Biotechnology Advances 69 (2023) 108273

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

3D biofabrication and space: A 'far-fetched dream' or a 'forthcoming reality'?

Nilotpal Majumder, Sourabh Ghosh *

Regenerative Engineering Laboratory, Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India

ARTICLE INFO

Keywords:
Space
3D bioprinting
Microgravity
Tissue engineering
Cell signaling pathway

ABSTRACT

The long duration space missions across the Low Earth Orbit (LEO) often expose the voyagers to an abrupt zero gravity influence. The severe extraterrestrial cosmic radiation directly causes a plethora of moderate to chronic healthcare crises. The only feasible solution to manage critical injuries on board is surgical interventions or immediate return to Earth. This led the group of space medicine practitioners to adopt principles from tissue engineering and develop human tissue equivalents as an immediate regenerative therapy on board. The current review explicitly demonstrates the constructive application of different tissue-engineered equivalents matured under the available ground-based microgravity simulation facilities. Further, it elucidates how augmenting the superiority of biomaterial-based 3D bioprinting technology can enhance their clinical applicability. Additionally, the regulatory role of weightlessness condition on the underlying cellular signaling pathways governing tissue morphogenesis has been critically discussed. This information will provide future directions on how 3D biofabrication can be used as a plausible tool for healing on-flight chronic health emergencies. Thus, in our review, we aimed to precisely debate whether 3D biofabrication is deployed to cater to on-flight healthcare anomalies or space-like conditions are being utilized for generating 3D bioprinted human tissue constructs for efficient drug screening and regenerative therapy.

1. Introduction

The concept of human space missions has progressed appreciably with the setting up of the International Space Station (ISS) in Low Earth Orbit (LEO)(Ghidini, 2018). Such concepts have been developed to envision the possibilities of permanent human colonization beyond the LEO (Hufenbach et al., 2011). With an increased amount of spaceflight beyond the LEO exposes the Astronaut to increased cosmic radiation, reduced or zero gravity conditions, as well as traumas or injuries due to unprecedented accidents (Ghidini, 2018). Such adverse extra-terrestrial circumstances cause moderate to severe medical problems like muscular atrophy, bone resorption, cardiovascular effects, ophthalmological issues as well as impaired wound healing process (Patel et al., 2016) (Chylack et al., 2009)(Cucinotta and Cacao, 2017)(Little et al., 2012). There are numerous reports that suggest the carcinogenic role of space radiation in promoting DNA breaks, chromosomal aberrations, oxidative stress and mitochondrial dysregulation, which are ultimately responsible for the malignant transformation of healthy cells(Huang et al., 2003)(Afshinnekoo et al., 2020) A combination of microgravity and cosmic radiation was also successful enough to induce a higher frequency of complex chromosomal aberration in human fibroblast cells, an inducer of carcinogenesis(Hada et al., 2019). Furthermore, the component of galactic cosmic radiations (high LET ^{56}Fe and ^{28}Si) have been shown to promote hyper and hypomethylation of the respective chromatin compartments (enhances, promoters or repressed heterochromatic region) of the CpG islands, thus affecting the epigenomic sites of lung cancer(Kennedy et al., 2018). Moreover, an upregulation in the Wnt/β-catenin and Ephrin B signaling pathway post ^{56}Fe irradiation exposure to intestinal epithelial cells delayed their migration, demonstrating oncogenic stress due to altered cytoskeletal dynamics(Kumar et al., 2018). Therefore, space radiation microenvironment plays a key role in triggering carcinogenesis. The short-term mild injuries can be easily cured using the first aid equipment on board or faster return to Earth in case of critical injuries that require immediate surgical intervention(Jemison and Olabisi, 2021). Therefore, during such missions at the Moon base, faster return is possible in case of a precarious medical emergency, but considering the distance, duration and 'no abort' condition of the Mars mission (500 days approx.), immediate return to Earth

* Corresponding author.
E-mail address: sourabh.ghosh@textile.iitd.ac.in (S. Ghosh).

https://doi.org/10.1016/j.biotechadv.2023.108273
Received 23 May 2023; Received in revised form 10 October 2023; Accepted 15 October 2023
Available online 18 October 2023

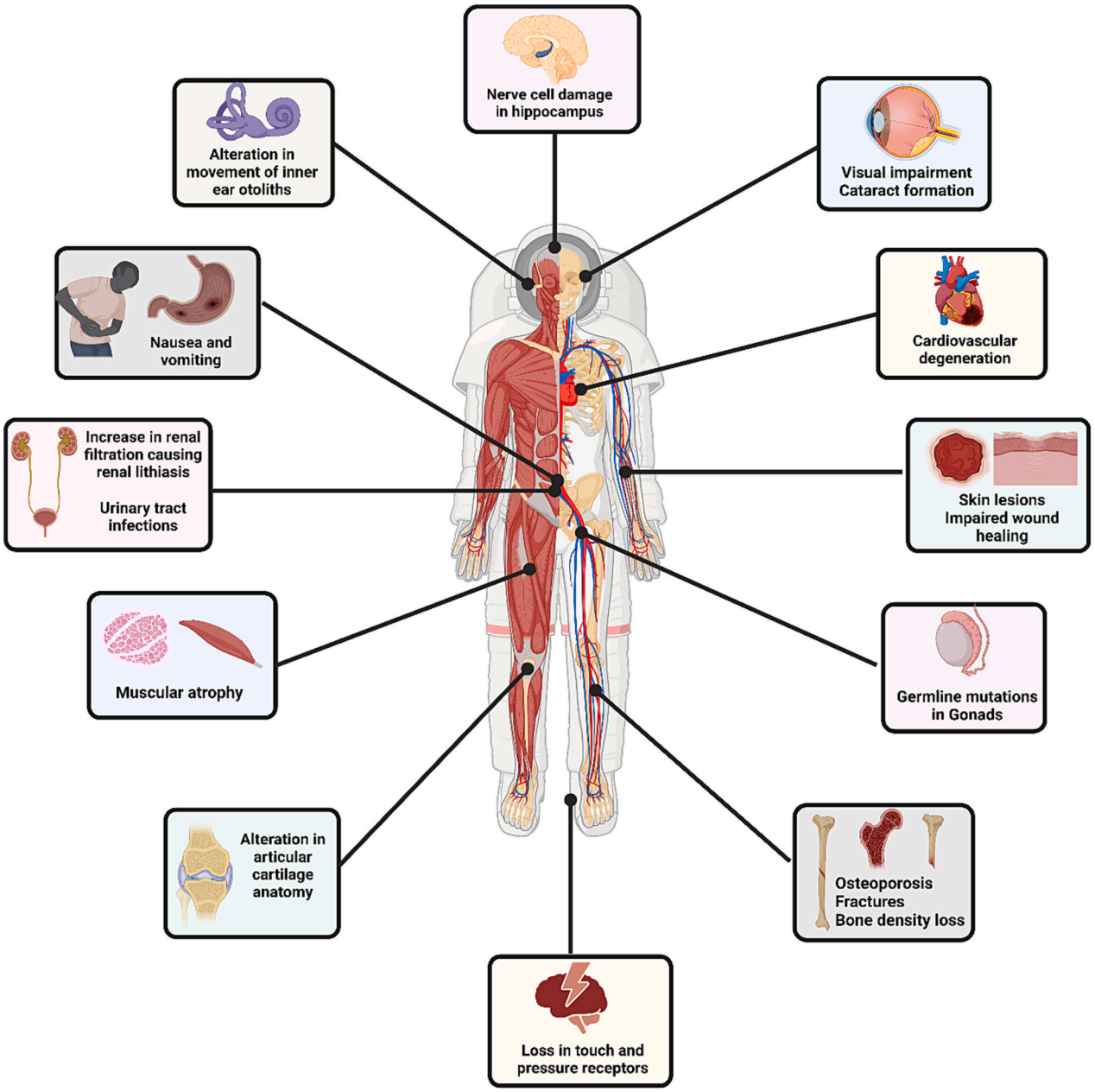

Fig. 1. A schematic representation demonstrating alterations in the normal physiological function of an astronaut under the influence of zero-gravity conditions during long-term spaceflights. A wide array of critical health deteriorations has been reported by the clinician post return to Earth-like cardiovascular degeneration, loss of motor functions, reproductive abnormalities and visual impairment besides the routine osteoporosis, bone density loss and fracture, muscular atrophy, and skin lesions. Researchers around the globe have been continuously investigating to decipher the intricate molecular mechanism that dwells underneath such alterations in the physiological functioning of the astronaut. They examined their cellular extracts or tissue specimens under different ground-based microgravity simulation facilities. This will allow space physiologists to unravel the exact cause of such physiological disturbances and devise advanced strategies to manage them. Despite incorporating cutting-edge technologies and approaches to cater to the superficial critical injuries on board, the major roadblock lies in nursing the internal anomalies by non-medico space travelers.

is practically not possible. In that case, the crew members must be self-sustained to alienate any medical emergency that can transpire (Ghidini, 2018)(Jemison and Olabisi, 2021). Thus, there is a compelling need for on-site reconstructive treatment facilities to combat chronic injuries due to the harsh extra-terrestrial conditions and successfully support hassle-free distant space missions (See Fig. 1, Table 1).

Fabrication of patient-specific tissue construct recapitulating the native tissue microarchitecture and physiological function is a major research gap in the intriguing field of tissue engineering. Traditional Tissue engineering techniques develop simplified scaffolds with random porosity and non-homogeneous cell distribution along with impaired diffusion of nutrients and oxygen, making it inefficient in replicating the biomimetic layout(Chawla et al., 2018a; Eltom et al., 2019; Zhang et al., 2009). 3D bioprinting, a state-of-the-art subset of 3D biofabrication thatcould be utilized by space travelers to generate onsite restorative therapy for various short- and long-term extraterrestrial missions. It has already proven to be a promising strategy in tissue engineering under Earth-like conditions using the directional deposition of biomaterials combined with cells and biochemical mediators to create tailor-made tissue constructs(Majumder et al., 2022; Murphy and Atala, 2014). There is also reported literature demonstrating the requirement of the human body as a bioreactor for tissue maturation by direct deposition of

Table 1
Effect of microgravity and cosmic radiations on the health profile of the Astronaut.

Organ System	Damage caused
1. Central Nervous System (CNS)	• Altered neurocognitive function, change in neuronal behavior and reduced motor functions(Roy-O'Reilly et al., 2021; Tays et al., 2021). • Optical disc edema with reduced retinal thickness, change in refractive error and chorioretinal folds with posterior subcapsular cataracts (Lee et al., 2020; Richardson, 2022). • Alterations in circadian rhythm due to damage in the hypothalamic-pituitary axis resulting in a change of mood and behavior with unusual stress response(Wu et al., 2018).
2. Cardiovascular System	• A sudden fall in blood pressure due to reduced peripheral vascular resistance (Orthostatic hypotension)(Fu et al., 2019). • Reducing central venous pressure causes limited plasma volume expansion, thereby altering stroke volume and cardiac output (Convertino, 2005).
3. Musco-skeletal system	• Loss of bone mineral density and osteoporosis due to an increase in calcium excretion with impaired intestinal calcium absorption, deficiency of Vitamin K and D and decreased levels of parathyroid hormone and calcitriol in serum(Comfort et al., 2021; Iwamoto et al., 2005). • Decrease in muscle mass (muscular atrophy) and reduced muscle fibre size due to hormonal dysregulation, lack of mechanical loading and nutritional imbalance during spaceflight (Lee et al., 2022)
4. Digestive System	• Reduced bone density of mandibular and alveolar bone and dissolution of masseter muscle fibers required for physically chewing food in the mouth (Yang et al., 2020). • Intensive secretion of gastric and pancreatic juices in the stomach due to altered hemodynamics of the associated abdominal organs(Afonin, 2013).
5. Excretory System	• Kidney damage pertaining to loss of glomerular and tubular integrity as a result of mitochondrial dysfunction due to cosmic radiation-induced oxidative stress(Pavlakou et al., 2018). • Renal stone formation due to supersaturation of stone-forming salts in the urine of the space traveler (Pietrzyk et al., 2007).
6. Reproductive System	• Inconsistency in maternal weight gain, and labor duration with increased lordotic contraction in female astronauts (Mishra and Luderer, 2019) • Decrease in sperm count, testosterone level and testicular weight in men due to microgravity and ionization radiations during spaceflight(Ahrari et al., 2022).

the cell-laden tissue mimic on the defect site of the patient, a process known as *in situ* 3D bioprinting(Chakraborty et al., 2022b)(Xie et al., 2022). Such direct printing of *de novo* tissues (skin, cartilage, and bone) in an anatomically defect site can be successfully utilized by the astronauts on board to cater to severe space conditions-induced tissue injuries.

3D bioprinting finds a widespread application in diseases concerning the need for tissue replacement and studying disease progression through various *in vitro* models. It also contributes to a large commercial application as an alternative to animal testing for various drugs and cosmetic companies(Bhattacharjee et al., 2014; Chawla and Ghosh, 2018a, 2018b). The microgravity condition is also considered a favorable environment to generate various bioinspired soft and hard tissues due to the absence of the gravitational pull(Cubo-Mateo et al., 2020). Using this principle of reduced gravity, scientists have already developed simplified tissue equivalents of cartilage, skin and bone that could emulate the biological niche of the native tissue. Furthermore, when evaluated for their physiological function, the microgravity-induced tissue equivalents depicted enhanced alkaline phosphatase activity in bone(Qiu et al., 1998) and elevated proteoglycan deposition in cartilage tissue(Wu et al., 2013). Microgravity conditions also increased the proliferation rate of endothelial cells (Carlsson et al., 2003) with enhanced nitric oxide synthase (NOS) secretion by PI3K-Akt pathway (Shi et al., 2012) and decreased expression of pro-angiogenic factor basic fibroblast growth factor (bFGF) (Griffoni et al., 2011). Parallelly, extensive research has been carried out in developing self-assembling organoids impeding the gravitational force of the Earth using human endothelial cells to develop into fully functional blood vessels(Grimm et al., 2018).

Although promising results have been achieved from these experimental observations, a major challenge is the functional stability and quality of the fabricated tissue construct under the influence of reduced gravity conditions. There is also existing evidence that ground-based microgravity simulation facilities displayed a conflicting role in regulating the biological functioning of the developed tissue equivalents. This is reflected in impeded osteogenesis of stem cells in bone tissues (Dai et al., 2007), redundant adherence of dermal fibroblast cells(Cialdai et al., 2017) or hindered proliferation of liver stellate cells(Fujisawa et al., 2022). The possibility of such unanticipated functional outcomes of the microgravity-led tissue-engineered constructs question the serendipitous role of zero-gravity conditions in tissue engineering. A plausible reason could be the erratic functioning of the intracellular signaling cascades under space-like conditions. Additionally, a lack of comprehensive simulation of the native tissue microenvironment also resulted in completely different physiological outcomes of the implanted grafts.

