Osteoblasts, Osteoclasts and Endothelial Cells in Microgravity

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Abstract

This paper will discuss the changes in osteoblasts, osteoclasts, and endothelial cells in a microgravity environment. According to NASA, microgravity is defined as a force of $1x10^{-6}$ g. Microgravity causes objects or humans in space to experience weightlessness. That weightlessness causes cell shape changes in bone and endothelial cells. In bone cells, microgravity causes an overall imbalance of these cells in the body, leading to major effects on their metabolic pathways. The metabolic pathways disrupted in microgravity cause an increase in reactive oxygen species. In endothelial cells, microgravity creates a stress response, which then causes an increase in caveolin1 gene expression. Ultimately, the changes caused in these cells lead to bone loss, imbalance, and a decrease in human cardiovascular health (Pietsch et al., 2011). In this text, we would also describe possible mechanisms to combat these negative side effects in the future and some experiments that could shed light on these topics.

Introduction

There are many challenges associated with long-term space travel, and the most urgent one is microgravity. Microgravity is defined as an environmental condition in which there is a minimal effect of gravity, and it can be experienced in space. Human bodies are not used to microgravity because they have evolved to thrive in Earth's gravity which is 9.81 N/kg. Thus, in an environment that has a different amount of gravity could cause physical stress. Currently, many scientists are exploring the negative effects of microgravity and searching for treatments to the damaging symptoms.

A negative effect that humans would experience is space motion sickness. The sickness is caused by the neurosensory system sending out stress signals. It leads to the astronauts experiencing headaches, nausea, and or vomiting (Figure 1). Another effect microgravity has on individuals is the change in hydrostatic pressure. In other words, the absence of normal gravity causes a shift in bodily fluids. With time the astronaut would experience homeostasis, but that will go along with blood loss, water loss, plasma volume shrinkage, and other neurohormonal reactions (Pietsch et al., 2011) (Figure 1). Another bodily symptom that is affected is the immune system. Various immune cells reduce their sensitivity and production due to the increase of stress from microgravity and other physical and psychological factors, which will be discussed more in-depth later. As the immune system becomes less efficient, there will be an overall decrease in autoimmune activity, thus an increase in susceptibility to infection, and cancer, all which are detrimental to the astronaut’s health (Figure 1). Lastly, there is a reduced tissue load due to the microgravity. Reduced tissue is an issue during space travel and unfortunately, it causes major problems (Figure 1). To recover from microgravity caused tissue and bone loss, a long exposure to Earth's gravity is required. Which ultimately is a major obstacle for astronauts that need to complete a mission in long term space travel. One factor that contributes, which will be discussed more in-depth later, is the change in calcium homeostasis and the imbalance of osteoblasts and osteoclast cells (Pietsch et al., 2011).

