Oxygen Tension Alters Cartilage Redox Balance in Response to Traumatic Impact

Jingyi Wang[1]; Greta E. Scheidt[1]; Corinne R. Henak[1]
[1]University of Wisconsin Madison, Madison, WI
chenak@wisc.edu

DISCLOSURES: Wang (N), Scheidt (N), Henak (N).

INTRODUCTION: Osteoarthritis (OA) is a common joint disease with limited treatment options. Cartilage redox balance, the balance between production and elimination of oxidants, is disrupted in OA. Excessive reactive oxygen species (ROS) trigger inflammatory responses, cause DNA damage, and lead to chondrocyte apoptosis[1,2]. Therefore, monitoring cartilage redox balance has potential for disease staging and treatment screening. Cartilage redox balance is responsive to altered oxygen tension. Chondrocytes cultured in 20% O_2 had more ROS production compared to 5% O_2[3]. Mechanical overloading can cause mitochondrial (MT) dysfunction[4,6] and therefore disrupt cartilage redox balance. Cell-permeable fluorescent dyes are used to detect ROS production in cartilage[5] and MT depolarization[4,6]. Although informative, these results have limited translational potential because of their use of exogenous dye. An alternative approach, optical redox imaging (ORI) allows label-free, real-time evaluation of redox balance in cartilage[7]. ORI utilizes the autofluorescence of vital cofactors in cellular redox reactions: flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) (together termed NAD(P)H). Although individual effects of oxygen tension or mechanical loading to cartilage redox are widely investigated, their interaction has not been studied thoroughly, leaving a gap in understanding of cartilage mechanobiological response at physiological oxygen tension. Hence, the objective of this study is to demonstrate the changes in cartilage redox balance in response to traumatic impact at differential oxygen tension using ORI.

METHODS: Forty 6-mm-diameter hemi-cylinder cartilage explants were collected from nine pigs (4-6 months old, sex unknown and assumed random) and randomly divided into four groups. Two groups underwent impact loading in either normal (Norm, 4.74±0.49 mg/L) or low (Low, 1.87±0.68 mg/L) oxygen tension, and two groups were unimpacted controls in matched oxygen tension. Impact was conducted using a wedge tip atop an inverted fluorescence microscope (Fig. 1). Loading was completed at 500 mm/s and the achieved maximum strain was 38%±10% within 12 ms. For each sample, ORI was performed at two baseline positions (Fig. 1) before impact. After 0-5 min (T1), 15-18 min (T2), and 28-31 min (T3), one position from each distance bin was randomly selected and ORI was performed in a random order. All images were acquired at 20×, and image intensity was obtained using ImageJ. The intensity in channel 1 (corresponding to NAD(P)H) and channel 2 (corresponding to FAD) at each position was normalized to averaged baseline intensity. Optical redox ratio (ORR) was calculated by dividing the intensity in channel 2 by the sum of two channels. The normality of the outcomes (intensity in each channel and ORR) were confirmed by Shapiro-Wilk normality test in Matlab[8]. A N-Way ANOVA tested the significance of the main effects (impact, oxygen, time, distance) and interactions, and post-hoc Tukey-Kramer was used for pairwise significance.

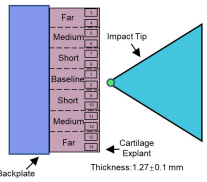

Fig 1. Schematic of impact loading and distance bins.

RESULTS: Impact and normal oxygen had significantly higher intensity than unimpacted and low oxygen in both channels, but did not affect ORR (Fig. 2A and B). Channel 1 intensity decreased as time increased, while ORR was higher in the third time bin (Fig. 2C). Distance significantly affected all outcomes (Fig. 2D). Channel 2 intensity and ORR decreased as distance increased. Impact in normal oxygen induced significantly higher channel 1 and 2 intensities compared to other groups (Fig. 3A). ORR was affected by the interaction between impact and time (Fig. 3B). All samples had visible cracks after impact.

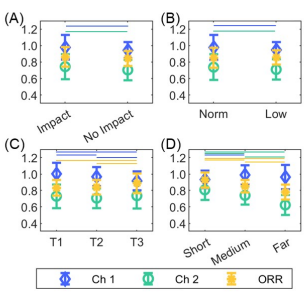

Fig 2. Individual effects relative to baseline (mean ±SD), lines indicate significance.

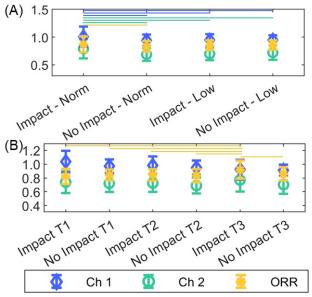

Fig 3. Interaction effects relative to baseline (mean ±SD), lines indicate significance.

DISCUSSION: This study demonstrated that impact loading increased FAD and NAD(P)H intensity, but this effect was eliminated at low oxygen tension. Over-production of ROS after impact has been reported in other studies[4,9]. The observed changes in fluorescence intensities are consistent with prior observations. Increased channel 1 intensity observed here could result from NAD(P)H accumulation due to MT dysfunction. NADH is reduced to its non-fluorescent form in MT[10] and NADPH can donate an electron to molecular oxygen, which produces ROS[11], so increased NAD(P)H can result from the MT dysfunction expected following impact loading. In addition, ROS production is linked to added NADH[10]. Increased FAD is often correlated to active MT function, but ROS are also generated as byproducts in the process in which FAD was produced. The increased channel 2 intensity may therefore be accompanied by ROS over-production. A previous study showed that cartilage ROS production was significantly increased after compression at 20% oxygen, but not at 5% or 1% oxygen[12]. This implies that lower oxygen tension is protective against oxidative stress, explaining why impact-induced redox changes in this study were not observed at low oxygen tension. Experiments that measure changes in ROS production, MT dysfunction, and ORI metrics should be conducted to establish a more reliable correlation between these variables.

SIGNIFICANCE: The findings in this study will aid the understanding of cartilage redox balance in response to stimuli of traumatic impact and altered oxygen tension, and the role of ORI as a tool to evaluate real-time cartilage redox status.

REFERENCES: [1]Lepetsos+, Free Radic Biol Med, 2019; [2]Zahan+, Med Pharm Rep, 2020; [3]Fermor+, Osteoarthr Cartil, 2010; [4]Delco+, JOR, 2018; [5]Arra+, Nat Commun, 2020; [6] Delco+, JOR, 2020; [7]Walsh+, Osteoarthr Cartil, 2019; [8]BenSaïda, MATLAB Central File Exchange; [9]Koike+, Sci Rep, 2015; [10]Kussmaul+, PNAS, 2006; [11]Schieber+, Curr Biol, 2014; [12]Fermor+, Osteoarthr Cartil, 2005.

ACKNOWLEDGEMENTS: Funding from UW-Madison VCRGE is gratefully acknowledged.