Disruption Of Articular Cartilage Extra Cellular Matrix Following Mechanical Trauma In An In-vitro Model

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INTRODUCTION: Traumatic joint injuries are known to leave lesions in articular cartilage which, left untreated, can progress to osteoarthritis. An in-vitro model mimicking a traumatic injury is therefore essential to characterize the effects of cartilage degeneration over a span of time and validate the efficacy of novel engineered solutions to treat those lesions. This study evaluates the consequences of a mechanical injury on osteochondral plugs by assessing the cartilage mechanical properties, GAG release, and cellular viability versus an intact-control group. As the underlying integrity of cartilage is mainly provided by the collagen network – which is imbedded with aggrecans (GAGs) – a mechanical injury is expected to damage the protein chains within the extra cellular matrix (ECM), leaving a compromised structure. Expected alterations from the injury include reduced fibril modulus, increased hydraulic permeability of the ECM, swelling of the cartilage, and GAG release from the matrix, among others.

METHOD: Osteochondral plugs (4 mm dia.) were harvested – with a trephine under constant irrigation – from equine femoral condyles a few hours postmortem. Four plugs were extracted from each condyle and distributed into the following groups: mechanical test (MT) injured, MT control, no mechanical test (no-MT) injured, and no-MT control. To mimic in-vivo conditions, osteochondral plugs were cultured in two separate compartments using a custom-made holder to restrict crosstalk between cartilage and bone (see **Fig. 1**) – and incubated up to 9 days at 37°C and 5% CO_2. Culture mediums were changed every 3 days and 1 ml aliquots were retained from the cartilage compartment to document GAG release with a DMMB colorimetric assay. Cartilage volume was characterized by measuring thickness and diameter under a dissection microscope upon harvest (3 days pre-injury), on day 0 (injury day), and day 6 (final timepoint). Mechanical injury was inflicted by a single unconfined compression of 50% deformation at a 100%/s deformation rate, as previously described in the literature. Cellular viability was evaluated for all no-MT samples in the surface (first 100µm depth) and the transitional (100-200µm depth) regions of thin cartilage slabs at day 6; a Live/Dead imaging kit (R37601, Invitogen) was utilized for the assessment. Mechanical tests were conducted on day 0 and day 6 in a bath of PBS containing 2% antibiotic kept at 37±1°C. Unconfined compression (UC) consisted of a quasi-static 10% initial deformation followed by four consecutive 2.5% deformations; cartilage stress was allowed to equilibrate after each deformation. Friction-on-glass was performed directly after UC by rotating the cartilage on its axial axis at an effective speed of 43 deg/s for two revolutions (0°-720°). Stress-relaxation curves were curve-fitted to a fibril-reinforced poroelastic model to extract mechanical properties and the coefficient of friction (COF) was approximated as the ratio of the rotational force to the loading force.

RESULTS: Experimental procedures were successfully conducted in a sterile manner with no sample contamination. Cellular viability showed distinct alterations in the surface region of the cartilage between injured and control group with 40% more cell death 6 days post-injury (see **Fig. 2**). However, no apparent discrepancies were observed in the transitional region. The injured group showed a decrease of fibril moduli with an increase of GAG release from the cartilage ECM (see **Fig. 3**). Inversely, the ECM hydraulic permeability increased with GAG release. Furthermore, injured cartilage volume increased nearly twice as much as the control group 6 days post-injury. The coefficients of friction were comparable between groups and timepoints and ranged between 0.2-0.47, n=5.

DISCUSSION: The results tend to show mild signs of cartilage degeneration 6 days post-injury. The disruption in the ECM was highlighted by the lowered strength of the collagen fibrils, the high GAG release, and the increase in hydraulic permeability of the cartilage tissue after the injury. Physically, the results indicate that the mechanical trauma damaged the collagen fibers within the ECM which in turn triggered cartilage swelling and GAG release from the matrix. GAG loss combined with cartilage swelling lead to an increase in ECM pore size which increased the hydraulic permeability of the cartilage. The differences in chondrocyte viability between the cartilage surface and transitional region suggest that the degeneration was superficial and had not yet progressed into the deeper tissue. Collectively, the results validate the proposed in-vitro model for cartilage degeneration. Forthcoming works shall focus on prolongating the study to reach a more severe state of cartilage degeneration, studying the effect of harvesting plugs from specific regions on the femoral condyles (e.g., distal, or proximal) to best normalize the data, and comparing the effect of different lubricants on cartilage properties.

SIGNIFICANCE: The significance of developing an in-vitro model that assesses the mechanical and biochemical properties of degenerated cartilage is to provide a method for improved screening of potential novel engineered solutions and perform well-targeted pre-clinical studies.

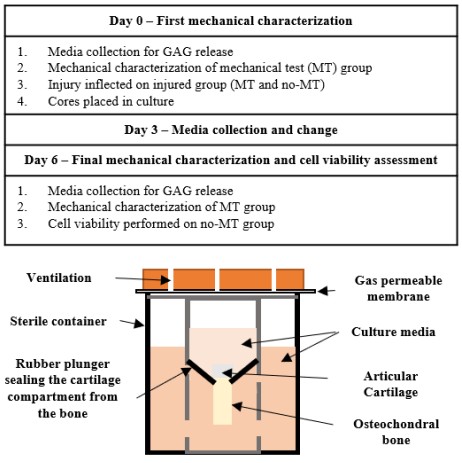


Figure 1: Study timeline (**top**) and cell culture setup used to separate the cartilage compartment from the bone (**bottom**).

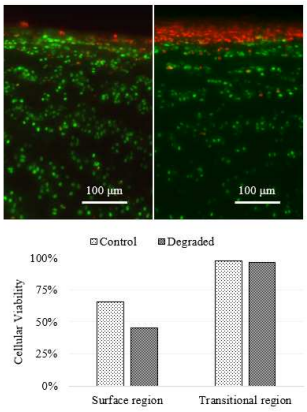


Figure 2: (Top) Illustration of the microscopic differences between a control sample (left) and a mechanically injured sample (right), and (**bottom**) their cellular viability within each region 6 days post-injury (n=2 per group).

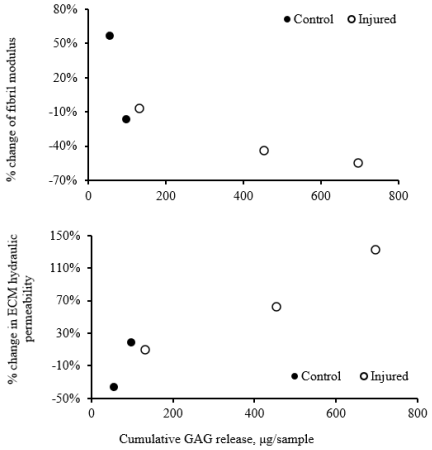


Figure 3: Mechanical properties of the control (n=2) and injured groups (n=3) at 20% deformation as a function of cumulative GAG release (sum of day 3 and 6). (**Top**) Percent change of the fibril modulus and (**bottom**) of the ECM hydraulic permeability with respect to their baseline value (day 0).