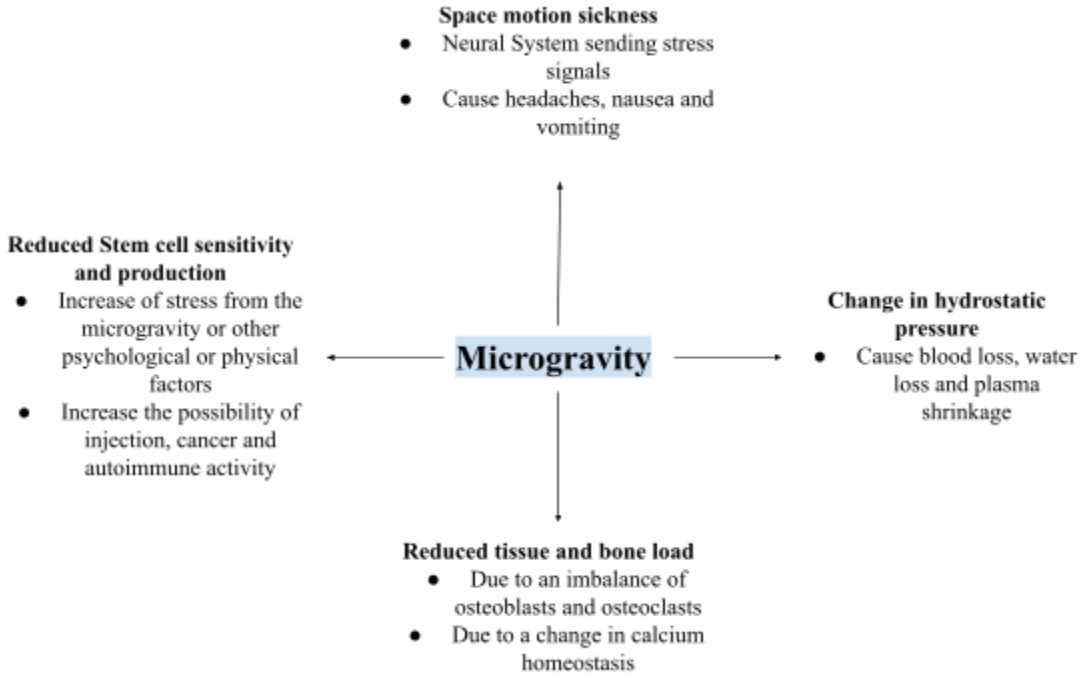

Figure 1. A diagram that visually lays out some of the effects that microgravity has on the human body. Information was from Piestch et al, 2011.

These negative consequences of microgravity manifest differently in each individual, thus adding multiple layers and complexity towards this topic. Furthermore, conducting experiments in a true microgravity environment, such as outer space, is difficult due to the cost and resources. Overall, that causes many scientists to experiment on Earth with various machines that can simulate the gravity spectrum. For example, the Rotating Wall Vessel, which is a machine that mimics an environment with microgravity (“Rotating Wall Vessel”, n.d). However, due to the many factors that contribute to outcomes and the complexity of each machine, there are many scientists that obtained conflicting research.

This writing will go into two main groups of cells, Endothelial cells, and bone cells. Endothelial cells line the blood vessels, where they regulate the exchange of nutrients between the tissue and the blood, as well as send signals to arrange connective tissue growth. Osteoblasts are cells that form a new bone and grow or heal an existing bone. Osteoclasts initiate bone loss; the balance between these two cells is to ensure bone growth.

This paper will explore endothelial, osteoblasts, and osteoclasts in microgravity, its effects, consequences, and possible solutions for these issues. These cells are all a significant part of our circulatory system and our bones.

Effects of Microgravity on Bone Cells

Osteoclasts and osteoblasts are created by stem cells. Stem cells can turn into any cell type, and are very helpful for recovery from injury (Kolios & Moodley, 2013). An overview of the differentiation process of bone cells is presented in Figure 2. The mesenchymal precursor cell differentiates into the osteoblasts, and the hematopoietic stem cells differentiate into the osteoclasts (Kim et al., 2020).

