Reviewer's report

Title: Viscoelastic properties of bovine knee joint articular cartilage: dependency on thickness and loading frequency.

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Reviewer: X. Lucas Lu

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Major Compulsory Revisions

Summary
This paper measured the viscoelastic properties of cartilage in bovine knee joints using a dynamic mechanical analysis (DMA) device. It was found that the storage modulus of cartilage, but not the loss modulus, is frequency dependent. Both storage and loss moduli are correlated with the thickness of cartilage.

General Comments
Articular cartilage can sustain the high frequency and high magnitude loading in host for several decades, and no engineering replacement exists at current stage. The unique function of cartilage is highly dependent on its ultrastructure. Fluid represents 65-90% volume in the tissue and is trapped in a dense solid matrix. When impact or high frequency loading is applied on cartilage, fluid pressure inside tissue can share 99% of the load, which endows the tissue an incompressible short time response. This is how the solid matrix in cartilage is protected from overloading or traumatic damage. The viscoelastic behavior of cartilage is largely related to the fluid flux in tissue and also known as fluid-depend viscoelasticity. A major flaw in the present study is the ignorance of this important mechanism, which further caused some major limitations in this study.

DMA was used to perform a frequency sweep on cartilage tissue, and two preload conditions were applied before testing with a 60s rest period. It usually takes hours for cartilage tissue to fully recover from a 10% thickness deformation. Thus the length of rest period could significantly affect the outcome of following tests.

The heel-strike can happen in less than 10 ms, but the impact loading is different with a 100 Hz dynamic loading. Under continuous high frequency loading, cartilage tissue can barely follow the loading platen. This is the famous “lift-over” problem in cartilage biomechanics. This problem is usually cautiously avoided by monitoring the force response curve of loading device. It is important for the author to demonstrate the actual force response curve and displacement curve over time during dynamic test. Although a force-control test was performed in this study, it is questionable whether the tissue can follow an 88 Hz vibration in complete sinusoidal curve.
A frequency sweep was performed on each sample. Does the sequence of frequency sweep affect the results? It often takes hundreds of cycles for cartilage to reach equilibrium state under 1 Hz dynamic loading. The loading history could significantly change the mechanical behaviors of cartilage if the tissue is not fully recovered. However, as indicated previously, the recovery time could be thousands of seconds.

The results demonstrated different moduli for cartilage at different locations. However, is this difference correlated with the thickness of tissue, or correlated with the strength and ultrastructure of solid matrix in cartilage? Without answering this question, the clinical meaning of this study is limited. It is widely accepted that intrinsic mechanical properties and ultrastructure of cartilage are regional-dependent in knee joint. However, current testing strategy showed that the properties are highly correlated with tissue thickness. Is the obtained result in this study the true intrinsic property of cartilage tissue? Or is it correlated with the geometry of the tissue?

At the early stage of OA, cartilage is under swelling. This is mainly due to the increase of proteoglycan and degeneration of collagen network, which results in high water content in tissue. The present study used healthy cartilage. Thus the results cannot be used to explain or predict the behaviors of OA tissue.

It is important to identify the significance and novelty of this study. What is the clinical relevance of the finding? What type of new knowledge does the result provide? A frequency dependent and thickness dependent modulus is not necessarily new findings.

What is geometry of tested cartilage-bone block? The indenter has a diameter of 5.2mm. What is the size of cartilage surface?

Is any technique employed to align the indenter tip normal to the articular surface?

In brief summary, the novelty and significance of this study should be clarified. Some major technique concerns in experiments should be addressed. The authors did an inadequate job in the explanation of results.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests