Microgravity and Radiation Effects on Astronaut Intervertebral Disc Health

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INTRODUCTION: The effects of spaceflight on the intervertebral disc (IVD) have not been thoroughly studied, despite the knowledge that spaceflight increases the risk of herniation of IVDs in astronauts upon return to Earth. However, as long duration missions become more common, fully characterizing the mechanisms behind space-induced IVD degeneration becomes increasingly imperative for mission success. This review therefore surveys current literature to outline the results of human, animal, and cell-level studies investigating the effect of microgravity and radiation exposure on IVD health. Overall, recurring study findings include increases in IVD height in microgravity conditions, upregulation of catabolic proteases leading to a weakening extracellular matrix (ECM), and both nucleus pulposus (NP) swelling and loss of annulus fibrosus (AF) fiber alignment which are hypothesized to contribute to the increased risk of herniation when reloading is experienced. However, the limitations of current studies are also discussed. For example, human studies do not allow for invasive measures of the underpinning biochemical mechanisms, correlating animal model results to the human condition may be difficult, and cellular studies lack incorporation of ECM and other complexities that mimic the native IVD microarchitecture and environment. Moving forward, the use of three-dimensional organoid culture models that incorporate IVD-specific human cells, ECM, and signals as well as the development of cell- and ECM-level computational models may further improve our understanding of the impacts that spaceflight has on astronaut IVD health.

KEYWORDS: intervertebral disc, microgravity, cosmic radiation, back pain, spaceflight.

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The impact of spaceflight on human health has been identified even before astronauts landed on the Moon or established long term residency on the International Space Station (ISS). The effect of the environmental conditions in space on human health can compromise mission success if astronauts are unable to carry out mission tasks, and can detrimentally impact astronaut health upon return to Earth. This has resulted in the establishment of NASA's Human Health and Performance Directorate and research organizations such as the Translational Research Institute for Space Health, which hosts the Red Risk School to expand the space medicine community's knowledge of the factors affecting humans while in space. The extreme environment experienced in space involves two main risks to human health: microgravity and ionizing radiation exposure.[54,64] Microgravity is characterized by the weightlessness felt by astronauts in space, as a very small gravitational force is experienced. Additionally, since all long-term spaceflight will extend beyond the Earth's geomagnetic field, astronauts are subjected to much higher doses of both solar particle events and galactic cosmic rays than while on Earth,[54] which subjects the body to a wide range of damaging radiation. Therefore, the two factors must be studied both independently and together to truly understand what the human body experiences while in space.

Overall, data has been gathered focusing on the physiological response of the human body to spaceflight in a variety of organ systems, showing the onset of debilitations such as cardiac deconditioning,[68] or worsening eyesight and changes in eye anatomy.[45] Spaceflight has also been shown to lead to a loss in bone density[39] and skeletal muscle atrophy,[18,25] especially in muscles involved in stabilization and balance.[10,14,39,48] However, investigation into the effects of spaceflight on intervertebral

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disc (IVD) health is relatively unexplored despite the known risk of IVD damage in space,[64] and that the incidence of IVD herniation is 4.3 times higher in astronauts compared to their Earth-bound counterparts.[10] It has been hypothesized that elongation of the spine and unloading of the IVD under microgravity leads to cell-mediated degradation of this complex tissue.[26] This degenerative process has been correlated to a loss of spinal curvature and IVD swelling, which is shown to contribute to localized low back pain during spaceflight[35] and a higher prevalence of lumbar IVD herniation upon return to Earth.[33] Taken together, spaceflight therefore poses a risk to overall human health and IVD health in particular, and warrants a comprehensive analysis of our current understanding of space-related IVD pathologies as well as the systems employed to study the mechanical, cellular, and biochemical influences. Ultimately, this knowledge can be used to develop future areas of research and countermeasures that could minimize the impacts of changes in IVD health and thus mitigate risk to mission success in future long-duration spaceflights.

Intervertebral Disc Anatomy and Physiology

The IVD makes up a third of the length of the spine[49] and supports the majority of the mechanical loads experienced by the torso during activities of daily living. The IVD serves to provide flexibility and maintain the mechanical integrity of the spine. The IVD is a unique avascular structure that consists of three distinct regions: the cartilaginous endplates (CEPs), nucleus pulposus (NP), and annulus fibrosus (AF) (**Fig. 1**). The CEPs are thin layers of hyaline cartilage positioned between the vertebral endplate and the NP.[50] This region acts as a mechanical transition region between the stiff vertebral bone and gelatinous NP within the IVD, but more importantly facilitates nutrient transport into the avascular IVD from adjacent blood vessels in the vertebral bone.[51] The NP is the gelatinous central core of the IVD that is comprised of collagen type II and the proteoglycan aggrecan.[52] The proteoglycan chains are comprised of highly sulfated glycosaminoglycans (GAG), which are covalently bonded to a core protein.[7] These molecules, primarily chondroitin sulfate and keratin sulfate, have net negative charges that create a high fixed charge density, allowing the influx of counter ions, which subsequently creates high osmotic potential, keeping the tissue hydrated.[75] This unique tissue composition allows the NP to generate osmotic swelling pressure. When the NP is compressed, the tissue's low permeability prevents fluid outflow, allowing the IVD to absorb mechanical loading and act as a shock absorber between the two vertebrae. The NP tissue also contains cells which display heterogenous phenotypes and morphologies.[71] While found in low densities within the tissue, these cells are responsible for the maintenance of the extracellular matrix (ECM), and thus the osmotic pressure of the NP,[11] by producing a GAG to hydroxyproline ratio of 27:1.[53]