Therefore, the current review addresses the pressing need to devise strategies to develop on-site advanced treatment facilities in spacecraft using approaches from tissue engineering and 3D bioprinting technology. Moreover, it also underlines the functional duality role of simulated microgravity conditions in regulating the functional outcomes of the tissue constructs. Therefore, through this current review, we have raised the following uncertainties:

- Where do we currently stand in engineering functional tissue equivalents under microgravity influence?
- Can 3D bioprinting be a possible solution to overcome the existing challenges that emerged while deploying traditional tissue engineering techniques in space conditions?
- Do the simulated microgravity conditions alter the normal functioning of the cell signaling pathways underlying the physiological outcome of the fabricated tissue construct? Is the role affirmative or detrimental?
- How far are we in providing on-site treatment facilities to space travelers during chronic space injuries?

A plethora of tissue engineering approaches have been comprehensively discussed to fabricate personalized tissue grafts (skin, bone, cartilage, liver, *etc.*) under various existing microgravity simulations and their implications in real-time space medical conditions. Additionally, the essential role of 3D bioprinting in enhancing the structural stability and physiological outcome of the fabricated constructs has also been highlighted. Moreover, the regulatory role of microgravity conditions in modulating the underlying signaling pathways governing tissue morphogenesis has been extensively reviewed. Furthermore, we explored the future possibilities of providing on-site regenerative treatment facilities to the astronauts on board in case of any chronic clinical insurgency. Therefore, this review aims to unravel the biggest uncertainty in *"In the current scenario, whether bioprinting is useful to space or space-like conditions aid in generating 3D bioprinted functional tissue equivalents?"*

2. The existing ground-based microgravity simulation facilities

Gravitational forces are thought to have a direct influence on the

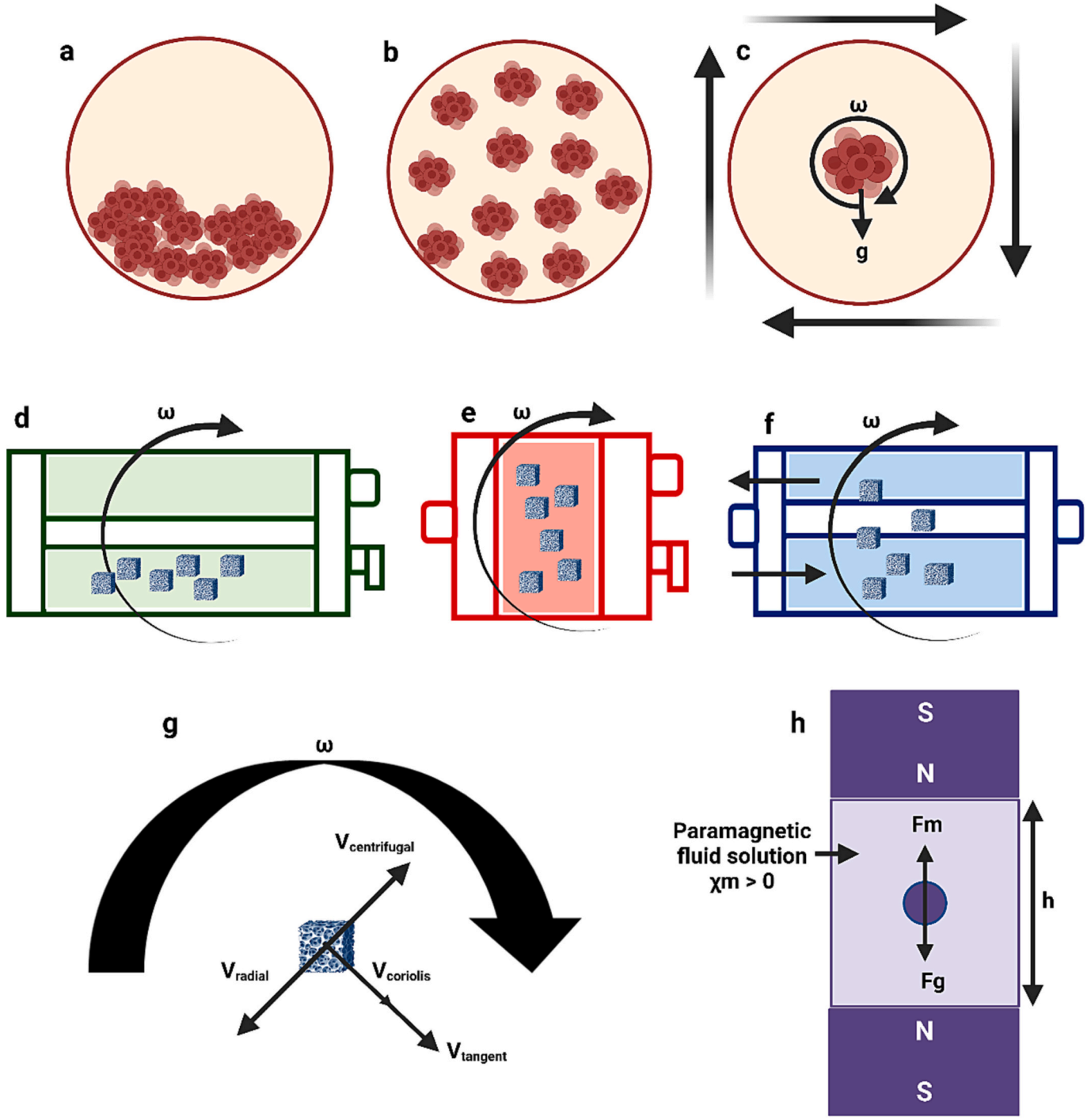

Fig. 2. A graphical representation illustrating the working of different ground-based microgravity simulators to emulate the zero gravity conditions of space. a b&c) 2D clinostat, d) Slow turning lateral vessel (STLV), e) High aspect ratio vessel (HARV), f) Rotating wall perfusion vessel (RWPV), g) the graphical representation of different forces acting on the tissue specimen within a microgravity simulator, h) Magnetic levitation. In a 2D clinostat (c), the sample is rotated at a high angular speed around the axis perpendicular to the gravitational vector to average the developed gravitational force and thus, allow the sample to remain suspended. In a rotating wall vessel (d,e,f &g), the continuous rotation generates a perfect balance between the centrifugal force, radial force, gravitational force and Coriolis force that allows the sample to remain in suspension while ensuing efficient transfer of nutrients and oxygen. However, the working principle of the magnetic levitation technique lies in balancing all the force vectors by the two magnets arranged at a certain levitation in a paramagnetic medium, making it an efficient technique in mimicking microgravity conditions. However, the primary question lies in the accuracy of these simulation facilities in mimicking the zero-gravity conditions like that of space. Although there lies a thin line of difference between 'zero-gravity' and 'microgravity' but that can impart a profound differential effect on the physiological outcome of the investigated tissue or cell specimen.

physiological function of any organism. Reduction in gravity or weightlessness as in space directly regulates the gene expression profiles (Carmeliet and Bouillon, 1999; Frigeri et al., 2008; Li et al., 2019) by regulating the mechano-transduction process(Bradbury et al., 2020; Li et al., 2018; Puca et al., 2012). Such alterations influence cellular functioning *in vivo* via differential activation and inhibition of associated signaling pathways. Performing real-time experiments to elucidate this altered cellular functioning requires access to the high-end technologies

of the International Space Station. This makes the process time-consuming and expensive(Bradbury et al., 2020; Eiermann et al., 2013). Simulating the zero-gravity environment of space on Earth is the best possible solution to study the alterations in such physiological and anatomical functionalities at cellular levels (See Fig. 2)

2.1. Clinostats

Clinorotation by *2D-Clinostats* is one of the first ground-based techniques researchers use to simulate microgravity conditions on Earth (Eiermann et al., 2013). The horizontally mounted sample is rotated around the axis perpendicularly to the gravitational pull to ensure it remains suspended without any sedimentation(Brungs et al., 2016; Eiermann et al., 2013). Therefore, clinorotation is useful in averaging the gravitational vector through the time-dependent rotation of its respective axes(Ferranti et al., 2021). The centrifugal forces generated by the clinorotation exhibited a profound effect on cellular morphology and physiological function. This has been elucidated by *Eiermann* et al., where a decreased metastatic potential of BLM melanoma cells and increased cell size of RAW 264.7 Macrophages were observed, possibly due to an increase in the number of focal contacts as compared to 1 g control (Earth conditions)(Eiermann et al., 2013). Although this experimental procedure demonstrated preliminary success, a pertinent challenge lies in the presence of residual acceleration due to the restricted rotation speed and radius. Hence, the sheer influence of gravitational force could be fortunately averaged but cannot be completely eradicated using this system(Ferranti et al., 2021). Moreover, there are high chances of bending stress due to the alteration in weight distribution. To overcome these challenges, *Fast Rotating Clinostats* have been experimentally designed by researchers for embryonic cell culture(Brungs et al., 2019). The objective lies in negating the gravitational force from all directions due to high rotation speed (Benavides Damm et al., 2014; Eiermann et al., 2013).

2.2. Rotating Wall vessel

The development of the *Rotating Wall Vessel* by the National Aeronautics and Space Administration (NASA) marked a new platform for carrying out experiments in modeled microgravity conditions on Earth (Klement et al., 2004; Schwarz et al., 1992a, 1992b). The design principle of the RWVs is mainly categorized as solid body rotation and oxygenation. While the former is achieved by a horizontal axis fitted to the cylinder, the oxygenation is achieved through silicon membranes (Schwarz et al., 1992a, 1992b). The cylindrical vessel comprising the fluids (media) and cells rotate about the horizontal axis, making the suspended particles align with the circular path as a function of rotational viscosity(Leach et al., 2001). The continuous rotation of the suspended cells and medium under a low-shear environment allows 3D aggregation to form tissue-like constructs. This enables the smooth uptake of oxygen and nutrients by the cells from the surrounding microenvironment(Gardner and Herbst-Kralovetz, 2016). The RWV is further classified as a) a High Aspect Ratio Vessel (HARV), b) a Slow Turning Rotating Vessel (STLV) and c) a Rotating Wall Perfusing Vessel (RWPV) based on their oxygen purging mechanism. The perfect balance between the gravitational force, buoyant force and the drag force keeps the tissue sample in a suspended form inside the bioreactor. Several studies have been carried out to validate the influence of the microgravity state developed using RWV on cytoskeletal morphology and functional dynamics. This demonstrates a direct implication in the development of multicellular 3D spheroids and multilayered tissue-engineered constructs (Barzegari and Saei, 2012; Goodwin et al., 1993; Klement et al., 2004; Schwarz et al., 1992a, 1992b).

2.3. Random positioning machine

The development of a three-dimensional clinostat, also known as a Random Positioning Machine (RPM), provided a more adequate simulation of weightlessness in Earth-bound conditions. It uses a computer-controlled two-axis rotation setup placed at a right angle to each other (Benavides Damm et al., 2014; Brungs et al., 2019; Russomano et al., 2005). The term '3D clinostat' can be rightfully used when the speed and direction of both the axes of rotation are constant. Conversely, the term 'RPM' is coined when both speed and directions are different(Herranz et al., 2013). The underlying operation principle is averaging the gravity vector acting on the sample from all directions to zero. The two sample mounting frames operated by two independent motors undergo constant reorientation powered by sophisticated algorithms to distribute the gravity vector in all directions (Wuest et al., 2015). The biological sample is mounted on frames that undergo continuous re-orientation. This causes a constant change of direction of the gravity vector's trajectory over time, influencing the cells to lose their 'sense of direction' and experience a 'weightlessness' condition known as microgravity (Brungs et al., 2016). Thus, even if a constant 1 g is acting on the sample, the average of the gravity vector is always zero, with the rotation time faster than the response time of the sample(Ferranti et al., 2021). The random high-speed rotation of the samples in 3D clinostat also marked an increased cell proliferative potential when tested for cord blood stem cells (CBSCs) and human cancer cells (hematopoietic, liver Chang and neural cells). It also positively regulated their differentiation potential with successful differentiation to hepatocytes, as evident from gene expression analysis. The sample placement is crucial in RPMs as the center of the intersection of both axes experiences the minimum force, whereas the outer periphery is exposed to the maximum thrust(van Loon, 2007). However, the induction of shear forces due to the random rotation of the samples in RPM affects the cell viability and reproducibility. This makes it a less efficient microgravity simulator when compared with 2D-Clinostat or real-time spaceflight experimental observations(Brungs et al., 2019).

2.4. Magnetic levitation

An exciting method to recapitulate the reduced gravity conditions is *Magnetic Levitation,* which balances the gravitational force with the magnetic force using a strong superconducting solenoid magnet. (Aleshcheva et al., 2016; Brungs et al., 2016; Parfenov et al., 2020). The underlying principle of magnetic levitation lies in the mechanism of 'magnetophoresis' where either the diamagnetic particles migrate in a paramagnetic medium (negative magnetophoresis) or *vice-versa* (positive magnetophoresis). The concept of 'negative magnetophoresis' have been utilized by researchers to simulate zero-gravity conditions using diamagnetic particle (cells/tissues) placed at a certain height (levitation). Such an arrangement allows all the force vectors acting on the sample to be nullified within a paramagnetic fluid(Ozefe and Yildiz, 2020). The strategy of diamagnetic levitation has been widely deployed in the engineering of skeletal muscle tissue using magnetic cationic liposome-based myoblast cells arranged in ring-shaped and string-shaped assemblies(Yamamoto et al., 2009). Furthermore, mouse 3 T3 cell lines and HCC827 lung cancer lines demonstrated enhanced deposition of extra-cellular matrices and forming 3D aggregates under the influence of the magnetic levitation setup (Türker et al., 2018). Russian scientists, also in collaboration with the ROSCOSMOS, have utilized this technique of magnetic levitation-based self-assembly process to culture human chondrocytes. They have successfully demonstrated chondrocyte re-differentiation, proving the efficiency of this technology in negating gravitational sensitivity(Parfenov et al., 2020). However, the major hurdle in magnetic levitation lies in the change of structural conformations of the proteins and nucleic acids within the cell with exposure to a strong magnetic field. The cytotoxic nature of the paramagnetic fluid used also adds up to the hurdle. Such strident experimental conditions exhibit a deleterious effect on the overall cell viability and functionality of the engineered tissue equivalent.

Table 2
The existing tissue-engineering approaches using ground-based microgravity simulation facilities.

