Topical: 3D Tissue Chips for Space Biology Studies

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Abstract
There is a fundamental gap in our understanding of how space environment modulates molecular signaling to effect changes in human tissue phenotype and function. Three-dimensional (3D) models for human cardiac, neurovascular, and vascular tissues exposed to space-like environments (e.g. radiation) will enable identification of functional changes to inform the utilization and design of targeted countermeasures that will allow timely intervention and tissue protection.

Introduction: It has been over 60 years since the first human flew into space, and humans have had a continuous presence in space for over 20 years on the International Space Station (ISS). There is also increasing interest in more extended duration spaceflight missions beyond low Earth orbit and even proposals for permanent extraterrestrial research stations. Despite this experience and interest, there are alarming deficits in our understanding of how the unique environmental conditions presented by spaceflight, including microgravity and galactic cosmic radiation (GCR), impact the human body.

Meta-analysis has shown that tissue complexity plays an essential role in mediating the effects of spaceflight. Due to the lack of biomimetic three-dimensional (3D) models and accurate radiation species, effective mitigation strategies are lacking. The impact on the cardiovascular and neurovascular systems is of particular concern due to the potentially lethal effects of spaceflight-induced tissue dysfunction.

Since the invention of reprogramming to generate induced pluripotent stem cells (iPSCs) from somatic cells, tremendous progress has been made in developing protocols for differentiating human iPSCs into various cell types, including cardiac, neural, and vascular cells. The iPSCs derived from patients are now widely used in disease modeling. However, traditional two-dimensional (2D) cultures are still limited in utilizing iPSCs to model disease processes, particularly cardiovascular and neurological diseases. These 3D tissue models (i) include anatomical and cellular complexity of the native tissue, (ii) are viable and functional for more than 90 days, and (iii) possess functionality similar to the native tissue. They are thus excellent models to study responses to space radiation.

Cardiac: The Bellagio Report on Cardiovascular Risks in Spaceflight noted that spaceflight could lead to a significant loss in cardiac muscle mass and cardiovascular function, which may lead to clinical risks. Cardiac arrhythmias have occurred in space in multiple astronauts with no prior history of cardiac arrhythmias. Orthostatic intolerance is evident in astronauts upon returning to normal gravity.

Despite these risks, little is known about the molecular determinants of spaceflight-induced cardiac dysfunction. Most studies on spaceflight-induced changes in the cardiovascular system have been

generally performed on animal models or cell lines, which may not adequately represent the effects of spaceflight on human physiology. The first study using human iPSC-derived cardiomyocytes (hiPSC-CMs) was only recently published and found differentially expressed genes involved in mitochondrial metabolism and alterations in calcium handling. However, recent work has illustrated the importance of the 3D microenvironment in mitigating the effects of spaceflight. Thus, we must utilize 3D human models of the myocardium to assess the effects of space radiation on the heart.

While ISS-based studies are adequate for assessing the effects of microgravity, the ISS is primarily shielded from GCR by the Earth's magnetic field. Most studies on the impact of GCR utilize monoenergetic single-ion beams, whereas GCR consists of high linear energy transfer protons and atomic nuclei traveling at relativistic speeds. Studies using GCR simulations are limited, as NASA only recently developed their ground-based GCR simulator. No studies to date have utilized 3D human models of the myocardium to assess the effects of GCR on the heart.

Neurovascular: The intricacy of the human nervous system, including the cell type diversity, cellular architecture, and functional connectivity, is one of the fascinating scientific topics. However, the difficulty in accessing human brain tissues greatly limited our investigation and understanding of various neurological disorders and conditions. In addition, in contrast to many mouse strains, humans are genetically diverse, affecting individual cellular and physiological differences in our body, including the neural system. Therefore, human neural diseases usually have complex and diverse etiologies and could be further complicated by variable genetic, epigenetic, and environmental factors that differ among individuals. One of the major limitations in studying neural diseases is the lack of an *in vitro* system that faithfully recapitulates the complexity and delicacy of the human brain. Although some animal model studies provide valuable mechanistic insights into the pathogenesis and causes of human neural disorders, their values are limited because of the species differences. Therefore, it is critical to develop an experimental neural system to reflect the human context to understand how the space environment affects the physiology of humanized neural model.