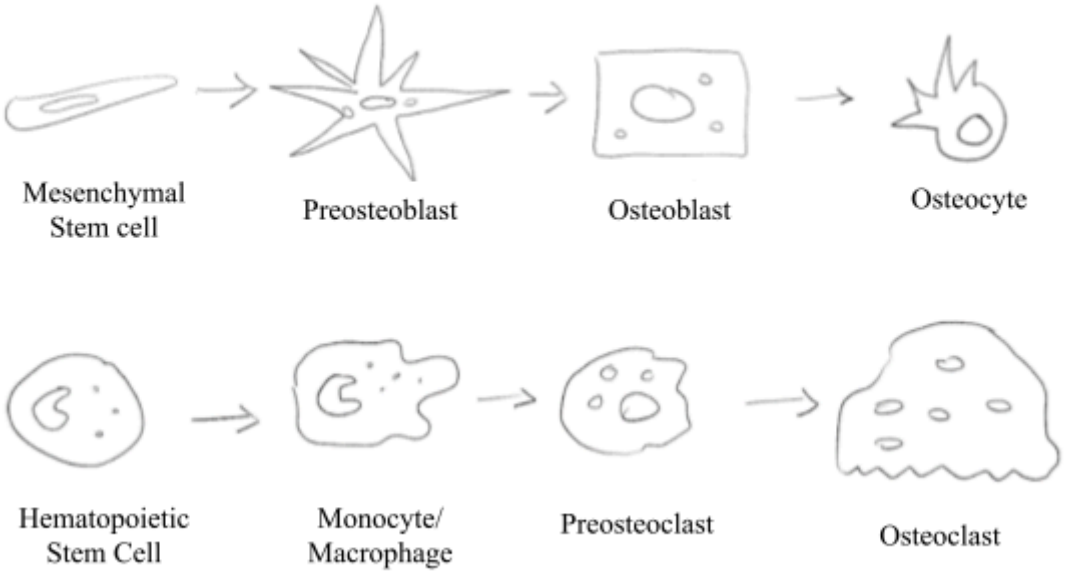

Figure 2. This is a diagram of the transformation from a mesenchymal stem cell, to an osteocyte, and a hematopoietic stem cell to an osteoclast. The circles in the cells represent nuclei and smaller granules.

A summary of the functions of osteoclasts and osteoblasts is presented in Figure 3. Osteoblasts are the cells that form the bone; they use a process that produces extracellular proteins that are then mineralized and filled with calcium. Osteoblasts can then be turned into an osteocyte or a bone-lining cell, or they could activate apoptosis (Kim et al., 2020). An osteocyte is a cell that stays inside the bones and would be reactivated when a repair is needed inside, and a bone lining cell stays at the surface and is inactive (Mohamed, 2008). An osteoclast is the opposite from osteoblasts, they contribute to bone reduction. The reduction is caused by a secretion of acids or proteolytic enzymes (Kim et al., 2020). These two cell types work closely together by communication of actual physical touch, or chemical signaling (Kim et al., 2020). They need to have clear communication or there could be an imbalance in bone formation and reduction and that can cause many problems for the body. This knowledge would also be important for studying osteoblasts and osteoclasts in microgravity because in space, bone loss is likely due to an imbalance of these two cell types and results from a loss of normal gravity-sensing mechanisms. There could be two reasons, either there are not enough osteoblasts working effectively, or the osteoclasts are too efficient and or being produced too much.