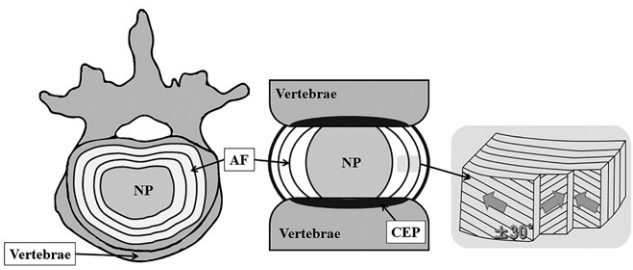

Fig. 1. Schematic of the IVD depicting distinct nucleus pulposus (NP), annulus fibrosus (AF), and cartilaginous endplate (CEP) regions.

The AF is composed of approximately 15–25 concentric rings of aligned collagen, which constrain the NP.[35,58] Specifically, these sheets of collagen type I are arranged in an alternating angle-ply structure, roughly 30° to the horizontal axis of the spine.[46] The unique collagen sheet structure of the AF is specifically designed to resist the radially directed forces generated by the osmotic pressure of the NP.[11] These tensile hoop stresses stabilize the spine during bending and torsion, illustrating the importance of proper AF structure on overall IVD function. Additionally, the mechanical properties of the AF change gradually, moving from the outer AF toward the NP, leading to different mechanical and biochemical cues in the inner to outer AF zones.[15] Similar to the NP, the AF contains a heterogenous population of AF cells with morphologies and phenotypes that change with location in the AF.[66,70] More specifically, the morphology of these cell types are elongated along the periphery and outer zones of the AF, and become increasingly spherical as the cells reside closer to the NP in the inner AF.

Etiology of Intervertebral Disc Degeneration and Herniation on Earth

When the IVD experiences prolonged or altered mechanical loading or injury, the tissue can undergo two complex, interconnected pathologies: IVD degeneration and herniation. Both manifestations reduce the IVD's ability to bear mechanical loads and can ultimately result in debilitating back pain. In regard to IVD degeneration, this process initiates within the NP region as an observed decrease in NP proteoglycan content, thus lowering the osmotic swelling capacity of the tissue.[44,69] This process leads to fibrosis of the NP, causing the tissue to lose its ability to support mechanical loading.[63] This loss of NP tissue reduces mechanical stability,[1] leading to loss in IVD height[22] and disruption or ingrowth of spinal nerves.[21] Additionally, the shift in homeostasis in the microenvironment of the IVD upregulates several senescent cellular responses, such as apoptosis or cell death, an increase in matrix metalloproteinase (MMP) expression, and a decrease in their inhibitors (tissue inhibitors of metalloproteinases: TIMPs), and an increase in a release of inflammatory cytokines such as IL-1β and TNF-α.[6] All of these cellular responses have negative consequences, causing further reduction in NP water retention and stiffening of the IVD, and acceleration of ECM degradation.[34]

IVD herniation is initiated by an accumulation of microdamage or an associated traumatic loading event.[57] These injuries can reduce the stability of the AF, as AF tears can develop and further propagate throughout the concentric layers.[31] The AF is primarily responsible for constraining the NP, so a disruption and overall weakening of the AF ultimately leads to the

protrusion of the NP through the AF. This process can ultimately lead to IVD collapse, progressive degeneration, pinched nerves, and can also result in discogenic low back pain.[49,56] Low back pain has been shown to affect up to 80% of the population globally and is the one of the leading causes of debilitation, costing the healthcare system up to $200 billion per year.[49] Within the United States, low back pain is the second most frequent reason for doctor visits and the most common cause of job-related disabilities leading to days missed on the job.[23,57]

While the etiology of IVD degeneration and herniation is well understood within the clinical environment on Earth, limited research has been done to elucidate the effects of microgravity and cosmic radiation on these IVD pathologies (**Fig. 2**). Therefore, the objective of this paper is to review current research including human, animal, and cell culture studies to further our understanding of how space impacts astronaut IVD health and to chart future directions for mechanistic studies.

Review of Human Studies

The effects of microgravity on IVD health. The potential risk of IVD degeneration during spaceflight has been identified for some time now,[38] which has led to the improvement of experimental capabilities for human studies on the ISS to study the long-term effects of unloading on both the human body and IVD in particular. One longitudinal study investigated whether astronauts are at a greater risk of NP herniation compared with an age/gender-matched control group on Earth. Overall, 321 astronauts and 983 control individuals were monitored pre-, during, and postflight. Out of these participants, the occurrence of herniation was found to be 4.3 times higher in the astronaut population compared to the Earth-based control group.[10] Herniation causes severe pain and often diminishes quality of life and patient mobility, and this debilitation could be even more severe for astronauts following extreme conditions experienced during spaceflight. Identifying the cause of increased herniation is complex, but vital to successful year-long missions beyond the ISS, especially as long-term missions result in terrestrial settlement with some degree of gravitational loading. One interesting result observed under microgravity conditions was an increase in IVD hydration during spaceflight, which was hypothesized to increase the pressure exerted on the IVD and tensile strain on the AF.[28,38] IVD swelling has also been observed in astronauts as microgravity causes changes in the spine, including increases in spine length and body height, which can contribute to degeneration upon return to Earth. Most recently, in-orbit ultrasound imaging of the lumbar IVDs of ISS astronauts throughout a 150-d mission demonstrated an increasing trend in IVD heights and loss of spinal curvature, which peaked at 90 d in orbit.[13,28] Interestingly, these values decreased back toward preflight values after 150 d in orbit; however, at this time point, IVDs often exhibited evidence of degeneration.[28] These findings suggest that a pattern of swelling during the first months of spaceflight may give rise to longer term degenerative changes in the IVD that could lead to herniation when loading is experienced upon return to Earth.

