Mechanical Testing of Irradiated and Non-irradiated Rat Vertebrae for Space Radiation Effects on Bone Properties: A Pilot Study

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ABSTRACT

Mechanical testing was conducted on rat vertebrae with the goal of assisting space radiation research in determining the effects of radiation on bone fatigue properties. This pilot study outlines the developed testing procedures used on both Instron and MTS machines to run cyclic fatigue tests on rat lumbar vertebra to determine the fatigue life of each specimen. The L5 bone from rat I.D. 722E was able to endure approximately 4,000 cycles before failure. However, a few samples failed immediately (under 30 cycles), while others never displayed signs of failing up to 150,000 cycles. This report addresses these inconsistencies, and offers potential corrections that can be made in future studies using the sample preparation and MTS experimental testing methods described below.

INTRODUCTION

In 2021, NASA intends to conduct the first planned, human-crewed space mission of the Orion spacecraft to Mars. However, there still exists uncertainty about the effects of radiation exposure on the health and safety of the astronauts. Berkeley Biomechanics is seeking to understand the effects of ionizing radiation exposure on astronauts, who would be travelling in space for up to 6-8 months on both ways of the journey. One major concern is the effect radiation has on the qualitative changes of the bone tissue and how this may contribute to an increase in fracture risk during a mission or upon return home.

The purpose of this study is to determine if radiation exposure has an effect on the fatigue properties of bone. Mechanical testing was conducted to obtain the Young's Modulus of each L5 segment of rat vertebrae, which could then be used to execute an appropriate fatigue, or cyclic loading, test to observe how many cycles each sample could withstand under a given load. We hypothesized that the radiation causes a change in bone tissue at the protein level causing a decrease in the energy-dissipation mechanisms of bone which would cause a decrease in the fatigue life, or number of cycles to failure. This semester has primarily been spent as a pilot study to refine the sample preparation and experimental testing procedures of fatigue using rat lumbar vertebra. The work the team put in this semester has worked towards designing an effective protocol of sample preparation and fatigue testing method that can be used on future samples to more seamlessly analyze the differences in mechanical properties of bone in irradiated and non-irradiated samples.

METHODS AND MATERIALS

Sample Preparation

As a continuation of the Fall 2015 semester, the first step in the testing process was to finish preparing the rat vertebrae prior to mechanical testing. Soft tissue was carefully removed from the received irradiated and non-irradiated rat spines with tweezers and scalpels, being careful to not damage the hard exterior cortical shell of the

Lumbar bones in the spine. The L3, L4, and L5 spine segments were then removed from the tissue-less spine through cuts through the intervertebral discs. All prostheses, excluding the dorsal prosthesis, were then removed with either a scalpel or dremel, again being extremely careful to not nick the vertebrae itself. Damage to the vertebra via a small scratch or cut could cause unintended crack propagation that would compromise the modulus and fatigue data collected later. It is key to note that the bone must be continuously dowsed in PBS to ensure the hydration of the bone.

The next step in the sample preparation process was to create uniaxial cuts on each now-separated segment of the spine. Fixtures, such as the one shown in Figure 1, were 3D printed to lodge the bone in order to more easily create the uniaxial cuts. A rod was placed through the spinal canal of the bone segments and suspended above the empty rectangular fixture with the dorsal prosthesis pointed into the fixture.

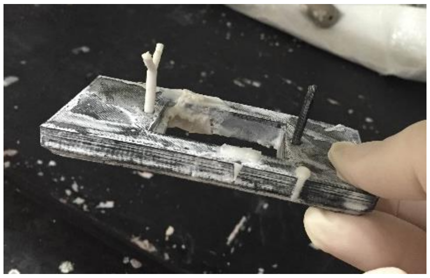

Figure 1: An example of a 3D printed fixture

PMMA was mixed and slowly added to the fixture and left to harden around the dorsal prosthesis, locking the bone into place with the vertebrae suspended above the hardened PMMA, to ensure no damage of the vertebrae itself due to the heat of the PMMA hardening reaction. One issue that was observed was excessive expansion by the PMMA, as seen in Figure 2.

Figure 2: Excessive PMMA bubbling with an example of a loaded bone into the fixture

While it didn't expand enough to encapsulate the vertebrae itself, it still presented a problem with both allowing the bone to sit straight as well as for mounting it for cutting. This problem became much less frequent by a combination of designing slightly refined fixtures, slowly stirring the PMMA mixture, and continuous pouring of the PMMA into the fixture to avoid trapped air bubbles.

