Microcrack Initiation and Propagation in Articular Cartilage

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Introduction: Articular cartilage is a heterogeneous tissue comprised of an interstitial fluid and solid constituents, including a network of collagen fibers. This network, which varies spatially in orientation and distribution, contributes strongly to mechanical function in health. Damage to the network of collagen may predispose cartilage to degradation and to osteoarthritis (OA) [1]. Microcracks in bone have been well described, e.g. [2], as have macro (millimeter) scale surface fissures in advanced OA [3]. Only now have we discovered that low-energy impact also creates and grows micron-scale cracks in the network of collagen [4-6]. In this study we investigate the extent that microcracks (<30 μm in width) propagate in collagen networks during cyclic, mechanical loading.

Materials and Methods: We extracted cylindrical cartilage-bone explants (3 mm Ø, full thickness) from load-bearing regions of five skeletally mature bovine knees (18-30 months old). We separated specimens from the lateral and medial femoral condyles, and assigned them to three different impact groups (none, low (1.5-2.5 mJ/mm^3), high (2.6-4.0 mJ/mm^3)) and thereafter three different cyclic compression groups (none, low (10%), high (15%)) which mimic walking 12,000 steps. We performed impact tests using a custom drop-tower, with impact energy density as the independent variable, and unconfined cyclic compression tests using a linear motor (Bose LM1 Electroforce). We performed Second Harmonic Generation (SHG) imaging (Carl Zeiss LSM 510, Nikon FN1) for three experimental phases (pristine, post-impact, and post-cyclic compression). We acquired full-surface tiled images, as well as axial *z*-stacks (surface to 200 μm deep, 2.5 μm slice increment) through the axial center of specimens and far from edges (to avoid edge effects). We measured the area density of cracks, as well as the crack lengths, widths, depths, and angles (relative to split-line direction) in the images using ImageJ. We calculated the input impact energy density, the first Piola-Kirchhoff stress, and the residual compaction. We applied a mixed model ANOVA (SAS Institute) including condyle, impact, and cyclic load as fixed main effects, and all possible interactions among these three terms. We included thickness as a covariate, and animal as a random effect.

Results and Discussion: We completed a total of 48 impact tests and 72 cyclic loading tests using 76 specimens from five skeletally mature bovine joints. We show representative SHG images in Fig. 1. Inter-condyle differences emerged in mean crack width, depth, and orientation. In the lateral condyle, cracks have a greater width under low vs. high cyclic loading ($p < 0.05$). In the medial condyle, the opposite was true. We found the largest mean crack width in the medial condyle after low impact and high cyclic loading vs. any other combination. In both condyles we found significant differences in crack density for low vs. high cyclic compression after low but not high impact. Crack density was substantially greater for low impact followed by low vs. high cyclic loading ($p < 0.005$). We found mean crack length was also greater under low vs. high cyclic loading ($p < 0.05$), although there is no difference in cracks formed under low vs. high single impacts ($p = 0.14$).

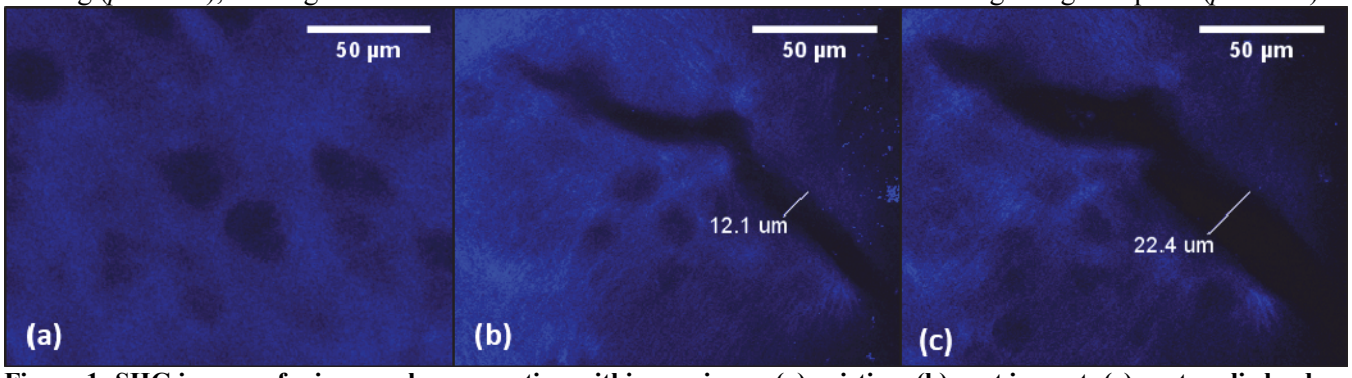

Figure 1: SHG images of microcrack propagation within specimen: (a) pristine; (b) post impact; (c) post cyclic load.

Conclusions: Microcracks primarily propagate by increasing in width. Our results show key differences in lateral and medial condyles, as well as significant differences in crack initiation and propagation behavior under diverse loadings. Specifically, effects of cyclic loads depend on prior impact loading. Understanding the mechanical mechanisms of cartilage microcracks may enable new treatment targets and detection of pre-clinical/early OA.

References: [1] Mori+, *Arch Pathol Lan Med* 117:196-8, 1993; [2] Burr+, *J Biomech* 18(3):189-200, 1985; [3] Pritzker+, *Osteoarthr Cartil* 14(1):13-29, 2006; [4] Kumar+, *Biomed Opt Express* 6(5):1895 -1903; [5] Kaleem+, *Osteoarthr Cartil* 25(4):544-53, 2017; [6] Kaplan+, *J Mech Behav Biomed Mat* 65(0):734-42, 2017.