However, these physical changes are not always detectable in astronauts, as studies have shown that reduction in IVD compressive loading results in water shifts, which is indicative of NP fibrosis, and atrophy of spine-stabilizing muscles,[13,39,61] both of which were determined independently from changes in IVD height.[9] Additionally, studies conducting pre- and postflight MRI imaging have shown no significant changes in IVD structure following spaceflight.[13,61] The lack of consistent physical indicators suggests changes in the IVD are occurring at the cellular and molecular level in response to unloading due to microgravity. Generally, it is hypothesized that the shift from gravitational loading to unloading initiates a catabolic cellular response, as the cells of the IVD recognize and convert extrinsic mechanical cues into intracellular signaling cascades that lead to ECM remodeling.[9] Therefore, when normal mechanical stress is taken away in the absence of gravity, these cells are not activated to carry out healthy metabolic pathways for IVD development and stability. The lack of mechanical stimuli can lead to the onset of degenerative pathways such as apoptosis and MMP upregulation,[16,59] weakening the astronaut IVDs while in space. Additionally, a lack of mechanical loading can inhibit the influx and efflux of nutrients and waste which can also detrimentally impact IVD health and cell function.[64] Indeed, bed rest studies,

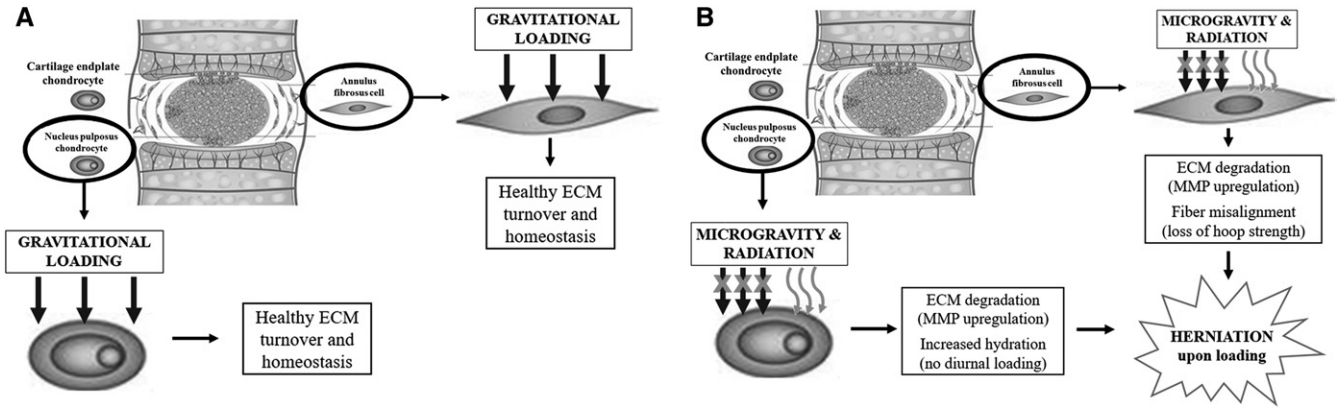

Fig. 2. Schematic depicting the effects of A) normal gravitational stresses and B) microgravity and cosmic radiation on the IVD, specifically highlighting changes in ECM turnover via upregulation of matrix metalloproteinase (MMP) by both NP and AF cells.

which have been used as Earth-based analogs for studying IVD degeneration during unloading, have shown cartilage ECM degradation at higher rates than when under normal loading conditions.[42] These findings suggest that unloading can initiate catabolic processes in cartilage metabolism, such as reductions in GAG content within the NP region of the IVD.[39] Therefore, the effects of microgravity are much more complex than simply spinal elongation, preventing an all-encompassing explanation for the effect of spaceflight on the IVD to be made when analyzing degeneration macroscopically, pointing directly to the importance of studying cellular mechanics and metabolism and the subsequent interactions with IVD ECM.