The rectangular fixture, now with the bone cemented into place by the PMMA, was able to be fixed and cut on either end with a diamond saw in order to remove the cartilage on either end of the vertebrae, leaving the vertebrae almost entirely intact (as seen in Figure 3).

Figure 3: L5 bone with uniaxial cuts by a diamond saw while fixed into the 3D printed fixture

When fixing it above the saw, it is crucial for the bone to be positioned carefully to ensure that parallel cuts are made. That way, when compressed during mechanical testing, uniaxial compression can be applied. In other words, to ensure that the load is distributed evenly across the top surface of the bone and not concentrated on a higher elevated peak of the bone. Of course, since the alignment is done by eye in our current method, there is often slight slope visible during the mechanical testing procedure. One step taken towards solving this issue this semester was designing a movable ball bearing fixture for the load cell compression machines, which would have the ability to flatly settle into place on the top surface of the bone. Once cut, the bone was removed from the PMMA fixture combination using a dremel, cutting just above the spinal canal to remove as much of the excess bone outside of the vertrbral body as possible. In addition to the preparation process, implemented changes from last semester also included improvements to administrative bookkeeping, such as more structured notes on the progress of each spine and more intensive scheduling.

Experimental Testing

While Sample Preparation followed similar protocol from the conclusion of last semester, the majority of progress and work made this semester for this pilot study was done in Experimental Testing. As previously mentioned, a movable fixture was designed to help correct for errors in parallel cuts made during the Sample Preparation process. Additionally, a new, smaller water bath was designed in order to decrease the amount of PBS needed to hydrate the bone during testing and allow for easier adjusting and tightening of the movable fixture during set-up for the tests.

Two testing machines were used during the Experimental Testing process of this study: the Instron and the MTS (set up for both can be seen in Figures 4a and 4b).

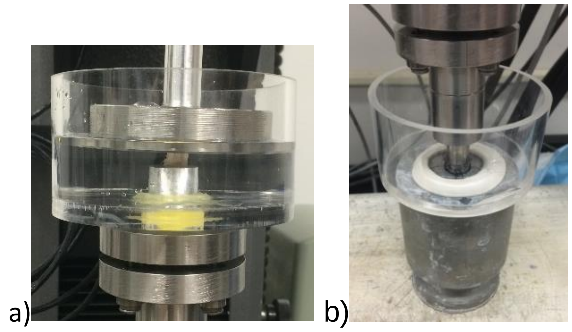

Figure 4: a) Instron machine set-up with loaded bone b) MTS machine set-up with loaded bone

Detailed Standard Operation Procedures of the following methods were authored and documented for each machine to assist correct use of the machines running and editing the methods desired for testing.

As observed in Figure 4, the bone was initially loaded onto the fixtures in each machine inside the newly created PBS bath, ensuring that the center axis of the bone lay directly underneath the adjustable fixture mounted above. After applying a preload of 30N, the screws on the fixture could be tightened into place once settled flat on the surface of the bone, and PBS could be added to the bath to maintain bone hydration. In order to test the fatigue life of the bone, a method was initially run to obtain the modulus of that specific bone. This was done by applying a sinusoidal load to bone 10 times from 30N to 80N. The data collected from this test provides a stress vs. strain curve, the slope of which is the modulus, or stiffness, of that particular bone. The multiple loading cycles provides homogeneity of the bone, and thus allows for the modulus to be recorded from the 10^{th} cycle. Using this modulus, the peak load of the cyclic loading fatigue test can be calculated using the following equation:

$$F = A * E * \varepsilon$$

The strain is ideally predetermined to remain constant for all samples. However, for the sake of this pilot test, the strain value was manipulated across the samples, anywhere from 0.007 to 0.012, in order to gather reasonable data that will help determine a set strain during the proceeding specimen batch received for testing this improved study protocol. The minimum force for the cyclic fatigue loading was set to correlate to a strain of 0.002. The cross-sectional area of the bone was determined through CT scanning of the specimen. To ensure quick transition from the Obtain Modulus test to the fatigue test, since the bone should not be repositioned in the water bath between the two tests, a new Matlab GUI was designed to output the maximum and minimum cyclic load necessary for the fatigue test using the Modulus data. From the fatigue test, data displaying the number of cycles until failure could be could be gathered and presented in graphical form for ease in comparison between the irradiated and non-irradiated samples.

RESULTS

The following graph shown in Figure 5 is created from the data obtain from the fatigue test of the L5 bone from rat specimen 722E.