Engineered tissue	Microgravity simulator	Cells used	Biomaterial	Key Experimental outcomes
Bone	HARV bioreactor	Secondary rat marrow stromal cells (MSC)	Microcarrier comprised of improved bioactive glass material and Cytodex-3 beads.	Expression of Alkaline phosphatase activity, Collagen Type-1 and Osteopontin(Qiu et al., 1998)
	Rotary Cell Culture System (RCCS) with HARV bioreactor	Bone marrow-derived Mesenchymal Stem Cells (BMSCs)	Ceramic biomaterial	An increased level of osteogenesis was observed in the dynamic culture system from ALP analysis and DNA content assay; *In-vivo* analysis revealed better bone repair in dynamically cultured tissue construct(Jin et al., 2010)
	Rotating Wall Vessel (RWV) and Spinner flask	Rat marrow stromal cells (MSC)	Porous degradable PLGA scaffolds	High osteocalcin secretion was observed in spinner flask conditions than in static culture; RWV culture conditions yielded higher calcium content than static culture(Sikavitsas et al., 2002).
	Rotary Cell Culture System (RCCS) equipped with HARV bioreactor	Human osteoblast and human pre-osteoclast cells	No scaffold	Successful differentiation of primary cells into matured construct consisting of a periphery populated by osteoblast and osteoclast and inner region comprising of osteocytes; Comparative gene expression of bone-specific markers observed in the construct with respect to human trabecular bone model (Clarke et al., 2013)
	Random Positioning Machine (RPM)	Human Bone-marrow derived Mesenchymal Stem Cells (h-BMSCs)	Nanostructured magnesium hydroxyapatite and Collagen-1	Osteoinductive characteristics were observed in the scaffold with osteoblastic lineage differentiation followed by bone-specific gene expressions ALP and Osteocalcin compared to the static culture system(Avitabile et al., 2020).
Cartilage	Rotational Wall Vessel (RWV) bioreactor	Rat Mesenchymal Stem Cells	Microcarrier beads	Chondrogenic cellular morphology and Alcian Blue positive matrix confirmed the differentiation of stem cells to chondrogenic lineage(Daane et al., 1991).
	HARV bioreactor	Bone marrow Mesenchymal Stem Cells (BMSCs)	Polyglycolic Acid (PGA)	Toluidine staining revealed a higher number of proteoglycans in a microgravity system than static; Enhanced expression of Collagen 2 and Aggrecan (ACAN)(Wu et al., 2013)
	Magnetically stirred spinner flask and STLV bioreactor	Cryogenic and normal human nasal septal cartilage cells	Vicryl (For cryogenic cartilage construct) PGA (Normal cartilage construct)	Formation of Hyaline Cartilage validated by H&E staining revealed a homogenous matrix and lacunae; the deposition of extracellular substance and Alcian Blue positive matrix confirmed the findings(Gorti et al., 2003).
	Rotating Wall Vessel (RWV)	Aged primary articular chondrocytes	No scaffold	Clustered chondrocytes with dense matrix confirmed by positive expression of Col2 and Articular proteoglycan; TEM analysis demonstrated cross-banded collagen fibrils in metabolically active chondrocytes(Marlovits et al., 2003).
	Magnetic Levitation Bioassembly	Human chondrocytes	Scaffold free. (Thermoreversible gel used as carrying agents for Chondrospheres to space)	Tissue spheroids fused to form a stable 3D cartilage construct; Good cell viability was observed(Parfenov et al., 2020).
Skin	Clinostat	Human adipose-derived mesenchymal stem cells	No scaffold	Upregulated ECM secretion with enhanced expression of COL-III(Ebnerasuly et al., 2018)
	Rotary Cell Culture System (RCCS)	Fibroblasts	No scaffold	Reduced α-SMA and E-cadherin expression(Cialdai et al., 2017)
Liver	Rotary Cell Culture System (RCCS) equipped with HARV bioreactor	Porcine hepatocytes	No scaffold	Exhibited hepatic cellular polarity and hepatic integrin expression(Nelson et al., 2010)
	Rotary Cell Culture System (RCCS)	HepG2 cells, human biliary tree/stem progenitor cells (hBTSCs)	No scaffold	The microgravity condition encouraged the formation of a 3D culture system with enhanced glycolytic metabolism, with hBTSCs showing the upper hand in hepatic differentiation(Costantini et al., 2019)
Blood vessels	Rotating Wall Vessels (RWV)	CD-34-positive human cord blood stem cells	Microcarrier – Cytodex-3 beads	Under microgravity conditions cells grown without microcarriers exhibited clustered morphology and expressed endothelial-specific markers(Carlsson et al., 2003)
	Clinostat	Human umbilical vein endothelial cells (hUVECs)	No scaffold	Enhanced secretion of endothelial nitric oxide synthase (eNOS) with tubular network formation(Shi et al., 2017a)
Neurons	Clinostats	Rat bone marrow-derived mesenchymal stem cells	No scaffold	Elevated expression levels of neuronal differentiation marker with increased secretion of neurotrophins(Chen et al., 2011)
	3D clinostat	Mouse bone marrow-derived mesenchymal stem cells	No scaffold	*In vivo* implantation depicted an enhanced survival rate besides promoted motor function(Yuge et al., 2010)
	Rotary Cell Culture System (RCCS)	Adipose-derived mesenchymal stem cells	No scaffold	Enhanced expression of neuron-associated genes(Luo et al., 2014)
In vitro cancer models	3D Clinostat or Random Positioning Machine (RPM)	Human follicular thyroid carcinoma cells ML-1 (Thyroid Cancer)	No scaffold	Cellular aggregation: upregulation of apoptosis-associated proteins; downregulation of fT3 and fT4 secretion(Svejgaard et al., 2015).
	Random Positioning Machine (RPM)	MC-7 breast cancer cells (Breast Cancer)	No scaffold	Multicellular spheroid formation showing relevance to ducts formed by epithelial breast cancer cells; demonstrated expression of cytoskeletal factors, cytokines, and apoptosis factors associated with three-dimensional aggregate formation(Nassef et al., 2020)

3. The utilization of space-like conditions to fabricate patient-specific tissue equivalents

With the introduction of tissue engineering, scientists worldwide are utilizing this advanced platform to address critical healthcare problems that were earlier considered untreatable or required complex surgical interventions.(Admane et al., 2019; Chawla et al., 2018b; Chawla et al., 2017; Henkel et al., 2013; Johnstone et al., 2013). One such critical health anomaly that the tissue engineers wanted to address is to devise strategies for providing on-flight treatment to space travelers suffering from similar critical injuries due to cosmic radiation or issues pertaining to cartilage degradation or bone resorption due to the weightless environment. (Cubo-Mateo et al., 2020; Jemison and Olabisi, 2021). There are numerous success stories of how ground-based microgravity simulation facilities have been exploited by engineers and researchers to generate a wide array of tissue equivalents (See Table 2). To name a few, Deavarasetty et al. successfully demonstrated the paracrine effect of mesenchymal stem cells in inducing liver-colorectal tumor organoid formation using an RWV bioreactor(Devarasetty et al., 2017). Similarly, Zarrinpour et al. reported enhanced neuronal differentiation potential of stem cells under simulated zero-gravity conditions using a fast-rotating clinostat(Zarrinpour et al., 2017). A comparative analysis was also carried out to evaluate the efficiency between HARV and STLV bioreactors as a microgravity simulator based on the formation of embryonic bodies (EBs), which demonstrated the upper hand of STLV in controlling cellular aggregation and consecutive formation of germ layers(Gerecht-Nir et al., 2004). Thus, these ground-based microgravity research facilities have efficiently provided a research platform that enabled researchers to confirm and validate the process of cellular differentiation to exhibit tissue-specific characteristics. The current section will discuss the advancements in developing major tissue-engineered body components utilizing the available microgravity simulation facilities, besides highlighting their functional duality role in tissue morphogenesis. The information from this section will allow space healthcare enthusiasts to understand how our body tissues behave differently in space conditions and, therefore, design strategies on how to regenerate them when degraded during spaceflight.

3.1. Bone

Bone loss is considered to be one of the most dangerous risks associated with both short- and long-term space missions(Ulbrich et al., 2014). Investigations from space travelers on reaching Earth have revealed a huge loss of calcium ions, one of the major regulators of osteogenesis(An et al., 2012; Pietsch et al., 2011). The activity of osteoblast is greatly reduced in microgravity conditions due to its reduced proliferation and differentiation potential as well as impaired response to biochemical mediators of osteogenesis in the surrounding environment(Arfat et al., 2014). Osteoclast cells are also considered to be responsible for bone resorption conditions in space, as evidenced by the increase in osteoclastogenic signaling pathways under microgravity influence (Lin et al., 2009; Tamma et al., 2009). Various tissue engineering approaches have been undertaken by researchers to construct anatomically relevant bone tissue equivalents using a wide variety of biomaterials and cell sources under the zero-gravity influence.

The first approach to study bone tissue engineering in weightlessness conditions was in 1988 when rat marrow stromal cells cultured in Cytodex-3 microcarrier beads exhibited adequate mineralization with enhanced osteopontin expression and Alkaline Phosphatase activity(Qiu et al., 1998). Consecutively, multiple approaches have been made to engineer bone tissue, such as the use of decellularized bovine bone scaffolds to culture bone marrow-derived mesenchymal stem cells (BMSCs), bioactive glass-polymer *c*omposites as well porous Polylactic-co-Glycolic acid (PLGA) scaffolds(Jin et al., 2010; Sikavitsas et al., 2002). The reports from their study clearly demonstrated the upper hand of the dynamic culture system in stimulating the differentiation of stem cells towards osteoblastic lineage (enhanced ALP activity and osteocalcin secretion). Moreover, the same dynamically cultured BMSC-loaded ceramic bone construct, when implanted *in vivo,* depicted efficient bone integration after 24 weeks (Jin et al., 2010). A similar *in vivo* bone tissue growth has been described by Nishikawa et al. when a BMSC encapsulated calcium phosphate-hydroxyapatite scaffolds post-culturing in 3D clinostat were implanted in a 7-week-old rat model (Nishikawa et al., 2005). Culturing human osteoblast and osteoclast precursors in randomized gravity conditions with very low shear in the HARV bioreactor also yielded bone-like tissue construct akin to human trabecular bone confirmed by enhanced mRNA levels of BMP proteins (Clarke et al., 2013). One of the recent approaches used a combination of nanocrystalline magnesium-doped hydroxyapatite and type-1 collagen as a scaffold and BMSCs as cell sources in RPM. They have successfully demonstrated the osteoinductive properties of nanostructured hydroxyapatite and collagen to negate the effect of microgravity on osteoblast dysfunction and differentiate into osteoblastic lineage. This is evident from the enhanced expression of alkaline phosphatase and osteocalcin genes with a reduction in surface marker expressions (CD29, CD44 and CD90) when hBMSCs were cultured in the RPM for 21 days (Avitabile et al., 2020).

Despite several successful approaches to developing bone tissue equivalent under microgravity conditions, there are reports that suggest the precarious role of microgravity conditions in inhibiting osteogenic differentiation. In that context, Dai et al. reported the redundant proliferation capacity and osteogenic potential of rat bone marrow-derived mesenchymal stem cells (rBMSCs) under simulated microgravity conditions. A detailed experimental and bioinformatic analyses revealed that the rBMSCs showed a tendency towards the G0/G1 phase instead of active proliferation with decreased levels of stem cell proliferation pathway downstream targets Akt and Erk1/2 post 3 days of clinostat culture (Dai et al., 2007). Furthermore, under microgravity influence, the stem cells displayed a neutral response to osteogenic growth factors, actin depolymerization and downregulation of osteogenic marker genes. The human BMSCs also demonstrated a tendency to transdifferentiate towards adipogenic phenotype over osteogenic lineage through decreased expression of osteogenic signaling targets BMP2 and SMAD1/5/8 (Zhang et al., 2018). However, when Gioia et al. cultured primary human osteoblast cells in a tabletop RPM, they inferred an uncanny observation with the primary osteoblasts undergoing de-differentiation to mesenchymal phenotype with loss of GTPase activities, Vitamin A metabolism and formation of filopodium, a typical mesenchymal characteristic(Gioia et al., 2018). Scientists have further justified the inhibitory role of microgravity in osteogenesis as the rapid loss of primary cilia from the cells when rat calvarial osteoblast cells were cultured in RPM as observed by Shi et al. (Shi et al., 2017a, 2017b) Such impaired osteogenic differentiation potential of BMSCs or the tendency of primary osteoblast dedifferentiation justifies the bone-tissue loss of the astronauts during spaceflight. Therefore, because of the ambiguity with the regulatory role of microgravity in governing osteogenesis, the pertinent question that arises is 'How to minimize osteoporosis and promote bone regeneration in space-like conditions?'. A plausible solution could be devising advanced strategies by manipulating the microgravity-modulated cellular signaling pathways (Chawla et al., 2018b; Midha et al., 2018) and cell culture niche besides using osteoinductive biomaterials to permit stable osteogenic differentiation under simulated zero gravity conditions.

3.2. Cartilage

Approaches to engineer cartilage tissue under simulated microgravity conditions began in 1991 when a group of researchers used rat mesenchymal stem cells in microcarrier beads in a NASA-developed RWV bioreactor. They could successfully differentiate those cells into chondrocytes validated through morphological analysis and Alcian Blue positive matrix(Daane et al., 1991). Consequently, Freed et al.

demonstrated enhanced proteoglycan synthesis and type-II collagen (COL-II) deposition when bovine articular chondrocytes embedded in PLGA scaffolds were cultured in rotational bioreactors for an initial 3 months, followed by culturing them in the space station or Earth conditions(Freed et al., 1997). Since then, rapid progress has been achieved in cartilage tissue engineering in space-like conditions using scaffolds made out of Polyglycolic acid (PGA) and Mesenchymal stem cells. They showed increased levels of chondrogenic marker (COL-II and Aggrecan) expression and toluidine blue staining in a microgravity setup when compared to the 1g control condition(Wu et al., 2013). Several other approaches were also carried out using cryogenic chondrocytes in serum-based chondrocyte media as well as aged articular chondrocytes inside rotating bioreactors. The results revealed enhanced chondrogenic marker expression and superior cellular morphology as compared to static Earth condition setup(Gorti et al., 2003; Marlovits et al., 2003). A detailed comparative analysis of chondrocyte cell morphology was carried out by Aleshcheva et al. when incubated in different ground-based microgravity simulation facilities. F-actin and Vimentin staining revealed the alteration of cellular morphology and formation of stress fibers in the chondrocytes when exposed to a fast-rotating clinostat for a period of 24 h(Aleshcheva et al., 2016). A recent approach made by scientists using a Magnetic Levitation-based scaffold-free biofabrication technique generated functional cartilage tissue constructs in real microgravity conditions. This widened the window of application of further cartilage tissue engineering research in space(Parfenov et al., 2020).

