Comparison of normal rat leg bone with those under simulated microgravity and cosmic radiations conditions

Background/preliminary results

In space, microgravity conditions and cosmic radiation have detrimental effects on the skeletal system of humans such as weakened bones, lowered elastic moduli and abnormalized the concentrations of calcium and phosphorus, as compared to bones not subject to these conditions.[1] Biologically, bone has both organic and inorganic components that are interwoven to create a sturdy yet flexible skeletal structure. The organic components are mainly collagen and long chains of protein which intertwine in flexible fibers. The inorganic component is the hydroxyapatite ($Ca_5(PO_4)_3(OH)$), a calcium-rich mineral that strengthens the collagen.[2] Calcium and phosphorus are the two major elements of the bone. Approximately 20% of bone mineral is comprised of phosphorus, which combines with calcium to form hydroxyapatite. In the bone formation process, osteoblasts build the bone while osteoclasts break down the bone. Osteoblastic activity relies heavily on local phosphate concentrations in the bone matrix.[3]

Elastic modulus is defined as a material's resistance to elastic deformation under a force.[4] A positive correlation between bone mineral density and elastic modulus has been established at the whole-bone scale and is commonly used in assessing fracture risk, diagnosing osteoporosis, and measuring the efficacy of therapies.[5-7]

There are three variables in this research: HLS (Hind Limb suspension) group, radiation groups and the control group. The hind-limb suspension group's rats were suspended by their tails for two weeks. Rats receiving radiation were exposed to 0.5- 2.0 GY X-ray radiation dose via a biological irradiator which was administered every other day over a 10-day period. The control group received neither treatments.

As previous studies showed that microgravity could decrease bone mineral density and elasticity. Radiation exposure has been shown to damage osteoblast precursors within the irradiated volume but the specific mechanisms and potential influences on bone elasticity are still unknown. [8]

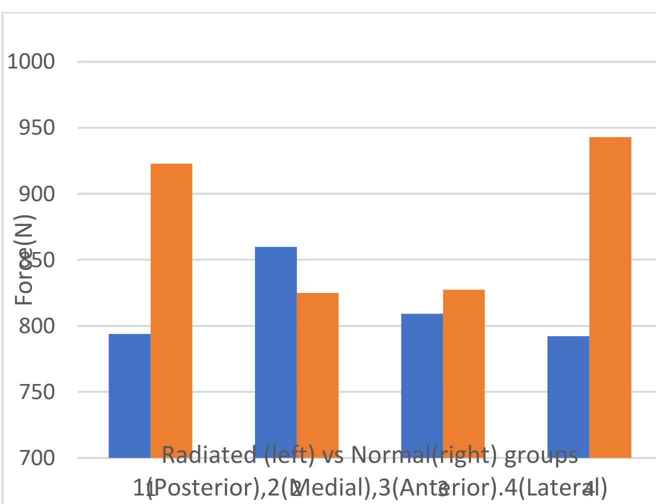

Figure1. Force applied to femur of radiated and normal group.

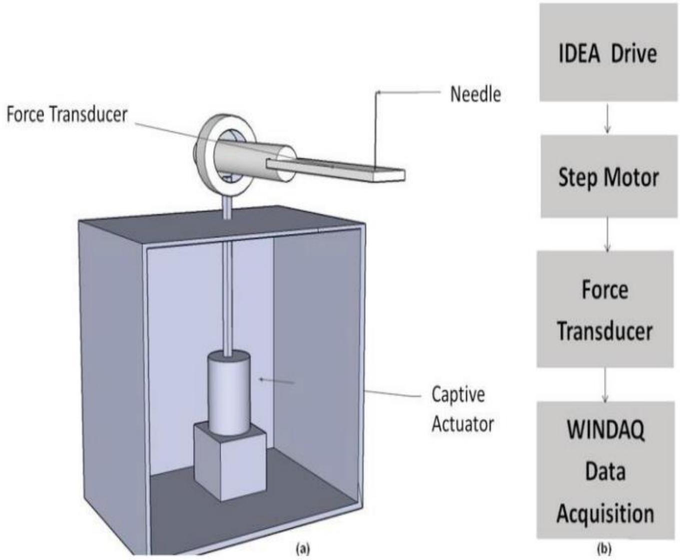

Figure2. (a) Sketch of the device showing the captive actuator, force transducer and the contact for bending. (b) A flow chart of the communication process to collecting and storing data.[9]

Question1: How are the elasticity differential in the control, HLS and radiation groups?