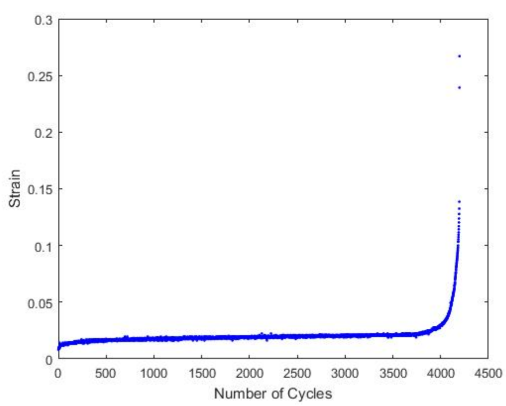

Figure 5: 722E L5 fatigue curve, showing strain vs. number of cycles

As observed from the graphical illustration, failure of this specimen occurred at approximately 4,000 cycles, denoted by the sharp increase in strain. However, throughout the testing process, there were a handful of specimen that either failed immediately (<30 cycles) or didn't show any signs of failure (>150,000 cycles). Potential explanations are explained further in the following discussion section.

DISCUSSION

The preliminary tests were conducted with the Instron machine on L4's, hoping that its potential limitations could be worked around because of its user-friendly interface. Unfortunately, the limitations proved too insurmountable for the tests to continue. This specific Instron machine was not designed for running fatigue tests, primarily because its screw-based system induced precision problems straining the bone to the appropriate amount. The MTS, on the other hand, is a hydraulic-based machine, allowing it to be much more accurate in reaching smaller strains. Problems also arose during the data accumulation phase of testing, as the Instron would consistently slow down from its 1Hz cycle speed during the multiple hour testing period, as well as occasionally not allowing the data that was accumulated to be saved. The MTS machine was able to show that it could maintain its speed and accuracy over long fatigue tests at cycle speeds up to 2Hz. Finally, the load cell for the Instron also maxed out at 100N, which proved to not be able to cause failure within 150,000 cycles, as demonstrated by MTS testing.

Once the methods were fully constructed, the L5's were tested using the MTS machine, which, while being much more successful in providing reasonable data, still included hiccups that are intended to be corrected over this summer and next semester. 4 of our 12 L5 samples tested experienced immediate failure under 30 cycles. Simultaneously, another set of our specimen ran for over 150,000 cycles and didn't exhibit signs of failure. There are a few potential reasons that hope to be addressed as next steps for possible causes. Most importantly, a consistent strain value needs to be chosen to run all of the samples at to provide constant conditions for testing. As a part of our pilot testing, we've run samples using strains anywhere from 0.007-0.012, depending on the plausibility of the loads they output from our initial modulus tests. When tested above approximately 180N, no matter of the percent strain correlating to that particular bone, immediately failure tended to occur, while loads under 145N showed no signs of failure after 150,000 cycles. It is important to note this very small sample size (12 samples in total, including 9 irradiated specimen and only 3 non-irradiated), but it is still worth noting in hopes of future corrections. Part of this variability, however, could be due to errors in the sample preparation process. Alterations may have to be put in place to ensure more careful tissue removal and cutting of the bone to prevent damage that could lead to premature propagation and fracturing. Additionally, some bones appeared slightly off-axis, which may have not allowed the bone to compress down its center axis, concentrating the load on a potentially weaker vertical axis of the bone.

A potential explanation for the specimen that never failed by 150,000 cycles could do with the fact that the limit for the MTS machine was set at 1.8mm. That is, the test would end only when the bone displaced 1.8mm from its original value. The outer cortical shell of these bones is very strong, and even though there might have been failure of the inner trabecular tissue, the cortex prevented the bone from displacing far enough to trigger the end of the test.

CONCLUSION

Overall, the designed testing methods outlined in this pilot study provide a more successful experimental testing process using the MTS machine that can be emulated, and further improved upon, for future studies. Given a larger sample size of rat specimen who have undergone higher dosages of radiation, it would now be possible to use the progress and procedures developed during this semester to gather more accurate, consistent, and reliable data to begin to analyze the effects radiation exposure actually has on bone properties.

Moving forward, more immediate analysis on can be done on the 12 spines we were able to test this semester, as we only finished conducting the testing this week. Additionally, there are further avenues of testing procedure improvement that will be explored. One inconsistency observed during the testing phase was that if the Obtain Modulus method was rerun, a slightly higher value was produced, possibly due to strength hardening on the bone. In future studies, it may be recommended that multiple modulus tests are run in order to find a more consistent modulus value to use when calculating the max load the cyclic fatigue tests runs to. Further, future research intends to explore the introduction of metal caps to surround the bone during testing. Not only would this provide flat, parallel surfaces for more uniform loading distribution, but would also allow the severed trabecula on either end to be attached to something, ideally in order to hold them in fixed place rather than in a frayed state.

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