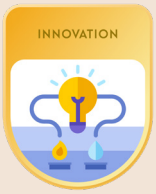
Bone Density in Microgravity Conditions Cured by IGF-1



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Due to the effects of microgravity in space, the bones of astronauts progressively deteriorate in density. When astronauts return to Earth, their weakened bones are at an increased risk of fracture. We proposed that by delivering a hormone called IGF-1 (Insulin-like Growth Factor) to astronauts, this situation would be ameliorated. Osteoblast cells, which are cells that are responsible for bone formation, exhibit receptors for IGF-1 and have been shown to be stimulated by the hormone. We will acquire the hormone by transforming baker's yeast cells to produce it. The IGF-1 gene, which we will extract from human skin cells, will be regulated by the constitutive promoter ACT-1 and the ADH1 terminator, along with other supplementary parts. We plan on establishing a system in which the modified yeast is grown and the secreted hormones are collected and purified. After the initial refining process, we will test if the effects on bone cells are positive. Not only can our treatment benefit astronauts, but it can also aid osteoporosis patients who suffer from similar bone mass issues. We believe that our system will enable astronauts to remain in space for extended periods and avoid long-term disability or even death due to decreased bone density.

Keywords: Space, osteoporosis, yeast, IGF-1, bone density, astronauts, ISS, NASA, medicine

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Watch a video introduction by the authors at http://bit.ly/2MitZny

Background

Bone loss is a widespread problem for astronauts to overcome as they are in space for extended periods of time. In a microgravity environment, due to the reduced stress on the bones, there is an increased rate of bone resorption and no corresponding increase in bone formation, causing a loss of mass that is about ten times that of osteoporosis, a condition in which bones lose density and become brittle. Each month in a microgravity environment, the proximal femoral bone loses 1.5 percent of its mass, leading to a 10% loss in bone mass over the course of six months. Recovery after returning to Earth can take as long as half a decade (Ohshima 2012).

Currently, one of the most popular methods of treating bone density is using bisphosphonates, which are drugs that decrease the production of osteoclasts, cells responsible for bone resorption, while maintaining regular production of osteoblasts (Tanaka et al. 2005; Jeffs 2012). It has been used to treat osteoporosis patients for over a decade and has been proven to improve bone mass. Astronauts currently take bisphosphonates weekly in space to prevent bone loss. Along with the bisphosphonates, astronauts exercise for two and a half hours daily, six days a week using ARED machines (Advanced Resistive Exercise Devices), which has also been proven to reduce the rate of bone loss in space (Jeffs 2012). However, these devices are heavy and bring extra weight onto rockets, which can prove to be expensive. Despite its apparent benefits, exercise alone has not been entirely successful in the prevention of bone loss in astronauts. Our group intends for our transformed yeast to serve alongside these pre-existing methods to solve the problem decisively.

IGF-1, originally called somatomedin C, is a 70-amino acid polypeptide hormone that is secreted naturally by the anterior pituitary gland in humans and produced primarily in the liver (Figure 1; Pang 2010). There have been numerous studies conducted to test the viability of IGF-1 as a treatment for bone density. Studies have shown that the hormone is critical to human bone development because it is essential for longitudinal bone growth and skeletal maturation (Locatelli and Bianchi 2014; Yawitz 2019). Furthermore, the importance of IGF-1 persists throughout adulthood, as it plays a vital role in the maintenance of bone tissue. Lower levels of IGF-1 in women are correlated with increased rates of fractures, by about 40% (Locatelli 2014). IGF-1 prolongs the life of osteoblast cells by reducing osteoblast apoptosis; additionally, it stimulates osteoblastogenesis, the process in which osteoblast cells are born. If IGF-1 leads to the formation of more osteoblast cells, then it must also have a positive effect on bone density because there are more cells to produce bone.

On Earth, deer antler velvet is available as an unregulated supplement. Early research proves that it can heal cartilage and tendon injuries while mitigating the damage in joints due to repetitive trauma. IGF-1 directly affects the body's ability to repair itself and it has shown pronounced promise for supporting those with stunted growth and healing injuries (Dell'Amore et al. 2013).

