow within the vessel. The bioreactor was connected to a recirculation loop consisting of an external silicone membrane gas exchanger (Avecor, Plymouth, MN) and a peristaltic pump (Randolph, Manchaca, TX). The system, which was maintained at 37°C, also contained reservoirs for fresh and waste media, and access for media and gas (10% CO_2 in air). Fresh medium was infused into the system once per day (50 to 100 mL/day), and medium was recirculated between the bioreactor and the gas exchanger four times per day (4 mL/min for 20 min every 6 hours). Under these conditions, metabolic parameters were maintained within previously established target ranges in both groups for the duration of the experiment (e.g. pH between 6.9 and 7.4; dissolved oxygen concentrations between 71 and 127 mm Hg, as assessed using portable cartridges) (I-Stat, Princeton, NJ). Concentration gradients within the bioreactor were minimized by differential rotation of the inner and outer cylinders at 10 and 1 rpm, respectively (on Mir), and by the convection associated with gravitational construct settling during solid body rotation of the bioreactor at 28 rpm (on Earth). On Mir, gas bubbles were observed in the bioreactor between flight days 40 and 130. The amount of gas stabilized at approximately 20 % of the total bioreactor volume, and the bubbles did not appear come into direct contact with the constructs, as assessed by videography. An equal amount of gas was introduced into the bioreactor on Earth, in order to match conditions on Mir as closely as possible.

<u>Post-flight (M.I.T.):</u> At the time of launch (i.e. after three months of culture) and after additional cultivation on either Mir or Earth (i.e. after seven months of culture), constructs were compared to native bovine calf articular cartilage with respect to: morphology (weight, histological and ultrastructural appearance), cell viability (trypan blue exclusion, intracellular esterase activity), biosynthesis rates (macromolecular incorporation of radiolabeled tracers), biochemical composition (DNA, glycosamino-glycan (GAG), total and type II collagen), and mechanical properties (aggregate modulus and hydraulic permeability in radially confined compression).

III. RESULTS:

<u>Construct shape:</u> Constructs grown on Mir tended to become more spherical whereas those grown on Earth tended to maintain their initial discoid shape (**Figure 2a**). These findings might be related to differences in cultivation conditions, i.e. videotapes showed that constructs floated freely on Mir but settled and collided with the vessel wall on Earth.

<u>Cell viability:</u> Cells isolated from post-flight constructs were alive and metabolically active, as demonstrated by attachment to Petri dishes, proliferation, and enzymatic conversion of a tracer substrate (**Figure 2b**). Cell viability post-flight was 95 – 99 %. Rates of macromolecular incorporation of radiolabeled tracers in Mir-grown constructs (post-flight) and Earth-grown controls were comparable.

<u>Construct structure:</u> Constructs grown on Mir and on Earth appeared histologically cartilaginous throughout their entire cross-sections (5 - 8 mm thick), with the exception of fibrous outer capsules (0.15 - 0.45 mm thick), as assessed using histological staining for GAG and immunostaining for collagen type II. Earth-grown constructs appeared to have a more organized extracellular matrix with more uniform collagen orientation than their Mir-grown counterparts, but collagen fiber diameters were similar in the two groups.

<u>Construct composition and function</u>: At the time of launch and after additional culture on Mir and on Earth, constructs contained thirteen, fourteen, and nineteen million cells, respectively. On Earth, construct wet weights increased 1.7-fold between three and seven months, which could be attributed to increasing amounts of collagen and GAG (**Figure 3 a&b**). On Mir, construct wet weights increased 1.3-

fold over the same time interval, due to deposition of collagen and unspecified components (**Figure 3 a&b**). The fraction of the total collagen that was the cartilage specific type II collagen decreased, but not significantly between the time of launch and landing, demonstrating good maintenance of the chondrocytic phenotype in microgravity. Construct mechanical properties improved both on Mir and on Earth as assessed by an increase in equilibrium modulus and a decrease in permeability. The equilibrium modulus of Earth-grown constructs was comparable to that of native calf cartilage and was 3-fold higher than that of Mir-grown constructs (**Figure 3c**).

IV. CONCLUSIONS:

The present study is the longest cell culture experiment ever carried out in space and the first to demonstrate the feasibility of microgravity tissue engineering. Final constructs were structurally and functionally cartilaginous and consisted of viable, metabolically active, differentiated cells, but constructs grown on Mir were smaller and mechanically inferior to those grown on Earth. The observed differences between the two groups might be attributed to the reduction in physical forces on Mir and/or to the related differences in construct cultivation conditions (i.e. free floating on Mir vs. gravity settling on Earth). In particular, the spherical shape, relatively lower weight and GAG fraction, and inferior mechanical properties of Mir-grown constructs were the observed effects of spaceflight. However, we could not distinguish between the relative contributions of launch, microgravity, local factors (e.g. radiation), and landing, emphasizing the need for the use of an in-flight, 1 G centrifuge for control groups in future flight experiments. Our results are consistent with previous studies showing that musculoskeletal tissues remodel in response to physical forces and are adversely affected by spaceflight. The same cell-polymer-bioreactor system could be used for a variety of controlled microgravity studies aimed at improving our fundamental understanding of how gravity affects cell function and tissue development. These results may have implications for human spaceflight (e.g. a Mars mission) and clinical medicine (e.g. improved understanding of the effects of pseudo-weightlessness during prolonged immobilization and intrauterine development).

V. PUBLISHED REPORTS OF FINDINGS:

L.E. Freed, R. Langer, I. Martin, N. Pellis, G. Vunjak-Novakovic. Tissue Engineering of Cartilage in Space. *Proceedings of the National Academy of Sciences USA* **94**: 13885-13890, 1997 (a commentary on this article appeared in the same issue on page 13380).

L.E. Freed, N. Pellis, N. Searby, J. deLuis, C. Preda, J. Bordonaro, G. Vunjak-Novakovic. Microgravity Cultivation of Cells and Tissues. *Gravitational Space and Biology Bulletin,* **12**: 57-66, 1999.

L.E. Freed and G. Vunjak-Novakovic. "Tissue Engineering Bioreactors" in: Principles of Tissue Engineering, 2nd edition (R.P. Lanza, R. Langer and J. Vacanti, eds., Academic Press) Ch. 13, pp. 143-156, 2000.

FIGURE LEGENDS:

Fig. 1: The Biotechnology System (BTS). The BTS is flight hardware consisting of a 125 mL rotating, perfused bioreactor that can house approximately ten tissue constructs each measuring 5-9 mm diameter x 4-8 mm thick. Medium recirculation between the bioreactor and gas exchanger, medium infusion from a reservoir, and rotation speeds of the inner and outer cylinders of the bioreactor can be controlled automatically.

Fig. 2: Spaceflight affects tissue structure but maintains cell viability. (a) full histological cross-sections of constructs grown on Mir and on Earth, stained for glycosaminoglycan, (**b**) cells isolated from

a Mir-grown construct after two days of monolayer culture demonstrating intracellular esterase activity (white color).

Fig. 3: Spaceflight affects tissue size, composition, and mechanical properties. Cartilaginous constructs cultured for three months in rotating bioreactors on Earth prior to launch (Launch) are compared to constructs cultured for an additional four months in space (Mir) or at 1 G (Earth) and to native tissue (Cartilage) with respect to **(a)** construct wet weight, **(b)** glycosaminoglycan content, as a percentage of wet weight, and **(c)** equilibrium modulus. Data are the average ± standard deviation of 2-7 independent measurements.