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A COMPREHENSIVE REVIEW ON IMPACTS OF MICROGRAVITY ENVIRONMENT ON THE STRUCTURE AND FUNCTION OF LIVING CELLS

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ABSTRACT
The concept and understanding the effects of microgravity on the functions of living cells (both eukaryotic and prokaryotic) is important as it accounts for the safety and health of astronauts during space flight. It's a fundamental question regarding the evolution of terrestrial life that how cells sense gravity and adapt in its absence and the mechanism of general mechanosensory responses of all living organisms. Other molecular organizations are also altered in the microgravity environment. The mass and architecture of the skeletal system adapt, to some extent, also influenced by their mechanical environment. Biochemical data of astronauts and histomorphometric analysis of rat bones show that the change in bone mass formation appears to be due in part to decreased osteoblast differentiation, matrix maturation and mineralization. Most of the microgravity researches on plant cells are concentrated on their cell root growth pattern, development of cell wall and gene regulation pattern. Most of the researches on plant cells have been done on *Arabidopsis thaliana*. Each of these studies has been found very important applications in space researches as well as for the future design of materials on earth.

Keywords: Histomorphometric, Osteoblast, Mechanosensory, Microgravity

INTRODUCTION
Gravity is a force that governs the motion throughout the universe. It holds us to the ground, and it keeps the moon in orbit around Earth and Earth in orbit around the sun. The nature of gravity was first described by Sir Isaac Newton, more than 300 years ago [1]. Gravity is the attraction between any two masses, most apparent when one mass is very large (like Earth). The acceleration of an object toward the ground caused by gravity alone, near the surface of Earth, is called "normal gravity," or 1g. This acceleration is equal to 32.2 Ft. /sec^2 (9.8 m/sec^2). The condition of microgravity comes about whenever an object is in free fall. Objects in a state of free fall or orbit are said to be weightless. Microgravity allows new materials to be developed which cannot be made on Earth due to gravity. These new materials can be used to speed up future computers, reduce pollution, improve fiber optics, and enable medical breakthrough to cure diseases. It is best illustrated by astronauts floating in their spacecraft. They are floating because they are in a microgravity environment. Besides astronauts, many people experience microgravity every day by riding roller coasters or jumping off diving boards. It is the "free fall" period of these activities when the microgravity occurs and of course only lasts

for a short period of time. All major physical components of gravity can have important effects on cell function. For instance, compression due to hydrostatic pressure, can affect the internal load-bearing structure of the cell (actin, microfilament, and microtubule cytoskeleton) making it more or less resistant to compression. Altered gene expression, reduction in transcription factors and a reduction in growth factor related proteins have been observed in microgravity. Specifically, it has been demonstrated that cell growth and differentiation of osteoblasts is inhibited or altered in the microgravity environment [2]. Cytoskeletal changes in actin, intermediate filament and microtubule networks have been found in microgravity as well. In the further sections of this review microgravity and its effects have been described[19-22].

HOW IS MICROGRAVITY ACHIEVED?

In a practical sense, microgravity can be achieved with a number of technologies, each depending upon the act of free fall. Microgravity can be created in two ways. Because gravitational pull diminishes with distance, one way to create a microgravity environment is to travel away from Earth. To reach a point where Earth's gravitational pull is reduced to one-millionth of that at the surface, you would have to travel into space a distance of 6.37 million kilometers from Earth (almost 17 times farther away than the Moon). This approach is impractical, except for automated spacecraft, since humans have yet to travel farther away from Earth than the distance to the Moon. However, a more practical microgravity environment can be created through the act of free fall (20).For research; microgravity is obtained by five methods [3].

1. *1. Drop tower*: The payload is simply dropped off the top of the tower and allowed to fall. This can provide 25 seconds of microgravity at a cost of pennies per pound.
2. *2. Sounding rocket*: The rocket goes up and comes down in the same general vicinity, never getting into orbit. Today's sounding rockets can provide between 3 to 9 minutes of microgravity. A sounding rocket can do much better, with a maximum duration of a quarter of an hour and microgravity that can be as low as 0.00001 g - one hundred-thousandth of Earth normal.

3. *Airplane flying parabolas*: The semi-famous example is the NASA Vomit Comet which is also used to train astronauts to work in a microgravity environment. This method can provide 25 seconds of microgravity at a cost in the range of a few dollars per pound.