Some studies have correlated high or low IGF-1 levels to risk of cancer, specifically prostate and breast cancer. This is because IGF-1 stimulates the growth of cells, which is the principle that cancer is based upon. We may have to test the hormone's effects on the human body in tissues other than bone cells to gather further evidence (Cohen 2019). Considering the danger of extreme levels of this hormone, it will be important to receive the correct dosage.

To combat the loss of bone mass, our findings suggest that if *Saccharomyces cerevisiae*, a particular strain of baker's yeast, can be modified and transformed to produce the IGF-1 protein, then we can test its effects on osteoblast cells and potentially move towards treating human patients in the future (Vai et al. 2000).

Although our system is focused primarily on aiding in long term space missions aboard the International Space Station (ISS) or other spacecraft, we believe our treatment can also be administered to patients suffering from osteoporosis. Osteoporosis is a prevalent condition, affecting 44 million men and women who are above the age of 50 in the United States alone. This number accounts for 55% of the people from that age group (International Osteoporosis Foundation Board Members 2019). If we can broaden the range of application for our treatment to include osteoporosis patients, the yeast cells could have a profound impact on elderly life as well as space exploration.

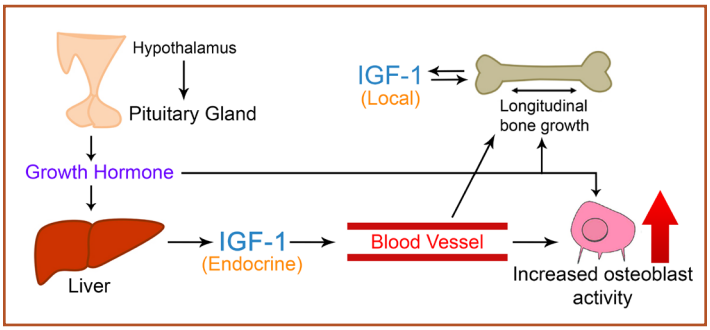


Figure 1. *How IGF-1 is distributed throughout the body*

Systems Level

Our ultimate goal is to be able to reverse bone density issues that become apparent once astronauts are exposed to microgravity conditions, as well as provide a reliable treatment for millions of people suffering from osteoporosis. The main component of our designed system to counteract this problem is our engineered yeast cell, which is intended to secrete the IGF-1 protein. The yeast will be grown in Petri dishes with nutrient agar where they can replicate quickly, thus maximizing the output of the IGF-1 protein. We chose agar plates as our medium because any liquid media would be ineffective in microgravity conditions. Upon secretion, we would be able to collect the proteins and inject them into a culture of bone cells, where they will mitigate the bone density problem.

The IGF-1 proteins produced by our yeast cells have more uses than just for astronauts. They can be used as a supplement for the millions of osteoporosis patients

worldwide once the effects have been determined to be beneficial. Another category of potential patients are children who have an IGF-1 deficiency. Due to the ease of quickly growing cultures of yeast cells, our transformation will provide a cheap source of the hormone if any patient requires it for whatever reason.

Our experimental plan consists of growing a culture of yeast cells, then separating ⅓ of them to serve as our negative control. In this state, they should be unable to reproduce and there should be no band that appears when they are put through gel electrophoresis. The other ⅔ of our cells will serve as our positive control and we will transform them with the IGF-1 and GFP genes. The cells that accept the transformation, now having the ability to produce GFP, will begin to multiply, forming colonies, while those that did not, will not. The colonies will be tested with PCR and if they express IGF-1, they will show a 500 base pair band. When transforming the cells of the positive control, it is possible that some colonies become truncated, meaning that they only take the GFP gene but not the IGF-1. This would result in visible growing colonies, but no band on the gel electrophoresis. PCR will be used throughout our experiment to copy genes for gel electrophoresis as well as reproduce the gene parts for transformations.