Hypothesis: The HLS (Hind Limb suspension) group and radiation groups will have lower elasticity than the control group. The HLS groups will be the lowest among the groups.

Methods: The bones will be subjected to the three-point bending technique which is used to measure the biomechanical paramount such as stress, strain, and stiffness. The three-points bending method fixes the bones at both ends while a force transducer exerts a known force upwards at a known speed, acting perpendicular to the bone. Each leg bone (tibia and femur) were bent with force prod acting on: posterior, medial, lateral, anterior points of center of the bone. The WINDAQ software records and displays the force applied to the bone. Also, the lengths of bones and inner and outer radii of the bone will be measured. All those measurements will be used to calculate Young's Modulus of elasticity. The two-way ANOVA will be used for data analysis. Factor A will be control groups vs treatment groups. Factor B will be the bone orientation (posterior, medial, anterior and lateral).

Expected results: If our hypothesis is supported, I might observe that the HLS group and the radiation groups have the lower force to change than the control groups. And the HLS group has the lowest force change. This might be due to microgravity acting little or none force to the rat's bone, so osteoclast breaks the calcium down from their bone into their blood to achieve homeostasis and its elasticity decreases through this process. [10]

Question2: How are the elemental differential in the control group, HLS (Hind Limb suspension) group and radiation groups?

Hypothesis: A trend of lowered calcium phosphate ratios will be in the HLS & irradiated bone cross-sections compared to the control group. The lowest ratio will be the radiation groups.

Methods: Bones will be cut in thin cross-section with a diamond tip saw. Then the bones are fixed to the slides, sputter-coated with gold for analysis using SEM (Scanning Electron

Microscope). In addition to imaging the bone, an energy disposed analysis (EDA) quantifies the relative percentages of carbon, oxygen, phosphorus, and calcium present. Data is summarized using graphs and the validity of data will be ascertained by using statistical analysis such as two-way ANOVA. Factor A will be control groups vs treatment groups. Factor B will be the bone orientation (posterior, medial, anterior and lateral).

Expected results: If our hypothesis is supported, the SEM data would indicate lowered calcium phosphate ratios in the HLS & in the irradiated bone cross-sections compared to the control groups. The irradiated groups would have the lowest ratio. This might be due to the 0.5- 2.0 GY X-ray radiation's effect on calcium, phosphorus and oxygen concentrations of the bone.[11]

Question3: What effect does radiation have on the cortical area and the cavity area of the tibia and femur.

Hypothesis: The cortical wall thickness will be decreased in the irradiated groups. Irradiation will induce a larger cortical and cavity areas compared to the control, non-irradiated group.

Methods: Bones mounted with epoxy onto glass slides and cut into thin (3 mm) cross-sections with a diamond tip saw blade. Then, the bone cross-sections will be imaged using a Leica MZ6 microscope (IL, USA) equipped with an OptixCam digital camera. The image analysis program, ImageJ (NIH, USA), will be used to measure the cortical and cavity areas. Using ImageJ, I will select an "area of interest" around the outside border of each bone's cortical cross-section, and the outside border of the bones' cavity. The pixel area will then be converted to mm square for each respective area.

