

THE EFFECTS OF FRICTION IN THE PRESENCE AND ABSENCE OF TRIBOLOGICAL REHYDRATION ON CHONDROCYTE HEALTH

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INTRODUCTION - Articular cartilage covering the ends of long bones protects joints during the thousands of articulations encountered daily. This tissue is unique in its composition, being a highly hydrated matrix (~80% fluid by weight) that is sparsely populated with resident cells called chondrocytes. These cells are embedded within a collagen matrix and experience shear, compression, and fluid forces during normal daily activity. As they are solely responsible for maintaining the homeostasis of our cartilage, is particularly useful to understand how chondrocytes respond to forces while in their natural environment.

That said, it has been historically difficult to study chondrocyte mechanobiology under controlled loading and articulation conditions on two accounts: first, the ability to apply highly defined loads and articulations to cartilage *in vivo* while also directly measuring tissue strains, shears, and frictions is extremely limited. Second, while *ex vivo* cartilage explant studies permit more rigorous control and assessment, classical explant testing strategies fail to replicate physiologically-consistent tribomechanical behaviors (high stress, low strain, low friction) over biologically-relevant time scales (hours to days).

Recently, our group has pioneered the use of a new cartilage testing configuration, the convergent stationary contact area (cSCA), which has allowed us to mitigate many of the difficulties encountered in previous *ex vivo* work¹⁻⁴. The cSCA promotes the maintenance of high fluid load fractions (>95%), low tissue strains (15-30%), and very-low friction values ($\mu=0.01-0.03$) in cartilage explants subjected to high, physiologically-relevant applied loads (5-7N) and sliding speeds (>60mm/s) over long experimental durations⁵. cSCA explants are unique in that they are larger in diameter than traditionally used explants ($\varnothing 19$ mm vs. $\varnothing 3-6$ mm), and thus retain the natural curvature of the joint, allowing for hydrodynamically-driven processes to occur during sliding. During sliding, fluid drawn into the convergent wedge of the cSCA is pressurized, facilitating the pumping of fluid into the articular

cartilage through a mechanism called 'tribological rehydration'^{1,4}. In the face of compression-induced fluid exudation, this process rehydrates the tissue, promoting strain recovery and driving physiologically-consistent frictional behaviors previously only seen *in vivo*.

While we have used the cSCA to extensively study tribological rehydration and sliding-tribomechanics in non-viable cartilage^{4,6,7}, the mechanical and biological consequences of tribological rehydration on live, metabolically active tissues has yet to be investigated. In live tissues, high-friction, driven by non-physiological sliding conditions (e.g. sliding at 1 mm/s), causes chondrocyte death and dysfunction⁸, supporting the hypothesis that friction may be a primary driver of osteoarthritis and disease. However, it is unclear how tribological rehydration, and its ability to actively mitigate tissue strain/friction at physiologically-relevant sliding-speeds, might influence this critical relationship. Because of the cSCA's unique ability to modulate friction magnitudes based on sliding speed⁷ and allow long-term sliding studies under very-low friction conditions⁶, it represents an ideal platform to study the effect of physiological friction on chondrocyte health and homeostasis. Thus, *the goal of this study was to use the cSCA to investigate the response of chondrocytes to sustained 'physiologically-consistent' low friction, and 'pathological' high friction conditions driven by sliding at high-speeds (80 mm/s) and low-speeds (1 mm/s).*

METHODS

Tissue Harvest: Extraction of $\varnothing 19$ mm cSCA osteochondral cores from equine femoral condyles was performed at the UPenn School of Veterinary Medicine, New Bolton Center. All samples were from animals enrolled in unrelated, non-musculoskeletal studies at New Bolton Center or from opportunistic necropsies of animals euthanized in the course of standard veterinary care; all cores were harvested within 2 hours of animal sacrifice⁴. After extraction, the underlying bone was

trimmed to ~12mm in height; the cores placed in high-osmolarity chondrogenic culture media (Low Glucose DMEM, 1% ITS+ Premix, 20 µg/mL ascorbic acid, 1% penicillin-streptomycin, 1% amphotericin-B, sucrose); and transported immediately to the University of Delaware. Upon receipt, explants were processed for immediate live-cell imaging, or 'Waterpik'ed with a high-osmolarity (380 mOsm) phosphate buffered saline containing antibiotics to remove bone marrow before being incubated in fresh high-osmolarity chondrogenic media at 37°C and 5% CO₂. Media was changed every 24 hours until study completion.

Preliminary Viability Assessment: Due to the limited number of explants that can be extracted from a single condyle and our relatively 'rough' extraction procedure (i.e. drilling), a preliminary study was conducted to map chondrocyte viability and health across the condyle/explant surfaces. Explants were harvested from the primary sliding axis of each condyle (5 y.o. male, 4 explants/condyle) with explants from the left stifle subjected to immediate Live/Dead staining-based viability assessment, and explants from the right stifle cultured for 36 hours before a similar assessment.