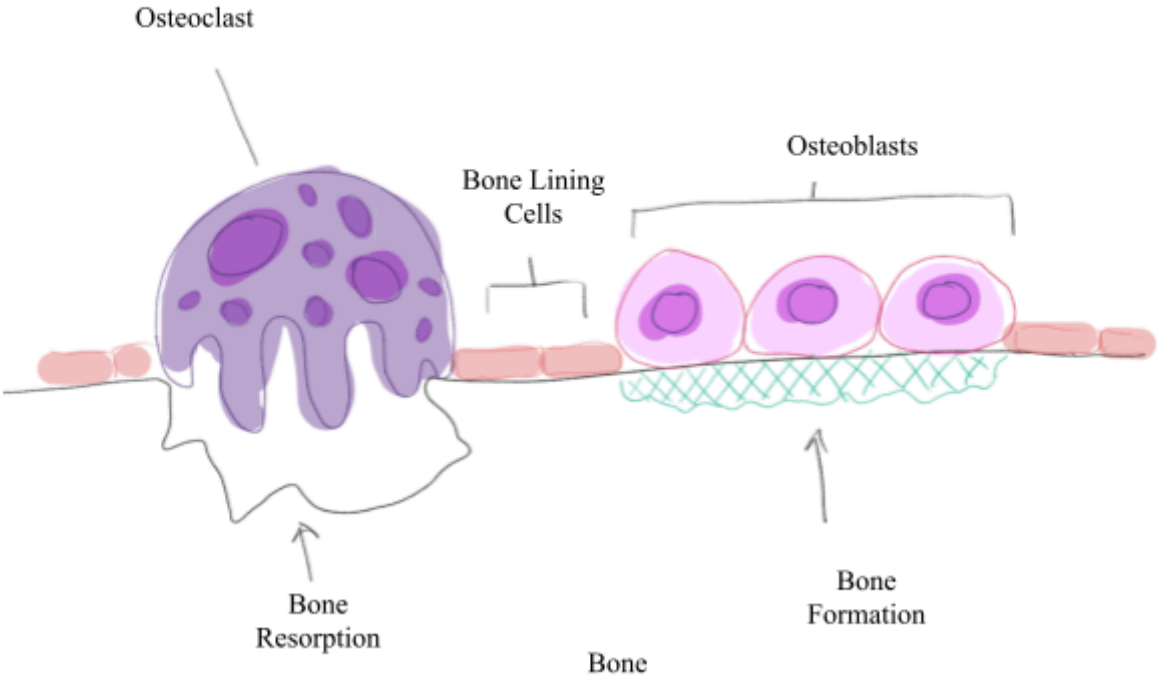

Figure 3. This diagram shows the function of bone formation of the osteoblasts and the bone resorption role of the osteoclasts. The circles inside the cells represent their nuclei. Osteoclasts have between 10 and 20 nuclei, meanwhile, osteoblasts have one to three (Feher, 2017; Qiu et al., 2019). The green marks indicate the newly synthesized bone.

Across a variety of studies of the effect of microgravity on bone, there is a consistent observation of an increase in bone resorption (Blaber et al., 2013; Morabito et al., 2020). The shift in body fluids and not having pressure constantly placed on the human body can cause further imbalance in osteoclasts and osteoblast cells. Previous research has shown that microgravity causes a change in osteoblast morphology, proliferation, differentiation, gene expression, and oxidative status (Morabito et al., 2020). Throughout the years, many studies focused on the cytoskeleton organization, because the cytoskeleton has been seen as the initial gravity sensor due to it being able to convert external mechanical forces into intracellular chemicals (Morabito et al., 2020). In the experiment done by Morabito and colleagues, they directly prove that the cytoskeleton is shifted. In the beginning hours of exposure to microgravity, the cell height increases, then after 72 hours, it develops its flattened shape (Morabito et al., 2020). Due to the changes in shape, researchers concluded that the cytoskeleton got reorganized because it directly influences cell morphology (Fletcher & Mullins, 2010).

To know more about the bone cells and to monitor signals created by weightlessness, MC3T3-E1 cells were used by Morabito and colleagues. This cell line is composed of osteoblast precursor cells. Through this study, the authors showed that microgravity has a huge impact

overall on cell growth, function, and structure. More specifically, during an STS-56 shuttle flight it was observed that the cell number, glucose utilization, and prostaglandin synthesis were reduced (Hughes-Fulford & Lewis, 1996). Through a random position machine (RPM), it is shown that the mineralized nodule creation and markers of osteogenesis were more suppressed in the microgravity-exposed cells, compared to the controlled group (Morabito et al., 2020). Their study showed that after 12 and 24 hours of microgravity exposure from RPM, there was a decrease in cell viability, and the cytoskeleton microfilaments were disrupted and reorganized (Morabito et al., 2020). In addition, the authors have noticed an increase in reactive oxygen species (ROS) and a development of oxidative stress, which means that microgravity also affects the metabolic pathway. ROS has been proven to increase levels of apoptosis and disrupt many biological cell processes, such as cell adhesion, proliferation, and survival (Domazetovic et al., 2017). The researchers, Morabito and colleagues, found that ROS increase can affect the function of the membrane glucose transporters. They concluded that would cause a decrease in glucose uptake, which can alter the cellular metabolic pathway, cause an increase in lactate production and a decrease in mitochondria function (Morabito et al., 2020). In addition, the ROS level increase can inhibit osteoblast functions. Their study has also shown that after 24 hours, there was an increase in integrin and actin expression levels (Morabito et al., 2020). Actin is a protein that is located in the majority of eukaryotic cells. Actin creates filaments and microfilaments (Dominguez & Holmes, 2011). This information suggests that the imbalance of bone cells is shifted in favor of the osteoclasts. The abnormalities in the osteoblast precursors and these negative side effects could mean that osteoblasts are not acting properly to synthesize new bone, thus leading to bone loss.

