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Review article

The effects of microgravity on the digestive system and the new insights it brings to the life sciences

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ABSTRACT

Background: Weightlessness is a component of the complex space environment. It exerts adverse effects on the human body, and may pose unknown challenges to the implementation of space missions. The regular function of the digestive system is an important checkpoint for astronauts to conduct missions. Simulated microgravity can recreate the changes experienced by the human body in a weightless environment in space to a certain extent, providing technical support for the exploration of its mechanism and a practical method for other scientific research.

Methods and materials: In the present study, we reviewed and discussed the latest research on the effects of weightlessness or simulated microgravity on the digestive system, as well as the current challenges and future expectations for progress in medical science and further space exploration.

Results: A series of studies have investigated the effects of weightlessness on the human digestive system. On one hand, weightlessness and the changing space environment may exert certain adverse effects on the human body. Studies based on cells or animals have demonstrated the complex effects on the human digestive system in response to weightlessness. On the other hand, a microgravity environment also facilitates the ideation of novel concepts for research in the domain of life science.

Conclusion: The effects of weightlessness on the digestive system are considerably complicated. The emergence of methods that help simulate a weightless environment provides a more convenient alternative for assessing the impact and the mechanism underlying the effect of weightlessness on the human body. In addition, the simulated microgravity environment facilitates the ideation of novel concepts for application in regenerative medicine and other fields of life science.

1. Introduction

Human space exploration is an ambitious endeavor, the results of which may have surprised several aviation scholars. However, with technological advancements, the “mysteries of space” are gradually being solved. The National Space Agency (NASA) is currently conducting a Mars exploration program, and its field of exploration is progressively becoming intensive. Furthermore, the International Space Program and some private spaceflight programs aim to conduct extensive space exploration, which implies that further research is required to be conducted by astronauts themselves. However, for the sake of the health of astronauts, the damage inflicted upon the body by the variable environmental factors in outer space will be the major obstacle in future exploration missions.

There are several significant differences between the terrestrial and space environments, including the presence of strong ultraviolet rays, altered gravity, and considerably high temperature differences. Microgravity has been shown to affect numerous organ systems in the body, including the musculoskeletal, hematopoietic, endocrine, and digestive systems (Graebe et al., 2004; Setlow, 2000). The major physiological effects exerted by microgravity on these organs include bone loss (Zhang et al., 2018; Grimm et al., 2016; Smith et al., 2015), muscle atrophy (Bettis et al., 2018; Radugina et al., 2018; Zhao and Fan, 2013), metabolic disorder (J. Yang et al., 2018; Tascher et al., 2017; Hughson et al., 2016; Wang et al., 2015), and immune dysfunction

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(Kennedy et al., 2014; Fitzgerald et al., 2009; Crucian et al., 2009).

The reduced energy intake and weight loss experienced during a space flight suggests that the space environment has a significant impact on the digestive system of astronauts (Smith et al., 2004). The digestive system consists of a group of organs that extend from the oral cavity to the anus and break down food products, assimilate nutrients, and eliminate waste. The dysfunction of the organs mentioned above may cause certain associated diseases. Ensuring the proper functioning of the digestive system in a weightless environment is related to the physical health of astronauts as well as to the success of space missions (Institute of Medicine (US) Committee on the Longitudinal Study of Astronaut Health 2004). According to the current researches, the main reason why weightlessness affects the digestive system may be the change of systemic hemodynamics. Secondly, weightlessness, as a source of stress, will cause the coordinated response of the body, thus leading to changes in the functions of various organs of the digestive system. However, as the digestive system involves multiple organs, current studies mainly focus on the changes of each organ, and the specific mechanism of the changes in its function needs further study.

Meanwhile, to investigate the effects of microgravity, different methods that simulate microgravity were developed. At the cellular level, random positioning machines (RPMs), clinostats, and levitating magnets are most frequently used. At the subject level, a -6° head-down bed rest in humans and tail suspension (TS) or hindlimb unloading (HU) in rodents are two of the most commonly used models.

In this review, we would first discuss the methods to simulate the microgravity environment and then focused on the changes in the digestive system under a weightless or simulated microgravity(μG) environment as shown in Figure 1, which may provide us with some theories pertaining to preventive interventions prior to the emergence of related diseases. In addition, new opportunities for developments in medical science under a microgravity environment will also be discussed.

2. Methods of simulating a microgravity environment

Since weightlessness is of great significance to human health and the cost of space flight experimental research programs are considerably high, it is of particular importance to use a variety of methods to simulate weightlessness or realize microgravity under laboratory conditions to investigate its effects on humans. Presently, researchers are unable to create a long-term microgravity environment on ground, although ground-based simulated microgravity can be facilitated for experiments, which can be regarded as “functionally near weightlessness”, to explore the physiological effects of weightlessness on the body. In the field of space biology, a variety of research methods have emerged for the investigation of ground models of weightlessness or simulated microgravity (Table 1), and these efforts have strongly supported our understanding of the mechanism underlying the biological effects of weightlessness.

2.1. Cellular level

At present, the ground-based microgravity cytology experiential methods primarily include a 2D-clinostats, RPMs, a rotary cell culture system (RCCS), and a superconducting magnet levitation (SMI).

2D-clinostats are well-established as tools for μG experiments. These rely on clinorotation, which generates centrifugal forces of intensities that depend on the distance of the cells from the rotation axes and speed (Afonin, 2013). The functioning of 2D-clinostats is based on the principle that during the cell rotation experiment, the cells rotate continuously about the horizontal axis under the impact of the rotary device, and the gravity vector changes continuously with respect to the direction of the cells, such that the cells constantly change their direction. In this field, with the appropriate rotational speed and limited centrifugal force, the cells always exhibit a delayed response to the gravitational force, thereby simulating a microgravity environment.