4. *Space Shuttle or International Space Station*: Days of microgravity can be achieved. The cost for putting a payload in orbit with the Space Shuttle is $10,000 per pound. Once available, the estimated cost for having commercial payloads on the International Space Station is $15,000 per pound.

5. *Slowly spinning an object*: This is not a true microgravity environment but simulates microgravity in certain processes. Those processes must have slow reaction times such as many biological processes. The axis of rotation must be parallel to the ground and only provides benefits in certain applications.

A common misconception is that gravity disappears when in low earth orbit (LEO), like the International Space Station. This is not the case. LEO is not far away enough from the Earth's surface to have decreased gravity - in fact, gravity there is similar to its intensity on the surface[4].The weightless effect is only caused because the objects on the space station and that space station are in constant free fall.

Microbial Growth Kinetics Under Conditions of Microgravity

Microbial Growth Kinetics under Conditions of Microgravity-4 (Biokin-4) will determine the characteristics of bacterial growth kinetics under microgravity conditions to develop a system for the removal and complete oxidation of gaseous and airborne contaminants originating in confined atmospheres with the use of microorganisms in order to purify and recycle air in manned space aircraft. Water and air are essential raw materials for manned space missions. Recycling of these materials is one of the biggest challenges in the further space exploration. The application of biotechnological techniques with as ultimate goal a fully closed ecological life support system is seen as the only solution[5]. The principle of the Biological Air Filter (BAF-system) is based on a support/sorbent membrane material colonized by selected micro-organisms in a near resting state, oxidizing the various contaminants. Two experimental BAF modules have already been designed, constructed and successfully tested. Laboratory experiments with the experimental models showed a high efficiency for the removal of the selected contaminants and demonstrated a good potential for space application. Main objective of the presented work is the determination of the influence of the space environment on the growth-kinetics of the biodegradation of the air-contaminant 1, 2-dichloroethane by micro-organisms at the degradation of organic volatile contaminants. The overall growth rate that the bacterial cells can reach depends on 1) the prevalent substrate concentration(s), 2) a cascade of biochemical reactions involved in the metabolism of the substrate, including the uptake and transport of the substrates, and 3) the kinetic properties of the enzymes involved in these reactions, and 4) the prevailing environmental conditions, such as temperature, pH and for instance the absence or presence of gravity. In the space where sedimentation of the bacterial cells is absent, it is thought that concentrations of physiologically important cellular metabolites will leak out of the bacterial cells. In the absence of gravity the cells will remain in close proximity to these excreted or diffused metabolites, leading to little energy loss. In the presence of gravity (1 g) the cells will sediment away from these metabolites leading to energy loss. Therefore it is hypothesized that bacterial cells modify their micro-environment in such a way resulting in (i) increased maximum specific growth rates/ substrate degradation rates in space as compared to growth on Earth, (ii) increased substrate affinities in space as compared to growth on Earth, (iii) increased molar cell yields in space as compared to growth on Earth and (iv) higher concentrations of excreted (by) products or metabolites on Earth as compared to growth in space [6].

The Effect of High Level Radiation on DNA Reparation Mechanisms in Microgravity Conditions

To study the influence of microgravity on radiobiological processes in space, space experiments have been performed, using an on-board 1×g reference centrifuge as an in-flight control. The trajectory of individual heavy ions was localized in relation to the biological systems by use of the Biostack concept, or an additional high dose of radiation was applied either before the mission or during the mission from an on-board radiation source [7, 8]. In embryonic systems, such as early developmental stages of *Drosophila melanogaster* and *Carausius morosus*, the occurrence of chromosomal translocations and larval malformations was dramatically increased in response to microgravity and radiation. It has been hypothesized that these synergistic effects might be caused by an interference of microgravity with DNA repair processes. However, recent studies on bacteria, yeast cells and human fibroblasts suggest that a disturbance of cellular repair processes in the microgravity environment might not be a complete explanation for the reported synergism of radiation and microgravity. As an alternative explanation, an impact of microgravity on signal transduction, on the metabolic/physiological state or on the chromatin structure at the cellular level, or modification of self-assembly, intercellular communication, cell migration, pattern formation or differentiation of the tissue and organ level should be considered [9].