Fortunately, during the study, the authors discovered that antioxidants, such as curcumin and Trolox, can have protective effects. Curcumin has been shown to inhibit ROS formation and enhance cell differentiation. Trolox has also been proven to restore the ROS and Calcium 2+ levels, the mitochondrial membrane potential, and glucose and lactate levels, which are all phenotypes that cells experience from the oxidative stress caused by microgravity. Trolox is a substance that can inhibit oxidation, the loss of electrons and can transfer through the cell membrane with passive diffusion (Distelmaier et al., 2012). In addition, Trolox is a water-soluble vitamin E analog. With those traits, Trolox can oxidize the reactive oxygen species (Distelmaier et al., 2012). In other words, Trolox is not able to change the rate of ROS production, instead, it seems to repair the proteins oxidized by ROS and lower cellular lipid peroxidation (Distelmaier et al., 2012). The use of Trolox has been quite hesitant due to Trolox's ability to have pro-oxidant abilities under certain conditions (Raspor et al., 2005). Additionally, in high concentrations, Trolox has been seen to produce cytotoxic effects (Distelmaier et al., 2012). Trolox is used as a standard, meaning that it is used to quantify and compare the antioxidant activity of other substances (National Center for Biotechnology Information, 2024). However, in a microgravity environment, Trolox has been seen to restore the proliferation of cells and counteract the biological changes of ROS (Morabito et al., 2020). This shows that Trolox could reverse the effects of ROS on the cells, therefore shows the significance this antioxidant holds. If astronauts were to be treated with an antioxidant it is possible that the chemical would restore some sort of balance to the bone cells, the astronaut's bones would not weaken as much in a microgravity environment and the astronauts would be able to carry out missions while living in microgravity long term. If they can do that, much more information about the long-term effects of microgravity could be found and human exploration into distant space could occur. In addition to a study by Morabito and colleagues, there are additional and contradicting results from other

labs. For example, another study has shown a loss of stress fibers and a decrease in actin mRNA levels when bone cells were exposed to microgravity (Qian et al., 2012). All of this further proves that this area in the field is still unknown and is complicated due to many exterior factors that can affect the results of the experiment, such as types of cell lines, cell types, time of day, stage of the cells, and machines.

A possible solution to these issues is having researchers do experiments on astronauts in space, such as observing the decay and pathway bones in the International Space Station. In other words, notice how the bone shape changes, and possibly if bone production is consistent throughout the whole body. Researchers could also observe the ROS levels, and the glucose uptake and lactate production to see if microgravity does affect the metabolic pathway. The ROS would be measured by using small molecular fluorescent probes because probes can provide information on redox changes in the cell environment (Winterbourne, 2014). However, if going to space cannot be achieved, researchers on Earth can conduct a long experiment. First, researchers take bone cells from the same batch and split them into different groups. Each group would go through a different microgravity simulation procedure. All the procedures would ideally be in the same lab, the parabolic flight would be the only exception. All would be occurring at the same time, temperature, and have all other factors to be constant. Then the differences and similarities between each strategy would be observed. The experiment could end there, but if it were to continue, the researchers could repeat all the previous steps and change one factor. For example, the researchers could raise the temperature or start at a different time in the day. Thus, all the effects of the different factors would be found, and the researchers can create an informed hypothesis about bone cells in space.