Despite the fact that adequate chondrogenesis was achieved using microgravity conditions, an investigation carried out by Stamenkovic et al. demonstrated poor cartilaginous characteristics when porcine chondrocytes were cultured in ISS and RPM. They observed a poor aggrecan/versican as well as collagen type-II/I gene expression pattern in the RPM cultured tissue with low cellular density and poor cartilaginous matrix staining as compared to ISS and 1 g control. (Stamenković et al., 2010). Furthermore, Fitzgerald et al. corroborated the previous findings when they observed ECM degradation in articular cartilage specimens with minimized proteoglycan content and mRNA expression profiles of cartilage ECM proteins when exposed to 30 days of microgravity condition in a spacecraft (Fitzgerald et al., 2019). A similar finding of mechanically inferior cartilaginous tissue development was reported by Freed et al. when bovine articular chondrocytes embedded PGA scaffolds were exposed to long-term microgravity conditions (4 months) in Mir Space station(Freed et al., 1997). Additionally, Wagner et al. evaluated the chondrogenesis of hBMSCs in an RWV bioreactor supplemented with TGF-β3 growth factor. They observed decreased staining intensity of Safranin O (proteoglycan) and COL-II with reduced COL-II mRNA expressions, signifying the heinous influence of microgravity on chondrogenesis(Mayer-Wagner et al., 2014). Thus, the above-mentioned observations highlighted the uncertainty of ground-based microgravity simulation facilities in promoting neochondrogenesis besides the irresolute role of mechanical loading and unloading on chondrogenic differentiation(Fitzgerald, 2017). Therefore, further investigations are currently in progress to abrogate the ambiguity and establish a stable microgravity facility to study chondrogenesis. This will help generate a high-quality, stable cartilage tissue construct that can be used to rapidly augment the damaged cartilage in case of chondrogenic dystrophies in space. Moreover, augmenting the advantages of 3D bioprinting, cell types, cartilage mimetic biomaterial, and developmental re-engineering strategies(Chakraborty and Ghosh, 2020a; Chawla et al., 2017) can help us produce permanent hyaline cartilage phenotype. Furthermore, a simultaneous inhibition of hypertrophic maturation *in vitro* during chondrogenesis under microgravity conditions will be another strategy that otherwise is a major roadblock in the success of articular cartilage tissue grafts.

3.3. Skin

Impaired wound healing is a major physiological outcome of microgravity conditions caused by rapid tissue degradation and change in the functional activity of cells associated with the healing process (Monici et al., 2019). There have been multiple incidents when an Astronaut, after returning to Earth, complained about skin quality deterioration during space missions(Riwaldt et al., 2021). Blaber et al. also demonstrated the reduced tissue regeneration capacity under microgravity conditions due to a possible constraint in the transformation from progenitor cells to a differentiated adult phenotype (Blaber et al., 2014). The impairment due to reduced gravity condition is generally attributed to delayed matrix production, cellular migration, collagen synthesis, and delayed neovascularization (Blaber et al., 2014; Blaber et al., 2010). An investigation by Ebnerasuly et al. portrayed the differentiation potential of adipose-derived mesenchymal stem cells (ADSCs) towards fibroblastic lineage under microgravity conditions. Incubating the ADSCs in a Clinostat for a period of 7 days significantly upregulated the ECM secretion and the expression profile of type-III collagen, indicating the development of fibroblast-like characteristics (Ebnerasuly et al., 2018). Furthermore, researchers have also reported the positive effect of microgravity conditions on keratinocyte proliferation and migration through epithelial-mesenchymal transition. This underlines the commencement of re-epithelialization protocol in wound healing(Bacci and Bani, 2022).

However, the same rotating cell culture system (RCCS) exhibited an opposing effect on fibroblast adherence and migration. This is evident from redundant α-SMA and E-cadherin expressions and rearrangement of the microtubule framework (Cialdai et al., 2017). The above finding was further supported by Sapudom et al. when they cultured human dermal fibroblast cells in 3D collagen matrices and incubated in an RPM for a period of 3 days with TGF-β1 supplementation. An enhanced tendency of fibroblast to myofibroblast differentiation was observed with elevated expression of α-SMA and reduced SMAD2/3 translocation to the nucleus irrespective of the presence of TGF-β1 growth factor. Therefore, it can be quintessentially hypothesized that simulated zero-gravity conditions endorse scar formation during the process of wound healing(Sapudom et al., 2021). Fedeli et al. further studied the impact of microgravity on a co-culture model of human dermal fibroblast and human keratinocyte cell line (HaCat) in an RPM setup. They reported a decreased proliferation potential of both keratinocyte and fibroblast cells with an inclination towards oxidative stress-mediated apoptosis and impaired cellular migration, as evident from the scratch wound healing assay(Fedeli et al., 2022). Thus, experiments conducted with fibroblasts and keratinocytes in zero-gravity conditions illustrated a contradictory role in regulating the entire wound healing protocol. Therefore, a comprehensive analysis must be undertaken to unfold the underlying mechanisms governing the impaired cellular proliferation and differentiation capacity of the skin tissue cells. Additionally, the mechanistic role of microgravity in stimulating scar formation (elevated α-SMA expression) during fibroblast differentiation needs to be studied. The observed hurdles can be further addressed with the introduction of 3D bioprinting technology(Cubo-Mateo and Gelinsky, 2021) and a cytocompatible scaffolding material to recapitulate complex cell-matrix interaction and intricate anatomical microarchitecture (rete ridges) (Admane et al., 2019; Lee et al., 2014). This will enable to fabricate anatomically and physiologically relevant full-thickness ready-to-use skin grafts for any chronic skin injuries during space flight.

3.4. Liver

The major hurdle in liver tissue regeneration *in vitro* lies in maintaining the physiological characteristics of the primary hepatocytes within the liver organoid/tissue equivalent due to its limited propagation capability and the deterioration of its functional attributes during the *in vitro* culture period. To its rescue, a wide variety of approaches

have been adopted by tissue engineers to speed up the differentiation of stem cells to hepatocyte phenotype. One such strategy adopted by Wang et al. using a rotational bioreactor demonstrated successful embryonic stem cell differentiation to hepatic cells with enhanced albumin production and P450 activity. Additionally, when implanted *in vivo* in mice models, the hepatic tissue constructs underwent further maturation displaying liver-like characteristics(Wang et al., 2012). The functionality of primary hepatocytes within a biodegradable scaffold under *in vitro* conditions was enhanced when a group of researchers cultured them in a microgravity bioreactor. Liver function tests reported an upregulated albumin secretion, p450 activity and urea secretion with a higher level of engraftment efficiency when transplanted in a mice model(Zhang et al., 2014). Several researchers have deployed a similar strategy of modeled microgravity conditions to maintain the native morphology(Nelson et al., 2010), and functionality and induce aggregate formation in primary hepatocytes (Khaoustov et al., 1999).

On the other hand, simulated microgravity conditions exhibited an inhibitory effect on the proliferation of Chang liver cells when cultured for 72 h in a 3D clinostat. The arrest at the G0/G1 phase due to minimized expressions of cell cycle regulators like cyclin A1, A2, D1 and Cdk-6 are assumed to be the major reasons for such redundant proliferative potential. A similar line of results has been reported by Fujisawa et al. with human hepatic stellate cells incubated in Clinostat for a period of 7 days. They demonstrated a reduced proliferation potential due to increased production of reactive oxygen species and expression of inflammatory targets (NF-κB, Smad3, *etc.*) Although hepatic stellate cells contribute to aggressive liver fibrosis, complete inhibition of its proliferation may foster hepatic dysfunction. Therefore, it can be inferred that the effect of microgravity influence on the hepatic differentiation protocol is cell-specific and that a perfect balance is sought to establish a complete harmony in regulating hepatic tissue homeostasis. Thus, strategies must be developed to fabricate liver tissue constructs under microgravity conditions with hepatophilic polymer blends and specific cell types that can cater to liver failure during spaceflight. The major objective should be to mimic the native 3D hexagonal cross-sectional morphology and co-culture with endothelial cells that can maintain the stable phenotype and physiological function of the *in vivo* liver tissue.

3.5. Blood vessels

Angiogenesis or neoblood vessel formation is a significant event in developing an anatomically relevant functional tissue construct. Endothelial cells are the key players that govern the process of angiogenesis. Although a wide array of biochemical mediators and signaling morphogens are required to induce neoangiogenesis, the effect of microgravity conditions is yet to be explored extensively. In a similar context, Shi et al. observed the angiogenic ability of human umbilical vein endothelial cells (HUVECs) when matured in a clinostat-based microgravity bioreactor. They observed an increased expression of endothelial nitric oxide synthase (eNOS) and blood vessel tube formation in Matrigel (Shi et al., 2012). Furthermore, when the conditioned medium of ASCs cultured in RPM was provided to the endothelial cells, the formation of a dense capillary network with elevated expression levels of VEGF-A significantly demonstrated the paracrine role of microgravity in angiogenesis(Ratushnyy et al., 2018). Additionally, several investigations have also deciphered the molecular mechanisms that regulated the induction of angiogenic potential in endothelial cells, such as RhoA-mediated cytoskeleton rearrangement(Shi et al., 2017a, 2017b) or iNOS-cGMP-PKG signaling pathway respectively(Siamwala et al., 2010). On the contrary, when porcine aortic endothelial cells were exposed to short-term (72 h) hypogravity conditions in an RPM, they illustrated an elongated morphology with a reduced proliferation rate. A detailed analysis revealed the pro-apoptotic characteristics of endothelial cells with elevated expression levels of BAX and p53. Furthermore, there are reports that demonstrated a redundant response of endothelial cells to the angiogenic stimulus under microgravity. Therefore, it underlines the paradoxical role of weightlessness conditions in controlling the entire process of angiogenesis. Thus, a deeper insight is needed to unravel the underlying signal transduction pathways governing the ambivalent role of weightlessness condition in regulating angiogenesis. Moreover, the covalent conjugation of several angiogenic signaling morphogens with the scaffolding material rather than exogenous addition in the media would enhance their bioavailability to the encapsulated endothelial cells. This would allow efficient vascular sprouting required for nutrient and oxygen transfer besides accelerating the wound healing process under zero gravity conditions.

3.6. Neurons

The process of mesenchymal stem cell differentiation is highly dependent on the mechanical microenvironment and topological cues, along with their exposure to tissue-specific morphogens. One such physical effect exerted by the reduced gravity conditions on the neuronal differentiation potential of mesenchymal stem cells was elucidated by Chen et al. with the objective of treating injuries of the central nervous system. They cultured rat BMSCs in both clinostat systems (0 g) as well as 1 g conditions and compared the differentiation potential of the stem cells through various biological characterizations. The BMSCs cultured in 0 g condition demonstrated enhanced expression levels of neuronal differentiation markers as well as elevated secretion of neurotrophins like nerve growth factor (NGF) and brain-derived growth factor (BGF)(Chen et al., 2011). A similar kind of inference was reported by Yuge et al. where the murine BMSCs cultured in simulated microgravity bioreactors assumed an ‘undifferentiated’ state. These cells, when implanted in a cerebral contusion mice model, displayed a higher survivability rate and improved motor function as compared to the 1 g control (Yuge et al., 2010). A three-dimensional tissue-engineered nerve (TEN) produced in an RCCS bioreactor using ADSCs further demonstrated the advantages of microgravity-based simulation facilities to promote neuronal differentiation of stem cells. (Luo et al., 2014). Furthermore, Mattei et al. illustrated the advantages of zero-gravity conditions in maintaining the phenotype and functioning of embryonic stem cell-derived neural organoids. However, the alteration in the expression pattern of several cortical markers and rostral-caudal genes questions the efficiency of the dynamic culture environment in stabilizing the neuronal phenotype of the generated organoids(Mattei et al., 2018). Hence, dynamic microgravity conditions provided a suitable niche for the stem cells to differentiate into neurons and maintain their stable neuronal phenotype in way better than static Earth-like culture conditions. This can help them accelerate the repair of the central and peripheral nervous system. However, there are conflicting reports that suggest that a combination of X-ray radiation and 24-h microgravity exposure of murine primary neural cells significantly impaired the functional attributes of the neural culture. This was justified by decreased neurite length and soma size, slow neurite growth and overall neuron survival (Pani et al., 2016). The increased tendency of neuronal apoptosis in rat models post-exposure to simulated weightlessness was also elucidated by Sun et al., further corroborating the conflicting role of hypogravity simulations in encouraging neuronal morphogenesis. Therefore, there is a dire need for a smart strategy to unfold the underlying molecular mechanism that governs the equivocal role of microgravity conditions in monitoring the neuronal differentiation process *in vitro*. Furthermore, the stability of the neural organoids developed using different biofabrication strategies can be maintained by strongly regulating the underlying cellular signaling cascades.

Therefore, the current ground-based microgravity simulation facilities have been successfully utilized by space physiologists and tissue engineers to demonstrate the tissue regeneration potential (skin, cartilage, liver, neurons) and physiological function of different cell types in monolayer or 3D aggregates. However, the poor osteoinductive influence of the microgravity conditions on the osteoblasts and osteocytes

