Mapping OA Progression in the Knee Cartilage: Insights from a Non-Invasive ACL Rupture Rat Model

Jasmine Doan[1], Colleen Mathieu[1], Sotcheadt Sim[1], Eric Quenneville[1] and Martin Garon[1]
[1]Biomomentum, 1980 Michelin, Laval QC H7L 5C2
garon@biomomentum.com

Disclosures: J. Doan (3A-Biomomentum), C. Mathieu (3A; Biomomentum), S. Sim (3A-Biomomentum), E. Quenneville (3A-Biomomentum; 4-Biomomentum), M. Garon (3A-Biomomentum; 4-Biomomentum)

INTRODUCTION: Traumatic injuries of the anterior cruciate ligament (ACL) pose a significant health concern due to the subsequent impairing pathological changes occurring within synovial joints. Osteoarthritis (OA) often develops in the knee after ACL tears and the medial anterior region of the knee is particularly vulnerable to cartilage degeneration as this area experiences increased varus torque in ACL-deficient knees (Ohori et al., 2017). Using a non-invasive *in vivo* ACL rupture in rat model, this study first aims to understand the temporal progression of OA at 4- and 7-weeks following ACL rupture, shedding light on the underlying pathophysiological mechanisms while making the medial anterior region a focal point of analysis. Secondly, this *in vivo* model was also used to investigate the ability of a standard-of-care intra-articular (IA) treatment to mitigate the progression of post-traumatic OA.

METHODS: In adherence to the Canadian guidelines for animal care and experimentation, a total of seventeen 14-week-old female Lewis rats were used in this study and distributed in two cohorts. The first cohort (T1) encompassed five animals, one served as a Control, while the remaining four underwent non-invasive ACL rupture in their right hindlimb and were euthanized 4-weeks after injury. For the second cohort (T2), twelve rats were divided into three groups of 4 animals: a Control group, an ACL Rupture Non-Treated group, and an ACL Rupture IA-Treated group. Eight rats underwent the non-invasive ACL rupture procedure in their left hindlimb. The Control group were euthanized 4-weeks after the start of the experiments, while the remaining were kept for an additional 3 weeks during which the ACL Rupture IA-treated group received weekly IA-injections of a Hyaluronan-based product. Non-invasive ACL rupture was achieved through a tibial compression method (**Figure 1**) through the application of a rapid vertical displacement of 2 mm at 8 mm/s to the flexed hindlimb of each anesthetized animal. Immediately following injury, the ACL rupture was ascertained through anterior drawer test, and later confirmed with joint dissection performed during necropsy. At necropsy, femoral condyles and tibial plateaus were collected and submitted fresh to mechanical characterization via normal indentation and thickness mapping of the articular cartilage using a mechanical tester (Mach-1v500css, *Biomomentum*). This assessment yielded mechanical data on cartilage thickness and instantaneous modulus (IM) acquired from 30-40 distinct positions on each cartilage surface. Statistical analyses were executed using the linear model with R software.

RESULTS: Mechanical testing results showed that the medial anterior femoral condyle in ACL ruptured joints had a notable increase in thickness observed at 4 weeks post-injury (Rupture T1) when compared to Control ($p = 0.0035$). Moreover, a subsequent significant reduction in thickness can be observed between the 4-week T1 and 7-week T2 post-injury Non-Treated groups ($p = 0.0077$). The Rupture T2 Non-Treated group is also accompanied with a significantly higher instantaneous modulus when compared to Control ($p < 0.0001$, **Figure 2A&B**). The Rupture T2 IA-treated group exhibited significant differences only when compared to the Rupture T2 Non-Treated group, with statistical significance established for both thickness ($p = 0.0067$) and IM ($p = 0.0006$). In the tibial plateaus, a noteworthy increase in thickness was only evident at the 7-week post-rupture mark (Control vs Rupture T2 Non-Treated, $p < 0.0001$) along with a significantly lower IM in comparison to the Control group ($p = 0.0301$) and the Rupture T1 group ($p = 0.0366$, **Figure 2C&D**). Furthermore, at the 7-week mark (T2), the IA-Treated group displayed comparable cartilage thickness to Control compared to the Non-Treated group ($p = 0.0215$). Colormaps of the cartilage surfaces (**Figure 3**) also evidently show that the instantaneous modulus of the condyle at 7 weeks when the injury is not treated is much higher than all other groups, while the stiffness of the anterior region of the tibial plateaus decreases.