Effect of microgravity on Endothelial Cells

Endothelial cells are the tissue cells that line the blood vessels and also exchange nutrients between the outer and inner environment of the vessels. The location of the endothelial cells is shown in Figure 4. Endothelial cells must have the ability to travel around the body. Endothelial cells are responsible for creating new blood vessels, providing the proper nutrients to wound sites, and removing waste – all of which are essential for cell regeneration (Morbidelli et al., 2020). To travel around the body, the endothelial cells use cell migration, which is very advantageous because the migration causes these cells to be able to provide support to the human body where needed, thus making them an adjustable life support (Bruce et al., 2002). In recent years, there has been an increase in research studying endothelial cells because it has been learned that cancerous tissues also depend on a blood supply. Thus, endothelial cells could also contribute to the increased rate of cancer in space (Bruce et al., 2002).

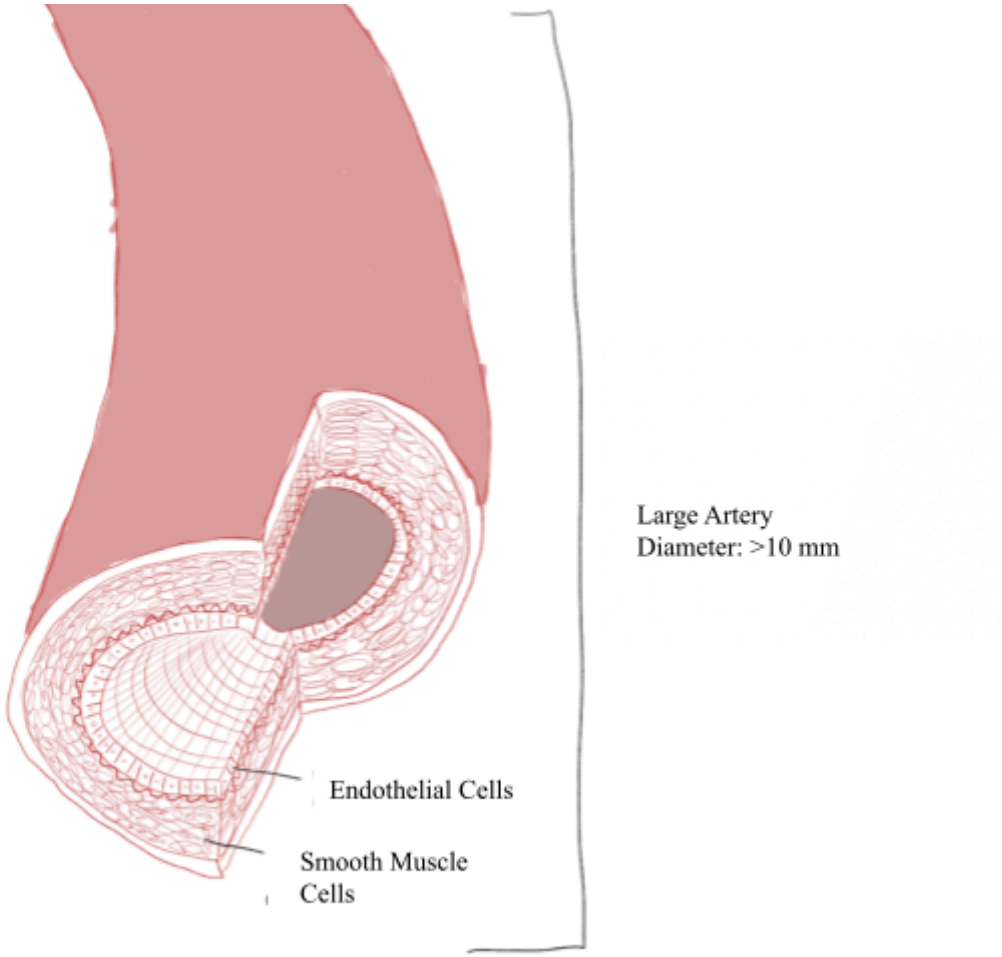

Figure 4. This diagram is of the cells inside an artery. An Endothelial cell's length is 50–70, width is 10–30 μm and diameter is 0.1–10 μm (Félétou, 2011).