RPMs systems are composed of an inner frame and an outer frame, both of which can rotate independently in random directions. Samples

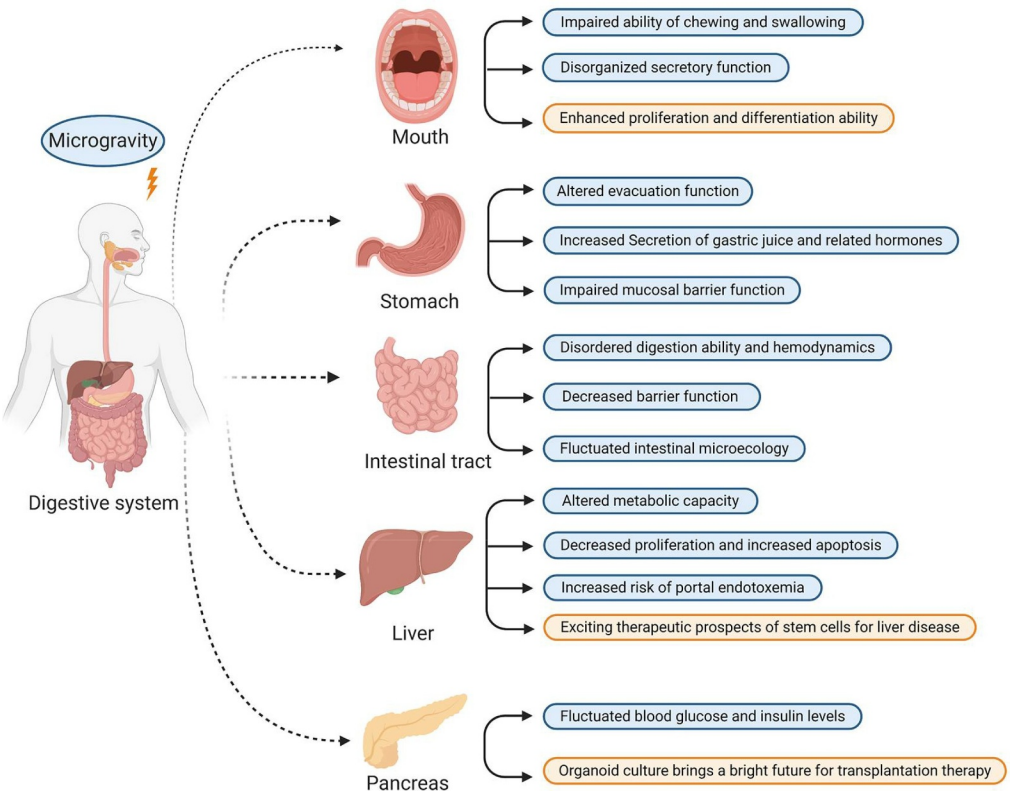

Figure 1. An overview of the changes of digestive system affected by microgravity environment.

Table. 1
Methods of microgravity environment simulation.

No.	Cellular level	Ref.	No.	Subject level	Ref.
1	2D-clinostats	(Afonin, 2013)	1	DI	(Tomilovskaya et al., 2019; Kermorgant et al., 2017; Treffel et al., 2016; Navasiolava et al., 2011)
2	RPMs	(Chen et al., 2019; Grimm et al., 2018; Ranieri et al., 2017)	2	HDT	(Hargens and Vico, 2016; Chouker et al., 2001)
3	RCCS	(Ethiraj et al., 2018; Penolazzi et al., 2016; Yang et al., 2016; Morabito et al., 2015)	3	TS or HU in rodents	(Sun et al., 2019; Andreev-Andrievskiy et al., 2018; Globus and Morey-Holton, 2016; Ohira et al., 2015; Chew and Segal, 1997)
4	SMI	(Afonin et al., 2007)	4	Anti-G suit	(Montmerle and Linnarsson, 2005)

contained in the inner frame are continuously reoriented; therefore, over time, the resultant gravity vector is averaged to zero (Chen et al., 2019; Grimm et al., 2018; Ranieri et al., 2017).

RCCS requires a dedicated container to be filled with a nearly homogeneous medium. The medium is directly inoculated with the cell suspension. The adherent cells are cultured on the microcarriers prior to their transfer to a container for cultivation. The container is then rotated about the horizontal axis. Concurrently, the culture, including the cells and the carrier, moves as the container rotates, and are simultaneously subjected to the action of gravity and liquid buoyancy to maintain a state of movement with a marginally circular trajectory in the container. During the rotation of the container, on one hand, the gravity vector continues to be randomly distributed, and on the other hand, the cells maintain a continuous state of free-fall motion, and the apparent gravity acting on the cell approaches zero, thereby creating the biological effect of simulated microgravity (Ethiraj et al., 2018; Penolazzi et al., 2016; Yang et al., 2016; Morabito et al., 2015).

In SMI cultures, the gravitational force is counterbalanced by a magnetic force, which impedes the sedimentation of molecules and atoms within cells. Under the environment created by this method, the cells aggregate in the interface between air and the culture medium, forming 3D spheroids. One of the major advantages of this approach is its compatibility with conventional multi-well plates and high-throughput formats. Nevertheless, the presence of a magnetic field may create certain artefacts (Afonin et al., 2007).

