Analysis of Mechanical Differences Between Healthy and Injured Bovine Intervertebral Discs

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Abstract

Back pain and other back problems continue to be issues affecting millions across the human population. It is important to characterize the mechanical property differences between a healthy disc and an injured disc to better understand what changes occur when a healthy disc degenerates.

The objectives of this study were to observe the differences in creep and recovery responses and applied mechanical loads between a healthy bovine intervertebral disc and a degenerated disc. Bovine disc samples were isolated from the spine and tested using a MTS machine. Each sample was initially tested twice as a healthy disc under a slow compressive ramp of 300N for two hours, and then unloaded for recovery for the 12 hours following. Then, the sample was injured using chABC enzyme to digest away the inner tissue layer of the disc. The injured sample was then tested using the sample parameters as the healthy sample using the MTS machine.

Some samples from the study suggest that the healthy disc compresses less than the injured disc does during the initial compressive loading conditions and recovers less than the injured disc does during the recovery process. This shows that the injured disc, because it has less disc tissue, compresses more than the healthy disc because of its inability to distribute and absorb loads and correlates with spine problems because the adjacent vertebrae in the spine are more likely to come in contact with one another in people with degenerated discs.

The fact that the injured disc recovers more than the healthy disc does indicates it absorbs more fluid from its surroundings, and is a phenomenon not well understood from the study in this point in time. Further testing and possibly biochemical analysis of the tissues found in healthy and injured bovine disc samples will provide better insight into characterizing the differences between healthy and degenerated intervertebral discs.

Introduction

The intervertebral disc is a structure found between vertebrae in the spine and is an important factor in the flexibility of the spine. It is composed of a gelatinous inner tissue called the nucleus pulposus and an outer tissue called the annulus fibrosis. Over time, the intervertebral disc degenerates and consequently loses its ability to distribute and absorb external loads applied to the spine, leading to back pain, inflexibility, as well as other serious problems. Since the intervertebral discs are sensitive to previous loading conditions, mechanical testing of the properties of healthy and degenerated disc samples must be strictly controlled.

The nucleus pulposus and annulus fibrosis introduce viscoelastic properties that should be accounted for in the mechanical analysis of intervertebral discs. A standard model of viscoelasticity composed of a Maxwell element in series and a Voigt element in parallel models the disc response to loading conditions. An initial load is applied and the spring elements in the standard model compress to

represent the instantaneous deformation, or displacement. Over time, the disc response to loading conditions change, represented by the dashpot element in the model and is known as the creep displacement. When the load is taken off, there is again both the instantaneous recovery and time-depending recovery.

Methods

Each sample was subject to three separate 14-hour creep and recovery mechanical experiments. The MTS test consisted of a 10-minute preload of 20N followed by a 300N compressive load for two hours, after which the sample recovered under a load of 20N. The samples were initially frozen but thawed in 1xPBS in a fridge for 24 hours before testing and kept outside at room temperature for an hour before testing in 1XPBS. While testing, the disc was kept hydrated in a 1xPBS bath that covered all tissue when the sample was uncompressed.

Tissue surrounding the bovine spine was originally removed leaving, only the intervertebral disc in tact between the vertebrae. The discs were set into flat samples the MTS machine was capable of testing uniformly by applying an uniaxial load using PMMA and then frozen in a gauze wrapping soaked in 1xPBS until needed.

Each sample was initially tested without altering the conditions of the discs, known as *Sample Number Healthy 1*. Because disc loading history affects its mechanical properties, the *Healthy* 1,the experimental results of the *Healthy 1* sample was disregarded. The *Healthy 2* sample was treated the same way as the *Healthy 1* sample and subject to the same conditions. After each disc sample was tested twice under healthy conditions, chABC enzyme at a concentration of 30u per disc was injected into the sample using a syringe. The sample was left outside at room temperature in 1xPBS for half an hour to an hour before hydrating in 1xPBS for 12-16 hours before being tested in the MTS.

Results

Three samples were tested using the methods described and their displacement values at different time points were compared to analyze differences between the conditioned healthy sample and the injured sample.

