The possibility of creating three-dimensional differentiated tissue-like assemblies by culturing cells in microgravity, either in space or on the ground, offers research opportunities that may lead to the generation of replacement organs for transplantation, and for studying multicellular responses in toxicology, radiation biology, tumorigenesis, and embryogenesis. Here, Brian Unsworth and Peter Lelkes review these opportunities, considering the practical and theoretical challenges of the microgravity environment.

Growing tissues in microgravity

BRIAN R. UNSWORTH[1] & PETER I. LELKES[2]

Advances in our scientific knowledge and biotechnological capabilities herald an exciting new betrothal between tissue engineering and space biology. In this mesalliance, microgravity may become a surprising, unconventional, and yet attractive venue for the generation of macroscopic tissue equivalents for a variety of basic and applied medical purposes.

In vitro culture of 3-D tissues

An alternative approach to donor organs for transplantation is the generation of replacement organs by means of 3-D *in vitro* cell culture (Fig.1). In the past, cell assembly in 3-D has resulted in formation of multicellular spheroids. In homotypic spheroids, comprised of one cell type only, a more "natural" level of cellular differentiation is attained than is possible when the cells are grown in two dimensions on culture dishes[1,2]. Heterotypic spheroids (composed of different cell types from the same tissue) have provided new insights into how multicellular assemblies respond to radiation injury[3], develop drug resistance[4] and undergo tumorigenesis[5,6]. Although such multicellular spheroids are a first step towards reconstruction of complex 3-D tissue equivalents, there are several inherent limitations, such as the restricted diffusion of nutrients and oxygen that limits their size. Spheroids larger than 1 mm in diameter generally contain a hypoxic, necrotic center surrounded by a rim of viable cells[7], thus mimicking closely the initial development of solid tumors.

In progressing beyond spheroids, creation of properly assembled and oriented tissues from single cells has attracted an interdisciplinary approach, collectively known as tissue engineering[8]. A major focus of tissue engineering is, how to best generate functional 3-D constructs large enough to serve as replacement organs or suitable for *in vitro* studies of, for example, drug toxicity or oxygen diffusion in tissues. In order to attain and maintain tissue-specific differentiation in these 3-D assemblies, dissociated cells are seeded either onto complex extracellular matrices, or inoculated as suspension cultures, with or without cell culture beads, in conventional stirred fermentors or rollers bottles.

Resorbable polymeric scaffolds (made of materials such as fibrous polyglycolic acid) have been used as provisional, biodegradable matrices for cell seeding[9]. Such scaffolds, which can be molded into complex geometric shapes, promote cellular proliferation and 3-D assembly. After the controlled biodegradation of the scaffolds, the 3-D cellular constructs form neo-tissues and organs, such as skin, cartilage, blood vessels and heart valves[10–14]. Similarly, peptides (containing cell adhesion-specific sequences) conjugated to polyethylene glycol promote aggregation and differentiation of neural cells in suspension, thus eliminating the need for microcarriers and facilitating the use of these aggregates as replacement tissues[15]. Some of these bio-engineered replacement tissues are currently being tested in animals[16–18].

The conventional fermentor approach has the disadvantage that high shear forces are generated, which damage the cells and hinder proper tissue-specific differentiation. Decreasing the stir rate while increasing the viscosity of the culture medium might partially reduce the hydrodynamic damage[19], however, aggregates formed under these conditions still exhibit necrotic centers. More recently, tissue constructs on biodegradable scaffolds have been successfully cultured in stirred bioreactors[20].