2.2. Subject level

Besides DI, a variety of methods of weightlessness simulation have been established, such as -6° head-down tilt (HDT) bed rest in human, TS or HU in rodents, and the anti-G suit.

The earliest experimental ground-based method used for microgravity simulation was DI (Tomilovskaya et al., 2019; Kermorgant et al., 2017; Treffel et al., 2016; Navasiolava et al., 2011), which is conducted in a special water tank. The subject is made to sit or lie down in the water, and the buoyancy and static pressure are utilized to simulate the physiological effects of fluid static pressure loss, such as body fluid redistribution, orthostatic hypotension, reduced muscle activity, and metabolic dysfunction.

HDT in humans and TS or HU in rodents are two of the most commonly used models that can mimic several physiological alterations in various organ systems caused during the actual spaceflight, including fluid shifts, muscle atrophy, bone demineralization, and suppressed cellular immunity. In HDT, the subject is required to be in a supine position or with their head in a low-laying position, such that the blood hydrostatic pressure in the body disappears partially or completely, which is similar to the physiological effect of microgravity (Hargens and Vico, 2016; Chouker et al., 2001). A large number of experiments have demonstrated that the response of multiple systems (including the circulatory, endocrine, and nervous systems) and organs (such as the heart, brain, and skeletal muscles) to a -6° head-down bed rest is similar to their response to microgravity. The TS or HU method is primarily used to simulate the physiological redistribution of blood to the head in a microgravity environment (Sun et al., 2019; Andreev-Andrievskiy et al., 2018; Globus and Morey-Holton, 2016; Ohira et al., 2015; Chew and Segal, 1997). In the study of simulated blood distribution, the positioning of the body axis at 30° from the ground is widely recognized.

An anti-G suit is used to exert pressure on the astronaut's abdomen and lower extremities to improve the properties of personal protective equipment against anti-overload capability, and is also known as the anti-load pant. The primary purpose of the equipment is to reduce damage to pilots while their body weight apparently increases under the given conditions (Montmerle and Linnarsson, 2005).

The existing ground-based methods of simulating microgravity are primarily used to recreate the effects of microgravity on particular organs, or to generate new forces that counteract the effects of gravity with the help of special devices; however, the subjects need to be in certain positions when such methods are used. The above methods of

simulating the microgravity environment needs to be further improved in order to realistically recreate the effects of weightlessness on the body. In addition, the simulated microgravity environment can also provide technical support for the co-culture of specific tissues to construct microorgans with certain functions, which we believe will contribute significantly to the field of life science in future.

3. Effects of microgravity on the digestive system

3.1. Oral cavity

The oral cavity, as the first organ of the digestive tract, realizes the initial physical digestion of food through the contraction of the masseter muscle and the chewing action of the teeth. During this process, the digestive juices secreted in the oral cavity also mix with the food and play a major role in the chemical digestion and lubrication. In addition, the combination of multiple muscle groups in the mouth also facilitate the transport of food to the stomach.

3.1.1. Ability to chew and swallow

The physical chewing action in the mouth is for grinding food adequately to further increase the contact area between the digestive juices and the food. This requires contraction of the masseter muscles to move the bones and the grinding of food by the shearing action of the teeth. The study in a microgravity environment demonstrated that the bone density and bone mineral content of the mandible and alveolar bone reduced significantly under microgravity compared to that in the control group (Rai et al., 2010). In addition, after a week under simulated microgravity, the muscle fibers of the masseter in rats were partially dissolved. The effect reduced in the second week and was completely alleviated in the fourth week (Zhang et al., 2013).

3.1.2. Secretory function

The fluid secreted by the salivary glands lubricates food and is also rich in digestive and immune enzymes. Mednieks et al. reported that the composition of salivary proteins changed in mice under microgravity, in particular, the levels of salivary amylase and proline-rich proteins reduced. Further studies have demonstrated that this may be related to the cAMP signaling pathway, and the specific protein content in saliva can be used as a potential biological marker for the occurrence of stress events during a space flight (Mednieks et al., 2014). In the study by Huai et al., subjects under simulated microgravity exhibited increased concentration and secretion rates of secreted immunoglobulin A (sIgA); this might be related to immune stress experienced under microgravity (Huai et al., 2012). Rai et al. demonstrated that the increased levels of matrix metalloproteinases (MMP) − 8 and MMP-9 may be related to the immune response induced by bacterial virulence under microgravity (Rai et al., 2010).

3.1.3. Proliferation and differentiation of stem cells

Periodontal ligament stem cells are one of the key seed cells in periodontal tissue regeneration engineering and plays a crucial role in the treatment of periodontal disease. The study by Li et al. revealed that microgravity can promote the osteogenic differentiation of human periodontal stem cells by activating the Smad signaling pathway (Li et al., 2012; Li et al., 2009). In addition, the study by Zhang et al. also confirmed that the proliferative capacity of human dental pulp stem cells followed a trend of enhancement under microgravity (Zhang et al., 2014).

In the above studies, the effects of microgravity on bone density, muscle tissue, and digestive enzyme content had adverse impacts on the initial digestive capacity of the oral cavity. However, the improvement of the ability of the stem cells to differentiate and proliferate under microgravity also provides novel ideas for the development of regenerative medicine. In addition, the increased secretion of sIgA is one of the manifestations of the body's response to microgravity stress. However, the specific mechanism underlying these changes requires further investigation.

