**Cover Letter**

On behalf of my co-authors, please find enclosed our manuscript titled “Real-time Visualization and Analysis of Chondrocyte Death Due to Mechanical Loading in Fully Intact Murine Cartilage Explants” for submission to Journal of Visualized Experiments (JoVE). In this manuscript, we introduce a method that allows for assessment of the spatial extent of cell death induced on the articular surface of mechanically loaded cartilage explants from murine synovial joints. This method requires careful dissections of mouse synovial joints without compromising chondrocyte viability, followed by mechanical testing of vitally stained cartilage explants using a custom microscope-mounted device. Importantly, this method enables testing on fully intact cartilage without compromising native boundary conditions. Moreover, it allows for real-time visualization of vitally stained articular chondrocytes and single image-based analysis of cell injury induced by application of controlled static and impact loading regimens.

Using this method, we have demonstrated that the spatial extent of cell injury depends sensitively on load magnitude and impact intensity. This method can be easily adapted to conduct comparative studies investigating the effects of different controlled environmental and mechanical conditions on the mechanical vulnerability of *in situ* articular chondrocytes. Additionally, the method can be used to screen treatments aimed at reducing the sensitivity of chondrocytes to mechanical stimuli when the tissue is exposed to chronic, abnormal loads (e.g., after a meniscal tear or other joint-destabilizing injury). Consequently, we believe that this manuscript and detailed visualization of the experiments/methods using JoVE’s unique multimedia format will be of a great interest to the readers of JoVE, particularly those specializing in cell mechanics, tissue mechanics and murine OA models.

We would also like to confirm that all the listed authors designed the study and helped write the manuscript. Additionally, Alexander Kotelsky and Joseph S. Carrier conducted the experiments.

Senior Science Editor, Nandita Singh, Ph.D., has assisted us in the submission process.

We would like to suggest Lin Han ([lh535@drexel.edu](mailto:lh535@drexel.edu), Drexel University), X. Lucas Lu ([xlu@udel.edu](mailto:xlu@udel.edu), University of Delaware), Corinne Henak ([chenak@wisc.edu](mailto:chenak@wisc.edu), University of Wisconsin-Madison), Gerard Ateshian ([ateshian@columbia.edu](mailto:ateshian@columbia.edu), Columbia University), Robert Sah ([rsah@ucsd.edu](mailto:rsah@ucsd.edu), UC San Diego) and Clark Hung ([cth6@columbia.edu](mailto:%20%3Ccth6@columbia.edu%3E), Columbia University) as referees for this manuscript.

Please feel free to contact us if you have any questions.

Sincerely,

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