The effects of radiation exposure on IVD health. Understanding space radiation and its effect on the human body will become increasingly important as missions to the Moon and Mars become more common. Radiation exposure research is often overlooked for applications on Earth, but analysis of patients exposed to low levels of ionizing radiation, such as events occurring in Three-Mile Island and Fukushima, have shown that ionizing radiation has potentially detrimental effects on the human body. Additionally, the amount of yearly radiation exposure here on Earth, measured in microGray (mGy), a unit of adsorbed radiation dose, has steadily increased over the last few decades,[77] from 5 mGy in 1982 to 30 mGy in 2006.[78] As research is conducted on Earth surrounding the effects of low dose radiation, these results may be able to be used as predictors of cosmic radiation effects on astronaut IVD health. Generally, radiation significantly hinders osteoblastic differentiation, affecting bone ECM and leading to osteonecrosis, weakening the spinal bones that support the IVD.[29] Because of the more extreme radiation experienced by astronauts traveling outside of Earth's magnetosphere, both acute and prolonged radiation exposure can pose risks to human health and overall mission success. An increased dose of radiation from both galactic cosmic radiation and solar particle events causes chemical reactions initiated by high energy deposition in cells, which impairs overall tissue integrity and function.[54] The general effect of radiation at the cellular level has been identified, as exposure to cosmic radiation causes genetic mutations that lead to programmed cell death,[60] which also contributes to alterations in IVD homeostasis. However, the potential cellular response and resulting IVD degeneration is generally unknown, especially when coupled with microgravity, as many studies do not look at the additive effects of both of these parameters. Therefore, there is a need for experimental models that can seamlessly integrate into current low Earth orbit culturing systems to be specifically analyzed for the effects of both microgravity and radiation exposure on IVD tissue during long-term spaceflight.

Review of Animal Studies

The effects of microgravity on IVD health. Animal models offer a unique opportunity to conduct whole organ studies similar to human studies, while also obtaining detailed data from histological or biochemical analyses. Microgravity animal models, therefore, offer a bridge between true microgravity data and the effects on the IVD on the cellular level. One method of conducting microgravity studies is through spaceflight payloads on biosatellites, for an average of 14 d.[27] Foldes et al. conducted semiquantitative analysis of histological and histochemical changes in the structure of lumbar IVDs of rats flown on the Kosmos 1887 biosatellite, which was flown in low Earth orbit for 13 d.[19] Macroscopic results of IVDs show lateral expansion in the NP region, as well as swelling of the entire IVD, which was synonymous with data gathered from human studies. There was also an observed decrease in fiber alignment in the outer AF regions, showing weakening of the hoop strength of this tissue, which could lead to a reduction in its ability to constrain the NP once reloaded. Overall, distinct histological differences could be used to pinpoint changes in IVD function and may provide an explanation of the back pain experienced by astronauts. Another murine study conducted on the Space Shuttle mission STS-118 by Gonzalez et al. showed diminished vertebral microarchitecture of the lumbar vertebrae, indicating less structural support for surrounding IVDs.[24] Additionally, IVD height was found to increase as expected through calculations of the IVD height index. It was concluded that lumbar IVD expansion in conjunction with reduction in lumbar spine boney structural integrity may be some of the most important characteristic features of IVD degeneration.[24] Therefore spaceflight offers true microgravity conditions and provides the most reliable test conditions for gathering data. However, it is often challenging to eliminate confounding variables from flight experiments, as payloads often overshoot landing positions and can sometimes lead to prolonged waiting times between reentry and vessel acquirement. This inaccuracy in landing can lead to prolonged fasting of the animals, exposure to low cage temperatures, and delayed death of the flown animals.[27] Therefore, spaceflight models are not always ideal experiments as all of these factors may contribute to the degeneration observed during postexperiment analysis on Earth.

When spaceflight is unavailable or unsuitable for a given study design, simulated microgravity studies are most commonly conducted using suspension methods, where the spine of a rat is unloaded by fixation of either the tail or pelvis above the ground.[14,41] One study conducting an 8-wk tail suspension test showed macroscopic IVD injuries resulting from simulated microgravity. In addition, disruptions to the microenvironment and the associated cellular response were determined, including the increase in proinflammatory cytokines IL-1β and TNF-α.[41] Quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis also showed the upregulation of both p53 and p16, indicative of degeneration.[41] Microgravity has also been shown to increase the upregulation of MMPs that degrade the ECM of the IVD, further weakening the microenvironment and making the IVD more susceptible to herniation. ECM degradation and subsequent herniation was therefore studied by Wu et al., who employed a rabbit model investigating both weightlessness and hypergravity.[79] Hypergravity, or increased gravitational loading, is experienced by astronauts during periods of takeoff and landing and was used to study the cellular response to the return to gravitational loading. Both micro- and

hypergravity showed significantly increased MMP upregulation after 90 d,[79] specifically MMP-1 and MMP-3. MMP-1 is also known as collagenase 1 and can specifically degrade collagen 1 found abundantly in the AF region of the IVD.[73] MMP-3 is a protease that can degrade laminin, proteoglycans, and collagen, all of which are important NP and AF ECM components in the IVD, respectively.[73] Therefore, this study highlighted the induced cellular response to changes in mechanical stress and the direct correlation between cellular responses and IVD weakening.

As shown, there has been data gathered on the effects of microgravity on the weakening of the IVD, specifically the induction of inflammatory pathways and ECM degradation, and the effects of reloading on the IVD. One study found that important ECM proteins, aggrecan, and proteoglycans decrease after a rat tail-suspension model is conducted,[47] specifically shown through a downregulation of mRNA expression for these ECM molecules.[81] The downregulation of aggrecan was correlated to decreases in GAG concentration normalized to DNA content, confirming a decrease in ECM deposition upon unloading. However, aggrecan mRNA levels and GAG/DNA content in the NP were recovered upon reloading, showing the ability to shift back to 'normal' ECM gene transcript expression. Additionally, TIMP1 mRNA levels in the NP were significantly lower in the tail-suspended mice compared to the control group, but TIMP1 expression was shown to recover to initial levels after reloading. No changes in catabolic genes were seen in the NP of the experimental groups compared to the control for both loading and reloading conditions.[81] In the AF, GAG content was not recovered during reloading and was accompanied by an increase in MMP-3 expression. In the AF, there was no significant change in TIMP1 upon reloading compared to the increase in catabolic expression.[81] This lack of recovery in the AF tissue may offer insight into the cause for herniation, as this differential response of NP recovery leads to applied stress on the weakened AF tissue and may ultimately result in NP protrusion. Additionally, the differential response of NP and AF cells when exposed to microgravity and reloading show the complexity of IVD degeneration, highlighting the need for a model and study design where these tissues can be easily distinguished and studied.