The Effect of Microgravity on Plant Cell Walls, Root Growth Patterns and Gene Regulation

The studies concentrated on the effects microgravity has on plant cell walls, root growth patterns and gene regulation within the plant *Arabidopsis thaliana.* The plant cell then detects the position of the statoliths and directs root cellular proliferation (growth) in that direction (gravitropism). Each of the studies has future applications on Earth and in space exploration[10.] The use of plants to provide a reliable oxygen, food and water source could save the time and money it takes to resupply the International Space Station (ISS), and provide sustainable sources necessary to make long-duration missions a reality. The molecular mechanism for the signal transduction of the gravity information in plants is still not well understood, even though this is one of the clearer examples of a biological gravity sensor. However, before plants can be effectively utilized for space exploration missions, a better understanding of their biology under microgravity is essential. Kennedy partnered with the three groups for four months to provide a rapid turnaround experiment opportunity using the BRIC-16 in Discovery's middeck on STS-131 and while research takes time, the process was accelerated at the end of the Space Shuttle Program neared.

Kiss and his group found that roots of space-grown seedlings exhibited a significant difference compared to the ground controls in overall growth patterns in that they skewed in one direction. Their hypothesis is that an endogenous response in plants causes the roots to skew and that this default growth response is largely masked by the normal gravity experienced on Earth's surface. Robert Ferlm, focused on comparing patterns of gene expression between *Arabidopsis* seedlings and undifferentiated *Arabidopsis* cells, which lack the normal organs that plants use to sense their environment -- like roots and leaves. Paul and Ferl found that even undifferentiated cells "know" they are in a microgravity environment, and further, that they respond in a way that is unique compared to plant seedlings. Elison Blancaflor, at the Samuel Roberts Noble Foundation, discovered that plant genes encoding cell-wall structural proteins were significantly affected by microgravity. Blancaflor has now extended his findings from BRIC-16 to generate new hypotheses to explain basic plant-cell function. For example, the BRIC-16 results led the Noble Foundation team to identify novel components of the molecular machinery that allow plant cells to grow normally. According to Levine, plants could contribute to bio regenerative life support systems on long-duration space missions by automatic scrubbing carbon dioxide, creating oxygen, purifying water and producing food [10, 11].

The Effect of Microgravity on Morphology and Gene Expression of Osteoblasts

Bone is a multifunctional organ and has to fulfill two main functions: the provision of mechanical integrity for both locomotion and protection, and the involvement in the metabolic pathways associated with mineral homeostasis. Mechanical factors are essential for the maintenance of skeletal integrity [12].

Spaceflight-Induced Alterations in Bone Metabolism of Humans and Rats

Skeletal unloading in humans and rats, as seen during spaceflight, induces a series of events in the bone, resulting in bone loss and compromised bone mechanical properties. It has been estimated that, in microgravity environment, on average about 1–2% of the skeleton is mobilized and lost each month. Bone mass changes are, however, site-specific rather than evenly distributed throughout the skeleton with the tendency that weight bearing bones are more affected by microgravity than non-weight-bearing bones. In addition, the duration of spaceflight and the level of bone remodeling before unloading also appeared to be factors able to modulate the bone response to microgravity. Although no pathological fractures have yet occurred, spaceflight-related bone loss may have potentially serious consequences in long-term spaceflight, especially because the recovery is a long-lasting process, if at all possible. In normal bone there is equilibrium between bone formation and bone resorption. Recent biochemical data from astronauts confirmed the previous findings in rats that microgravity induces an uncoupling of bone remodeling between formation and resorption that could account for bone loss. All bone formation parameters (bone alkaline phosphatase, osteocalcin, and type 1 procollagen propeptide) were decreased, whereas bone resorption markers (procollagen C-telopeptide, deoxypyridinolines) were increased when measured during flight. In conclusion, decreased osteoblast function likely plays an important role in the process of spaceflight induced bone loss [12, 13]. One possible underlying mechanism is that the levels of systemic hormones or local growth factors are altered. On the other hand, osteoblasts themselves may be sensitive to altered gravity levels as has been shown for other cell types.

Effect of Mechanical Stimulation on the biology of bone-derived cells

An important aspect of osteoblast activity is the production of bone matrix during the process of osteoblast differentiation [12]. Based on in vitro studies, osteoblast differentiation has been characterized as a process of sequential expression of the genes for collagen type I and alkaline phosphatase during the phase of matrix formation and maturation followed by gene expression for osteocalcin and osteopontin concurrent with the stage of mineralization.