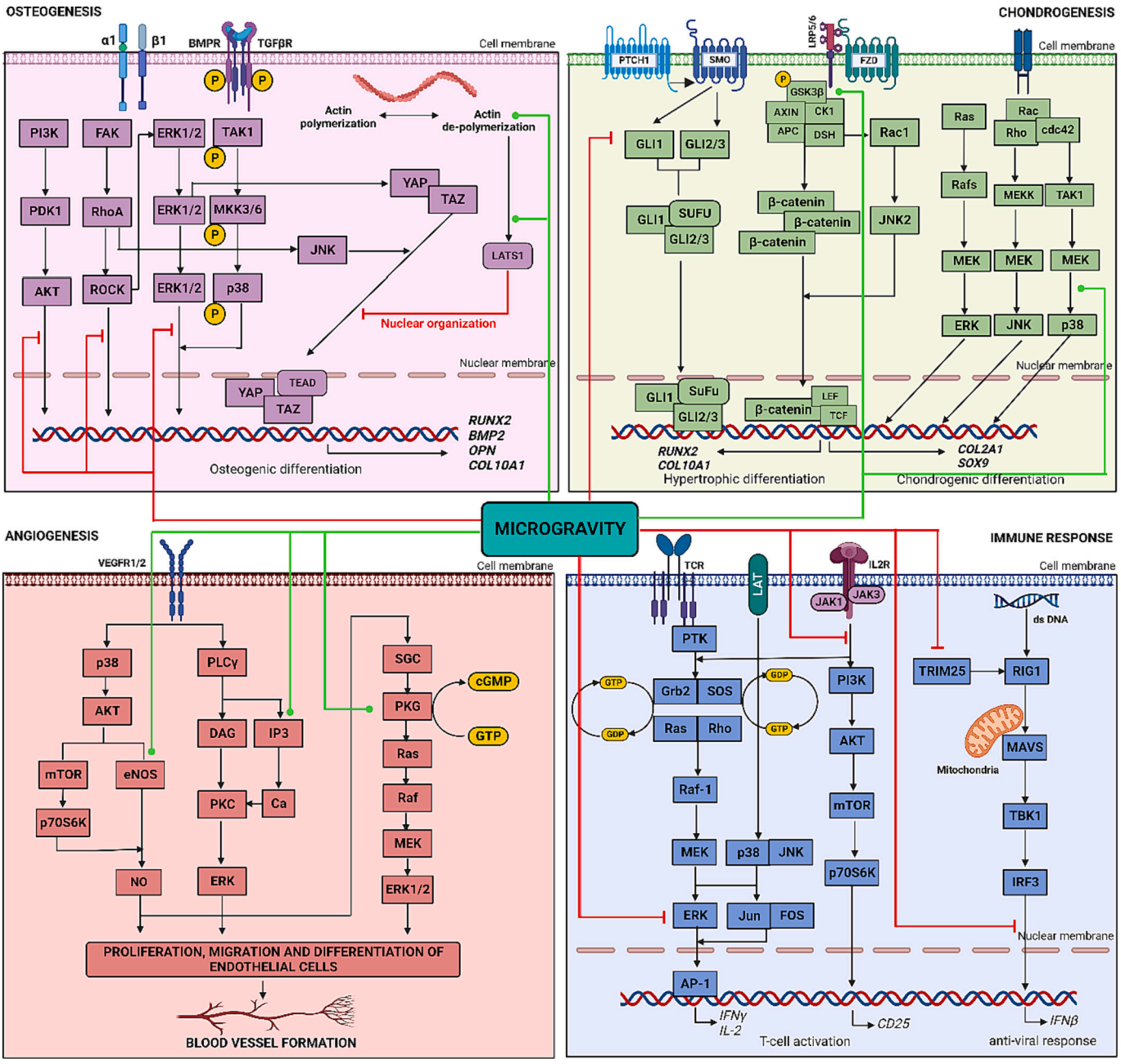

Fig. 3. The regulatory role of microgravity influences the activation and inhibition of the underlying signal transduction pathways governing tissue morphogenesis. Microgravity exhibits a positive role in governing the process of chondrogenesis by activating the canonical Wnt/β-catenin and non-canonical p38 pathways, respectively, while impeding the pro-hypertrophic IHH/SHH pathway. Additionally, it encourages neoangiogenesis *via* the production of eNOS and PKG pathways, thereby contributing to accelerated wound healing in space-like conditions. On the other hand, microgravity exerts a negative regulatory role in osteogenesis and immune response by suppressing their key underlying molecular pathways (ERK1/2, PI3K, *etc.*). Therefore, microgravity influence is a 'boon' to several physiological processes (blood vessels, cartilage) and a 'curse' for the rest (bone, T-cell activation). The contradictory role of microgravity condition is further demonstrated with chondrogenesis as an example, wherein it promotes the neocartilage formation and simultaneously enhances blood vessel formation, a key hallmark feature of chondrocyte hypertrophy. Thus, a critical checkpoint needs to be established to continuously examine the extent and time period of microgravity exposure to the experimental tissue specimens.

has been well demonstrated when cultured in ground-based simulation facilities. This further authenticates the progressive osteoporosis and bone fractures of astronauts during long-term spaceflights, strongly affecting their natural motor functions on Earth. Thus, the incorporation of 3D bioprinting technology into the currently existing approaches would allow space researchers to not only regenerate organs or tissue constructs but also decipher the physiological outcomes of tissue regeneration under space.

4. The pressing need for incorporating 3D bioprinting in space tissue regeneration

The existing tissue engineering approaches using microgravity simulation techniques are majorly focused either on monolayer culture or 3D spheroids. Only a handful of research groups investigated the effect of zero-gravity conditions on scaffold-based 3D tissue constructs. Nevertheless, with a huge amount of effort governing stem cell differentiation and the molecular pathways that govern the process, the

ultimate biological system developed through these approaches becomes physiologically irrelevant when compared with the *in vivo* tissue specimen. This is because:

- Monolayer and 3D aggregate-based cell culture platforms lack homogeneity in nutrient and oxygen exchange, affecting the overall differentiation process.
- The lack of efficient intracellular and cell-matrix interactions in 2D culture fails to simulate the *in vivo* tissue microenvironment required for stable phenotype maintenance and generate desirable physiological outcomes of the fabricated tissue equivalent. The significance of such communications is known to have direct implications in gene expression profiles of a 3D multicellular tumor spheroid model compared to the 2D melanoma NA8 cell line, as elucidated by Ghosh et al. (Ghosh et al., 2005).
- The inadequacy in the cell spreading and cell migration with response to the biochemical cues in the 3D spheroid system.
- The absence of any topological or mechanical cues in both the scaffold-free 2D and 3D culture models exhibits a profound effect on the functional characteristics of the embedded stem cells. The interaction between the cell adhesion RGD motifs on the ECM component and the cell surface integrin receptors is a prime requirement to initiate cell proliferation and differentiation signaling cascades(Sainio and Järveläinen, 2020). Moreover, the ECM provides additional biochemical signals through its embedded signaling morphogens or mechanical stiffness that can be used to further mimic the native physiology of the desired tissue. (Lv et al., 2015). Furthermore, the structural support provided by the scaffolding material ensures the long-term stability of the tissue-engineered constructs when cultured for a prolonged duration *in vitro*. The essential role of such cell-ECM interaction on chondrogenesis has been elucidated by Chawla et al. They have reported that the BMSC-loaded silk fibroin-gelatin 3D bioprinted constructs cultured portrayed an increased level of chondrogenic gene expressions as compared to their 3D aggregate and 2D control with minimized tendency of chondrocyte hypertrophy(Chawla et al., 2017).

Therefore, the advent of 3D bioprinting technology surpassed all the above-mentioned challenges and allowed the fabrication of complex tissue assembly mimicking the native tissue microarchitecture and physiological function. It is a semi-automated robotic dispensing technology that utilizes layer-by-layer fashion to deposit biomaterials, living cells and bioactive factors. Through this layered 3D structure, it aims to recapitulate native tissue microarchitecture and function to produce patient-specific tissue constructs(Chawla et al., 2018a; Das et al., 2015). It comes with some added advantages over traditional scaffolding techniques:

- Ability to control the inter-filament porosity of the printed scaffold, allowing efficient transfer of nutrients and oxygen throughout the scaffold.
- Providing architectural and topographic cues that are required for cellular alignment, spreading, migration and tissue morphogenesis within the construct.
- The establishment of cell-ECM interaction with the biomaterial allows the activation of several tissue-specific signaling pathways required for cellular differentiation(Chakraborty and Ghosh, 2020a).
- Homogenous cell distribution throughout the 3D construct allows efficient cell-cell as well as cell-ECM interaction.

Plenty of research papers have already been published by various research groups, including ours, in demonstrating successful tissue morphogenesis in a 3D bioprinted construct using different biomaterials and cell types(Chakraborty et al., 2022a; Chawla et al., 2018b). Furthermore, by combining the concept of developmental biology and 3D bioprinting, tissue engineers have developed biomimetic articular cartilage and bone tissue equivalents. They not only replicate the anatomical architecture of the native tissue but also exhibit functional relevance like load-bearing capacity in the case of bones and hindered chondrocyte hypertrophy in cartilage construct (Chawla et al., 2018b; Chawla et al., 2017; Daly et al., 2016). Additionally, our laboratory has also recapitulated the regulatory signaling pathways and the undulated morphology of the native skin required for the development of an *in vitro* full-thickness skin model within a silk fibroin-gelatin 3D bioprinted skin construct(Admane et al., 2019). Thus, inspired by the above examples, an on-flight wound healing strategy could be to synthesize an artificial skin graft using astronauts' own plasma as a biomaterial supplemented with his own fibroblasts, keratinocytes and melanocytes (isolated previously and carried along). Therefore, these advantages can be widely utilized by gravitational biologists and tissue engineers to implement 3D bioprinting as a major handy tool in space expeditions to address the on-site rapid treatment of chronic space injuries.

5. Modulation of cell-signaling pathways under microgravity conditions

Microgravity exhibits a profound effect on the cellular anatomy and function at the level of cytoskeletal arrangement, which is very different from that in Earth conditions. This modulation of cell signaling and consequent gene expression patterns affects the quality, stability, and physiological function of the tissue constructs (See Fig. 3).

5.1. Osteogenesis

Different signaling morphogens tightly regulate the process of osteogenesis or osteogenic differentiation. When hMSCs in the osteogenic medium were exposed to both microgravity and normal gravity conditions, it depicted a decrease in osteogenic potential under microgravity conditions. This is evident with a marked decrease in ALP activity and RUNX2 expression and an increased level of PPARγ2 expression. Furthermore, an accumulation of lipid vacuoles was also observed, indicating an increased adipogenic differentiation potential of hMSCs. Evaluation of the underlying signaling pathways revealed a decrease in phosphorylated ERK that regulates the expression and activity of RUNX2 with an increase in activated p38 that described the increased tendency towards adipogenic differentiation(Zheng et al., 2007). Another reason for the decreased osteogenic differentiation ability of hMSCs under simulated microgravity conditions can be due to the Filamentous actin (F-actin) depolymerization that activates a tumor suppressor gene LATS1. This further inhibits the translocation of TAZ protein to the nucleus, a prime regulator of RUNX2 expression and osteogenic differentiation. The former observation was further corroborated by treating the cells with an F-actin depolymerization drug that also showed similar results. Further, the loss-of-function of LATS1 showed increased translocation of TAZ protein, demonstrating the role of the F-actin/TAZ pathway in osteogenic differentiation under microgravity conditions(Chen et al., 2016). One of the major factors that can be attributed to impaired osteogenesis in microgravity conditions is the lack of cell adhesion. This was confirmed with the redundant expression of osteopontin (OPN) in rat osteoblasts under 0 g conditions that mediate the attachment of bone cells to their respective substrates (Kumei et al., 2006). Furthermore, the loss of β1 integrin expression with a mesenchymal phenotype conversion(Gioia et al., 2018), vinculin spots, disrupted F-actin fibers (Saxena et al., 2007) and disorganized cytoskeleton arrangement(Guignandon et al., 2001) was observed under microgravity conditions. This contributed to the reduced expression of focal adhesion molecules in primary osteoblast cells, impairing the activation integrin signaling pathway required for adequate osteogenesis in microgravity conditions.

5.2. Chondrogenesis

A wide constellation of cellular signaling pathways has been described by Chakraborty et al. that modulate the central route of chondrogenesis and hypertrophy under normogravity conditions. (Chakraborty and Ghosh, 2020b). These chondrogenic signaling cascades might have an altered physiological outcome under the influence of microgravity conditions. When IHH and SHH viral transfected BMSCs were encapsulated in a microcarrier and cultured in RCCS, it showed increased levels of expression of cartilage-specific marker genes (SOX9, COL-II, Aggrecan) than control 2D cell culture. Also, it showed a decreased expression profile of hypertrophic marker genes (COL-X, Annexin-V and ALP), further confirmed by ALP activity. An important role of the Hedgehog (HH) signaling cascade is elucidated in stimulating the process of chondrogenesis *via* IHH and SHH, as is evident from the increased level of expression of their respective genes in the transfected groups. This is in contrast with the pro-hypertrophic role of the HH signaling pathway during chondrogenesis. Moreover, a zero-gravity environment with low fluid shear regulates the mechanotransduction process, ultimately alleviating the onset of chondrocyte hypertrophy and enabling stable maintenance of cartilage architecture and function (Liu et al., 2016)(Chen et al., 2019). The role of TGF-β- induced p38 MAPK pathway in chondrogenesis is elucidated under simulated microgravity conditions by culturing Adipose-derived Stem Cells (ADSCs) in the RCCS bioreactor. The microgravity conditions facilitate faster activation of the p38 MAPK pathway marked by enhanced gene expression profiles of the respective cartilage-specific marker genes(Yu et al., 2011). Additionally, Nordberg et al. significantly demonstrated the enhanced expression of LRP proteins (LRP4/5/6) in chondrocytes when cultured in a microgravity bioreactor(Nordberg et al., 2019). An enhanced expression of LRP corresponds to simultaneous activation of the Wnt signaling pathway following β-catenin nuclear translocation, a key mediator for successful chondrogenesis(Hartmann, 2016)(Yates et al., 2005). A detailed transcriptomic analysis of a human cartilage specimen in a parabolic flight for 120 s illustrated elevated expression levels of several unknown proteins like Activity Regulated Cytoskeleton-Associated (ARC) protein (cytoskeletal rearrangement in chondrocytes), Ephrin A-1 (Eph A signaling in early chondrogenesis) and Repulsive Guidance Molecule BMP Co-receptor A (a coreceptor of TGF-β and BMP) (Aissiou et al., 2023). Thus, simulated microgravity technologies regulate the process of cartilage homeostasis through various early to late-stage chondrogenic cell signaling cascades.

5.3. Angiogenesis

A plethora of signaling pathways govern the microgravity-induced activation of angiogenesis in endothelial cells. When HUVECs were cultured in a clinorotation-based bioreactor system, they exhibited enhanced expression of eNOS, which was immediately reversed when incubated with the eNOS inhibitor. Additionally, the regulatory role of the PI3K signaling pathway in promoting angiogenesis in HUVECs was also demonstrated when a PI3K inhibitor significantly downregulated the expression of eNOS as well as Akt. Thus, a synergistic action between PI3K, Akt and eNOS pathway regulated the process of angiogenesis in the endothelial cells under simulated microgravity conditions(Shi et al., 2012). Similar signaling crosstalk has been elucidated by Siamwala et al. with the activation of macrovascular endothelial cells under exposure to microgravity conditions. Such exposure significantly contributed to the decoupling of iNOs to produce maximum nitric oxide production, stimulating angiogenesis. Moreover, the additional activation of the cGMP-mediated PKG pathway further escalated the activation of the endothelial cells towards angiogenesis(Siamwala et al., 2010). Furthermore, blocking of RhoA by microgravity conditions showed random disorganization of the actin filaments within the endothelial cells (marked by phalloidin staining). This can be attributed to efficient vessel tube formation and encouraging early wound closure(Shi et al., 2017a, 2017b). However, there are also reports where endothelial cells, when cultured in RPM, upregulated reactive oxygen species (ROS) production, thereby subjecting the cell population towards oxidative damage-induced apoptosis, thereby inhibiting angiogenesis (Huyan et al., 2022). Therefore, there exists a paradoxical role of 'microgravity' in regulating the process of angiogenesis through complex signaling crosstalk between a wide constellation of signaling pathways.

5.4. Immune response

During long-term space explorations, astronauts under exposure to different microgravity conditions and chronic radiation suffer from various immunological dysfunctionalities. Among these, monocytes, macrophages, lymphocytes (B & T-cells), and dendritic cells are the major immune cells whose function is influenced by weightlessness conditions(Ludtka et al., 2021; Sun et al., 2021). Microgravity impairs the polarization of the M0 macrophage phenotype to classically activated M1 and, alternatively, activated M2 macrophage phenotype. The former was confirmed by the lower level of expression of M1-associated TNF-alpha and M2-associated Arg-1 and CD-206, respectively(Lv et al., 2023). Further validation was carried out using RNA sequencing followed by Gene ontology and KEGG pathway analysis of bone marrow-derived macrophages cultured in an RCCS incubator for 12 days. The results demonstrated the involvement of RAS, ERK and NF-kB in reducing the proliferation and differentiation of the cultured macrophages (Shi et al., 2021). Additionally, microgravity plays an ambidextrous role where it decreases the production rate for dendritic cells when cultured in RCCS for 12 days (Savary et al., 2001), whereas it increases the production rate for human splenocytes when cultured in a Rotary bioreactor for 16 h(Chen et al., 2015). The proliferation capacity of B-lymphocytes with a simultaneous increase in cellular apoptosis has been observed in HMy2.CIR cells, when cultured in RWV bioreactor for 30 min (Dang et al., 2014). However, the underlying molecular mechanism regarding the alteration of the effector function of a particular component of the immune system is rarely discussed.