Expected results: I expect to observe a larger cortical and cavity area with irradiation. Similarly, irradiation will decrease the cortical wall thickness. My rationale for these expected results is because human bone gets weaker due to losing calcium and phosphate when people age. To withstand the body weight and potential force, human bones will get bigger to increase the area and decrease the pressure by following the formula P=F/A(P(pressure), F(force), A(area)). [12]
If observation suggests that the cortical area did not enlarge, it might be because the bone did not adapt weight-bearing since the gravity in near earth orbit got 5-10% smaller but more importantly in orbit the falling satellite is in apparent weightlessness condition.

Significance of research

In the future, it won't be just astronauts who could go to space but probably the regular people all around the earth. The SpaceX company has proposed the development of Mars transportation infrastructure in order to land the first humans on Mars by 2024 and facilitate Mars colonization eventually.[13] However, there are still mainly unknowns about the human skeletomuscular system reactions under the space condition. Further experimentation is needed to confirm the results on the compounding effects of cosmic radiation and microgravity on the composition and characteristics of leg bones. Furthermore, this peculiar summer, I will investigate the effects of hindlimb suspension and radiation exposure on the structural and chemical properties of the rat leg bones via determination of elastic moduli, elemental concentrations and anatomical structure. Accompanying with statistical analysis to discover the relations between these variables. I will try to use 3D graphs techniques to integrate and illustrate the variability of the results of elasticity, elemental composition and anatomical structure of HLS (Hind Limb suspension) groups, radiation groups and the control groups simultaneously.

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Appendix A. Gantt Chart of activities.

Activities	Weeks #									
	1	2	3	4	5	6	7	8	9	10
1. Bending the bone.	X	X								
2. Cutting the bone and fix to slides		X	X							
3. The two-way ANOVA data analysis				X	X	X	X			
4. Microscope imaging		X	X							
5. ImageJ analysis		X	X	X						
6. Coated with gold			X	X						
7. SEM			X	X	X	X				
8. EDS					X	X	X			
9. 3D graph							X	X	X	
10. Poster							X	X	X	
11. Presentation								X	X	X

Appendix B: Details of activities shown in Gantt Chart (Appendix A).

1-Bending the bone- The bones will be subjected to the three-point bending technique which is used to measure the biomechanical paramount such as stress, strain, and stiffness. The three-points bending method fixes the bones at both ends while a force transducer exerts a known force upwards at a known speed, acting perpendicular to the bone. Each leg bone (tibia and femur) were bent with force prod acting on: posterior, medial, lateral, anterior points of center of the bone. The WINDAQ software records and displays the force applied to the bone.

2- Cutting the bone and fix to slides- Bones mounted with epoxy onto glass slides and cut into thin (3 mm) cross-sections with a diamond tip saw blade.

3- The two-way ANOVA data analysis- Factor A will be control groups vs treatment groups. Factor B will be the bone orientation (posterior, medial, anterior and lateral).

4- Microscope imaging- the bone cross-sections will be imaged using a Leica MZ6 microscope (IL, USA) equipped with an OptixCam digital camera.

5- ImageJ analysis- The image analysis program, ImageJ (NIH, USA), will be used to measure the cortical and cavity areas. Using ImageJ, I will select an "area of interest" around the outside border of each bone's cortical cross-section, and the outside border of the bones' cavity. The pixel area will then be converted to mm square for each respective area.

6- Coated with gold- Bones will be cut in thin cross-section with a diamond tip saw. Then the bones are fixed to the slides, sputter-coated with gold for analysis using SEM (Scanning Electron Microscope).

7- SEM- Scanning Electron Microscope to provide the image of the posterior, medial, anterior and lateral of the rat's bone.

8-EDS- Energy dispersive system is used to separate the characteristic x-rays of different elements into an energy spectrum, and EDS system software is used to analyze the energy spectrum in order to determine the abundance of specific elements.

9-3D graph- A graph in 3 dimensions is written in general: $z = f(x, y)$. That is, the z-value is found by substituting in both an x-value and a y-value. I will be trying to show how my 3 groups interact with each other.

10- Poster- Integrate all my data and illustrate the results by making the poster.

11-Presentation- I will be making presentation at INBRE or Arkansas Space Grant Consortium or equivalent regional conference.