Response Of Cartilage Impact Between Multiple Joints And Implications For The Development Of Post Traumatic Osteoarthritis.

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Disclosures:

Introduction: Cartilage properties, including gene expression, thickness, and biomechanics vary between joints. These differences suggest site specificity of cartilage biology and why some joints are more susceptible to post-traumatic osteoarthritis (OA) than others. Most studies only compare a small subset of joints¹,² or compared cartilages between different species.³ The purpose of this study was to do a comparison of cartilage in multiple joints within a single species, to evaluate the differences among joints both in the baseline characteristics and in the response to traumatic injury.

Methods: Explant harvest and injury Full thickness DIA=6 mm cartilage explants were harvested aseptically from equine (ages 2.5-4 years) articular surfaces: caput humeri of the shoulder joint (SH), condylus lateralis radii of the elbow joint (EL), lateral proximal surface of os carpale III of the carpus (CA), condylus lateralis metacarpi III of the distal metacarpus (MC), condylus lateralis phalanx proximalis III of the proximal phalanx (PP), trochlea osis femoris of the femoral-patellar (FP), distal surface of os tarsi centrale of the tarsus (TA), condylus lateralis metatarsi III of the distal metatarsus (MT). Half of the explants were subjected to a single 30 MPa4 compression within 1 sec using a DIA=2.25 mm indenter on an EnduraTEC ELF3200 mechanical test frame (EnduraTEC, Minnetonka, MN), while the other half remained as controls.

Imaging with multiphoton microscopy (MPM) After 60 min at 37°C, explants were cut in cross-section and imaged in 1 μM sodium fluorescein (Akorn, Inc., Lake Forest, IL) in PBS to quantify cell death. A Tsunami titanium:sapphire laser (Newport Corp., Irvine, CA) at 780 nm wavelength was used for image collection. Control images were thresholded to binary data in Fiji and processed with custom MATLAB (MathWorks, Natick, MA) code to quantify cell density and fit to an exponential decay equation. Cell density decay length was compared between joints using an AOV with block by horse. Superficial zone chondrocytes were identified if demonstrating an orientation within [-16°, 16°] to determine histogram distribution. Cell death was manually quantified in control and injured images and compared using an AOV with block by horse.

Gene expression and analysis At 48 hours, RNA was isolated from explants using the RNEasy kit (Qiagen, Germantown, MD). Quantitative PCR was performed with a LightCycler® Real-Time PCR System (Roche Diagnostics, Indianapolis, IN) to quantify expression of collagen type 2 (COL2A1), aggrecan (AGG), cartilage-derived retinoic acid-sensitive protein (CD-RAP), serum amyloid A (SAA), matrix metalloproteinase 1 (MMP-1), MMP-13, and heat shock proteins 90 (HSP90). Results were normalized to 18S and calculated with the Roche Applied Sciences E(efficiency)-method. 6 Baseline control expression and percent difference change after injury were each evaluated with a Kruskal Wallis AOV.

Results: Cell density decay length was not statistically different between joints (Table 1). The cellular density within the superficial zone varied between 0.5-1.8x10³ cells/µm², and the depth to which the superficial extended typically did not exceed 200 μm (Figure 1). Cell death was significantly decreased after injury, with a significant interaction effect from joint; however, most joints post-injury shared similar cell death (p=0.0148, Table 1).

Differences between joints in COL2A1 and CD-RAP were found, with COL2A1 being 13.9x lower in FP (p=0.0114) and 9.6x lower in SH (p=0.0460) than PP, and CD-RAP being 16.3x higher in PP (p=0.0098) and 14.6x higher in TA (p=0.0202) than FP (Figure 2). After injury, the change in CD-RAP expression was 204% increased in FP over MC (p=0.0460) (Figure 2).

Discussion: Overall, few differences in cartilage properties were found when comparing each joint grouping from the sample population. Cell death after injury was significantly increased, with most joints responding similarly to trauma. Gene expression within the FP appeared the most consistent. Decreased basal gene expression and an increased post-injury expression in the FP, a region with low clinical prevalence of OA, suggesting a lower basal metabolism and a stronger response to injury. This overall between-animal variability implies that between-animal biological variability is as great as between-joint biological variability within a single animal. In the clinical setting, this suggests that because of individual biological variation, personalized OA treatment based on a genetic or biomarker screen may have a greater implication than a single standard treatment.

Significance: Cartilage properties vary highly both between joints and between animals when evaluating the same joint. The variation in properties between joints is as great as between animals, suggesting that individualized osteoarthritis treatment based on genetic or biomarker screening may be more relevant for patient success than a standard treatment for all patients.

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**Figure 1.** Histogram distribution of superficial zone chondrocytes.

**Figure 2.** Baseline COL2A1 was significantly lower in FP and SH than PP (**p=0.0114 and *p=0.0460, respectively, Figure 2). CD-RAP was increased in PP and TA over FP (**p=0.0098 and *p=0.0202, respectively). After injury, CD-RAP was significantly increased in FP over MC (*p=0.0460).

### Table 1.

<table>
<thead>
<tr>
<th></th>
<th>SH</th>
<th>EL</th>
<th>CA</th>
<th>MC</th>
<th>PP</th>
<th>FP</th>
<th>TA</th>
<th>MT</th>
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</thead>
<tbody>
<tr>
<td>Mean decay length, µm (SE)</td>
<td>537 (75)</td>
<td>422 (58)</td>
<td>254 (42)</td>
<td>765 (278)</td>
<td>545 (173)</td>
<td>1116 (341)</td>
<td>335 (50)</td>
<td>368 (46)</td>
</tr>
<tr>
<td>Control mean cell death, % (group)</td>
<td>4.5 (E)</td>
<td>9.1 (DE)</td>
<td>3.1 (E)</td>
<td>1.4 (E)</td>
<td>0.7 (E)</td>
<td>5.1 (E)</td>
<td>9.6 (DE)</td>
<td>2.7 (E)</td>
</tr>
<tr>
<td>Injured mean cell death, % (group)</td>
<td>16.5 (CD)</td>
<td>22.8 (ABC)</td>
<td>19.0 (BC)</td>
<td>17.5 (BCD)</td>
<td>20.5 (BC)</td>
<td>16.5 (CD)</td>
<td>30.6 (A)</td>
<td>25.0 (AB)</td>
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