The field of microgravity on endothelial cells is more well researched compared to the bones cells. In a paper researched by Spinshni et al., they have found that even mild microgravity can activate endothelial cells' stress response, which causes a caveolin1 gene expression increase (Spishni et al., 2003). With the gene expression increase it inhibits endothelial cell growth and increases prostacyclin and nitric oxide (Spishni et al., 2003). The reason is due to the enzymes: prostacyclin synthase, cyclooxygenase 2 and nitric oxide synthase. These enzymes are located in the caveolae, which are cup-shaped pits that are located in the plasma membrane in vertebrate cells (Parton et al., 2023). Since the caveolin1 gene expression is enhanced in a microgravity environment, that causes an increased distribution of caveolae in the cell to occur (Spishni et al., 2003). Nitric oxide synthase will be further explored in this paper.

Nitric oxide, also known as nitrogen monoxide, stimulates the dilation of blood vessels and the release of hormones, such as the human growth factor (Kiani et al., 2022). Nitric oxide is a soluble gas created from the amino acids inside endothelial cells by nitric oxide synthase, eNOS (Tousoulis et al., 2012). Prostacyclin's role in the body system is to be a powerful vasodilator and platelet aggregation inhibitor (Stitham et al., 2011). The enzyme prostacyclin synthase creates the prostacyclin, and the synthase converts prostaglandin H2 into the prostacyclin (Li et al., 2008). Cyclooxygenase 2 could also produce prostaglandin by converting arachidonic acid into prostaglandins (Turini & Dubrois, 2002). In conclusion, if microgravity causes stress in endothelial cells, the levels of these molecules will increase and there would be a larger release of hormones, which is an inhibitor to platelet aggregation, and more stimuli to blood vessel dilation. All of these effects could lead to abnormal or delayed wound healing when a human is in a microgravity environment, which could be very dangerous on long space journeys.

Many other factors could affect endothelial cells, such as Hsp70, HGF, and actin that are disrupted in microgravity. In the following section, a group of scientists did experiments on the human umbilical vein endothelial cells with the previously mentioned factors.

Heat shock proteins are a group of proteins expressed in response to stress, and Hsp70 is one of those proteins. Hsp70 could increase the survival of endothelial cells. It is seen that microgravity conditions cause an increase in Hsp70, therefore create an increased amount of stress in endothelial cells (Versari et al., 2007). Another factor these authors researched is the hepatocyte growth factor (HGF), which is a protein that can promote the survival of its target cell. Therefore, an increase in HGF can encourage the survival of the endothelial cells. During this study, microgravity caused HGF to be overexpressed. However, the protein did not affect the endothelial migration (Versari et al., 2007). eNOS, also called endothelial nitric oxide synthase 3, is known for its central role of maintaining Endothelial homeostasis by regulating cellular functions (Heiss et al., 2015). These authors also looked at eNOS and saw that it increased in microgravity, which is consistent with the findings described previously. The increase can be a factor in the cardiovascular system weakening (Versari et al., 2007). Actin is also another important factor that could affect endothelial cells. In the Rotating Wall Vessel machine with fluorescence microscopy, it is shown after a few hours that the machine disorganized their actin. And within a few days, with the western blot technique, the scientist detected a decreased amount of actin following exposure to microgravity. (Versari et al., 2007).