Another unique way to study IVD degeneration under simulated microgravity is the use of extracted mice IVDs cultured in rotary wall vessel bioreactors.[32] These rotating conditions offer a ground-based analog for spaceflight and allow a more accurate simulation compared to the hind-limb suspension method. IVD organ culture carried out for 8 wk resulted in significant loss of proteoglycan content as measured through quantification of GAG to hydroxyproline ratios, and these decreases in proteoglycans were evident as early as 2 wk into the culture period.[32] Using immunofluorescence, MMP-3 expression was found to significantly increase in both AF and NP tissues compared to the static condition.[32] These results suggest a degenerative response due to microgravity and this conclusion was supported by apoptotic analysis through TUNEL assays, showing that more cells underwent apoptosis in the rotary wall vessel bioreactor compared to a static culture.[32] This study therefore provides valuable information on the effects of microgravity on cultured IVDs as well as highlights robust methods of characterizing IVD degeneration using organ culture methods.

The effects of radiation exposure on IVD health. Animal models are also useful in elucidating the effects of radiation exposure on the IVD. A study conducted at the NASA Space Radiation Laboratory (NSRL) in Brookhaven, NY, USA, showed interesting results when studying the additive effects of both space radiation and unloading on bone and muscle loss.[37] This mouse model subjected six groups to a variety of conditions, such as only unloaded, unloaded and proton radiation, and unloaded with both high atomic number energy (HZE) and proton radiation, compared to normal gravitationally loaded control groups. This study is unique in that it used the NSRL's ability to subject the radiation groups to multi-ion beam radiation, which includes both HZE and proton radiation. This multi-ion radiation is the closest ground-based simulation of space radiation behind Earth's geomagnetic field, and therefore offers the best data to combat potentially detrimental radiation damage during long-term missions. Overall, unloaded groups showed the most significant bone and muscle loss compared to loaded groups, which supports the hypothesis of microgravity's severe effects on spinal function.[37] Interestingly, no significant degeneration of bone or muscle was found in the loaded groups subjected to proton and HZE + proton radiation. However, when unloaded groups were subjected to this same space radiation analog, there was an observed amplification of bone loss above the unloading control group.[37] These results therefore highlight the potential additive effects between microgravity and radiation exposure, showing that these two parameters should be studied together, particularly for studying the effects of these environmental factors on IVD health. Overall, it was shown that specifically HZE + proton radiation resulted in bone sensitization to microgravity, resulting in reduced stability of the spine and further exacerbating the degeneration occurring due to spinal unloading during spaceflight. Thus, this confirms the need to assess the combinatorial effects of both parameters on IVD health, which has yet to be studied.

Translatability of animal study results to human data. Currently, it is unclear whether results obtained from animal models can be directly correlated to what occurs in the human body. Results from animal models must be cautiously interpreted when considering the significant differences that exist between animal IVD physiology and that of humans. For example, rat models offer a high degree of feasibility for microgravity simulation; however, their IVDs contain notochordal cells, which rapidly disappear from human IVDs after the first decade of life.[3] Moreover, their disappearance correlates with the onset of IVD degeneration in humans and, thus, their persistence in rat IVDs adds a confounding variable when studying injury and degeneration.[43] More specifically, notochordal cells have been shown to favorably influence the metabolism of proteoglycans within the NP,[9] and demonstrate the ability to promote IVD

regeneration via in-situ stimulation of NP cells.[8] In addition, rodents differ from other mammals in that the anatomical shape, profile, and relative size of rodent IVDs vary compared to humans,[3] making it hard to conclude that rodent IVDs are an accurate representation from which to extrapolate findings to humans.

A Review of Cell Culture Studies

The effects of microgravity on IVD health. Recent advancements in cell culture techniques have allowed for the development of microfluidic models that mimic human IVD cellular environments without the limitations of animal models. The development of a "spine-on-a-chip" is a useful model for mimicking the native environment of the IVD (**Fig. 3**), with lower resource costs and ability to test the cellular response to inflammatory agents without the use of a whole IVD organ culture.[30] These cell culture studies are commonly used for space research, both on the ISS and here on Earth, using ground-based analogs to simulate microgravity. One study conducted by Freed et al.[20] investigated bovine articular chondrocytes grown on polyglycolic acid scaffolds in rotating bioreactors (3 mo) followed by 4 mo aboard the Space Station. Results showed a fourfold decrease in the cellular function of chondrocytes grown in microgravity as determined by sulfate and proline incorporation.[20,65] The aggregate modulus of the scaffolds cultured in space were threefold lower than Earth-grown scaffolds, showing reduced ECM mechanical properties of the tissue in space-grown cultures. The space-grown scaffolds also had significantly reduced GAG content, which may be related to the observed decrease in osmotic pressure of the NP, thereby reducing its ability to bear load and absorb shock during reloading.