Spaceflight-Related Alterations in Osteoblast Characteristics

A decreased osteoblast function is claimed to play a role in the process of spaceflight-induced bone loss. Recent experiments using several osteoblastic cell types show that cell morphology as well as gene expression of growth factors and matrix proteins are altered under microgravity conditions. Most of the studies refer to ground samples as controls. Ideally, an in-flight unit-gravity (1 g) centrifuge should be used as an internal control as described in certain experiments [12-14].

Effects of Microgravity on Cell Cytoskeleton and Embryogenesis

Embryonic processes such as cell sorting, the intercalation and embryonic waves need to be studied in microgravity to see what changes occur and why. Despite the altered cell morphology and functions observed in many of the experiments performed in microgravity, embryos of some animal species Experiments that have been done with cells in microgravity show changes in morphology, cytoskeleton and function[10-14, 15]. Changes in cytoskeleton have been noted and studies on microtubules in gravity have shown that they are gravity sensitive. Further study of basic chemical reactions that occur in cells should be done to shed some light on the underlying processes leading to the changes that are observed in cells and embryos in microgravity can still develop into living organisms that are able to reproduce [15].

Embryos in Microgravity

There have been many studies done on embryos in microgravity and a variety of species have been used. Comparing variations in embryo development over a broad range of species can be difficult. Each species has differing rates of development and has different sensitivities to perturbations in their environment. There have been difficulties studying development in microgravity in space when using the whole developmental period from fertilization to hatching. Experiments have been done on rats in microgravity but, due to a lack of standardization, it is difficult to compare results Rats were allowed to mate in a Cosmos 1129 biosatellite experiment and the females failed to become pregnant, although fertilization had occurred Human pregnancy is counter indicated by NASA with microgravity listed as one of the factors. The reasons for this are that microgravity "May have an impact on in utero embryonic development and reproductive physiology in both males and females as evidenced by animal studies" [15, 16]. Amphibian egg orientation in gravity is important to their survival. They are normally orientated yolk down and undergo cortical rotation of 30 degrees during development. Eggs that are rotated greater than 175 degrees from perpendicular show abnormal development.

Cytoskeleton Abnormalities and Genetic Changes, Gravitational fields and Cytoskeleton Organization

Cells rely on microtubules for their structure, the transport of organelles and for the process of cell division. Adhesion sites on the cell surface also incorporate microtubules and actin into their structure, a process that may influence cell-cell interactions. Intermediate filaments also determine cell shape and support the other cytoskeleton structures of microtubules and microfilaments. Cells respond to their environment within a three dimensional tissue structure. The number of adhesive structures in a cell and its resulting cell polarity may change the way it will behave. For instance cells that are not polarized are more likely to undergo apoptosis Microgravity has effects on both cell shape and cytoskeleton. Microtubules typically grow (polymerize) into long tubular polymers consisting of alpha- and beta-tubulin dimers bind together in a specific orientation. Microtubule polymerization is that of an out of equilibrium chemical reaction-diffusion process. The microtubules are continually forming and disintegrating so that when a microtubule depolymerizes it leaves behind components to build another microtubule [14, 15].

Tissue Engineering in Microgravity

Treatment for organ-loss and tissue-loss problems due to disease and accident costs in excess of $400 billion a year. When we look at transplantation, there are tremendous shortages of the various tissues and organs that we use. Microgravity provides an advantageous environment because it gets gravity as a force out of the picture, allowing intercellular forces to be more evident or more effective[23]. If cells are not driven to sediment against a surface and are suspended in fluid, the attractive forces on molecules between those cells have a greater chance to act (Pellis,R; NASA Johnson space center). NASA scientists have developed a rotating culture vessel called a bioreactor that simulates a microgravity environment [16, 17].Also, the shear forces on the cells are very small, which is another advantage [18]. Nanobiotechnology Based Tissue Engineering in Microgravity has also shown promising future in this field.

CONCLUSIONS

Astronauts experience profound physiological changes as they adjust to the microgravity environment in space. We know that all physiological processes in the body are governed by the activity of diverse and specialized cells, which comprise the different tissues. Thus, spaceflight exerts its detrimental effects on astronauts via changes in cellular structure and or functions. Numerous studies have shown that all types of cells from many organisms behave differently in space than on Earth. Because there is a great diversity of cell types in nature, the effects of microgravity on those cells are extremely diverse and often complex.

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