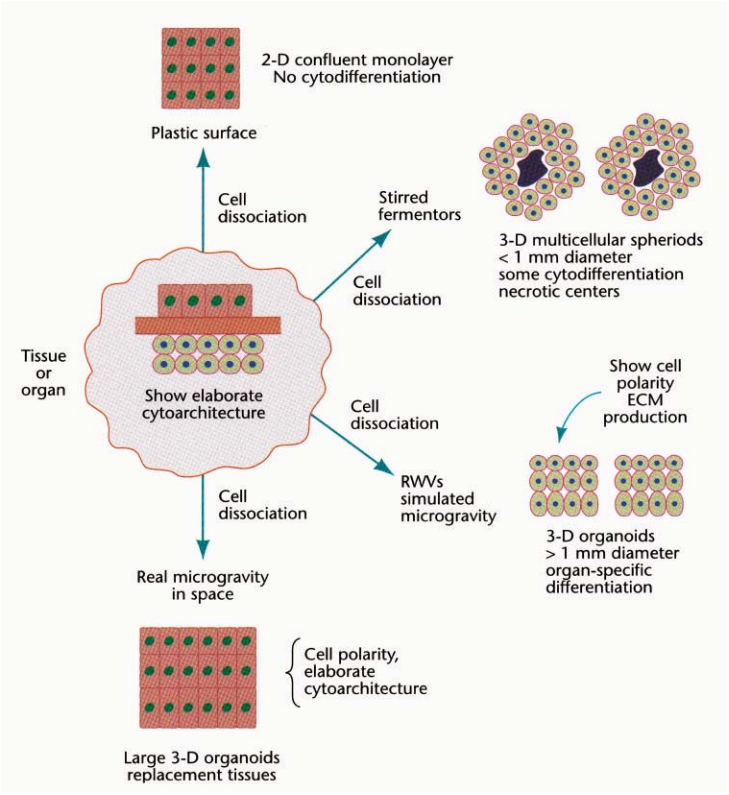

Fig.1 The advantages of growing tissues in microgravity. Dissociated cells, cultured on plastic, form a confluent 2-D monolayer and do not differentiate. Some cytodifferentiation is achieved using conventional stirred fermentors in which 3-D multicellular spheroids, of limited size and complexity, are formed. The microgravity culture conditions in the Rotating Wall vessel (RWV) bioreactors enhance cytodifferentiation and promote assembly of individual cells into 3-D organoids which show cell polarity, extra cellular matrix (ECM) production and organ-specific differentiation.

Fig. 2 Several incarnations of the RWV bioreactor. Due to its geometry and the use of a large surface area, flat membrane oxygenator at the rear of the chamber, the oxygenation capacity of the High Aspect Ratio Vessel (HARV) (*a*) is higher than that of the Slow Lateral Turning Vessels (SLTV) (*b*). Therefore, HARVs are mostly used for cell types that require more oxygen per unit volume of culture medium. STLV-type bioreactors, consisting of a cylindrical growth chamber that contains an inner co-rotating cylinder with a gas exchange membrane, are suited for cells with low oxygen requirements. Because sampling from individual vessels may yield inconsistencies, multiple samples can be grown simultaneously in a new batch culture system which employs a "Four Station Rotor Base" (*c*). In the most advanced variant of the STLV, a fully automated, computer controlled system (*d*) continuously monitors flow through the rotating vessel, allowing for on-line monitoring of vital signs such as, pH, oxygen and glucose levels. A further advantage is the capability to change, sample or modify the medium without stopping the vessel. (Courtesy of Synthecon Inc.)

Growing tissues in microgravity

Efforts at tissue reconstruction would benefit from a venue which promotes cell–cell association while avoiding the detrimental effects of high shear stress. Such a venue might be provided by microgravity. Indeed, it was observed many years ago that cells in suspension tend to aggregate when exposed to microgravity in space[21,22]. In an effort to derive the potential beneficial effects of microgravity and low fluid shear for cell culture here on earth, scientists at the National Aeronautics and Space Agency (NASA) introduced the Rotating Wall Vessel bioreactor (Fig.2). Briefly, RWV bioreactors are horizontally rotated, fluid-filled culture vessels equipped with membrane diffusion gas exchange to optimize gas/oxygen-supply. The initial rotational speed is adjusted so that the culture medium and the inoculum—individual cells, pre-aggregated cell constructs or tissue fragments—rotate synchronously with the vessel, thus providing for an efficient, low-shear mass transfer of nutrients and wastes. As the cell aggregates grow in size, the rotational speed is increased to compensate for increased sedimentation rates. Under these conditions, at any given time, gravitational vectors are randomized and the shear stress exerted by the fluid on the synchronously moving particles is minimized. These simulated microgravity conditions[23] facilitate spatial co-location and three-dimensional assembly of individual cells into large aggregates. The time-averaged gravitational vector acting on these cellular assemblies (which at any given time are actually in a state of free fall in a rotating flow field) is reduced to about 10^{-2} g (ref. 24). This is in contrast to "real" microgravity (reduced gravity) in space. In a typical experiment on board a space craft or on the space station circling in near-earth orbit, the gravitational force is approximately 10^{-4}–10^{-6} g. The shear stress acting on single cells is vanishingly small, because their sedimentation velocity is negligible compared with the rotational speed and the movement of the bathing fluid. Hence, single cells are practically always in suspension, rotating quasi-stationary with the fluid. The shear stress acting on tissue culture beads covered with cells or on cell aggregates or tissue fragments depends on their size, density and settling rate. As these constructs grow in size and exhibit increasing sedimentation velocities, the shear stress can approach up to 0.5 dynes/cm^2.