Despite the information gathered from these studies, there is a limited number of IVD cell culture studies investigating mechanical unloading. Most commonly, studies on cartilage are conducted to analyze cartilage-specific mechanisms which can be used to predict IVD pathologies during spaceflight due to the similarity between the cells and ECM components of the two tissues. Stamenkovic et al. investigated the deposition of cartilage ECM and cellular organization across cultures in microgravity on the ISS, simulated microgravity using random positioning clinostats, and normal 1-g loading.[65] Overall, aggrecan deposition and cell densities greatly decreased in ISS cultures compared to both Earth-cultivated tissues. Higher collagen ratios were observed in ISS samples, showing the onset of fibrosis in cartilaginous tissue. Overall, proper cartilage metabolism was disrupted in samples cultured on the ISS. Additionally, tissue cultured in Earth-based clinostat systems only showed tissue characteristics between that of microgravity and 1-g conditions, demonstrating the systems inability to sustain true microgravity conditions.[65] Therefore, as future developments are made in microfluidic models, it is important to create a system that can fit within current ISS capabilities for further investigation of IVD degeneration in the context of spaceflight. Aleschcheva et al. investigated the short-term effects of microgravity on morphology and gene expression in chondrocytes cultured in a random positioning machine (RPM) to simulate microgravity conditions.[2] The RPM offers two independently rotating frames, as shown in Fig. 3, compared to traditional single-axis clinostats, creating an environment that randomly alters the position of an experiment. This mechanism enables researchers to simulate microgravity through 3D changes in orientation, essentially eliminating the effect of gravity. Exposure to simulated microgravity in this system resulted in alterations of the cells' cytoskeleton, and changes in growth, proliferation, differentiation, migration, and adhesion in human cells after 24 h.[12] These results show how quickly cartilaginous cells can recognize and respond to microgravity.

The effects of radiation exposure on IVD health. While there are not many studies conducted specifically studying cosmic radiation's effects on the cellular level, more radiation research studies are being conducted due to the increased attention given to ground-based radiation exposure such as radiotherapy and diagnostic procedures. One study isolated chondrocytes from both humans and pig donors and irradiated these monolayers with 2 or 10 G_y gamma rays.[77] Overall, the human and pig cartilage responded similarly to radiation, as proteoglycan synthesis was reduced even when stimulated with IGF-1, potentially revealing that radiation exposure may impair important intracellular signaling pathways involving ECM anabolism.[77] Additionally, catabolic proteins, such as ADAMTS5, MMP-1, and MMP-13, were observed in the culture media following both radiation conditions.[77] Overall, studies such as these show that radiation induces an active degeneration of cartilage and a reduction in proteoglycan synthesis. These degenerative results of radiation exposure could ultimately be heightened when the IVD is exposed to the wide range and high dose of cosmic radiation. However, future research of similar study designs with both NP and AF cells would need to be conducted to verify these effects

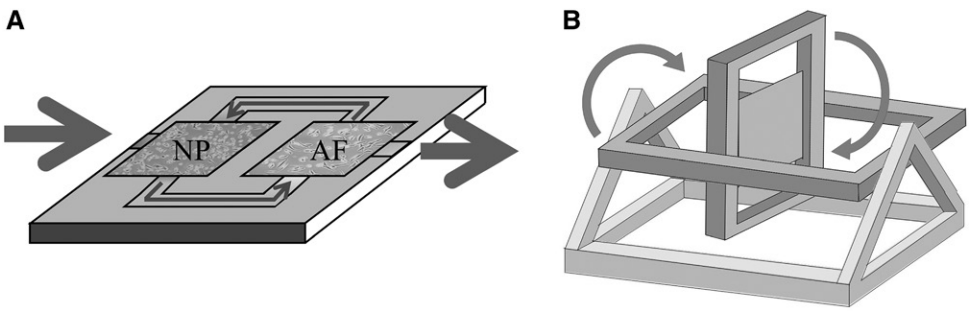

Fig. 3. Schematic of A) theoretical spine-on-a-chip microfluidic device, showing complexity of IVD cellular composition and subsequent communication between the distinct cell types, and B) random positioning machine (RPM) which simulates microgravity by changing the cell culture's 3D orientation through systemic rotations of each axis (indicated by arrows).

specifically within the IVD compared to cartilage studies. Therefore, an IVD-specific model is necessary in order to gather valuable data regarding IVD degeneration in future cell culture studies.

IVD-specific organoid models. The emerging gold standard for 3D cell culture studies on both the tissue- and organ-level is the use of a 3D organoid culture, which mimics the native multicellular and complex ECM environment found in the target organ or disease.[17] Additionally, there are several factors that make organoid culture an ideal platform for investigating the effects of spaceflight on the IVD. For most organs and tissues, the microscale of the organoid culture must be limited to allow for nutrient access to the interior of the construct, as methods for introducing vasculature and other supporting tissues is not well developed.[17] However, these limitations are irrelevant when applied to the IVD, the center of which is innately low in nutrients and which lacks nerves and blood vessels. IVD cells are, therefore, great candidates for culturing in organoid systems because of clear parallels between the organoid and native IVD microarchitecture.