Tribology Friction Study: Slow- and fast-speed sliding is being used to investigate the effect high- and low-friction articulation has on cell health in the cSCA. After 36 hours of culture, explants are assigned one of four groups: (1) *free swelling*, (2) *static compression*, (3) *slow-speed sliding*, or (4) *fast sliding*. Explants in groups 2 – 4 are placed in the tribometer and subjected to 30-minutes of compression at 7N (~0.25 MPa), followed by 30-minutes of reciprocal sliding at either 0- (*static*), 1- (*slow sliding*, high friction), or 80-mm/s (*fast sliding*, low friction) while the 7N load is maintained. Tested explants are then further divided into two groups: *immediate assessment* and *moderate-term culture*. Explants in the *immediate assessment* group are bisected after testing and stained for cell viability and mitochondrial function. Explants in the *moderate-term culture* group are cultured in fresh media for 24 hours, then bisected and stained for cell viability and apoptosis.

Viability, Mitochondria Function, and Apoptosis Assessment: After bisection, one-half of each explant is stained with 5 µg/mL Hoechst (nuclear stain), 1 µM Calcein AM (Live stain), and 1 µM Ethidium homodimer-1 (Dead stain) for 90 minutes before being imaged on a Carl Zeiss AxioObserver.Z1 microscope. The remaining half of the explant is either stained with (1) 10 µM JC-1 (indicator of mitochondrial health) + 5 µg/mL Hoechst [*immediate assessment* group], or (2) 5 µM Caspase-3/7 substrate (indicator of apoptosis) + 5 µg/mL Hoechst [*moderate-term culture* group]. Z-stacks are captured at the edges and center of each explant for assessment. Following imaging, samples are processed and reserved for histology.

RESULTS

Preliminary Viability Study: Live/dead staining revealed increased cell death and matrix-fissuring at the outer edges of each explant (**Fig. 1A,B**), which we suspect to be an artifact of extraction. We are working to reduce shear generated by the core-drill during extraction by using a punch-isolation-system, where the 'central' cartilage of interest is isolated from the surrounding cartilage using a punch before drilling out the 'isolated' osteochondral core, thereby protecting the 'central' cartilage from shearing. Qualitatively, we also observed increased cell death in the explants from the most posteriorly isolated cores as well as the medial condyles (**Fig. 1C,D**). No marked changes in cell viability were seen between the immediate imaging group and the group that was cultured for 36 hours. From this initial study, we concluded that extraction of cSCA cores from the middle-to-anterior regions of the condyles provides the healthiest tissue, and that 36 hours of culture prior to testing is not detrimental to chondrocyte health.

Sliding Friction Study: Data collection is ongoing following initial trouble-shooting, and we will assess cell viability, and mitochondrial

function or apoptotic death, in the high-, low-, and no-friction groups compared to free-swelling controls. Additionally, frictional and strain outcomes will be assessed (e.g. start-of-sliding, end-of-sliding, and time-average strains and frictions; and strain recovery) to confirm that high-speed sliding can drive tribological rehydration (seen by positive strain recovery) and physiologically low friction coefficients ($\mu < 0.05$), while low-speed sliding drives pathologically high friction coefficients.

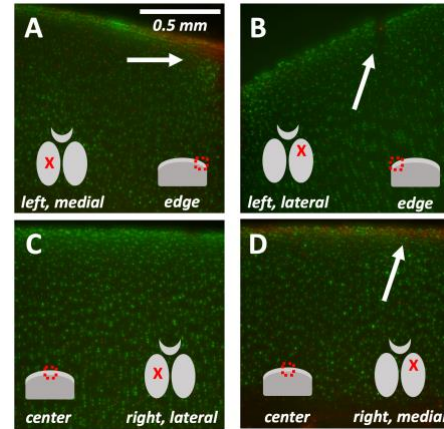


Figure 1. Representative Live/Dead staining images show A) cell death, and B) fissures towards edge in explants immediately assessed; and C) live chondrocytes in the center of an explants from the lateral condyle, and D) scattered cell death in the center of an explant from the medial condyle in explants cultured for 36 hours.

DISCUSSION – Based on our preliminary work, we have identified improvements to our extraction protocol to reduce shearing of the tissue of interest. Additionally, we have successfully maintained chondrocyte health throughout several days of culture and are able to use those explants for tribomechanical testing without inducing contamination. In the opportunistic equine stifle joints we are using, there is a need to consider anatomical position within the joint to obtain the highest quality cartilage, which we will continue to do moving forward. While our friction-based studies are nascent, we expect to show that absent tribological rehydration (i.e. those samples in the low-speed sliding group) induces high-friction sliding, which drives chondrocyte dysfunction in agreement with previous studies⁸. However, given that sliding in the cSCA can drive tribological rehydration at high speeds, we expect to see chondrocytes *protected* from detrimental shear at high sliding-speeds, which we have shown can drive more physiologically-consistent, low-friction outcomes. If this is indeed true, it suggests that we can use the highly-controlled nature of the cSCA to investigate articular cartilage mechanobiology and wear under a variety of sliding/friction conditions, as well as testing outcomes in tissues experiencing impact, degradation, and other changes indicative of disease. Lastly, the cSCA may help inform explant handling techniques that have the potential to increase the length of time that osteochondral explants remain viable after being extracted from the joint.

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