Cell differentiation in rotating wall vessels

Cell culture conditions in the "simulated microgravity" environment of RWV bioreactors combine two beneficial factors: low shear stress, which promotes close apposition (spatial co-location) of the cells; and randomized gravitational vectors, which either directly affect gene expression or indirectly facilitate paracrine/autocrine intercellular signaling, for example, through restricted diffusion of differentiative humoral factors[25]. Close apposition of the cells in the absence of shear forces presumably promotes cell–cell contacts and the initiation of differentiative cellular signaling via specialized cell adhesion molecules. This process then might lead to the rapid establishment and expansion of aggregate cultures, which unlike in conventional fermentors, are not disrupted by shear forces. In addition, the low-shear environment, in concert with randomized gravitational vectors, might restrict the diffusion of mitogenic and/or differentiative growth factors, which are secreted by the cells. These autocrine/paracrine feedback mechanisms might further enhance the aggregation and differentiation, and contribute to the observed capability of this environment to maintain high density cell cultures[24].

A definite answer as to whether the aggregation- and differentiation-promoting effects of the RWV culture conditions are due to the (simulated) microgravity, the low-shear environment or a combination of the two will have to wait until controlled experiments can be carried out in real microgravity during space flight. As a first step, we and others have studied the assembly and tissue-specific differentiation of homotypic and heterotypic cell cultures under low shear stress conditions, approaching those maximally encountered for large cell aggregates in RWV Bioreactors[26]. Clearly, fluid shear stress, via shear stress responsive elements (SSRE), can modulate the pattern of gene activation[27]. However, in order to activate SSRE, shear stress must exceed threshold values[28] (approximately 2–4 dynes/cm^2). Results obtained so far suggest that the low shear stress environment in gently mixed conventional stirred fermentors (in the order of 0.5 dynes/cm^2) facilitates tissue-like aggregation and assembly, but might not be sufficient to induce organotypic differentiation[29].

Differentiated 3-D constructs in simulated microgravity

Many groups are using RWV bioreactors to facilitate the assem-

MIR space station

bly of macroscopic, differentiated, tissue-like 3-D constructs (called variously proto-tissues, tissue-equivalents or organoids) from a variety of normal and transformed cell types[30–37] (see Table for a partial listing). These studies range from using RWV bioreactors as a suitable *in vitro* venue for culturing cells such as viruses[38], human tumor cells[33,35] and cells producing valuable bio-products including antibodies and antigens[34].

The feasibility of the RWV bioreactors for the generation of macroscopic replacement tissue equivalents has been tested by culturing dissociated bovine articular cartilage-derived chondrocytes and heart cells from neonatal rats and chick embryos on resorbable polymer scaffolds in the bioreactors[39]. After five weeks of cultivation in the RWV, cartilaginous constructs had a composition reminiscent of native cartilage in terms of their collagen and glycosaminoglycan content (the components required for mechanical stability), and cardiac tissue constructs consisted of elongated cells that contracted spontaneously and synchronously. However, not all cell types will form true 3-D aggregates in the RWV environment. For example, the insect ovary cell line SF-9 will not aggregate in the RWV, and yet the cells respond to the unique culture conditions by enhanced cell proliferation[40].