Specifically related to IVD cultures, IVD cells have also been shown to exhibit native morphology and phenotype when seeded in a three-dimensional pellet culture system.[40] IVD cells have been successfully grown in spheroid cultures, demonstrating that centrifugation and subsequent culturing of these cells is a reproducible procedure that results in pellet formation. This culturing technique also resulted in native collagen Type II and proteoglycan synthesis similar to the native microenvironment of the IVD. A simple spheroid culture system can, therefore, be used to study IVDs in vitro while maintaining native cellular responses to external influences that may lead to inflammation, degeneration, or herniation. Coculture pellets of mesenchymal stem cells and nucleus pulposus cells have also been commonly used as cell-based therapies for IVD regeneration. However, these cocultures can also be used as a model to develop bilaminar cultures to mimic the anatomy of the IVD. Specifically, Allon et al. achieved a novel spherical bilaminar cell pellet where mesenchymal stem cells were enclosed in a shell of NP cells.[5] This unique bilaminar pellet system was achieved through a multistep centrifugation protocol, where the inner cell type was centrifuged at low speeds for 5 min, followed by the addition of the second cell type and centrifugation again at the same low speed conditions.[4,5] This method created clear bilaminar cell pellets shown through histological analysis. These pellet cultures were then tested under inflammatory conditions, specifically exposed to IL-1β and TNF-α cytokines in addition to applied pressure and hypoxia, indicative of normal IVD conditions at rest. The coculture of mesenchymal stem and NP cells accelerated the differentiation of these stem cells into mature NP cells, allowing these pellets to be used as a therapeutic strategy for degenerating IVDs.[4] While these pellet cultures are created for applications other than disease modeling, this study successfully showed stable NP cell phenotype and viability when cultured in a 3D environment.

However, most pellet culture models do not consider the importance of the presence of native ECM components or microarchitecture. The culturing of cells in spheroid systems allows for correct morphology, but often increases cell-cell interactions that are not indicative of true tissues, preventing the creation of a truly mimetic IVD culture. Therefore, a small-scale model incorporating ECM structure could provide an improved in vitro model to understand how IVD cells respond to changes in their native environment. ECM incorporation for the formation of organoid cultures is an increasingly popular practice for the development of injectable cultures for therapeutic and regenerative applications. Ong et al. showed success in initiating hepatic differentiation through the incorporation of small intestinal submucosa into mesenchymal stem cell pellet cultures.[55] Although this method did not lead to accelerated hepatic differentiation in this specific therapeutic study, the incorporation of microscopic sections of ECM into the organoid during initial centrifugation shows the success of this method for other application such as tissue modeling. The incorporation of ECM, and specifically ECM found in the IVD, could provide both cell-cell and cell-matrix interactions that allow for the creation of microscale organoid models that are mimetic of native IVD tissue.

Therefore, the need for an organoid culture system that incorporates both native NP and AF cell types and ECM to create an IVD-mimetic microenvironment has been identified. Currently, our lab has been working to develop such an IVD organoid model; the overview of our fabrication strategy is shown in **Fig. 4**. Acellular NP scaffolds, previously developed in our lab[74] and resembling native NP microarchitecture, are cryo-milled into microparticles of a defined size. These microparticles are centrifuged together with NP cells and cultured to create NP organoids. These organoids are then encapsulated in a collagen hydrogel to emulate native AF microarchitecture. Secondary centrifugation is then used to create direct surface seeding of AF cells onto the collagen surface with the intent of facilitating elongated AF morphologies typical of native AF cells. These organoids can be cultured for multiple weeks and demonstrate excellent viability, maintenance of NP cell morphology, and distinct NP and AF regions. In the future, this model will be validated for use as a disease model to help outline and predict IVD function and pathology.

DISCUSSION

There are many avenues for IVD research within the space community, illuminating potential mechanisms of IVD degeneration due to spaceflight. Specifically, microgravity and radiation exposure have been shown to increase IVD hydration and IVD height, correlating to debilitating back pain for the majority of astronauts. Microscopically, decreases in AF fiber alignment in conjunction with NP swelling shows a possible mechanism of accelerated herniation once returned to gravitational loading. Results obtained from animal models demonstrate increases in proinflammatory cytokines and MMP expression, which have been correlated to occur jointly with