Device Level

When considering the potential hosts for our design, there are many conditions and environments that must be met in order for our chassis to survive and reproduce:

1. Our chassis should be repressed from mutating too rapidly and should prevent any major significant changes to the device
2. The device must survive in a microgravity environment with increased radiation exposure

The main functional component of our organism is the system we designed to secrete the IGF-1 hormone, called Astro IGF. The chassis we are using for our organism is *Saccharomyces cerevisiae,* more commonly known as baker's yeast. We chose this chassis because of its relative prevalence, detailed documentation, and basic cell machinery which we need to produce the hormone. After we transform the yeast cells with Astro IGF, it will begin to secrete the IGF-1 hormone. Green Fluorescent Proteins will confirm the expression of the hormone as it is being continuously produced.

Another possible way of separating transformed colonies from others is by growing populations of yeast cells with an amino acid deficiency, and then transforming them with the gene to produce this amino acid along with the IGF-1 gene. We prefer to simply use GFP because it would be easier to use fluorescent lighting to confirm successful transformation rather than having to grow a specific culture of yeast.

One of the most prevalent obstacles we face is how to keep random mutations under control while in space. The *mean time to fail* is the average minimum time until random mutations cripple the system. This measurement assists designers and researchers to predict when a system may cease to function properly. By using this measurement, we can perfect our system to have the longest *mean time to fail* so that it may remain in space for the longest possible time without having to transform more yeast cells.

Multiple unique environmental factors, particularly microgravity and space radiation, pose a continuous risk to the DNA integrity of living organisms. We are particularly concerned with the effects of these factors because they may play a significant role in our plasmid to produce our specific protein. Increased amounts of radiation will cause damage through the interaction of charged particles with the DNA molecules or indirectly through the production of free radicals (Moreno-Villanueva 2017). Furthermore, it may result in an increased rate of mutation which shortens our system's *mean time to fail*. These conditions will be taken into consideration throughout our testing.

Parts Level

Astro IGF (Figure 2) will contain the following: the ACT-1 promoter, the ribosome binding site BBa_K792001 (a Kozak Sequence), the IGF-1 gene, the GFP (Green Fluorescent Protein) gene, and the ADH1 terminator (Registry of Biological Parts 2003).

ACT-1 Promoter

The ACT-1 Promoter is constitutive and serves to continuously create and transcribe proteins to secrete the IGF-1 protein. It is important that this promoter is constitutive because we want to maximize our system's output of IGF-1. Allowing the gene to be expressed constantly allows for the highest possible secretion of our desired products.

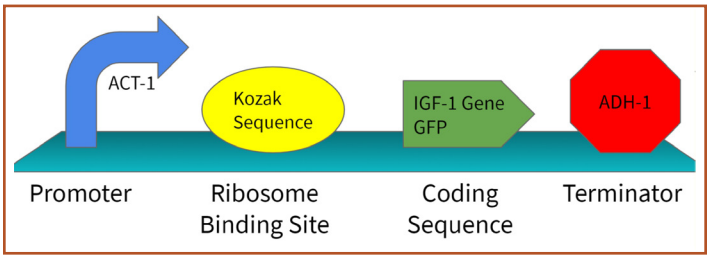
BBa_K792001 Ribosome Binding Site

The Kozak sequence is the eukaryotic analog of the bacterial RBS. It is a short sequence that includes the codon ATG and is critical for the initiation of the translation process. The 5'UTR sequence of the MFα1 gene in yeast cells is responsible for fulfilling this role. BBa_K792001 is a specific region of the MFα1 gene (Registry of Standard Biological Parts 2003). It is important that we include a suitable ribosome binding site along with our parts because it is critical to initiating the translation process.

We chose BBa_K792001 as our ribosome binding site because of its compatibility with the yeast cell, as well as its important role in the expression of IGF-1.

The IGF-1 and GFP Gene Coding Sequences

Our coding sequence consists of two genes: the IGF-1 gene and the GFP gene. The IGF-1 gene codes for the IGF-1 protein, and begins expressing IGF-1 during the translation process. The Green Fluorescent Protein (GFP) will serve as a reporter. Because it is alongside the IGF-1 gene in our coding sequence, it will be expressed alongside the IGF-1. As the yeast produces the IGF-1 protein along with the active GFP, the products should glow green, indicating that our system is working. This will allow us to differentiate between working and nonworking colonies in our positive control.



