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

Novakofski, Kira; Berg, Lise Charlotte; Bronzini, Ilaria; Fortier, Lisa

Publication date:
2014

Document version
Early version, also known as pre-print

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

Kira D. Novakofski[1], Lise C. Berg[2], Ilaria Bronzini[3], Lisa A. Fortier[1].
[1]Cornell University, Ithaca, NY, USA, [2]University of Copenhagen, København, Denmark, [3]University of Padova, Padova, Italy.

Disclosures:
K.D. Novakofski: None. **L.C. Berg:** None. **I. Bronzini:** None. **L.A. Fortier:** 3B; Consultant for Arthrex.

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 osteoarthrit (OA) than others. Most studies only compare a small subset of joints1,2 or compared cartilages between different species. 3 Th 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 ossis 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 Fiji5 and processed with custom MATLAB (MathWorks, Natick, MA) code to quantify cell density and fit to an exponential decay equatio 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 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 0.5-1.8x10-3 cells/µm2, and the depth to which the superficial extended typically did not exce 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 lowe 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 expressior within the FP appeared the most consistent. Decreased basal gene expression and an increased post-injury expression in the FI 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.
Acknowledgments: The authors would like to Jesse Silverberg for his assistance in image analysis, Hussni Mohammed and Laur Goodman for their assistance with statistics, and Lawrence Bonassar for his expertise in injury model development.
References: 1. Treppo et al. J Ortho Res. 2000;18(5):739-748. 2. Eger et al. J Ortho Res. 2002;20(3):526-534. 3. Stockwell. J Ana 1971;109(Pt 3):411. 4. Milentijevic et al. J Orthop Trauma. 2005;19(7):466. 5. Sauvola, Pietikäinen. Pattern Recognit.

2000;33(2):225-236. 6. Tellmann, Geulen. BIOCHEMICA-MANNHEIM-.

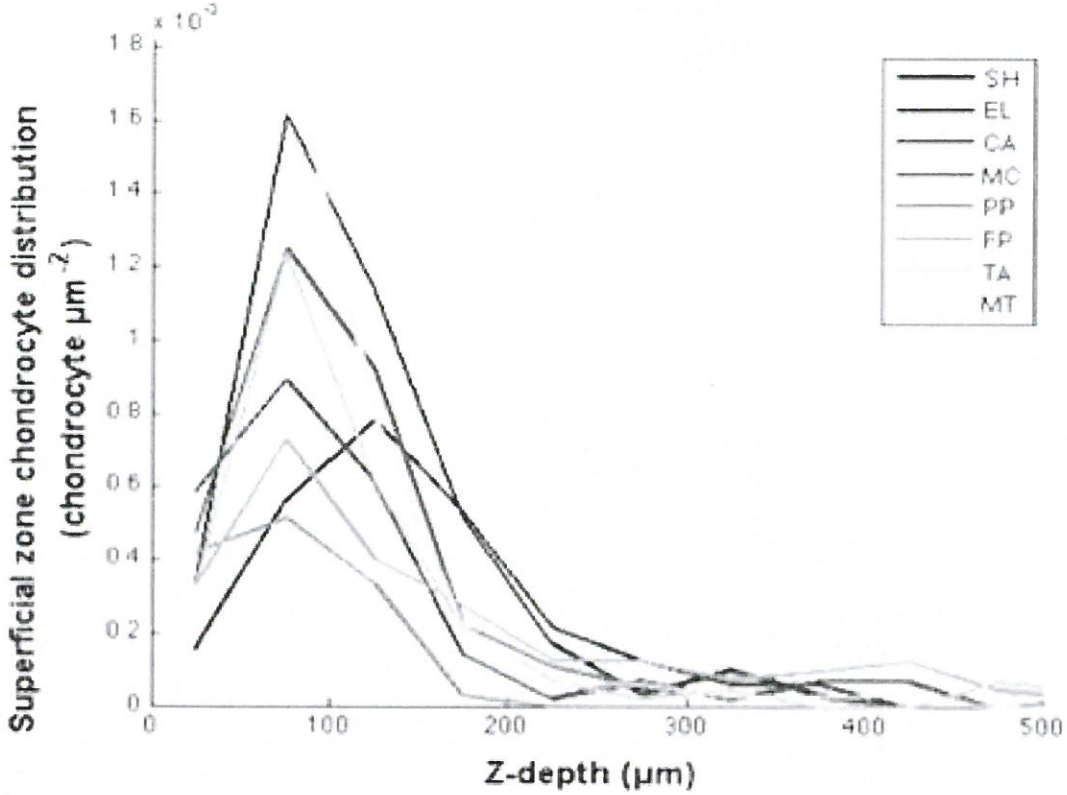

Figure 1. Histogram distribution of superficial zone chondrocytes.

2006;4:16.

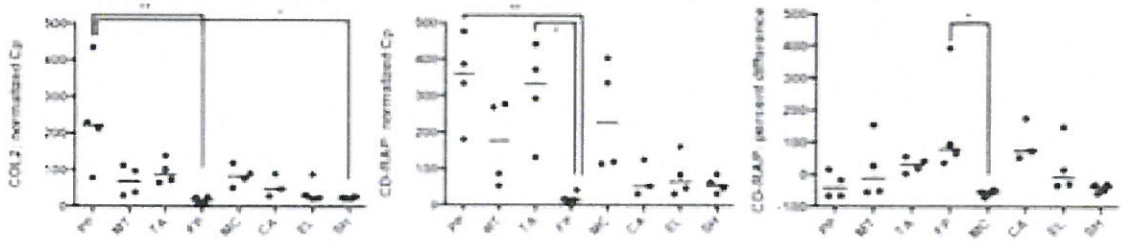

Figure 2. Baseline COL2A1 was significantly lower in FP and SH than PP (**p=0.0114 and *p=0.0460, respectively, Figure 2). CD-RAP was increased in PP and TA over FP (**p=0.0098 and *p=0.0202, respectively). After injury, CD-RAP was significantly increased in FP over MC (*p=0.0460).

Table 1.

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