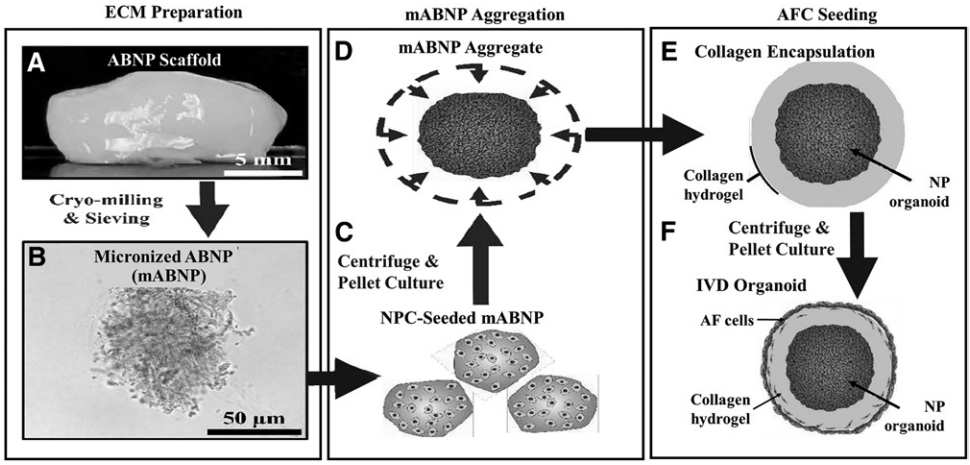

Fig. 4. Overview of the fabrication strategy to create intervertebral disc (IVD) organoids. ECM preparation begins with A) an acellular bovine nucleus pulposus (ABNP) scaffold previously developed in our lab.[69] The scaffold is cryo-milled and sieved to collect B) micronized ABNP (mABNP) microparticles 80–100 μm in diameter. These mABNP are then seeded with NP cells (NPCs) to create C) NPC-seeded ABNP cultures that are centrifuged together to create D) a mABNP aggregate mimicking native nucleus pulposus (NP) microarchitecture and cell morphology. AF cell (AFC) seeding begins with E) collagen encapsulation around the mABNP aggregate culture. Direct surface seeding of AFCs then result in the completed F) IVD organoid culture, which incorporates ECM to model the distinct NP and AF regions of the IVD.

observed spinal elongation, further characterizing possible causes of degeneration. Additionally, animal models studying IVD recovery upon reloading showed quick recovery in the NP (increase in GAG and water content, TIMP1 upregulation), but less recovery capability in the AF to return to its original mechanical strength (loss of hoop strength, no change in TIMP1 expression, but an increase in catabolic expression). This differential recovery makes the NP more likely to herniate through the weakened AF tissue, which is hypothesized to correlate to the increase in occurrence of herniation once astronauts experience gravitational loading again on Earth.

However, while these results provide insights regarding potential mechanisms of spaceflight-induced IVD degeneration, human studies cannot provide biochemical analysis and animal results cannot be directly correlated to mechanisms in the human body. Additionally, the characterization of potential additive effects due to the long-term exposure to microgravity and radiation exposure has not been studied extensively. Many of these limitations can be met by the use of IVD organoid studies, which offer a microenvironment mimetic of the native IVD and are compatible with cell culture capabilities aboard the ISS, allowing for deeper study of microscopic mechanisms occurring in low Earth orbit. With further development of this IVD organoid model through the continued validation of native cell morphologies, and both incorporation and production of ECM during culture, microscale IVD organoids could be an advantageous model for studying the effects of microgravity and radiation exposure moving forward. This model could then be used to potentially confirm cellular and biochemical changes specific to humans in parallel to macroscopic research conducted through astronaut studies, ultimately creating a holistic investigation into IVD degeneration in space and herniation upon return to Earth.

In the future, research specifically investigating the effects of microgravity and space radiation could be improved to fully characterize the complex environment astronauts experience, especially as longer duration missions to the Moon and Mars are expected. As an alternative approach to trial-and-error experimentation, computational modeling enables the quantitative integration of many interconnected reactions and is being increasingly utilized in basic biological research. Many finite element models exist for a ground-based spine, but a spaceflight-exposed spine differs significantly in morphological and physiological changes. These distinct changes create the need for altered material models, boundary conditions, and loading conditions within the current ground-based spine computational models.[67] However, computational modeling is an advantageous tool in that these specific model parameters can be tailored to spaceflight conditions, creating a useful model from what is currently known about space-induced biological changes. In spaceflight-exposed spine models run uniquely without gravitational loading, spinal elongation was observed after a 9-d spaceflight.[67] This finding was mainly due to increases in individual IVD heights, which parallels results obtained from previous human studies. Computational models therefore provide a new method to obtain valuable insight about the effects of spaceflight on the spinal column without having to conduct human studies. Additionally, the development of in silico models allows computational modeling to be tailored to the ECM- and cell-levels using agent-based and intracellular signaling network models, respectively. Agent-based systems provide a unique framework for the integration of both intracellular molecular processes with the native organization of ECM and cells in multicellular tissues.[72] Chondrocyte cell models have also been developed using gene expression analysis and previous knowledge from proteomic pathway informatics to identify seven signaling pathways important to cartilage development and maintenance.[36,62] Models have also been developed to outline the biochemical composition of the IVD specifically,[80] which can account for the high degree of complexity in molecular changes as the IVD undergoes degeneration and herniation. This microscale computational modeling therefore offers a unique capability to integrate microgravity, radiation, and other space-specific environmental parameters to efficiently predict IVD tissue and cell responses.[76]

Finally, it is hypothesized that the next stage of IVD research will be to expand into studies done in both micro- and partial

gravity on the ISS and the Moon as colonies are established. As more data is collected continuously at colonized settlements in space through both human and cell culture studies, the threat of herniation to mission success will become better understood. In addition, the data from organoid cultures grown in space could be used to validate and refine the biochemical changes outlined in spaceflight computational models to create injury predictions and develop in-flight countermeasures. Additionally, a well-developed organoid model could be used for high-throughput drug screening to identify effective therapeutics to mitigate IVD pathologies for both astronauts during long-term spaceflight and patients here on Earth. Elucidating the effects of microgravity and cosmic radiation will, therefore, allow for space exploration toward Mars to expand scientific knowledge without compromising human health.

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