3.2. Stomach

As the most inflated part of the digestive system, the stomach stores food temporarily by receptive relaxation, and also facilitates the complete mixing of chime with the digestive enzymes through gastric peristalsis. In addition, the gastric wall also contains a large number of endocrine cells, and the hormones released from these cells play a key role in regulating the secretion of digestive juices.

3.2.1. Evacuation function

Afonin et al. used DI to simulate the effects of microgravity on the stomach evacuation function. The results indicated that there was no significant effect on the gastric emptying of liquid food (Afonin et al., 2011). Prakash et al. also confirmed that there was no significant delay in gastric emptying under simulated microgravity (Prakash et al., 2015). However, notably, the study by Pei and Chang demonstrated that the considerably slow gastric movement rhythm increases under the -6° head-down position of a simulated microgravity environment, indicating a dysfunction in the gastric movement rhythm (Pei and chang, 1997). The study by Li et al. showed that the interstitial cells of Cajal in the rat stomach were affected by the simulated weightlessness, which may induce gastric pacing and slow wave disorder, leading to gastric motility disorder (Li et al., 2013).

3.2.2. Secretion of gastric juice and related hormones

In the study by Afonin, the monitoring of data received from studies conducted on astronauts revealed that under microgravity, the activity of gastric and pancreatic secretion (insulin and C-peptide) increased during the early stages after space flight. The secretory changes in the stomach, characterized by more intensive secretion of gastric juices, lowered pH, and elevated pyloric tone under the microgravity environment, may be related to changes in the venous hemodynamics of the abdominal organs (Afonin, 2013). In another study by Afonin and Sedova, after 4 months of bedrest, the subjects displayed increased blood and urine levels of pepsinogen, indicative of gastric hypersecretion (Afonin and Sedova, 2009).

Guo et al. observed that in the early period under weightlessness (6–12 h), the serum GAS content increased in tail-suspended rats; however, with the extension in TS time, the serum GAS content gradually reduced (Guo et al., 2013). In the study by Riepl et al., certain experiments were conducted using the fasting blood samples of the astronauts on the EUROMIR-94 mission; the fasting plasma levels of motilin, pancreatic polypeptide (PP), vasoactive intestinal peptide (VIP), and secretin increased and the levels of plasma cholecystokinin (CCK) decreased under acute exposure of the astronauts to microgravity. Chronic (4 week) exposure induced an enhancement of plasma CCK, motilin, neurotensin, VIP, and insulin, whereas the plasma concentrations of PP, secretin, gastrin, and somatostatin exhibited no changes. During the 25 day stay at the MIR station, the plasma levels of CCK, motilin, and neurotensin increased. Short-time body rotations caused an elevation of the plasma levels of PP, while it reduced the levels of plasma motilin (Riepl et al., 2002). In a study Wang et al., the number of EC cells in the gastric antrum and duodenum increased significantly, and the secretion and function of gastrin and 5-hydroxytryptamine (5-HT) were enhanced under simulated microgravity (Wang et al., 1995). Chen et al. further confirmed the reduction in plasma ghrelin levels and the increase of VIP content in rats under simulated microgravity (Chen et al., 2012). The changes in the levels of these hormones reflect the levels of digestive juice secretion, and further affect the digestive ability of the body.

3.2.3. Mucosal barrier function

The mucosa is a membrane-like structure composed of epithelial and connective tissue. Mucus secreted by the gastric epithelial cells can form a barrier with bicarbonate to protect the gastric wall.

In the study by Atiashkin and Bykov, microfocal lesions in the mucus coat, dystrophic developments in the acid glands, dissociation of the mucus barrier function, and deterioration of its biosynthetic function were observed in the stomach of the subjects after the 12 day Foton-M3 flight

(Atiashkin and Bykov, 2012). Guo et al. reported that the gastric mucosal barrier of tail-suspended rats under may suffer local and systemic stress-induced damage during the early stage. The specific manifestations were nuclear shrinkage and deformation, mitochondrial swelling, and endoplasmic reticulum expansion in certain gastric mucosal adenocytes, caused by the increased expression of superoxide dismutase (SOD), malondialdehyde (MDA), and nitric oxide (NO) (Guo et al., 2014). In addition, Zhang et al. reported that the healing rate of experimental gastric ulcers induced by acetic acid significantly reduced under simulated microgravity (Wang et al., 1997; Zhang et al., 2012).

From the above findings, we can infer that under microgravity, the stomach exhibits gastric motor dysfunction and hypersecretion, and is more vulnerable to injury. However, existing studies also suggest that the gastric emptying function is not significantly affected under a weightless environment, possibly owing to the body's self-regulatory function. More importantly, gastric hypersecretion and impaired mucosal barrier function will further aggravate the incidence of related diseases. These experimental results can provide theoretical support to the medical care of astronauts; however, the specific mechanism underlying abnormal gastric function needs to be investigated further.

3.3. Intestinal tract

Intestinal mucosal cells are responsible for the absorption of nutrients, and also serve as a natural barrier between the host and the colonizing flora of the intestinal tract. Additionally, the intestinal microbiota is essential for regulating epithelial integrity, immune system maturation, and energy metabolism (Henning et al., 2014; Sommer and Bäckhed, 2013). The disruption of or defects in epithelial integrity may influence microbial colonization and allow free passage of microorganisms or other substances across the epithelial barrier where they stimulate the immune system and induce inflammation, autoimmunity, allergy, and even carcinogenesis (Zhang et al., 2015; Peterson and Artis, 2014).

3.3.1. Changes in digestion and hemodynamics

Rabot et al. showed that SD rats aboard the US space shuttle exhibited changes in the intestinal short-chain fatty acid spectrum and concentration after 9 and 14 days of flight (Rabot et al., 2000). Afonin et al. and Pei and Chang reported that the rate of intestinal content emptying was accelerated (Afonin et al., 2011, 48). This may be related to the alterations in gastrointestinal hemodynamics. Relevant studies have demonstrated that the effects of microgravity on blood circulation in the gastrointestinal tract primarily manifest as increased vascular volume and reduced flow velocity in the resting state. Zhang et al. reported that the contractility of the mesenteric arterioles in rats reduced significantly under simulated weightlessness (Zhang and Ma, 1998), while Dunbar et al. reported that the mesenteric vessels exhibited reduced reactivity to vasoconstriction induced by sympathetic nerve stimulation (Dunbar et al., 2000).

3.3.2. Changes in intestinal mucosal cells and intestinal permeability

Studies by Shi et al. have demonstrated the reduction of the regeneration rate of colonic epithelial cells, the number of goblet cells, and the expression of genes associated with defense and inflammatory response under microgravity (Shi et al., 2017). Rabot et al. also reported the reduction in the number of intestinal goblet cells in mice aboard a spacecraft (Rabot et al., 2000). Bai et al. confirmed that under simulated microgravity, rats underwent observable pathological changes in the cecal epithelium, characterized by disordered and sparse arrangement of epithelial villi (Bai et al., 2001). Chen et al. confirmed that the expression of tight junction proteins between small intestinal mucosal epithelial cells in tail-suspended mice was reduced significantly, which would subsequently damage the intestinal mucosal barrier, increase intestinal permeability, and increase the risk of colonization by opportunistic pathogens (Chen et al., 2011). Li et al. confirmed that the levels of NF-κB increased significantly in the ileal mucosal tissue of tail-suspended rats, and proposed that this may have resulted from a stress reaction in the intestinal mucosa cells under microgravity and would further regulate the expression of downstream genes and resultantly, damage mucosal cells (Li. et al., 2008). Bao et al. observed that the mucosal damage induced by abdominal infection in mice was further aggravated under microgravity than under normal gravity (Bao, 2015). In addition, Yao et al. showed that the inflammatory response in mice infected by E. coli was enhanced in a simulated microgravity environment, thereby causing further damage to the intestinal mucosal barrier (Yao et al., 2016). Liu et al. reported that the expression levels of microRNAs (miRNAs) in macrophages infected by E. coli under simulated weightlessness had significant differences, and proposed that these differentially expressed miRNAs may further aggravate the inflammatory response by regulating MAPK, WNT, TGF-β, and other signaling pathways (Liu et al., 2017).

3.3.3. Changes in intestinal flora and microecology

There are a large number of microbial parasites that colonize the human intestinal tract, which play an important role in the nutritional and metabolic process of the body under normal conditions; however, alterations in the homeostasis between the host and the colonizing bacteria may also lead to the incidence of diseases (Okumura and Takeda, 2018; Tian et al., 2018). Huang et al. investigated relevant studies and concluded that the morphological structure, metabolic activity, virulence, and pathogenicity of bacteria and other microorganisms changed significantly in a microgravity environment (Huang et al., 2014). Shi et al. reported that under microgravity, the intestinal flora of mice underwent significant changes that manifested as an increase in the concentration of Firmicutes and a reduction in that of Bacteroides (Shi et al., 2017). In addition, some studies have suggested that the growth rate of intestinal flora, such as E. coli and Clostridium difficile, is significantly accelerated in a weightless environment, whereas the protective flora, such as bifidobacteria and lactobacillus, reduced in numbers or were even eliminated (Y. Yang et al., 2018). Shao et al. demonstrated that under a simulated microgravity environment, L. acidophilus exhibited enhanced acid resistance and reduced sensitivity to cefuroxime, gentamicin sulfate, penicillin sodium, and other antibiotics (Shao et al., 2017). In addition, Chopra et al. confirmed that the virulence of Salmonella sp. was significantly enhanced when cultured in a microgravity environment using a biofilter, and the expression of heat-resistant enterotoxins in pathogenic E. coli increased as well under similar conditions (Chopra et al., 2006). Theoretically, all of these changes to the intestinal flora are harmful to the body to an extent.

Notably, NASA's space experiment confirmed the increased resistance of certain intestinal microbes to antibiotics (Rivera et al., 2003). This undoubtedly increases the challenges of treating infections in a microgravity environment. Li et al. suggested that the intestinal microflora and the innate immune system both respond to simulated microgravity and contribute to the proinflammatory shift in the gut microenvironment. The data also emphasize the necessity of evaluating the susceptibility to inflammatory bowel diseases in astronauts (Li et al., 2015). As astronauts execute space missions, intestinal disorders in them may affect their health adversely, and may severely affect the implementation of space missions. Although direct evidence is lacking, astronauts are recommended to maintain a regular intake of probiotics to reverse these changes and maintain a microecologic balance (Barzegari and Saei, 2012; Somova and Pechurkin, 2005). In addition, Yang et al. suggested that estrogen may reduce the susceptibility to autoimmune diseases such as colitis by regulating gut microbiota under microgravity (Yang et al., 2018). This conclusion may provide a solution for future challenges.