Paulsen et al. described the effect of short-time clinorotation (5 min) on the signal transduction pathways of Jurkat T-cells and monocytes both in microgravity stimulated and non-stimulated form. They observed a higher degree of phosphorylation of the MAPK kinases ERK1/2 (2.8-fold), p38 (slight change), MEK (1.7-fold) and a 2.2-fold higher phosphorylation of protein kinase C (PKC) in the non-stimulated microgravity cultured T cells. Moreover, for monocytes, only an enhanced PKC phosphorylation was denoted (3.7-fold). The phosphorylation profile, however, underwent a rapid alteration when the cells were exposed to simulated weightlessness conditions for an extended period of 15 min. This underlines the importance of time-of-exposure of simulated microgravity conditions to the immune cells (Paulsen et al., 2010). On the contrary, simulated weightlessness exerted a malevolent effect on the expression pattern of IL-2 and its corresponding receptor (IL-2R) required for the overall functioning of T-cells and its further differentiation into effector T-cells (Cogoli, 1996; Cogoli et al., 1984). A study by Wang et al. further illustrated how the 'weightlessness' condition suppressed the generation of immune response by the macrophage cells when infected with a pathogen. A model comprising *E. coli*-infected U937 macrophage cells was cultured in an RCCS bioreactor for a period of 72 h. They reported reduced secretion and expression levels of TNF-α and IL-6 (pro-inflammatory cytokines), necessary for the activation of macrophages during bacterial infection. Moreover, RNA sequencing analysis data further revealed the suppression of ERK-1/2 and p38 signaling cascade activation, a key requirement during an immune response to a pathogen(Wang et al., 2020). The deleterious effect of simulated zero-gravity conditions on the anti-viral response by the immune system in a zebrafish model system in an RCCS bioreactor has been elucidated by Zhu et al. They have shown that the impairment in actuating the retinoic-acid-inducible gene (RIG)-I-like-receptor (RLR) signaling cascade following the dysregulated

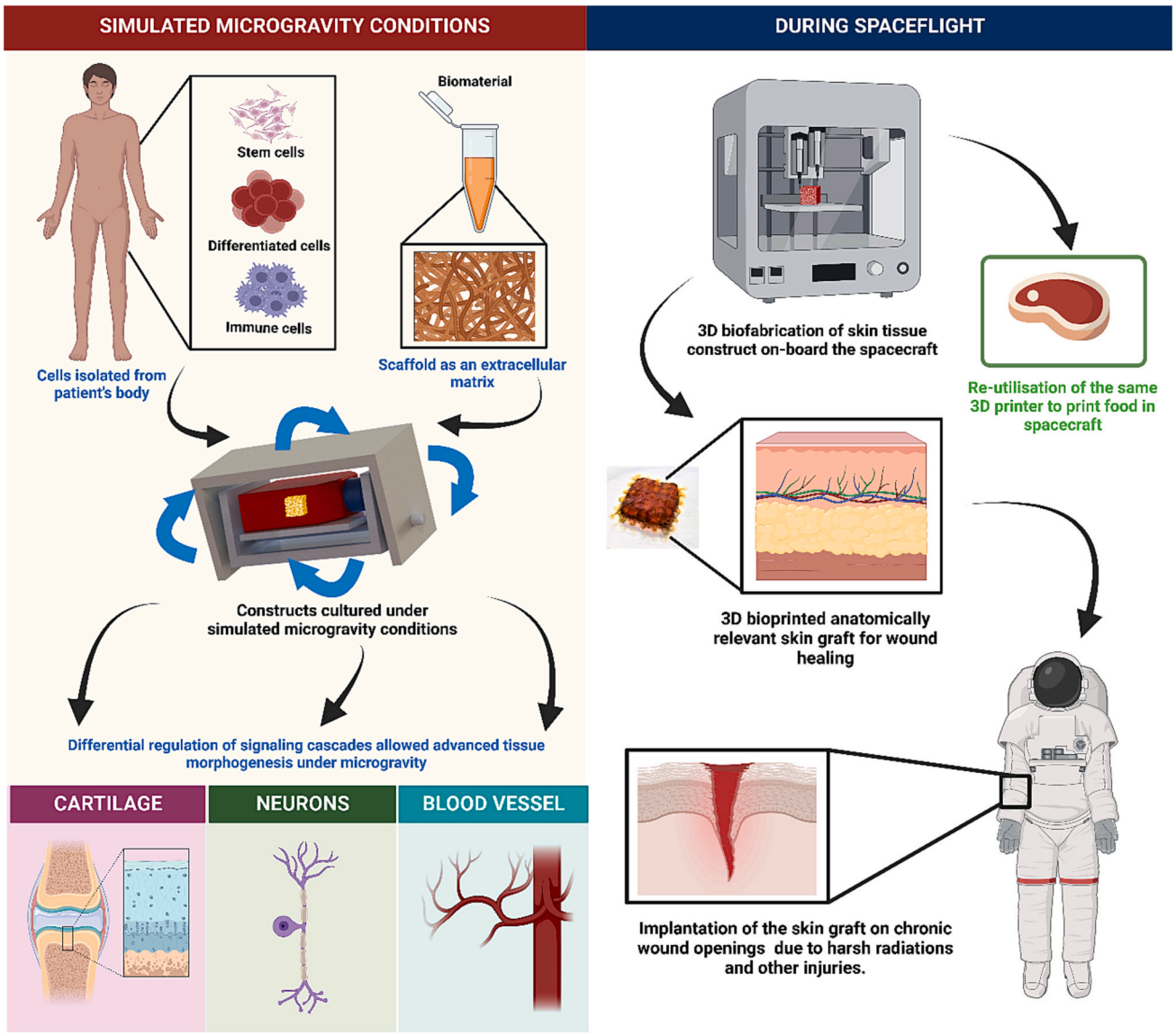

Fig. 4. An illustrative representation depicting the current scenario (left) in how the researchers are utilizing the ground-based microgravity simulation facilities to fabricate advanced tissue-engineered constructs augmenting principles from space physiology, 3D bioprinting, cell and molecular biology and materials engineering while space neither benefiting nor harmed, a symbolic of *commensalism.* However, in the near future, it is anticipated that both space-like conditions will be utilized by astrobiologists and tissue engineers to enhance the quality of engineered human tissue equivalents and further delve deep into the molecular mechanisms governing the aberrant physiological function of the human body in space than in Earth. Additionally, the biofabrication facility can be used as a multipurpose tool to cater to chronic wound management as well as print edible food for spacecraft travelers for long-term space missions demonstrating *synergism*.

expression of TRIM25, a key effector molecule for RLR pathway activation is mainly responsible for delayed anti-viral response(Zhu et al., 2021). Hence, an equivocal role of microgravity exists for regulating different innate and adaptive immune reactions in response to different foreign body invasions.

Therefore, it is clearly evident that the cellular signaling cascades exhibit an ambivalent role under the influence of microgravity if compared with the physiological outcome in Earth conditions. In contrast, the simulated weightlessness condition further demonstrated arguable characteristics in simultaneously encouraging or inhibiting the activation of several tissue-specific signaling pathways. For instance, *in vitro* chondrogenic differentiation of BMSCs in normal gravity conditions exhibits a natural tendency towards hypertrophic differentiation despite showing adequate chondrogenesis (Majumder and Ghosh, 2023). However, under microgravity influence, it portrayed a minimized hypertrophic maturation, a highly desirable outcome for cartilage tissue engineering to maintain stable articular cartilage phenotype (Bhattacharjee et al., 2015; Jubeck et al., 2008). Thus, the differential signaling mechanism exhibited by a specific cell under microgravity influence poses a major challenge to achieving the desired physiological functioning and stability of the engineered tissue construct. Therefore, the current need of the hour is to unravel the intricate signaling crosstalk that mediates the physiological outcome of the fabricated tissue construct in space-like conditions. This signaling morphogen can then be used either in soluble form or covalently conjugated to our desired biomaterial and 3D bioprinted. Thus, utilizing this approach, we can successfully generate synthetic biomimetic tissue assemblies recapitulating the native tissue microenvironment.

6. Does the relationship between 3D bioprinting and space demonstrate 'commensalism' or 'synergism'?

Space agencies all around the world are planning various short- and long-term space missions to explore vivid survival opportunities for

human beings beyond Earth. This is mainly an attempt to make interplanetary travel as easy and natural as traveling between countries and continents(Ghidini, 2018). Moreover, various attempts are being made by commercial entities and space agencies to create permanent extraterrestrial outposts beyond Earth(Levchenko et al., 2019). The critical medical injuries that are associated with long-term manned space missions or medical treatments in future extraterrestrial human colonization require on-site treatment facilities where immediate return to Earth is not possible. Moreover, the presence of different forms of radiation outside the magnetic field of Earth, namely ionizing radiations, cosmic radiations, solar flares, *etc.*, exhibits a detrimental effect on the health profile of the astronauts to such an extent that it may lead to organ failures in several cases. Hence, strategies must be developed to explore the possibilities of regenerating organs or tissue specimens under such a harsh microenvironment.

With the advent of tissue engineering, scientists have attempted to fabricate engineered tissue constructs that may travel along with the space travelers in the space shuttle and may use it when needed. However, storage and quality maintenance of these tissue equivalents pose a major drawback to the process. Proliferative and metabolic signals get affected in microgravity, which can negatively impact their ECM formation, tissue (re)generation and pathways related to tissue remodeling. These reasons led scientists to install 3D bioprinters in space stations that use different methods to deposit the bioink onto the substrate with advanced strategies like the magnetic field and acoustic vibrations (Ahlfeld et al., 2018; Parfenov et al., 2020). There are certain challenges that are needed to overcome to enable smooth outcomes: a) The printing force must alleviate the zero-gravity condition to enable smooth extrusion and stable tissue fabrication, b) The printing process must be performed under very limited resources on board, c) Effect on cellular signaling pathways post-transplantation for long distant space missions during extra-terrestrial colonization must be carefully evaluated as it might be very different than it is demonstrated on Earth, d) Stringent regulatory screening procedures as it involves direct human use of the fabricated tissue model may be a challenging factor, and e) the common challenges of bioprinting in terms of rheological and machine optimization to achieve high print fidelity, cell source, fabrication of vascularized constructs, *etc.* are needed to overcome microgravity (Cubo-Mateo et al., 2020). Parallelly, the signaling pathways related to tissue (re)generation and tissue remodeling also need to be optimized in microgravity (Cubo-Mateo et al., 2020), The major limitation is very limited resources on-flight and lack of telecommunications with experts on Earth due to distance. Hence, the crew members must be well trained with all scientific and technical demonstrations required. This can be resolved by incorporating 'multi-functionality' in the design domain, like a 3D bioprinter which can be repurposed to print edible meat for consumption by the crew members(Jemison and Olabisi, 2021)(Handral et al., 2020). *In-situ* 3D bioprinting of skin has also emerged as a new tool to deposit the bioink at the injury site and using the patient's own body as the bioreactor for cells to mature and develop a full-thickness skin (Hakimi et al., 2018).

However, in the current scenario, 3D biofabrication technology (mainly 3D bioprinting) largely requires space-like conditions to generate complex 3D tissue morphology and signaling pathway activation emulating the native cellular niche, as discussed in earlier sections. Thus, the current relationship between 3D biofabrication and space can be rightfully termed as *'commensalism',* where 3D biofabrication is getting all the benefits from space, but space is neither benefited nor harmed by 3D bioprinting. It is in the near future that we can envisage the *'synergistic'* interactions between both space and 3D biofabrication (See Fig. 4). A scenario where an astronaut is receiving an on-site 3D bioprinted skin graft using his own cells as well as the spacecraft itself carrying 3D bioprinted skin tissue analogs to biomonitor the effect of real-time zero gravity conditions on the functionality of the bioassembly. Thus, overcoming all regulatory and technological odds, 3D biofabrication and microgravity research aim to allow a hassle-free colonization procedure beyond the visible horizon.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Admane, P., et al., 2019. Direct 3D bioprinted full-thickness skin constructs recapitulate regulatory signaling pathways and physiology of human skin. Bioprinting 15, e00051.

Afonin, B.V., 2013. Analysis of possible causes of activation of gastric and the pancreatic excretory and incretory function after completion of space flight at the international space station. Hum. Physiol. 39.

Afshinnekoo, E., et al., 2020. Fundamental biological features of spaceflight: advancing the field to enable deep-space exploration. Cell 183. https://doi.org/10.1016/j.cell.2020.10.050.

Ahlfeld, T., et al., 2018. Bioprinting of mineralized constructs utilizing multichannel plotting of a self-setting calcium phosphate cement and a cell-laden bioink. Biofabrication 10, 45002.

Ahrari, K., Omolaoye, T.S., Goswami, N., Alsuwaidi, H., du Plessis, S.S., 2022. Effects of space flight on sperm function and integrity: a systematic review. Front. Physiol. 13 https://doi.org/10.3389/fphys.2022.904375.

Aissiou, A.K., et al., 2023. Transcriptomic response of bioengineered human cartilage to parabolic flight microgravity is sex-dependent. NPJ Micrograv. 9, 5.

Aleshcheva, G., et al., 2016. Tissue engineering of cartilage on ground-based facilities. Micrograv. Sci. Technol. 28, 237–245.

An, S., Gao, Y., Ling, J., Wei, X., Xiao, Y., 2012. Calcium ions promote osteogenic differentiation and mineralization of human dental pulp cells: implications for pulp capping materials. J. Mater. Sci. Mater. Med. 23, 789–795.

Arfat, Y., et al., 2014. Physiological effects of microgravity on bone cells. Calcif. Tissue Int. 94, 569–579.

Avitabile, E., et al., 2020. Bioinspired scaffold action under the extreme physiological conditions of simulated space flights: osteogenesis enhancing under microgravity. Front. Bioeng. Biotechnol. 8, 722 Preprint at.

Bacci, S., Bani, D., 2022. The epidermis in microgravity and unloading conditions and their effects on wound healing. Front. Bioeng. Biotechnol. 10, 666434.

Barzegari, A., Saei, A.A., 2012. An update to space biomedical research: tissue engineering in microgravity bioreactors. Bioimpacts 2, 23–32.

Benavides Damm, T., Walther, I., Wüest, S.L., Sekler, J., Egli, M., 2014. Cell cultivation under different gravitational loads using a novel random positioning incubator. Biotechnol. Bioeng. 111, 1180–1190.

