Impact-induced Fissuring at High Strain Rates in Adult Equine Hock Cartilage

Cornell University, Ithaca, NY, USA.


Introduction:
Osteoarthritis (OA) affects approximately 10% of the US population, causing a large economic burden [1]. Post-traumatic osteoarthritis (PTOA) represents a unique subset of OA, wherein damage follows traumatic injury. An estimated 12% of all OA is PTOA [2]. Further, PTOA is particularly relevant for both the human ankle and the equine hock, as these joints rarely exhibit OA without a known injury, but frequently exhibit PTOA following traumatic or repeated injury [2,3].

Injury is thought to cause PTOA in part through damage to the extracellular matrix of the articular cartilage. Both fissuring and softening of the cartilage matrix have been observed following impact (e.g., [4]). Evaluating the immediate aftermath of impact injury on matrix damage can provide insight into the mechanical parameters necessary to create such damage in-vivo.

Previous evaluations of impact injury have found that the mechanical parameters necessary to create damage vary widely, depending on the animal, joint and type of sample. For example, cartilage impacted across the intact joint results in larger peak stresses before damage occurs than cartilage impact on explants [5,6]. These discrepancies may be due to differences in strain rate, rather than differences in the amount of stress required to cause damage [7]. The effect of impact strain rate on signs of matrix damage, such as fissuring or softening, has not been evaluated. Recently, our laboratory has developed a spring-loaded impact apparatus that mounts on a confocal microscope equipped with a high speed camera. This enables the application of impact loads and direct measurements of tissue strains [8]. Therefore, the purpose of this study was to evaluate the effects of strain, strain rate, force, and force rate on the presence of matrix damage (fissures) in adult equine hock cartilage.

Methods:
Adult equine hock cartilage with subchondral bone was removed from the joint. Samples were stained using 5-DTAF for 30-60 minutes and rinsed briefly in PBS. Samples were attached to a custom impact apparatus [8]. The subchondral bone was glued to the backplate, which acts as a cantilevered beam. The displacement of the backplate was calibrated with known forces to enable force measurement. Different amounts of spring tension were used to impact the samples, resulting in different peak strains, peak forces, strain rates and force rates. During impact, samples were imaged at 1000 frames per second using a Phantom V7.1 digital video camera. Camera data were used to determine strain and force from the cartilage displacement under the impact tip and the movement of the backplate, respectively. Following impact, samples were divided into groups based on the presence of a fissure. Four samples each were analyzed in the intact and the fissured groups. Axial strain was calculated by comparing the undeformed cartilage thickness to the cartilage thickness under the impact tip in each frame. Force was calculated from the movement of the backplate relative to the position prior to impact. Rates were calculated from the difference in strain or force between subsequent 1 ms video frames. Peak strain rate, peak force rate, peak strain and peak force were determined for each sample.
For the four fissured samples, fissure length, width, area and angle with the articular surface were measured using ImageJ. Differences between the fissured and intact groups were tested using t-tests. Linear regression analysis was used to compare fissure dimensions with strain rate, force rate, strain and force. All p-values were corrected with Bonferroni’s method. Significance was set at $p \leq 0.05$, and near significance at $0.05 < p \leq 0.1$.

**Figure 1.** Impact methods. A – cartilage was mounted in a custom device and impacted while imaged with a high-speed camera. B – video frames of cartilage under impact, articular surface is to the right.

**Results:**
Strain rate was significantly larger in fissured samples than in intact samples (Fig. 2A), while force rate and peak strain were nearly significantly different between the two groups (Figs. 2B, 2C). Peak force was not different between fissured and intact samples (Fig. 2D).

**Figure 2.** Mechanical results for fissured versus intact samples. A – peak strain rate, B – peak force rate, C – peak strain, D – peak force. Diamonds indicate each sample, black bars with error bars show averages plus/minus one standard deviation for each group. * indicates $p \leq 0.05$, † indicates $0.05 < p \leq 0.1$.  

Fissure morphology varied between samples (Fig. 3). Fissure area significantly decreased with increasing strain rate (Fig. 3A). Fissure angle was significantly less oblique to the articular surface as the force rate increased and was nearly significantly less oblique as the force increased (Fig. 3B). Fissure width nearly significantly increased with strain. Some fissures tended to close following impact (Fig. 3C), while others were large and remained open (Fig. 3D).
Discussion:
In this study, strain rate was an important variable for both the presence of fissures and fissure area. Other variables were also significantly correlated with fissure morphology, and nearly significantly different between fissured and intact samples. These results suggest that strain rate is an important predictor of the matrix damage that precedes PTOA, but that multiple variables should be used to obtain a more complete understanding of the mechanics that cause damage. Interestingly, the resulting fissure area decreased with increasing strain rate, potentially suggesting different failure mechanisms.

There are several limitations in the present study that merit attention. Because the area of each sample was not measured, the stress rate could not be calculated. Also, there were only four samples in each group. Despite small sample sizes, this pilot study demonstrates the potential for these methods to be used to determine specific thresholds in strain rate separating fissured and intact samples.

Building on the present study, there are several interesting potential areas for ongoing research. These experimental methods could be combined with logistic regression methods [9] to establish specific strain rate thresholds that cause fissure. Additionally, these methods can be extended to the human ankle, for which the adult equine hock is an appropriate animal model. Finally, the experimental set-up with a high-speed video camera allows for the evaluation of microscale strains and strain rates [8]. This may provide more detail regarding the local mechanics of fissuring.

Significance:
This research provides the basis for understanding the mechanical variables that cause PTOA, and presents an analysis of the effects of strain rate of matrix fissuring.

ORS 2015 Annual Meeting
Poster No: 1196