Under microgravity, the digestive capacity of the intestinal tract was observed to reduce and the state of emptying was accelerated; this was possibly related to a disorder in intestinal hemodynamics. The results of studies on intestinal mucosal cells and intestinal permeability suggest that the permeability of the gastrointestinal tract increased and the mucosal barrier function reduced in a weightless environment. In addition, the increased resistance, virulence, and pathogenicity of bacteria and other microorganisms under weightlessness form a vicious circle along with increased intestinal permeability, thereby increasing the susceptibility to intestinal infection. At present, there are no reports on the specific

mechanism underlying the above changes in a weightless environment.

3.4. Liver

The liver is an important organ associated with metabolism and detoxification. Multiple studies have reported that the levels of proteins and glycogen, as well as the functional structure of various enzymes, changed in the liver under a simulated microgravity environment. The oxidative stress in the liver also increased. The blood flow in the portal veins reduced and the levels of endotoxins increased (Merrill et al., 1992).

3.4.1. Metabolism

As the largest human organ associated with metabolism and detoxification, the functions of the liver depends on the complex metabolic processes in hepatocytes. Any factor that affects the integrity of hepatocytes or metabolic processes will have a significant impact on the detoxification capacity of the liver. Anselm et al. performed proteomic analysis on the liver cells of mice flown on "Bion-M1″ and observed that 218 proteins related to the gluconeogenesis process were upregulated, while proteins related to the lipid peroxidation stress response of the liver were downregulated (Anselm et al., 2017). Jonscher et al. reported that mice flown on the Space Transportation system-135 Space flight experienced weight loss and lipid redistribution (Jonscher et al., 2016). Abraham et al. observed that rats flown on Cosmos 936 exhibited a change in the expression of 30 carbohydrate and lipid metabolism-related enzymes, including a significant reduction in the activity of glycogen phosphorylase, α-glycerol phosphate acyltransferase, diacylglycerol acyltransferase, aconitic acid enzyme, and glucose-6-phosphate dehydrogenase, and a significant increase in the activity of the soft fatty acyl coenzyme A desaturation enzyme. The results indicated the accumulation of glycogen in hepatocytes and reduction in the levels of hexadecanoic and hexadecenoic acids (Abraham et al., 1981). Merrill et al. also revealed the changes in the expression of proteins and glycogenase-related enzymes in the hepatocytes of rats flown on Cosmos 2044 (Merrill et al., 1992). Notably, Chen et al. showed that under microgravity, the levels of cytochrome P450 (CYP450) isoform proteins and mRNAs related to drug metabolism changed in rats (Chen et al., 2018). Lu et al. also confirmed that the activities of CYP2C11, CYP2E1, and P-glycoprotein were significantly inhibited (Lu et al., 2002).

3.4.2. Proliferation and apoptosis of liver cells

The metabolic and detoxification potential of the liver are closely related to the remarkable regenerative ability of the liver. Specifically, the liver can rebuild 70% of lost tissue within a few weeks, which depends on the activation, transdifferentiation, metaplasia, or compensatory proliferation of quiescent hepatocytes (Joan et al., 2015). Therefore, the increase in hepatocyte apoptosis would reduce the number of hepatocytes and further affect the normal function of the liver. The regeneration of the liver is controlled by coordinated signaling events. The increasing number of evidence-based experiments conducted under normal gravity suggest that cell proliferation, apoptosis, tissue remodeling, and tumorigenesis are tightly regulated by microRNAs (miRNAs) (Chen et al., 2016; Bei et al., 2016; Tanimizu et al., 2014; John et al., 2014). On one hand, a series of studies have demonstrated that under a weightless environment, the proliferative potential of hepatocytes undergoes reduction. Chen et al. showed that the expression of proliferation markers Ki67, PCNA, and PH3 was significantly inhibited, and concurrently, the cell cycle regulatory molecules (CCNA2, CCND1, CDK1, and CDK2) were downregulated in the liver of tail-suspended rats. RT-qPCR analysis revealed that only the levels of miR-223 were significantly elevated in the liver of the tail-suspended groups (Chen et al., 2017). Chen et al. speculated that the miR-223 may play a key role in the regulation of hepatocyte proliferation under microgravity. On the other hand, Cui et al. showed that weightlessness was closely related to hepatocyte apoptosis, and the expression of p53 in the hepatic tissues of rats under weightless conditions was consistent with that observed during hepatocyte apoptosis (Cui et al., 2010). Cui et al. also reported that under a simulated microgravity environment, rat hepatocytes underwent detrimental changes, such as chromatin concentration and marginalization, mitochondrial swelling, endoplasmic reticulum expansion, and the appearance of apoptotic bodies (Cui et al., 2010; Cui et al., 2008). Du et al. also confirmed that prolonged exposure to microgravity induced significant damage to the liver and triggered hepatocyte apoptosis (Du et al., 2015). Notably, a study by Tian et al. revealed that in a simulated microgravity environment, the proliferative capacity of mice liver Kupffer cells reduced during stress injury (Tian et al., 2016).

