Spatial Pattern of Cellular Response of Articular Cartilage to Mechanical Injury Depends on Impact Magnitude

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA) is the result of physical trauma to joints, which causes tissue and cellular damage to cartilage. Joint trauma occurs from a wide variety of injuries, ranging from mild sprains to vehicular accidents. Resulting in adverse cellular responses include cell death, mitochondria (MT) depolarization from excessive intracellular calcium, and MT-driven caspase activation through cytochrome C release, leading to apoptosis. Previous work has shown the tissue surface is most prone to cellular damage, however it is unknown how varying levels of injury affect spatial patterns of cell damage in cartilage. We hypothesize that higher level injuries will cause a greater magnitude of adverse cellular response that penetrates deeper zones of the cartilage. The objective of this study was to analyze spatial patterns of cellular response to increasing impact energies.

METHODS: Femoral condyle cartilage of 7 neonatal bovids were collected within 24 h of sacrifice and cylindrical plugs (6 mm diameter by 2 mm thick) were extracted using sterile practices. A cylindrical indenter was used to impact cartilage plugs in unconfined compression at varying energy levels, using a previously described spring-loaded impactor. Impacts were characterized at 50 kHz using a load cell and linear variable differential transducer (LVDT) to measure impact force and cartilage deformation. Impact energy ranged from 0.35 MJ/m³ to 2.35 MJ/m³, with non-significant cartilage cracking occurring below 0.59 MJ/m³ and ~50% probability of cracking at 2.93 MJ/m³. Samples were axially bisected after impact, stained for either chondrocyte viability (Calcien AM and Ethidium homodimer), apoptosis (CellEvent Caspase-3/7 Green), or MT polarization (MitoTracker Green, Sytox Blue Nucleic Acid Stain, and Tetramethylrhodamine) and the cross-section was imaged using confocal microscopy. Depth-dependent cellular responses were quantified using confocal images and a custom MATLAB code. Statistical analysis was done using a two-way ANOVA and significance was evaluated at p < 0.05.

RESULTS: Figure 1A indicates impacts result in cell death, apoptosis, & MT depolarization respectively. Graphs in Figure 1B, C, and D show all assessments of cellular damage were most pronounced at the cartilage surface zone with magnitude of damage decreasing with distance from surface. All impact levels increased adverse cellular responses to at least 140 µm below cartilage surface. Higher levels of impact energy propagated cellular damage into areas of the middle zone (~460 µm) (Figure 1B) compared to lower energy levels, which did not pass the surface zone (~300 µm).

DISCUSSION: This study shows MT depolarization can be detected at much greater depths into the tissue for all impact energies (Figure 1D); revealing MT depolarization, compared to cell death or apoptosis, is more sensitive to injury affecting at minimum ~220 µm of cartilage. Cellular damage is exacerbated by impact energy, with damage being primarily concentrated in the cartilage surface zone, indicating the surface layer acts as a protective layer to limit damage propagation through the tissue. Depth-dependent tissue assessment can reveal finer details on cartilage mechanics and tissue damage, compared to gross anatomy assessment. Limitations in this study include cartilage impacts having to be performed in open air as opposed to a more sterile environment, which can make the cartilage tissue viability decrease throughout the testing process. Future work includes tribological assessment of damaged cartilage to evaluate the compounding effect of motion when mechanical injury is present, in order to simulate walking after injury; and the use of mitoprotective therapies early in the disease process in order to restore MT bioenergetics after joint injury.

SIGNIFICANCE: These data show that depth of penetration of cellular damage in cartilage depends on impact magnitude. As such, therapeutic strategies that target cartilage in PTOA patients may need to be tailored to the extent of injury.


Figure 1. A) Confocal images before and after impact show (left to right) cell death, apoptosis, and MT depolarization B) Cell viability through tissue depth C) Number of apoptotic cells through tissue depth D) Percent MT depolarization through tissue depth. Lines denote significant difference from respective impact group to control group for a given depth. n=12.