RWV bioreactors have also been used to promote further differentiation of preassembled bioengineered tissue equivalents, such as skin (D. Dimitrijevich, pers. comm.), cartilage[20] and fetal neuronal cells[16], and to culture intact tissues, such as fragments of liver[41]. (See NASA web site for the latest information on the more than three dozen cells being cultured in RWVs).

Culture conditions in the RWV provide an excellent *in vitro* system for studying the effects of microenvironmental cues, especially intercellular communication on tissue-specific cell assembly, differentiation and function[30]. Thus, homotypic organoids formed from only one cell type in the RWV express tissue-specific differentiation markers beyond the levels seen in conventional tissue culture[34]. However, these proto-tissues still do not faithfully recapitulate the phenotypic diversity of the parental tissues. Important components, notably mesenchymal/interstitial cells and tissue-specific matrices are absent. Organotypic differentiation of the 3-D constructs, both normal and neoplastic, is enhanced in heterotypic co-cultures comprising phenotypically diverse cells[36,42,43]. Specifically, co-culture of tumor cells and normal stromal cells in RWV bioreactors leads to the formation of differentiated tumor masses. For example, when transformed prostate epithelial cells were cultured with normal bone stromal cells, heterotypic cell–cell and/or cell–matrix interactions lead to phenotypic and genotypic alterations of the tumor cells[43,44]. In the RWV, but not in the static controls, the tumor cells grew in an androgen-independent manner and became metastatic. Remarkably, in these heterotypic 3-D assemblies, the tumor cells invaded the bone matrix and ceased to produce prostate-specific antigen[44]. Similarly, in 3-D cultures of mixed Mullerian tumors of the ovary grown in High Aspect Ratio Vessels (HARVs), both tumor cells and mesenchymal cells expressed *in vivo*-like tissue-specific characteristics, including the expression of oncogenes such as HER-2/neu[35]. Culturing dissociated cancer-derived cells in RWV bioreactors results in assembly of tumor organoids that could serve as *in vitro* models for drug screening and for studying phenotypic instability, drug/radiation-resistance, and the inter-and intracellular signaling pathways involved in clonal expansion.

In the case of normal tissues, heterotypic co-cultures yield organotypically differentiated proto-tissues, as shown for co-cultures of isolated human small intestinal epithelial and mesenchymal cells[30]. These 3-D assemblies displayed cellular polarization and differentiation, as assessed by the presence of apical brush borders. Furthermore, when human kidney tubular epithelial cells were cultured in RWVs in heterotypic co-cultures containing at least 15 per cent non-epithelial cell "contaminants", the epithelial cells re-expressed numerous microvilli and showed other differentiated characteristics[45]. Remarkably, these markers of cellular differentiation were either not expressed or expressed to a much smaller degree in homotypic cultures containing greater than 99 per cent epithelial cells. Besides their potential usefulness as replacement organs, differentiated, 3-D constructs from normal tissue could also serve as tissue equivalents for toxicity testing.

Vascularization of 3-D tissue constructs

Cell aggregates generated *in vitro* (in either suspension cultures or stirred fermentors) that exceed about one millimeter in size, invariably develop necrotic cores. Culture conditions in RWV bioreactors are unique in that the fluid dynamics of the system allow for efficient mass transfer of nutrients and oxygen diffusion. In this environment, dissociated cells can assembe into macroscopic tissue aggregates several millimeters in size, which are largely devoid of such necrotic cores. With the possible exception of avascular tissue of low cellularity and slow metabolism, such as cartilage, all tissues, including those growing in RWV bioreactors, will eventually require internal, blood-vessel like conduits for the delivery of oxygen and nutrients as well as for the removal of waste products[46]. Inclusion of endothelial cells in the mixture of cells cultured in the RWV bioreactors, with the stated purpose of generating vessel-like conduits[30], has so far failed to yield spontaneous formation of blood vessels *in situ*[47]. In these co-cultures, endothelial cells remain viable and organize into homotypic clusters without forming tubular structures. Attempts to generate macroscopic 3-D constructs as replacement tissues will necessitate combining RWV technology with innovative methods for creating bioengineered blood conduits such as growing endothelial cells on the inside and outside of tubular scaffolds[12].