3D brain organoid technology development expands the already diverse possibilities of using iPSCs to study human neurobiology. Compared with adherent 2D neural differentiation, the process of brain organoid generation preserves the spatial architecture that forms as the primitive neural tissues self-organize and allows the investigation of early human brain formation, a process difficult to observe in vivo and that diverges significantly between human and experimental organisms. It has been shown that brain organoids from human iPSCs develop stratified structures resembling the ventricular zone, subventricular zone, intermediate zone, and cortical plate. This recapitulates the presence of the outer subventricular zone *in vivo* in humans. Within the germinal zones of human brain organoids, proliferative precursors resembling ventricular and outer radial glial cells are generated in a temporal and spatial pattern consistent with in vivo human cortical development. To model long-distance neuronal migration between distinct brain regions, brain organoids can be pre-patterned to develop region-specific identity, after which different organoids can be cocultured to allow physical contact and fusion. This approach has observed directional migration of inhibitory neurons from ventral cortical organoids into dorsal cortical organoids. As brain organoids mature with prolonged culture and co-culture with non-neural cells (such as endothelial cells), functional neuronal connections are formed, allowing the observation of

neuronal activity on the levels of cells and network. Spontaneous and evoked neural activity can be measured using calcium imaging, and multielectrode and different types of glial cells can also be seen, allowing the investigation of the development and function of these glial cell types.

Another advantage of human iPSC-derived human brain organoids is the possibility of genetic editing. Gene editing tools, such as TALEN and CRISPR/Cas9, have made it possible to modify specific genes or locus precisely. This approach enables us to understand the genetic factors involved in toxicity or pathology caused by space radiation and other environmental factors in human brain organoids, which any other model could not achieve.

Due to many advantages, it could be prudent to incorporate brain organoids into space neurobiology to pursue pertinent biological questions about space radiation.

Vascular: Radiation has been shown to damage the human vascular system and cause subsequent adverse health effects in individuals subjected to it. However, studies of the systemic damages to the vasculature are limited to rodent models, and studies of damage to human cells have been limited to monolayer culture of endothelial cells or microvascular constructs in gels. Rodent models fail to recapitulate the effects of radiation on human cells on a genetic level, and current standard cell culture models fail to incorporate other vascular cell types, ignoring the impact of cell-cell signaling, an essential component of tissue damage response. In addition, the native mechanical stimuli that these cells would experience are ignored, providing an insufficient model for truly understanding how native vasculature responds to radiation.

In recent years, tissue-engineered microvascular networks or vascular grafts made of biocompatible materials have been developed for therapeutic applications. They have subsequently been used in conjunction with human cells to study disease states in blood vessels better. In these in vitro hydrogel materials or cellularized grafts, tubular scaffolds have been used successfully to study multiple vascular cell types in the correct 3D structure and anatomical orientation, and geometry. These constructs can then be attached to a flow system, mimicking the shear force and circumferential stretch that induces endothelial cells and perivascular cells, including pericyte and smooth muscle cells, to behave more similarly to native cells in the vasculature. By subjecting this physiologically relevant model to radiation, we can gain a more accurate picture of the specific radiation damages cells undergo and how they can be mitigated.

Furthermore, the use of iPSCs for these blood vessel models also allows for the personalized analysis of the damage mechanisms in each patient of interest. This then promotes the translational potential of the model to tailor mitigation strategies for radiation damage to best help each individual.

One promising mitigation strategy focuses on the expression of HMGB1, a secreted factor involved in activating inflammatory pathways, resulting in long-term vascular pathogenesis. The shift in nuclear vs. cytoplasmic expression of HMGB1 in response to radiation exposure can be analyzed using these 3D tissue-engineered vascular constructs. Subsequently, a pre-developed protein antagonist has shown promise in preventing the activation of HMGB1. The effectiveness of this antagonist can be evaluated by treating the tissues with this antagonist in conjunction with

radiation exposure and examining any mitigation of damage in the cells as decreased expression of activated HMGB1.

Another mitigation strategy that can be explored due to the use of iPSCs as a cell source is optogenetics to activate specific signaling pathways that provide protective measures against radiation. For this approach, an iPSC line carrying the photoactivatable system is developed through gene editing. These iPSCs can be incorporated into the various 3D tissue models to investigate the protective properties of this system after radiation exposure.

These strategies could not be adequately characterized or utilized at all without using hiPSC-derived 3D *in vitro* tissue structures. These *in vitro* systems provide great utility by filling the current gaps in understanding the mechanisms underlying radiation damage to the human body. In addition, they can be used to test damage mitigation strategies against specific astronaut cells before the person of interest is even exposed to damaging radiation. This can provide valuable insight into how to protect future astronauts on missions into deep space, and it can simultaneously be used to protect the many individuals on earth subjected to ionizing radiation, including cancer patients undergoing radiotherapy.