3.4.4. Portal endotoxemia

The liver's remarkable metabolic function is inseparable from its unique dual blood supply system comprising the hepatic artery and portal vein. The liver receives approximately two-thirds of its blood supply from the portal vein, nearly 75% of which is routed through the mesenteric vein. This implies that once the intestinal barrier is damaged, a large number of bacteria and their products can enter the liver via the portal vein. The imbalance between intestinal flora and the intestinal mucosal barrier in a weightless environment has been described earlier. Zhou et al. demonstrated that subjects who were continuously under -6° head-down bedrest exhibited a reduction in the maximum portal vein flow velocity with time, and the portal vein flow velocity gradually recovered after they returned to the upright position, and was nearly normalized on the 7th day (Zhou et al., 2010). Riviera et al. conducted experimental studies on animals under ground-based simulated microgravity and confirmed that intestinal peristalsis disorder, bacterial flora displacement, and intestinal mucosal barrier damage induced by environmental changes causes endotoxin components to enter the portal vein, which subsequently damage the liver (Rivera et al., 2003). Zhou et al. observed that the levels of portal endotoxin in rats under simulated weightless conditions increased significantly at 6 h and peaked at 12 h of exposure. The endotoxin levels gradually reduced till 5 days, and re-elevated at 7 days (Zhou et al., 2011). Further research revealed abnormal changes in the ultrastructure of rat hepatocytes under microgravity, including changes to the chromatin, mitochondria, and endoplasmic reticulum, a finding consistent with the above mentioned reports of hepatocyte injury in a weightless environment.

3.4.4. Stem cell therapy for liver

Stem cells are relatively undifferentiated cells that retain the ability to divide and proliferate throughout postnatal life, and resultantly generate progenitor cells that can differentiate into specialized cells. Based on the developmental stages, there are two major types of stem cells; embryonic stem cells, derived from embryos, and adult stem cells (also referred to as somatic stem cells), which can be isolated from tissues such as bone marrow and fat.

In recent years, several studies have focused on stem cell differentiation for tissue repair or organ transplantation. Currently, the only treatment for patients with end-stage liver disease is liver transplantation. However, the number of organ donations does not match up to the clinical requirement, which has led to the death of several patients due to organ failure or serious complications encountered during the waiting period. As an emerging technology, stem cell differentiation is expected to function as a useful alternative to liver transplantation in patients with end-stage liver disease. Microgravity helps subjects achieve a floating state by creating non-effect of acceleration. It has been reported by various groups of researchers that microgravity promotes proliferation and differentiation of stem cells (Ko et al., 2007; Herrera et al., 2006; Lelkes et al., 1998).

Majumder et al. cultured murine oval liver stem cells in a microgravity environment simulated by an indigenously fabricated 2D-clinostats. Microgravity affects the BMP4/Notch1 signaling in stem cells, inducing the differentiation of stem cells into hepatocytes. The study also denoted that microgravity treatment for 2 h enhanced the proliferation of stem cells by two-fold without inducing apoptosis and compromising cell viability. In addition, microgravity alone can induce the differentiation of stem cells within 2–3 days (Majumder et al., 2011). The PICM-19 pig liver stem cell line was cultured in space for nearly 16 days during the STS-126 mission to

assess the effects of spaceflight on the differentiation capacity of the cells. However, when the flight and ground-control cultures were compared 17 h after the space shuttle returned to Earth, no differences were observed, with the exception of some genes that were differentially expressed (Talbot et al., 2010). Talbot et al. analyzed the possible causes of this phenomenon and suggested that the STO feeder-cell-mediated three-dimensional growth effects on PICM-19 cells might have overpowered the gravitational effects.

The reduced proliferation and increased apoptosis of hepatocytes in a weightless environment directly affect the metabolic and detoxification functions of the liver, a finding which is consistent with the results of relevant studies. In addition, the increase in portal endotoxin levels further confirms the high risk of intestinal infections under weightlessness. The enhanced proliferation and differentiation of stem cells under a microgravity environment provides a novel research idea for regenerative medicine and organ transplantation. However, the existing studies only involve two cell lines, and the investigation of the differentiation ability of other cell lines requires further study. Moreover, the safety and functional assessment of humanized animal models associated with stem cell differentiation is yet to be conducted. In addition, the specific mechanism underlying the contrasting proliferation capacity of hepatocytes and stem cells in a weightless environment needs to be explored further.

3.5. Pancreas

As the major secretory gland in the human body, the function of the pancreas primarily consists of the secretion of pancreatic juice necessary for digestion and various hormones necessary for the regulation of homeostasis in the internal physiological environment. In addition, pancreas transplantation has received more and more attention in the treatment of diabetic patients. But the current treatment is limited to islet tissue that can be transplanted. The promotion effect of weightlessness on organ culture shows a good prospect. If progress in this direction is carried out, the treatment of islet transplantation will bring good news to the majority of diabetic patients.

3.5.1. Changes in glandular status and function

The primary function of the pancreas is to secrete pancreatic juice, which contains a variety of digestive enzymes, and insulin, which regulates blood sugar levels. MIyake et al. reported the shrinking of pancreas in SD rats flown on the Columbia spacecraft (Miyake et al., 2004). In addition, Macho et al. observed a significant increase in blood glucose levels in Wistar rats flown on Cosmos 2044, along with an increase in plasma insulin levels (Macho et al., 1991). Liu et al. observed that the blood glucose levels in tail-suspended rats fluctuated during a 7-day experiment, and the insulin levels increased during the early stage, peaked at 6 h, and then reached a trough, along with the levels of C-peptide, at 3 days; meanwhile the glucagon levels remained high (Liu et al., 2012). Further research revealed that HSP70 expression increased in the pancreatic tissues of tail-suspended rats, especially in the pancreatic β cells (Liu et al., 2013). These findings suggest that microgravity can affect the state of the pancreas and glucose metabolism.

3.5.2. Pancreatic islet transplantation therapy

Pancreatic islet transplantation therapy has garnered significant attention as a useful method for the long-term alleviation of diabetic symptoms. The major problems currently encountered are insufficient number of donors, rejection of recipients, and realization of functionality after transplantation. If these obstacles are removed, islet transplantation therapy will greatly improve the cure rate of diabetic patients.