Bhattacharjee, M., Chameettachal, S., Pahwa, S., Ray, A.R., Ghosh, S., 2014. Strategies for replicating anatomical cartilaginous tissue gradient in engineered intervertebral disc. ACS Appl. Mater. Interfaces 6, 183–193.

Bhattacharjee, M., et al., 2015. Tissue engineering strategies to study cartilage development, degeneration and regeneration. Adv. Drug Deliv. Rev. 84, 107–122.

Blaber, E., Marçal, H., Burns, B.P., 2010. Bioastronautics: the influence of microgravity on astronaut health. Astrobiology 10, 463–473.

Blaber, E., Sato, K., Almeida, E.A.C., 2014. Stem cell health and tissue regeneration in microgravity. Stem Cells Dev. 23 (Suppl. 1), 73–78.

Bradbury, P., et al., 2020. Modeling the impact of microgravity at the cellular level: implications for human disease. Front. Cell Dev. Biol. 8, 96.

Brungs, S., et al., 2016. Facilities for simulation of microgravity in the ESA ground-based facility Programme. Micrograv. Sci. Technol. 28, 191–203.

Brungs, S., Hauslage, J., Hemmersbach, R., 2019. Validation of random positioning versus Clinorotation using a macrophage model system. Micrograv. Sci. Technol. 31, 223–230.

Carlsson, S.I.M., Bertilaccio, M.T.S., Ballabio, E., Maier, J.A.M., 2003. Endothelial stress by gravitational unloading: effects on cell growth and cytoskeletal organization. Biochim. Biophys. Acta 1642, 173–179.

Carmeliet, G., Bouillon, R., 1999. The effect of microgravity on morphology and gene expression of osteoblasts in vitro. FASEB J. 13 (Suppl), S129–S134.

Chakraborty, J., Ghosh, S., 2020a. Cellular proliferation, self-assembly, and modulation of signaling pathways in silk fibroin gelatin-based 3D bioprinted constructs. ACS Appl. Bio Mater. 3, 8309–8320.

Chakraborty, J., Ghosh, S., 2020b. Cellular proliferation, self-assembly, and modulation of signaling pathways in silk fibroin gelatin-based 3D bioprinted constructs. ACS Appl Bio Mater 3, 8309–8320.

Chakraborty, J., Mu, X., Pramanick, A., Kaplan, D.L., Ghosh, S., 2022a. Recent advances in bioprinting using silk protein-based bioinks. Biomaterials 287, 121672.

Chakraborty, J., Majumder, N., Sharma, A., Prasad, S., Ghosh, S., 2022b. 3D bioprinted silk-reinforced alginate-Gellan gum constructs for cartilage regeneration. Bioprinting 28, e00232.

Chawla, S., Ghosh, S., 2018a. Establishment of in vitro model of corneal scar pathophysiology. J. Cell. Physiol. 233, 3817–3830.

Chawla, S., Ghosh, S., 2018b. Regulation of fibrotic changes by the synergistic effects of cytokines, dimensionality and matrix: towards the development of an in vitro human dermal hypertrophic scar model. Acta Biomater. 69, 131–145.

Chawla, S., Kumar, A., Admane, P., Bandyopadhyay, A., Ghosh, S., 2017. Elucidating role of silk-gelatin bioink to recapitulate articular cartilage differentiation in 3D bioprinted constructs. Bioprinting 7, 1–13.
Chawla, S., Midha, S., Sharma, A., Ghosh, S., 2018a. Silk-based bioinks for 3D bioprinting. Adv. Healthc. Mater. 7, 1701204.
Chawla, S., Sharma, A., Bandyopadhyay, A., Ghosh, S., 2018b. Developmental biology-inspired strategies to engineer 3D bioprinted bone construct. ACS Biomater Sci. Eng. 4, 3545–3560.
Chen, J., et al., 2011. The simulated microgravity enhances the differentiation of mesenchymal stem cells into neurons. Neurosci. Lett. 505, 171–175.
Chen, H., et al., 2015. The distinctive sensitivity to microgravity of immune cell subpopulations. Micrograv. Sci. Technol. 27, 427–436.
Chen, Z., Luo, Q., Lin, C., Kuang, D., Song, G., 2016. Simulated microgravity inhibits osteogenic differentiation of mesenchymal stem cells via depolymerizing F-actin to impede TAZ nuclear translocation. Sci. Rep. 6, 1–11.
Chen, L., Liu, G., Li, W., Wu, X., 2019. Chondrogenic differentiation of bone marrow-derived mesenchymal stem cells following transfection with Indian hedgehog and sonic hedgehog using a rotary cell culture system. Cell. Mol. Biol. Lett. 24, 1–16.
Chylack, L.T., et al., 2009. NASA study of cataract in astronauts (NASCA). Report 1: cross-sectional study of the relationship of exposure to space radiation and risk of lens opacity. Radiat. Res. 172, 10–20.
Cialdai, F., et al., 2017. Modeled microgravity affects fibroblast functions related to wound healing. Micrograv. Sci. Technol. 29, 121–132.
Clarke, M.S.F., Sundaresan, A., Vanderburg, C.R., Banigan, M.G., Pellis, N.R., 2013. A three-dimensional tissue culture model of bone formation utilizing rotational co-culture of human adult osteoblasts and osteoclasts. Acta Biomater. 9, 7908–7916.
Cogoli, A., 1996. Gravitational physiology of human immune cells: a review of in vivo, ex vivo and in vitro studies. J. Gravit. Physiol. 3, 1–9.
Cogoli, A., Tschopp, A., Fuchs-Bislin, P., 1984. Cell sensitivity to gravity. Science 1979 (225), 228–230.
Comfort, P., et al., 2021. Effects of spaceflight on musculoskeletal health: a systematic review and meta-analysis, considerations for interplanetary travel. Sports Medicine (Auckland, N.Z.) 51, 2097–2114. https://doi.org/10.1007/s40279-021-01496-9.
Convertino, V.A., 2005. Consequences of cardiovascular adaptation to spaceflight: implications for the use of pharmacological countermeasures. Gravit. Space Biol. Bull. 18, 59–69.
Costantini, D., et al., 2019. Simulated microgravity promotes the formation of tridimensional cultures and stimulates pluripotency and a glycolytic metabolism in human hepatic and biliary tree stem/progenitor cells. Sci. Rep. 9, 5559.
Cubo-Mateo, N., Gelinsky, M., 2021. Wound and skin healing in space: the 3D bioprinting perspective. Front. Bioeng. Biotechnol. 9, 720217.
Cubo-Mateo, N., et al., 2020. Can 3D bioprinting be a key for exploratory missions and human settlements on the moon and Mars? Biofabrication 12, 43001.
Cucinotta, F.A., Cacao, E., 2017. Non-targeted effects models predict significantly higher mars mission cancer risk than targeted effects models. Sci. Rep. 7, 1–11.
Daane, E., Duke, P.J., Campbell, M., 1991. Chondrogenesis of limb mesenchymal cells cultured on microcarrier beads. TSEMJ 22, 52.
Dai, Z.-Q., Wang, R., Ling, S.K., Wan, Y.M., Li, Y.H., 2007. Simulated microgravity inhibits the proliferation and osteogenesis of rat bone marrow mesenchymal stem cells. Cell Prolif. 40, 671–684.
Daly, A.C., et al., 2016. 3D bioprinting of developmentally inspired templates for whole bone organ engineering. Adv. Healthc. Mater. 5, 2353–2362.
Dang, B., et al., 2014. Simulated microgravity increases heavy ion radiation-induced apoptosis in human B lymphoblasts. Life Sci. 97, 123–128.
Das, S., et al., 2015. Bioprintable, cell-laden silk fibroin-gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs. Acta Biomater. 11, 233–246.
Devarasetty, M., Wang, E., Soker, S., Skardal, A., 2017. Mesenchymal stem cells support growth and organization of host-liver colorectal-tumor organoids and possibly resistance to chemotherapy. Biofabrication 9, 21002.
Ebnerasuly, F., Hajebrahimi, Z., Tabaie, S.M., Darbouy, M., 2018. Simulated microgravity condition alters the gene expression of some ECM and adhesion molecules in adipose derived stem cells. Int J Mol Cell Med 7, 146–157.
Eiermann, P., et al., 2013. Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells. Micrograv. Sci. Technol. 25, 153–159.
Eltom, A., Zhong, G., Muhammad, A., 2019. Scaffold techniques and designs in tissue engineering functions and purposes: a review. Adv. Mater. Sci. Eng. 2019.
Fedeli, V., et al., 2022. Microgravity modifies the phenotype of fibroblast and promotes remodeling of the fibroblast–keratinocyte interaction in a 3D Co-culture model. Int. J. Mol. Sci. 23 https://doi.org/10.3390/ijms23042163. Preprint at.
Ferranti, F., Del Bianco, M., Pacelli, C., 2021. Advantages and limitations of current microgravity platforms for space biology research. Appl. Sci. (Switzerland) 11, 1–18.
Fitzgerald, J., 2017. Cartilage breakdown in microgravity—a problem for long-term spaceflight? NPJ Regen Med 2, 10.
Fitzgerald, J., Endicott, J., Hansen, U., Janowitz, C., 2019. Articular cartilage and sternal fibrocartilage respond differently to extended microgravity. NPJ Micrograv. 5, 3.
Freed, L.E., Langer, R., Martin, I., Pellis, N.R., Vunjak-Novakovic, G., 1997. Tissue engineering of cartilage in space. Proc. Natl. Acad. Sci. 94, 13885 LP – 13890.
Frigeri, A., et al., 2008. Effect of microgravity on gene expression in mouse brain. Exp. Brain Res. 191, 289–300.
Fu, Q., et al., 2019. Impact of prolonged spaceflight on orthostatic tolerance during ambulation and blood pressure profiles in astronauts. Circulation 140, 729–738.
Fujisawa, K., et al., 2022. Evaluation of the effects of microgravity on activated primary human hepatic stellate cells. Int. J. Mol. Sci. 23.
Gardner, J.K., Herbst-Kralovetz, M.M., 2016. Three-dimensional rotating wall vessel-derived cell culture models for studying virus-host interactions. Viruses 8, 304.
Gerecht-Nir, S., Cohen, S., Itskovitz-Eldor, J., 2004. Bioreactor cultivation enhances the efficiency of human embryoid body (hEB) formation and differentiation. Biotechnol. Bioeng. 86, 493–502.
Ghidini, T., 2018. Regenerative medicine and 3D bioprinting for human space exploration and planet colonisation. J. Thorac. Dis. 10, S2363.
Ghosh, S., et al., 2005. Three-dimensional culture of melanoma cells profoundly affects gene expression profile: a high density oligonucleotide array study. J. Cell. Physiol. 204, 522–531.
Gioia, M., et al., 2018. Simulated microgravity induces a cellular regression of the mature phenotype in human primary osteoblasts. Cell Death Dis. 4, 59.
Goodwin, T.J., Schroeder, W.F., Wolf, D.A., Moyer, M.P., 1993. Rotating-wall vessel coculture of small intestine as a prelude to tissue modeling: aspects of simulated microgravity. Proc. Soc. Exp. Biol. Med. 202, 181–192.
Gorti, G.K., et al., 2003. Cartilage tissue engineering using cryogenic chondrocytes. Arch. Otolaryngol. Head Neck Surg. 129, 889–893.
Griffoni, C., et al., 2011. Modification of proteins secreted by endothelial cells during modeled low gravity exposure. J. Cell. Biochem. 112.
Grimm, D., et al., 2018. Tissue engineering under microgravity conditions-use of stem cells and specialized cells. Stem Cells Dev. 27, 787–804.
Guignandon, A., et al., 2001. Cell cycling determines integrin-mediated adhesion in osteoblastic ROS 17/2.8 cells exposed to space-related conditions. FASEB J. 15, 2036–2038.
Hada, M., et al., 2019. Increased chromosome aberrations in cells exposed simultaneously to simulated microgravity and radiation. Int. J. Mol. Sci. 20.
Hakimi, N., et al., 2018. Handheld skin printer: in situ formation of planar biomaterials and tissues. Lab Chip 18, 1440–1451.
Handral, K., Hua Tay, S., Wan Chan, W., Choudhury, D., 2020. 3D printing of cultured meat products. Crit. Rev. Food Sci. Nutr. 1–10.
Hartmann, C., 2016. Wnt signaling in cartilage development. Cartilage: Physiol. Develop. 229–252. https://doi.org/10.1007/978-3-319-29568-8_10.
Henkel, J., et al., 2013. Bone regeneration based on tissue engineering conceptions—a 21st century perspective. Bone Res 1, 216–248.
Herranz, R., et al., 2013. Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. Astrobiology 13, 1–17.
Huang, L., Snyder, A.R., Morgan, W.F., 2003. Radiation-induced genomic instability and its implications for radiation carcinogenesis. Oncogene 22. https://doi.org/10.1038/sj.onc.1206697.
Hufenbach, B., et al., . The global exploration roadmap. In: 62nd International Astronautical Congress 2011, **4**. *IAC*, pp. 3266–3275.
Huyan, T., et al., 2022. Simulated microgravity promotes oxidative stress-induced apoptosis in ARPE-19 cells associated with Nrf2 signaling pathway. Acta Astronaut. 198, 161–169.
Iwamoto, J., Takeda, T., Sato, Y., 2005. Interventions to prevent bone loss in astronauts during space flight. Keio J. Med. 54, 55–59.
Jemison, M., Olabisi, R., 2021. Biomaterials for human space exploration: a review of their untapped potential. Acta Biomater. https://doi.org/10.1016/j.actbio.2021.04.033.
Jin, F., et al., 2010. Establishment of three-dimensional tissue-engineered bone constructs under microgravity-simulated conditions. Artif. Organs 34, 118–125.
Johnstone, B., et al., 2013. Tissue engineering for articular cartilage repair—the state of the art. Eur. Cell. Mater. 25, e67.
Jubeck, B., et al., 2008. Promotion of articular cartilage matrix vesicle mineralization by type I collagen. Arthritis Rheum. 58, 2809–2817.
Kennedy, E.M., et al., 2018. Galactic cosmic radiation induces persistent epigenome alterations relevant to human lung Cancer. Sci. Rep. 8.
Khaoustov, V.I., et al., 1999. Induction of three-dimensional assembly of human liver cells by simulated microgravity. In Vitro Cell. Dev. Biol. Anim. 35, 501–509.
Klement, B.J., Young, Q.M., George, B.J., Nokkaew, M., 2004. Skeletal tissue growth, differentiation and mineralization in the NASA Rotating Wall vessel. Bone 34, 487–498.
Kumar, S., Suman, S., Fornace, A.J., Datta, K., 2018. Space radiation triggers persistent stress response, increases senescent signaling, and decreases cell migration in mouse intestine. Proc. Natl. Acad. Sci. U. S. A. 115.
Kumei, Y., et al., 2006. Microgravity signal ensnarls cell adhesion, cytoskeleton, and matrix proteins of rat osteoblasts: osteopontin, CD44, osteonectin, and alpha-tubulin. Ann. N. Y. Acad. Sci. 1090, 311–317.
Leach, J.E., et al., 2001. Plants, plant pathogens, and microgravity–a deadly trio. Gravit. Space Biol. Bull. 14, 15–23.
Lee, V., et al., 2014. Design and fabrication of human skin by three-dimensional bioprinting. Tissue Eng. Part C Methods 20, 473–484.
Lee, A.G., et al., 2020. Erratum: author correction: spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. NPJ Micrograv. 6, 23.
Lee, P.H.U., Chung, M., Ren, Z., Mair, D.B., Kim, D.H., 2022. Factors mediating spaceflight-induced skeletal muscle atrophy. Am. J. Phys. Cell Phys. 322, C567–C580. https://doi.org/10.1152/ajpcell.00203.2021.
Levchenko, I., Xu, S., Mazouffre, S., Keidar, M., Bazaka, K., 2019. Mars colonization: beyond getting there. Global Chall. 3, 1800062.
Li, N., et al., 2018. Microgravity-induced alterations of inflammation-related Mechanotransduction in endothelial cells on board SJ-10 satellite. Front. Physiol. 9, 1025.
Li, L., et al., 2019. Effects of simulated microgravity on the expression profiles of RNA during osteogenic differentiation of human bone marrow mesenchymal stem cells. Cell Prolif. 52, e12539.