DISCUSSION: This study revealed compelling insights into the temporal progression of cartilage degradation in the anterior regions of both the femoral condyles and tibial plateaus following ACL rupture. The study demonstrated that, at 4 weeks post-rupture, the condyles' cartilage displayed signs of swelling, indicative of early onset OA. In contrast, the 7-week timepoint unveiled a considerably more advanced stage of cartilage degeneration with notable increase in stiffness, signaling a transition into a medium to severe OA state. In contrast, the administration of IA-treatment appeared to exert a protective influence on the cartilage following ACL rupture. This intervention not only mitigated the severity of OA progression but also allowed articular cartilage to maintain properties like those observed in the Control groups or in the 4-week post-ACL rupture group. These findings underline the validity of this ACL rupture-induced OA model in assessing the progression of the disease as well as the effectiveness of potential treatments.

SIGNIFICANCE: This clinically relevant ACL injury model offers non-invasive means to faithfully replicate the evolving pathological features of post-traumatic OA over time and stands out as a powerful tool for efficiently screening potential therapeutic interventions.

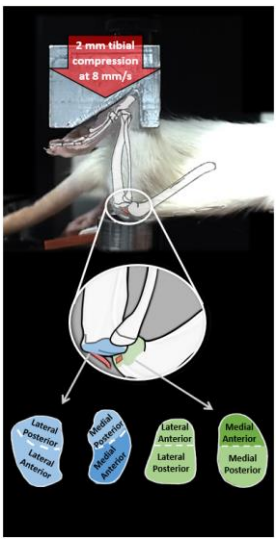

Figure 1 – Non-invasive ACL rupture by tibial compression method.

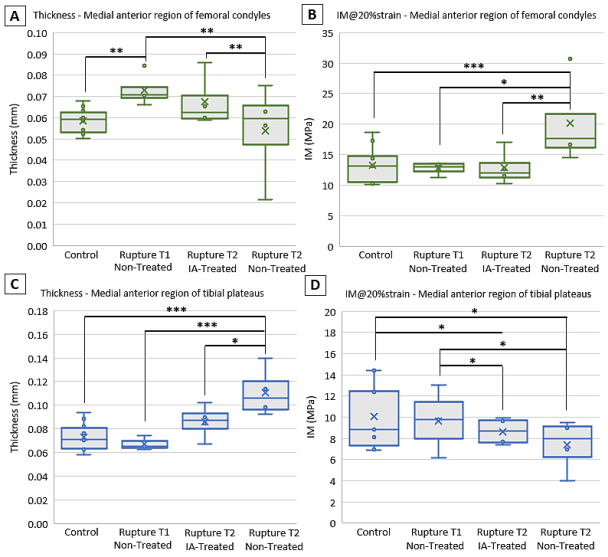

Figure 2 – Cartilage thickness and instantaneous modulus box and whisker plots for the medial anterior region of rat femoral condyles and tibial plateaus in all groups: Control (n=10), ACL Rupture T1 at 4 weeks (n=4), ACL Rupture T2 at 7 weeks IA-Treated (n=4) and at 7 weeks Non-Treated (n=4). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

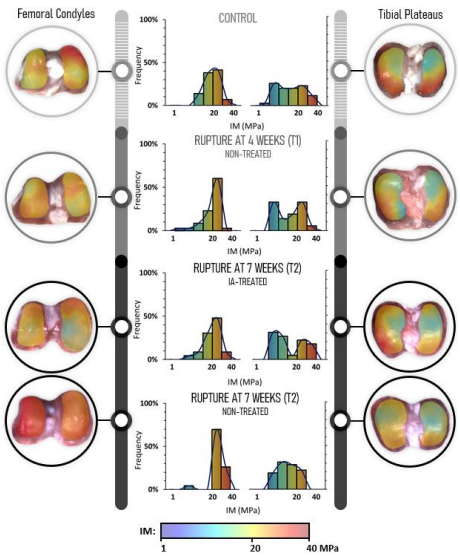

Figure 3 – Representative colormaps of the instantaneous modulus with their respective frequency graph for femoral condyles (FC) and tibial plateaus (TP) of all groups.