The in vitro culture of islet cells was followed by a stationary culture step, in which cells only grow in a single layer along the horizontal plane, the cell density is low, and there is no differentiation. Studies have shown that microgravity can induce 3D clump formation in cells. Song et al. confirmed that the simulated microgravity environment can increase the survival rate of rat islets cultured in vitro, increase the number of cells, and enhance the secretory function of the islet cells (Song et al., 2004). In a study by Luca et al., insulin secretion was observed in rat–pig islet constructs following elevated glucose stimulation. However, it was not significantly higher than that obtained from islets cultured alone. This indicated that the apparent encapsulation of the neonatal porcine cell clusters by Sertoli cells created a barrier to the optimal media and inhibited oxygen perfusion to the centrally-positioned islet cells, evidenced by necrosis in the central tissues (Luca et al., 2006). Rutzky et al. demonstrated that microgravity can reduce the immunogenicity of islets by eliminating the class II MHC-expressing dendritic cells (Rutzky et al., 2002). Han et al. co-cultured tissue-like Sertoli-islet cell aggregates under microgravity in an rotating wall vessel bioreactor (Han et al., 2009). Co-cultivation in this bioreactor is advantageous as it reduces the immunogenicity of the islets and enhances the attachment of the islets to the Sertoli cells. Notably, these aggregates were more active in terms of insulin secretion and had higher graft survival rates.

It is well known that the intercellular interactions within the islets of Langerhans are important for their functional competence. Tanaka et al. used a clinostat to optimize the culture conditions, which resulted in the induction of a large number of pancreatic β-cell spheroids of the required size, depending on the cell density (Tanaka et al., 2013). They produced a large number of β-cell spheroids of a certain size that were optimal for islet transplantation. This culture system in a simulated microgravity environment may maximize the self-assembly of cells and subsequent spheroid formation. It also provides more opportunities for future islet cell source selection. In addition to identifying more sources of cells, improving the current cell culture conditions can also make up for the shortage of donors. The polyglycolic acid scaffold (PGA) is believed to confer certain advantages to cell culture. Song et al. reported that under PGA-μG conditions, the islets had a purity $\geq$85%, a higher survival rate, and an enhanced ability to secrete insulin compared to islets cultured alone under static, μG, or PGA conditions (Song et al., 2013).

The fluctuations in blood glucose levels and irregularities in the insulin and glucagon levels in weightless environments may be the eventual result of the body's self-regulatory activity in a stressful environment. The specific regulatory mechanism and the energy utilization state of the organism under weightless conditions need to be investigated further. The microgravity environment can provide a greater number of patterns for the culture of islet cells and ensure specific secretory activities in these cells. The reduced immunogenicity of islet co-culture under microgravity can also reduce the difficulty of islet transplantation. With respect to the insufficiency in the number of islet transplantation donors, the current research results may provide certain promising methods for islet transplantation; however, the specific mechanism needs to be explored further to provide new ideas for the transplantation of other organs.

4. Conclusions and perspectives

As a critical factor in the space environment, microgravity widens the gap between the space and ground environments, and moreover, its adverse effects on the human body cannot be ignored. In the process of exploring and further understanding the effect of weightlessness on the human body, the development of aerospace medicine can help improve the physical and mental health of astronauts, and also provide a theoretical basis for the treatment of certain diseases. Based on the above studies, we observed that the experimental subjects could undergo a variety of reactions in the process of adapting to a new microgravity environment. On one hand, microgravity and the changing space environment may exert adverse effects on the human body. First, the abnormal secretory activity of the oral cavity, stomach, pancreas, and liver may primarily result from a compensatory response related to rising hormone levels while the body is in a state of stress. This may be partly responsible for the disruption of biorhythms in the body of an astronaut. Second, the weakening of gastrointestinal motility is a direct consequence of hemodynamic disorder in a weightless environment. Meanwhile, the imbalance in the intestinal microecology and the weakening of the intestinal barrier function may increase inflammation along with hemodynamic disorder, or even directly cause liver damage. In addition, as the largest human organ associated with metabolism and

detoxification, the reduced proliferative capacity, increased apoptosis of hepatocytes, and the changes in the expression levels of metabolism-related enzymes also have significant effects on the generation and utilization of energy in astronauts. On the other hand, the weightless environment also provides novel ideas for life science research. The enhancement of the ability to induce differentiation of stem cells under microgravity may provide new ideas for stem cell research as well as solutions to the most common problem encountered during transplantation—the shortage of donors. Similarly, islet transplantation has been regarded as a novel idea in the treatment of diabetes in recent years. Notably, the combined culture under microgravity can also solve the issues related to immune rejection to a certain extent.

In summary, with continuous advancements in aerospace engineering, the impacts of the space environment on the human body will be gradually understood. Exploring the damage to the human body caused by the space environment and its underlying mechanisms could result in more comprehensive preparations prior to the execution of the mission. Further efforts should be made to explore additional ways to overcome the unfavorable environment in space, which will help realize the “aerospace dream” of humankind. Concurrently, the surprising advantages offered by the environmental change will bring forth new ideas and opportunities to several disciplines of life science. The exploration of space is difficult and tedious; however, researchers continue with their efforts in the pursuit of science. Moreover, to complement the developments in the aerospace industry, the space medicine industry requires greater efforts from medical workers. In future, we believe there will be more useful findings from laboratories worldwide that would strongly support human space exploration missions.

Declaration of Competing Interest

There is no conflict of interest that should disclose.

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