Figuew 2. *Our coding sequence*

ADH1 Terminator

The ADH1 terminator, specifically the BBa_K2637012 part, is a naturally occurring terminator sequence found in yeast. It also works as a terminator for foreign genes expressed in yeast, which is the function we plan on utilizing in our system.

Safety

Our proposed system has a number of possible health risks and tests that must be undergone to determine the safety of our system. First and foremost, our yeast must be constructed and tested with proper lab equipment, as should be the case when dealing with any genetic transformation. Once the yeast has been transformed and is growing within Petri dishes, we will want to ascertain that it remains in the Petri dish to avoid any unnecessary contamination. Another thing we will need to keep sterile are the collected hormones. The hormones will be tested on human bone cells *in vitro*, and if contaminated, will compromise the test results. We wish to observe the effects of the outside environment, and how IGF-1 positively affects bone cells. Additionally, drug delivery poses a difficult obstacle and currently the best possible implementation of our treatment would be through injection (Fitzpatrick 2019).

The final aspect that we will need to be particularly careful with is the hormone itself. Before injecting it into human beings, we will need extensive testing on bone tissue to prove that there are no negative side effects of our treatment. Dosing is another important aspect because studies have shown extreme levels of IGF-1 correlates with an increased chance of developing various cancers. Hopefully, after positive results in the bone tissue, we will begin to test on live mammals, such as mice. Currently, IGF-1 supplements are used as bone and muscle growth stimulants for athletes and patients. Possible side effects of our treatment could result in excessive bone growth, and/or malignant or rapid duplication of bone cells. Additionally, there is potential to unknown side effects of the treatment which will require lengthy and careful testing on bone tissue. We don't have a comprehensive knowledge of the side effects of the treatment until we do lengthy and careful testing on bone cells.

Discussions

As space exploration becomes more expansive, scientists are looking for ways for humans to safely and economically venture out in the universe. Thus, the health of astronauts is of the utmost priority. Furthermore, because there is such a large population suffering from osteoporosis, it is also an urgent issue. The proposal of using yeast cells to produce IGF-1 synthetically may be more sustainable compared to current treatments such as bisphosphonates and other bone morphogenetic protein supplements. Pills containing these treatments take up space in the rocket payload and will eventually run out when aboard the ISS or on deeper interplanetary missions. Having yeast cells continuously secreting IGF-1 could theoretically serve as an infinite source of bone-strengthening supplements, as long as they make it to space safely. On the spaceship, we will need to freeze-dry yeast cells and bring them back to life when necessary for the astronauts. There are many ways that an error may occur in the process of bringing these cells into space, such as the heat thawing them out. This may present a problem in the later stages of our project.

Once the device has secreted proteins, another challenge that presents itself is the collection of the IGF-1 hormone itself. It is extremely important that we collect a pure form of the enzyme. Any possible contaminants will affect the livelihoods of the astronauts. We have been researching two possible methodologies to isolate enzymes, each based on filter-sandwich assays. (Heinis et al. 2002) Human blood has a slightly basic pH level at 7.4. IGF-1 works in the blood, so in order for us to have the correct structure of the hormone, we need to ensure that this pH level is adhered to in our petri dishes.

The conditions in space may also prove to be inhospitable for our transformed yeast. The heightened levels of radiation along with the lack of gravity will cause unforeseen effects. Aboard the ISS, radiation levels are significantly greater than those on Earth—about 30 times the amount (Ohshima 2012). This speeds up the natural process of mutations, which could cause rapid death through apoptosis. There is a possibility that the transformation would simply mutate out after a short period of time (MTF), and the yeast would cease to produce IGF-1. There aren't many ways to recreate these conditions on Earth. The one situation in which microgravity can be imitated without going to outer space is in a diving aircraft, but it only lasts for a few minutes. We can also bombard the yeast cells with radiation at a similar level as the radiation aboard the ISS. However, in order to test all of the conditions with the most accuracy, the best way is to perform the testing aboard the ISS itself.

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