Lin, C., et al., 2009. Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. J. Bone Miner. Res. 24, 1651–1661.
Little, M.P., et al., 2012. Systematic review and meta-analysis of circulatory disease from exposure to low-level ionizing radiation and estimates of potential population mortality risks. Environ. Health Perspect. 120, 1503–1511.
Liu, P.-C., et al., 2016. Transfection of the IHH gene into rabbit BMSCs in a simulated microgravity environment promotes chondrogenic differentiation and inhibits cartilage aging. Oncotarget 7, 62873.
Ludtka, C., Silberman, J., Moore, E., Allen, J.B., 2021. Macrophages in microgravity: the impact of space on immune cells. NPJ Micrograv. 7, 13.
Luo, H., Zhu, B., Zhang, Y., Jin, Y., 2014. Tissue-engineered nerve constructs under a microgravity system for peripheral nerve regeneration. Tissue Eng. Part A 21, 267–276.
Lv, H., et al., 2015. Mechanism of regulation of stem cell differentiation by matrix stiffness. Stem Cell Res Ther 6, 103.
Lv, H., et al., 2023. Microgravity and immune cells. J. R. Soc. Interface 20, 20220869.
Majumder, N., Ghosh, S., 2023. Unfolding the mystery behind the onset of chondrocyte hypertrophy during Chondrogenesis: toward designing advanced permanent cartilage-mimetic biomaterials. Adv. Funct. Mater. https://doi.org/10.1002/adfm.202300651. Preprint at.
Majumder, N., Mishra, A., Ghosh, S., 2022. Effect of varying cell densities on the rheological properties of the bioink. Bioprinting 28, e00241.
Marlovits, S., Tichy, B., Truppe, M., Gruber, D., Vécsei, V., 2003. Chondrogenesis of aged human articular cartilage in a scaffold-free bioreactor. Tissue Eng. 9, 1215–1226.
Mattei, C., Alshawaf, A., D'Abaco, G., Nayagam, B., Dottori, M., 2018. Generation of neural organoids from human embryonic stem cells using the rotary cell culture system: effects of microgravity on neural progenitor cell fate. Stem Cells Dev. 27, 848–857.
Mayer-Wagner, S., et al., 2014. Simulated microgravity affects chondrogenesis and hypertrophy of human mesenchymal stem cells. Int. Orthop. 38, 2615–2621.
Midha, S., Chawla, S., Chakraborty, J., Chameettachal, S., Ghosh, S., 2018. Differential regulation of hedgehog and parathyroid signaling in mulberry and nonmulberry silk fibroin textile braids. ACS Biomater Sci. Eng. 4, 595–607.
Mishra, B., Luderer, U., 2019. Reproductive hazards of space travel in women and men. Nat. Rev. Endocrinol. 15 https://doi.org/10.1038/s41574-019-0267-6.
Monici, M., et al., 2019. Tissue repair and regeneration in space and on earth. ISGP & ESA Life Sci. 118.
Murphy, S.V., Atala, A., 2014. 3D bioprinting of tissues and organs. Nat. Biotechnol. 32, 773–785.
Nassef, M.Z., et al., 2020. Breast Cancer cells in microgravity: new aspects for Cancer research. Int. J. Mol. Sci. 21.
Nelson, L.J., Walker, S.W., Hayes, P.C., Plevris, J.N., 2010. Low-shear modelled microgravity environment maintains morphology and differentiated functionality of primary porcine hepatocyte cultures. Cells Tissues Organs 192, 125–140.
Nishikawa, M., et al., 2005. The effect of simulated microgravity by three-dimensional clinostat on bone tissue engineering. Cell Transplant. 14, 829–835.
Nordberg, R.C., Mellor, L.F., Krause, A.R., Donahue, H.J., Loboa, E.G., 2019. LRP receptors in chondrocytes are modulated by simulated microgravity and cyclic hydrostatic pressure. PLoS One 14, e0223245.
Ozefe, F., Yildiz, A.A., 2020. In: Sahu, D.R., Stavrou, V.N. (Eds.), Magnetic Levitation Based Applications in Bioscience. IntechOpen. https://doi.org/10.5772/intechopen.92148. Ch. 8.
Pani, G., et al., 2016. Combined exposure to simulated microgravity and acute or chronic radiation reduces neuronal network integrity and survival. PLoS One 11, e0155260.
Parfenov, V.A., et al., 2020. Magnetic levitational bioassembly of 3D tissue construct in space. Sci. Adv. 6, eaba4174.
Patel, R., Welford, S., Gerson, S.L., 2016. The effects of galactic cosmic radiation exposure on hematopoietic stem cell dysfunction and oncogenesis. Blood 128, 5297.
Paulsen, K., et al., 2010. Microgravity-induced alterations in signal transduction in cells of the immune system. Acta Astronaut. 67, 1116–1125.
Pavlakou, P., Dounousi, E., Roumeliotis, S., Eleftheriadis, T., Liakopoulos, V., 2018. Oxidative stress and the kidney in the space environment. Int. J. Mol. Sci. 19 https://doi.org/10.3390/ijms19103176.
Pietrzyk, R.A., Jones, J.A., Sams, C.F., Whitson, P.A., 2007. Renal stone formation among astronauts. Aviat. Space Environ. Med. 78.
Pietsch, J., et al., 2011. The effects of weightlessness on the human organism and mammalian cells. Curr. Mol. Med. 11, 350–364.
Puca, A., Russo, G., Giordano, A., 2012. Properties of mechano-transduction via simulated microgravity and its effects on intracellular trafficking of VEGFR's. Oncotarget 3, 426–434.
Qiu, Q., Ducheyne, P., Gao, H., Ayyaswamy, P., 1998. Formation and differentiation of three-dimensional rat marrow stromal cell culture on microcarriers in a rotating-wall vessel. Tissue Eng. 4, 19–34.
Ratushnyy, A., Ezdakova, M., Yakubets, D., Buravkova, L., 2018. Angiogenic activity of human adipose-derived mesenchymal stem cells under simulated microgravity. Stem Cells Dev. 27, 831–837.
Richardson, R.B., 2022. The role of oxygen and the goldilocks range in the development of cataracts induced by space radiation in US astronauts. Exp. Eye Res. 223, 109192.
Riwaldt, S., et al., 2021. Role of apoptosis in wound healing and apoptosis alterations in microgravity. Front. Bioeng. Biotechnol. 9, 679650.
Roy-O'Reilly, M., Mulavara, A., Williams, T., 2021. A review of alterations to the brain during spaceflight and the potential relevance to crew in long-duration space exploration. NPJ Micrograv. 7, 5.
Russomano, T., et al., 2005. Development and validation of a 3D clinostat for the study of cells during microgravity simulation. Conf. Proc. IEEE Eng. Med. Biol. Soc. 2006, 564–566.
Sainio, A., Järveläinen, H., 2020. Extracellular matrix-cell interactions: focus on therapeutic applications. Cell. Signal. 66, 109487.
Sapudom, J., et al., 2021. Fibroblast Differentiation and Matrix Remodeling Impaired under Simulated Microgravity in 3D Cell Culture Model. Int. J. Mol. Sci. 22 https://doi.org/10.3390/ijms222111911. Preprint at.
Savary, C.A., et al., 2001. Characteristics of human dendritic cells generated in a microgravity analog culture system. In Vitro Cell. Dev. Biol. Anim. 37, 216–222.
Saxena, R., Pan, G., Mcdonald, J.A.Y.M., 2007. Osteoblast and osteoclast differentiation in modeled microgravity. Ann. N. Y. Acad. Sci. 1116, 494–498.
Schwarz, R.P., Goodwin, T.J., Wolf, D.A., 1992a. Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. J. Tissue Cult. Methods 14, 51–57.
Schwarz, R.P., Goodwin, T.J., Wolf, D.A., 1992b. Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. J. Tissue Cult. Methods 14, 51–57.
Shi, F., et al., 2012. Effects of simulated microgravity on human umbilical vein endothelial cell angiogenesis and role of the PI3K-Akt-eNOS signal pathway. PLoS One 7, e40365.
Shi, W., et al., 2017a. Microgravity induces inhibition of osteoblastic differentiation and mineralization through abrogating primary cilia. Sci. Rep. 7, 1866.
Shi, F., et al., 2017b. Simulated microgravity promotes angiogenesis through RhoA-dependent rearrangement of the actin cytoskeleton. Cell. Physiol. Biochem. 41, 227–238.
Shi, L., et al., 2021. Spaceflight and simulated microgravity suppresses macrophage development via altered RAS/ERK/NFκB and metabolic pathways. Cell. Mol. Immunol. 18, 1489–1502.
Siamwala, J.H., et al., 2010. Simulated microgravity promotes nitric oxide-supported angiogenesis via the iNOS–cGMP–PKG pathway in macrovascular endothelial cells. FEBS Lett. 584, 3415–3423.
Sikavitsas, V.I., Bancroft, G.N., Mikos, A.G., 2002. Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. J. Biomed. Mater. Res. 62, 136–148.
Stamenković, V., Keller, G., Nesic, D., Cogoli, A., Grogan, S.P., 2010. Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng. Part A 16, 1729–1736.
Sun, Y., Kuang, Y., Zuo, Z., 2021. The emerging role of macrophages in immune system dysfunction under real and simulated microgravity conditions. Int. J. Mol. Sci. 22.
Svejgaard, B., et al., 2015. Common effects on Cancer cells exerted by a random positioning machine and a 2D clinostat. PLoS One 10, e0135157.
Tamma, R., et al., 2009. Microgravity during spaceflight directly affects in vitro osteoclastogenesis and bone resorption. FASEB J. 23, 2549–2554.
Tays, G.D., et al., 2021. The effects of long duration spaceflight on sensorimotor control and cognition. Front. Neural Circ. 15. Preprint at.
Türker, E., Demirçak, N., Arslan-Yildiz, A., 2018. Scaffold-free three-dimensional cell culturing using magnetic levitation. Biomater. Sci. 6, 1745–1753.
Ulbrich, C., et al., 2014. The impact of simulated and real microgravity on bone cells and mesenchymal stem cells. Biomed. Res. Int. 2014, 928507.
van Loon, J.J.W.A., 2007. Some history and use of the random positioning machine, RPM, in gravity related research. Adv. Space Res. 39, 1161–1165.
Wang, Y., et al., 2012. Rotating microgravity-bioreactor cultivation enhances the hepatic differentiation of mouse embryonic stem cells on biodegradable polymer scaffolds. Tissue Eng. Part A 18, 2376–2385.
Wang, J., et al., 2020. Simulated microgravity suppresses MAPK pathway-mediated innate immune response to bacterial infection and induces gut microbiota dysbiosis. FASEB J. 34, 14631–14644.
Wu, X., Li, S., Lou, L., Chen, Z., 2013. The effect of the microgravity rotating culture system on the Chondrogenic differentiation of bone marrow mesenchymal stem cells. Mol. Biotechnol. 54, 331–336.
Wu, B., et al., 2018. On-orbit sleep problems of astronauts and countermeasures. Mil. Med. Res. 5, 17.
Wuest, S.L., Richard, S., Kopp, S., Grimm, D., Egli, M., 2015. Simulated microgravity: critical review on the use of random positioning machines for mammalian cell culture. Biomed. Res. Int. 2015, 971474.
Xie, M., et al., 2022. In situ 3D bioprinting with bioconcrete bioink. Nat. Commun. 13, 3597.
Yamamoto, Y., et al., 2009. Preparation of artificial skeletal muscle tissues by a magnetic force-based tissue engineering technique. J. Biosci. Bioeng. 108, 538–543.
Yang, J.-Q., et al., 2020. The effects of microgravity on the digestive system and the new insights it brings to the life sciences. Life Sci Space Res (Amst) 27.
Yates, K.E., Shortkroff, S., Reish, R.G., 2005. Wnt influence on chondrocyte differentiation and cartilage function. DNA Cell Biol. 24, 446–457.
Yu, B., et al., 2011. Simulated microgravity using a rotary cell culture system promotes chondrogenesis of human adipose-derived mesenchymal stem cells via the p38 MAPK pathway. Biochem. Biophys. Res. Commun. 414, 412–418.
Yuge, L., et al., 2010. Simulated microgravity maintains the undifferentiated state and enhances the neural repair potential of bone marrow stromal cells. Stem Cells Dev. 20, 893–900.
Zarrinpour, V., Hajebrahimi, Z., Jafarinia, M., 2017. Expression pattern of neurotrophins and their receptors during neuronal differentiation of adipose-derived stem cells in simulated microgravity condition. Iran. J. Basic Med. Sci. 20, 178–186.
Zhang, L., Hu, J., Athanasiou, K.A., 2009. The role of tissue engineering in articular cartilage repair and regeneration. Crit. Rev. Biomed. Eng. 37.
Zhang, S., et al., 2014. Three-dimensional culture in a microgravity bioreactor improves the engraftment efficiency of hepatic tissue constructs in mice. J. Mater. Sci. Mater. Med. 25, 2699–2709.

Zhang, C., et al., 2018. Space microgravity drives transdifferentiation of human bone marrow-derived mesenchymal stem cells from osteogenesis to adipogenesis. FASEB J. 32, 4444–4458.
Zheng, Q., et al., 2007. Could the Effect of Modeled Microgravity on Osteogenic Differentiation of Human Mesenchymal Stem Cells Be Reversed by Regulation of Signaling Pathways?.
Zhu, L., et al., 2021. Attenuation of antiviral immune response caused by perturbation of TRIM25-mediated RIG-I activation under simulated microgravity. Cell Rep. 34, 108600.