In contrast to our current inability to neovascularize proto-tissues from a mixed inoculum containing endothelial cells, the expansion of tissue fragments, such as of liver[41] and kid-

Table Partial list of cells and tissues cultivated in microgravity in space and on the ground

Cell/tissue	Environment	Observation	Reference
Lymphocytes	Several shuttle missions, Maser sounding rockets	Inhibited locomotion, impaired immunocompetence, impaired mitogenicity, changes in cytokine production, altered cellular signaling	71, p
Chondrocytes	RWV in space (MIR/ STS-79) for 4 months	In space culturing of pre-assembled 3-D aggregates, results in constructs which are mechanically inferior to similar aggregates grown in RWV on the ground. Effect might mimic microgravity- induced loss of cartilage	64
PC12 cells	Six weeks serial passaging of cells in culture bags on MIR Space Station (STS- 86)	Establishes feasibility of long term, serail passaging of cells in space, formation of large aggregates with epitheloid morphology	p
MIP 101 leukemia cells	EDU-1 on STS- 70	Increased proliferation, enhanced CEA production	66
Osteoblasts	Four days on STS-56 in Materials Dispersion Apparatus minilabs (MDA)	Inhibition of growth, reduction in serum growth activation, changes in microfilament structure	51
Myoblasts	Ten days on STS- 45 in space Tissue Loss Flight Module "A" (TLMA)	Several permeant phenotypic alterations, including failure to fuse into myotubes	72
Neonatal rat heart cells	HARV	3-D organization, synchronous beating	39,73
Rodent skeletal muscle satellite cells	HARV, STLV	Enhance proliferation, 3-D organization, attenuation differentiation	74,75
MIP 101 leukemia cells	STLV	Increased proliferation, enhanced CEA production	34
Human Ovarian Tumor Cells	STLV	Capability of growth out of the body, organization into differentiated tissue-like constructs, enhanced oncogene expression	35
Diverse human tumor cell	HARV	Organization of tissue-specific epitheloid structures, enhanced production of cell adhesion molecules	33
Human prostatic cancer cells	STLV, HARV	Enhanced differentiation, reduced proliferation in 3-D aggregates. Upregulation of growth factors and basement membrane proteins in co-cultures mimic physiological growth conditions of prostate epithelial cells with stromal cells, altered responses to sex hormones and growth factors	43,76
PC12 cells	STLV, HARV	Formation of large aggregates exhibiting neuroendocrine differentiation, altered cellular signaling mechanisms	67
Normal human kidney cells	STLV	Re-expression of tissue-specific morphology (microvilli) and differentiation markers	45
Bovine cartilage	STLV	Macroscopic large 3-D constructs with enhanced tissue specific differentiation (ECM proteins e.g., chondroitin sulfate, and cytoskeletal proteins (e.g. vimentin)	20,31,39
Murine osteoblasts	Clinostat	Decreased of differentiated phenotype (reduction of alkaline phosphatase and osteocalcin)	77
Lymphoid tissue HIV	HARV	Repopulation of human tonsil fragments with exogenously added T and B lymphocytes; capable of infection by HIV	37
Liver	HARV	Expansion of tissue from microscopic fragments, angiogenesis	41

p, personal communication

ney (P.I.L., unpublished work), in the RWV environment is accompanied by a commensurate increase in the density of microvessels. This process, perhaps suggestive of angiogenesis, indicates that it may be possible to grow blood vessels in RWVs if a suitable environment can be established.

From cells on earth to tissues in space

It has been known for more than 20 years that single cells respond to microgravity in space with changes in cellular morphology and function[48,49]. According to a recent review, more than 25 different cells types have been flown in space[50]. Strikingly, microgravity during space flight seems to affect the organization of the cytoskeleton (microfilaments)[51] and intracellular signaling mechanisms related to protein phosphorylation. The cytoskeleton is presumably one of the prime sites for microgravity sensing[52]. At the single cell level, alterations in the cytoskeletal architecture might explain changes in the vectorial pathway of intracellular phosphorylation cascades[53]. The functional impairment of space-flown lymphocytes, such as altered capping/patching and locomotion[54,55],

