SHEAR-STRESS-INDUCED CHONDROCYTE DEATH IS REDUCED BY ANTIOXIDANT TREATMENT


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INTRODUCTION
A history of joint injury is among the most significant risk factors for osteoarthritis (OA). Injury-induced, or post-traumatic, OA often develops within five to ten years of the injury, and the process depends in part on mechanical stress conditions in the healing joint (Beaupre et al., 2000; Stevens et al., 2001). The mechanisms linking joint trauma with post-traumatic OA are poorly understood. However, surface incongruities arising from incompletely-reduced joint fractures can lead to excessive mechanical stress at and around step-offs. Excessive shear stress appears to promote cartilage destruction and arthritis (Wilkins et al., 2000). Preliminary studies in our laboratory suggested that elevated shear stress leads to the release of reactive oxygen species (ROS) by chondrocytes. Oxidative damage resulting from chronically elevated ROS can accelerate cell senescence and induce apoptosis, suggesting a link between chondrocyte damage and subsequent cartilage degeneration (Toussaint, 2000).

Based on these findings, we hypothesized that excessive mechanical shear stress causes chondrocyte death via an oxidative mechanism. To test this, we cultured human cartilage explants in a mechanically active bioreactor, the Triaxial Compression Vessel (TCV), which is capable of modulating shear stress levels (Heiner and Martin, 2004). This device was used to test the effects of shear stress on chondrocyte viability. We also tested the effect of adding an antioxidant, n-acetyl cysteine (NAC), to determine the role of oxidants in mediating shear stress effects.

METHODS
Human cartilage explants (4 mm diameter) were harvested from non-osteoarthritic ankle joints from four donors. A subset of the explants was pretreated overnight with 2.5 mM NAC, and all explants were placed in the TCV for mechanical stress treatment. The TCV is a novel culture device capable of imposing variable shear stress states at quasi-physiologic levels. A water chamber applies transverse compression, and a piston applies axial compression. The interplay between axial and transverse compression determines shear stress levels. In this study, four different treatments (900 cycles at 1 Hz) were applied: 1) non-stressed control, 2) 5 MPa axial compression only (maximum shear stress), 3) 5 MPa axial plus 5 MPa transverse compression (minimal shear stress), and 4) 5 MPa axial plus 2.5 MPa transverse compression (intermediate shear stress). At least six explants were used for each treatment group. Following mechanical stress treatment in the TCV, explants were removed from the device and incubated overnight in calcein AM to stain viable cells. After cryosectioning, slides were mounted with DAPI to stain cell nuclei. Micrographs of the superficial, middle, and deep zones of the cartilage sections were taken under UV light (to image DAPI-stained nuclei) and under 488 nm light (to image calcein-stained cells). Total cells (DAPI-stained) and live cells (calcein-stained) were counted to determine percent viability. One-way
ANOVA and Tukey’s test were used to evaluate the statistical significance of differences between treatment groups.

RESULTS AND DISCUSSION
Chondrocyte viability in all zones was high (>95% of unstressed controls) when explants were treated with low or moderate shear stress (5 MPa axial compression with either 5 MPa or 2.5 MPa transverse compression), but there was loss of viability when explants were treated with high shear stress (5 MPa axial compression alone). The highest loss of chondrocyte viability occurred in the superficial zone, where viability was reduced to less than 75% of unstressed controls (Figure 1).

Pre-incubation of explants with the antioxidant NAC significantly reduced shear-stress-induced chondrocyte death in the superficial zone. NAC-treated explants exposed to high shear stress showed >85% viability in the superficial zone, which was not significantly different from the value for unstressed controls (93% viable) and significantly greater than in explants without NAC (<75% viable) (Figure 2).

SUMMARY
High shear stress induces chondrocyte death in the superficial zone of cartilage explants, and an antioxidant reduces this effect. These findings support the hypothesis that the harmful effects of shear stress on chondrocytes are mediated by oxidative stress. Also, antioxidant use may represent a possible preventative or treatment for post-traumatic OA.

REFERENCES

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