Tunable osteoarthritis *in vitro* model based on equine osteochondral plugs

Colleen Mathieu[1], Yasaman Pooshidani[1], Renato Castillo[1], Jasmine Doan[1], Sotcheadt Sim[1], Eric Quenneville[1], Martin Garon[1]
[1]Biomomentum Inc., Laval, QC
garon@biomomentum.com

Disclosures: C. Mathieu (3A-Biomomentum), J. Doan (3A-Biomomentum), Y. Pooshidani (3A-Biomomentum), R. Castillo (3A-Biomomentum), S. Sim (3A-Biomomentum), E. Quenneville (3A/4-Biomomentum), M. Garon (3A/4-Biomomentum)

INTRODUCTION: Mechanical overloading of synovial joints is known to cause cartilage lesions that can lead to chronic diseases such as osteoarthritis (OA) if left untreated. Several *in vitro* OA models are being developed on osteochondral plugs with numerous mechanical loading protocols, culture durations and culture medias. However, high sample-to-sample variability is a reoccurring limitation in published work. Thus, the purpose of this study was to investigate whether employing nondestructive assessment of cartilage electromechanical properties to guide plug extraction could reduce sample-to-sample variability and lead to a robust and tunable OA model.

METHODS: Equine femoral condyle blocks (N=4) were harvested and submitted to electromechanical quantitative parameter (QP) assessment with a hand-held probe (Benchtop Arthro-BST, Biomomentum) used on 40+ positions of the surface. (**Figure 1A**). Bone-cartilage plugs (D=4.8mm) were then extracted from each block with a drill press under constant irrigation (**Figure 1B**). Based on internal quality scores, 17 plugs were retained and distributed in 3 test groups: Control (n=5), Injured (n=6), and Injured+TNFα (n=6). Plugs were fitted in custom 2-chamber systems (**Figure 1C**) for 6 days incubation at 37oC and 5% CO_2. Both chambers were supplied with DMEM/F12 media with 0.1%BSA and 50ng/mL Dexamethasone, and TNFα (100 ng/mL) was only added to the cartilage chamber of the Injured+TNFα samples. Culture media was sampled at day-3 and day-6 to quantify levels of glycosaminoglycans (GAG) released. To determine the percentage change in cartilage mechanical properties, a sequence of unconfined compression and rotational friction-on-glass was performed aseptically at day-0 prior to injury and again at 6-days post-injury. Mechanical injury was inflicted on samples (n=12) at day-0 via a compressive deformation of 50% strain at 100%/s rate, except for the control group. Stereomicroscope images were used to measure the cartilage thickness and record macroscopic alterations to the cartilage surface. Once the final mechanical tests were completed, cartilage samples were taken to determine cell viability and GAG tissue content, and to generate Safranin-O/Fast Green-stained paraffin sections for GAG. ANOVA with Tukey Post-Hoc test was used for statistics.

RESULTS: The electromechanical quantitative parameter (QP) of cartilage confirmed a high variability between osteochondral blocks harvested and between plugs from the same block (**Figure 1**). Images of the cartilage surfaces taken immediately after injury revealed that the severity of cracks was strongly correlated to the plugs' QP values (**Figure 2**) with deeper cracks apparent in plugs with High-QP compared to superficial ones for plugs with Low-QP. Percentage changes in cartilage mechanical properties in unconfined compression and friction were also accentuated in High-QP plugs compared to Low-QP plugs. However, the most drastic changes were observed for Permeability (**Figure 3A**) which increased by 67-80% in Low-QP plugs but increased by 221-300% in High-QP plugs. High-QP plugs were also more responsive to the effect of TNFα that lead to a 2-fold higher in GAG release (**Figure 3B**) compared to injury alone and translated in a visible gradient of GAG depletion in histology (**Figure 3CD**).

DISCUSSION: As expected various levels of alterations were observed for 1) the mechanical and tribological properties, 2) the GAG levels released in the media and 3) the GAG matrix distribution visible in histology sections. Moreover, these alterations were not only dependent on the mechanical injury and the presence of TNFα in the culture media but also strongly dependent on the electromechanical quantitative parameter (QP) measure before the cartilage plug extraction. Consequently, the addition of the QP measure to our protocol successfully helped lower sample-to-sample variability. Future studies will investigate the effect of longer culture duration, various levels of mechanical injuries & the use of other pro-inflammatory cytokines.

SIGNIFICANCE: OA is a debilitating disease with a slow progression where the patient' diseased joints advance through different stages of cartilage degradation. A tunable OA model would help scientists to develop therapeutics that could target specific stages of cartilage degradation.

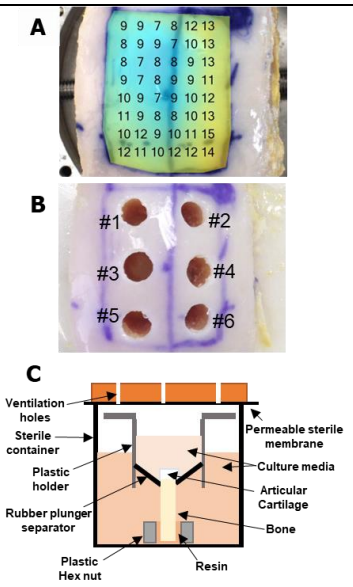

Figure-1: Example of (A) mapping of cartilage's electromechanical properties prior to the (B) extraction of osteochondral plugs and the schematic of (C) the *in vitro* plug culture set-up of these plugs.

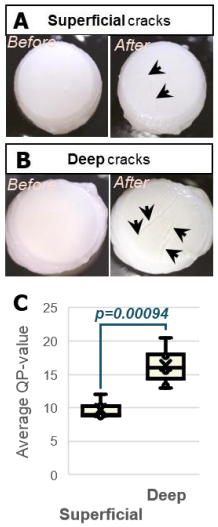

Figure-2: Representative top-view images of cartilage plugs' surfaces showing macroscopic (A) superficial or (B) deep cracks following the mechanical injury and the related (C) average QP-value for each category of cracks.

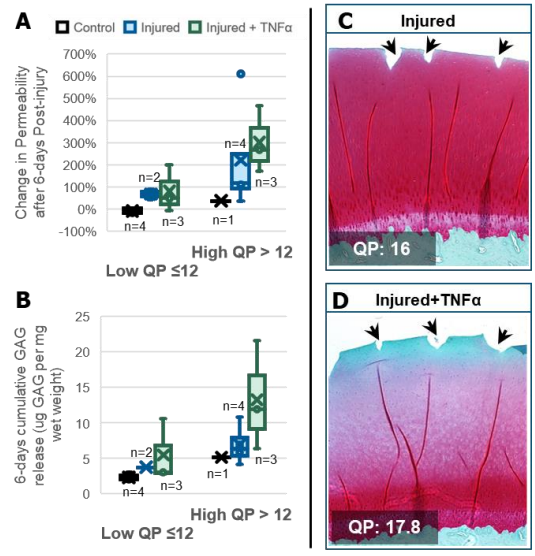

Figure-3: Final percentage change in (A) cartilage permeability, (B) cumulative GAG released in media at 6-days post-injury depending on test group (control, Injured, Injured + TNFa) and eletromechanical properties (QP-value categories) and representative images of GAG distribution with cartilage section of (C) **injured** and **(D) injured+TNFa** plugs.