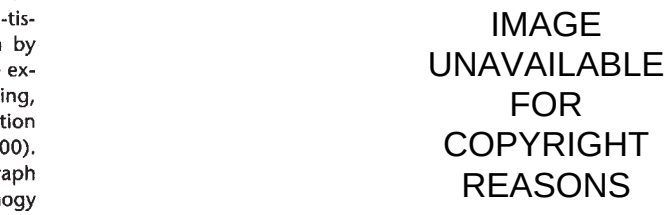

Fig. 3 Formation of proto-tissues from PC12 pheochromocytoma cells cultured in space. ***a***, Photograph of live proto-tissue cultured in space taken by astronaut D. Wolf during the experiment. ***b***, Nuclear staining, showing tissue-like organization (original magnification ×200). ***c***, Scanning electron micrograph showing epitheloid morphology and differentiation of closely apposed cells. (Courtesy of NASA.)

which has been attributed to alterations in protein kinase C-dependent signaling[56], may also result from cytoskeletal rearrangement in microgravity.

In order to mass culture cells in space, several kinds of bioreactors, including the RWV system, have been tested[57–59]. The severe constraints of space flight, such as limitations on space, permissible weight and crew time, have led to the development of several protocols that should enable cells to be grown in space under limited supervision. Ideally the cultures would be pampered in a fully automated system with a computer controlling the rate of, for example, media exchange and waste removal[60,61].

It has been reported that during space flight, cell suspensions preferentially aggregate[21] and yield higher cell densities than on the ground[59]. One reason for enhanced cell–cell interactions may be that microgravity induces a tissue-specific up-regulation of cell adhesion molecules, extracellular matrix proteins and their respective receptors. In line with this notion, studies on board Spacelab indicated a significant increase in collagen synthesis in human dermal fibroblasts in microgravity[62]. However, in rats, spaceflight decreased the levels of osteocalcin as well as the prepro-alpha2 chain of type I precollagen mRNA in long-bone and calvarial periosteum[63]. Increased expression of "glue proteins" such as collagen by certain cell types cultured in space may yield larger and/or more differentiated organoids than parallel cultures maintained at 1 g on earth (although an abundance of glue protein does not necessarily translate into more highly organized tissue). For other cells, however, which are adversely affected by microgravity (such as bone, cartilage or muscle) a space-walk might yield inferior results. This hypothesis was tested, in part, on a recent Shuttle/Mir mission: STLV type RWV bioreactors were used as a long-term (four months) cell culture venue for the generation of 3-D cartilaginous constructs from chondrocytes that had been pre-aggregated on resorbable scaffolds and then cultured for three months in RWV bioreactors on the ground[64]. In parallel ground control experiments, the constructs were maintained for four months in RWVs. The cartilaginous constructs that were flown for four months in space proved to be mechanically inferior to constructs grown on earth. Although, disappointing, this was not unexpected. What is surprising is that cartilaginous constructs obtained in the simulated microgravity environment of the RWV bioreactors are quite similar in their composition and mechanical strength to natural cartilage[20,36]. The finding that the constructs grown in space were mechanically inferior might reflect a direct adverse effect of microgravity on cellular processes involved in the synthesis, secretion and assembly of extracellular matrix proteins. This is in keeping with the well known adverse effects of space flight on bone and cartilage[65]. On the other hand, the superiority of cartilaginous constructs on earth might reflect the effects of the physical forces at 1 g on the differentiation of the bioengineered constructs[64]. The question remains: what are the fundamental differences between simulated microgravity and the real thing? Are the differences due to distinct g levels (10^{-2} g in RWV versus 10^{-4}–10^{-6} g in space), or due to residual physical forces (sedimentation, low but finite shear stress) at 1 g? A definite answer will have to wait until the experiments can be repeated on board the International Space Station, in microgravity, as well as under conditions in which gravitational forces can be artificially restored in space.

The results of growing cartilage in space suggest that space culture may not be advantageous for each and every cell type in terms of generating functional replacement tissues. In contrast to the cartilage experiment, other investigators such as the NASA cell culture biotechnology group, who have flown MIP 101 Leukemia cells in the RWV aboard various Shuttle missions[66], reported during the NASA Space Shuttle Investigators Workshop enhanced responses (aggregation and secretion of bioactive products) for cells cultured in space RWVs compared with cells cultured in terrestrial RWVs (N. Pellis & J.M. Jessup, pers. comm.).