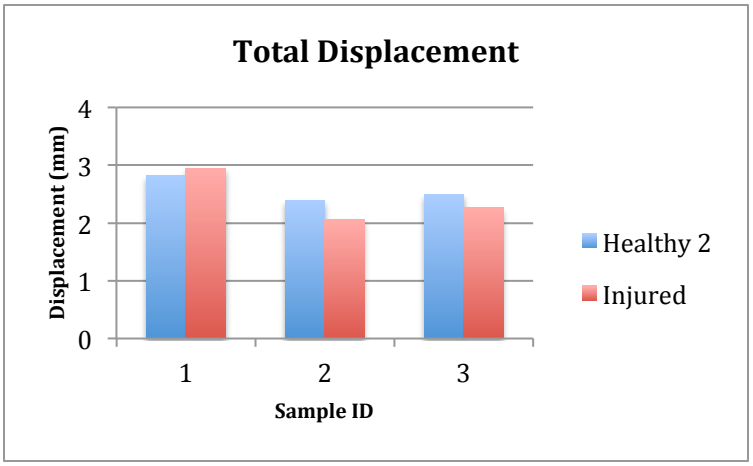

Fig 1 – The total change in disc height of each sample between the healthy condition and injured section

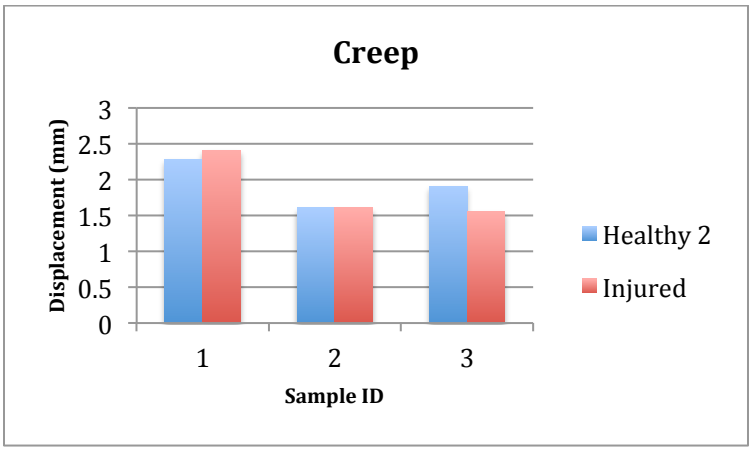

Fig 2 – The changes in disc height during two hours of constant 300N load

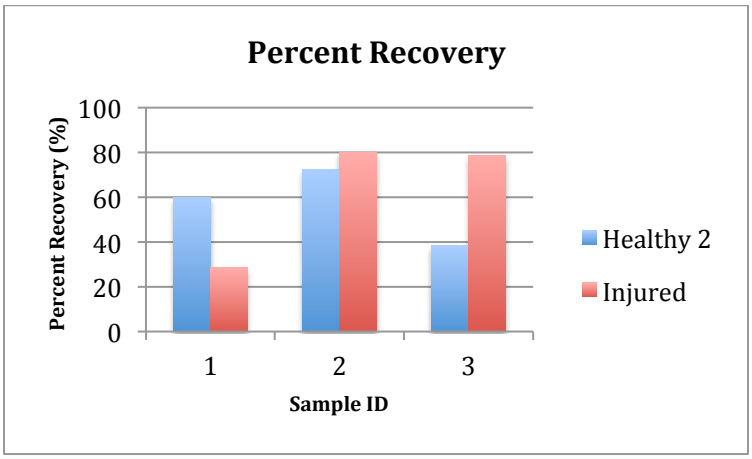

Fig 3 – The total recovery displacement compared to the total displacement of the sample under loading conditions

Between the healthy and injured samples, the p-value of the total displacement, creep, and percent recovery are respectively 0.40223274, 0.647718522, and 0.809480663.

Discussion

The results are inconclusive because the p-values do not indicate a significant difference between the healthy and injured samples. This is likely due to the human error in making samples: if the disc and two surfaces of PMMA are not parallel and flat, then testing in the MTS is not entirely accurate because the load is not uniaxial. In addition, the water bath used to test the samples started leaking and temperature sensitive hypoxy was used to create a temporary seal that seemed to have affected the data.

One potential source of the seemingly similar properties of the healthy and injured discs could be the digestion of the nucleus pupolsus. Although a consistent

amount of chABC enzyme was used each time, the enzyme may not have been given proper time to digest away the tissue or it may simply not have worked well enough to digest enough tissue to create an injured sample different from a normal healthy sample. Future testing will include methods to extract the nucleus pulposus using a small tool.

Acknowledgements
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References

O'Connell, G. D., Jacobs, N. T., et al., 2010. Axial creep loading and unloaded recovery of the human intervertebral disc and the effect of degeneration. Journal of the Mechanical Behavior of Biomedical Materials 4 (2011) 933-942.