Discussion:

In this paper, many topics about cells in microgravity were discussed. The bone cells, osteoblasts, and osteoclasts have been observed to become unbalanced while in a microgravity environment. The reason is due to an altered calcium homeostasis, a change in inhibition of proliferation and differentiation, changes in the cytoskeleton and the nucleus, increased bone resorption and decreased bone formation, and changes in the metabolic pathway. Additionally, endothelial cells also experience changes. One example is microgravity causing an increase in stress, which then causes an inhibition of the caveolin1 gene expression. The inhibition then causes many other problems to occur, such as the increase in blood vessel dilation. In addition, other factors such as the increase in eNOS and Hsp70, and disorganized actin contribute to the overall change of endothelial cells in a microgravity environment. The results of the endothelial cell experiments have supported the idea that microgravity causes an increase in stress and

negatively affects the human body. As mentioned in the introduction, the immune cells' decrease in production and sensitivity, as well as space motion sickness are all linked to stress.

Even though both of these cells are quite different, the cell microgravity reactions do have some similarities. For example, both bone cells and endothelial cells exhibited a change in shape due to disorganization in actin and a resulting change in the cytoskeleton. This result makes sense because in a microgravity environment, there is no force pushing the individual onto the surface (gravitational force), and everything can freely move wherever it desires. The cytoskeleton of a cell is used to the gravitational force on Earth, but with the shift of gravity, the microtubules and the actin would mechanically unload thus causing disorganization (Bradbury et al., 2020).

Even though the experiments described in all the papers referenced here could bring the understanding of cells in space one step closer, it has to be acknowledged that many factors could have affected the results. For instance, the different microgravity simulation devices, they all have the same goal but have different strategies to reach them. That can cause inconsistent results from each device and cause the conclusion to be more confusing. Other factors that contribute to the result are the type of cell line and culture being used, the environment temperature, and measurement.

The next step in understanding human bodies in space would be to start another experiment. The experiment would first be to determine which microgravity simulator device provides the most accurate results. To reach that goal is to send one type of cell culture into space and observe the results. Simultaneously on Earth, to do experiments on all the different devices, except the parabolic flight, in one lab. The lab would have a consistent temperature that is the same as on the International Space Station. After all the results were observed, then the comparison would provide the answer of which device is most accurate. The most accurate results would be compared to the space station's results and the difference would be observed. The differences would be acknowledged every time a result from that device is shared, and the result would then be interpreted and theorized about what the cell would be like without that difference. Another option would be to improve the most accurate device, so the results would be identical to the ones in space. Even though this procedure would be quite costly, it could provide valuable answers and would save future researchers both effort and money. After finding which procedure is the most accurate, the scientist can review past results and analyze the accuracy. With the result of these experiments, a new guideline can be made, so the most accurate answers can be provided.

For treatments, a possible next step could be experimenting with Trolox because it can reverse some of the effects on the bone cells in microgravity. The experiment could also include other antioxidants, and then the chemicals can be compared and their effectiveness evaluated. Therefore, antioxidants could be a possibility for bone cell treatments. Another possible treatment could be using engineering. To specify, the use of engineering would create a chamber that can stimulate Earth's gravity, and it would be located on the space station for long term astronauts to use. The chamber would work by applying force to one wall, and the astronauts could work and sleep in these rooms. The purpose of the chamber is to let the astronaut's body take a break from the stress microgravity applies to them and would hopefully delay a majority of the effects of microgravity on the body. There could also be experiments held to determine the minimum amount of gravity that is needed to avoid the consequences of microgravity. Therefore, after the result is found, the chamber would apply the minimum amount of gravity. In general, the knowledge of the minimum amount of gravity needed can cause life on

other planets and the International Space Station to become safer because scientists would have more knowledge on what is damaging to humans.

Overall, the discoveries about the human body in space can have a positive impact on the people on Earth too. For example, new pharmaceuticals that are found to reverse some of the effects of bone loss in space can also be used to treat osteoporosis. Scientists may find that a certain lifestyle, pharmaceuticals, or treatment that further benefit the body can be applied back on Earth. These discoveries can be helpful for many illnesses that currently do not have a solid cure or treatment.

In conclusion, the human body in space is still an area that needs to be further researched; there is still much to learn. Future discoveries would cause humans to gain a better understanding of their cells, and how they react in space. Finally, it would push human space travel forward, and the deep unknown space would slowly be discovered.

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