Recently, we participated as guest investigators in a study aimed at exploring the feasibility of using simple, gas-permeant cell culture bags as an alternative in which to grow and serially passage PC12 pheochromocytoma cells over a prolonged period of time in space. In comparison to ground controls, the degree of aggregation and the rate of proliferation of the samples in space was significantly accelerated. For example, the rate of glucose consumption, a measure of cellular metabolic activity and, at least for PC12 cells, also of proliferation, was about five times higher in space than on the ground (P.I.L., C. Waters and B.R.U., unpublished work). Similarly, large cellular aggregates were observed in space, but not under static conditions on the ground (Fig. 3). These results, although preliminary, suggest that the space environment might be advantageous for generating neuronal/neuroendocrine tissue equivalents using PC12 cells[15,67].

These data prompt a cautionary approach towards the potential use of "space factories" for producing medically/scientifically useful tissue equivalents. The outcome might be

highly cell-type specific. On the other hand, the complex responses of cells and tissues to space flight call for well-designed scientific studies aimed at a better understanding of the cellular and molecular mechanisms involved in the adaptation to microgravity[68].

Caveats

Although microgravity, either simulated or in space, may be useful for the mass production of replacement tissues, for the time being the usefulness of this approach is just a promise yet to be realized. Similarly, the advantages of RWV-generated, 3-D tumor cell organoids have to be substantiated, scientifically and clinically. And yet, while this field is still in its infancy, microgravity-based organoid formation should soon find its niche in the wider field of tissue engineering.

Microgravity-derived 3-D constructs produce abundant cell adhesion molecules, interstitial collagen and other basement membrane proteins together with their integrin receptors. However, it is not known whether these 3-D constructs from space, once removed from suspension culture, will remain intact and function long enough to become fully vascularized and integrated into the host tissue in a transplant setting.

The potential advantages of using microgravity in space for generating replacement tissues must be balanced against several possible problems of space flight. Using the space environment, rather than the simulated microgravity facilities on the ground, requires a round trip ticket. The beginning and the end of this voyage are particularly stressful. The extreme conditions at the launch of a space craft, and probably at reentry, go far beyond the temporary discomfort experienced by the astronauts. Recent results suggest that these launch-associated stresses elicit responses at both cellular and molecular levels. For example, exposure of mouse osteoblast cells to g forces and vibrational forces that simulated the conditions of a space shuttle launch induced the rapid expression of several genes[69]. In some of the recent joint MIR/NASA missions, we observed long-term (in the order of days) negative effects of simulated launch conditions on vascularization of the extra-embryonic chorioallantoic membrane in developing quail embryos (P.I.L., unpublished work). Therefore, when conducting cellular experiments in space, it is mandatory to include both an additional set of ground controls that simulate the conditions of a shuttle launch, and an onboard 1 g centrifuge to control for these deleterious forces. At present it is not clear how cellular responses induced by launch conditions will impact subsequent experiments carried out in space.

Another problem may be DNA damage caused by elevated levels of radiation in space. Enhanced DNA damage is caused by the synergistic action of the direct effect of radiation on DNA integrity and impairment of cell signaling pathways[70]. This synergism might lead to radiation damage that could translate into cytogenetic alterations.

In terms of "bigger is better" the microgravity environment in space may be superior to that of simulated microgravity in ground-based RWV bioreactors. However, some of the caveats raised might pose significant threats to the goal of obtaining functional proto-tissue and multicellular 3-D constructs. Some thirty years ago man first set foot on the surface of the moon. Today, space still beckons and what started as an adversarial space race has evolved into an intense international scientific and technical collaborative effort. The advent of the International Space Station early in the next millennium will provide laboratory facilities in which the promise of tissue engineering in space may eventually be realized. The advent of the International Space Station might alleviate at least some caveats, by enabling the assembly of tissues from cells adapted to the space environment through several passages.

Acknowledgments

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[1]*Department of Biology*
Marquette University
Milwaukee, Wisconsin 53233, USA
[2]*Laboratory of Cell Biology*
Department of Medicine
University of Wisconsin Medical School
Milwaukee, Wisconsin 53201, USA