HIGH SHEAR STRESS INDUCES P53 EXPRESSION AND APOPTOSIS IN CARTILAGE EXPLANTS

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INTRODUCTION

Joint injuries leading to articular surface incongruities (e.g., step-offs) can cause excessive mechanical shear stress, which promotes cartilage destruction and post-traumatic osteoarthritis (OA). Here we test the hypothesis that excessive shear stress induces chondrocyte stress responses that lead to apoptosis. We cultured human cartilage explants in a mechanically active bioreactor, the Triaxial Compression Vessel (TCV), which is capable of modulating shear stress at physiologically relevant loading levels. This device was used to test the effects of shear stress on chondrocyte viability, apoptosis, and expression of p53, a protein that is involved in the early stages of apoptosis.

METHODS

Cartilage explants harvested from non-osteoarthritic ankle joints from 2 donors were placed in the TCV for mechanical stress treatment. The TCV imposes variable shear stress states at quasi-physiologic levels by applying transverse compression and axial compression simultaneously. The interplay between axial and transverse compression determines shear stress levels. In this study, 2 different treatments (900 cycles, 1 Hz) were applied: 1) 5 MPa axial compression only (high shear stress), 2) 5 MPa axial + 5 MPa transverse compression (minimal shear stress). Treated explants were incubated overnight in calcein AM to stain viable cells (green signal), then were cryoembedded and sectioned. Replicate sections were stained for apoptosis by TUNEL assay using TMR-red-labeled nucleotide (orange signal) or with an anti-p53 antibody using HRP/NBT detection (black signal). Whole sections were scanned using a 10x objective to generate composite images composed of 10-20 frames. Composite images were analyzed by scoring the number of cells stained with calcein AM, TUNEL assay or p53 antibody as a percent of total cells present. At least 3 entire sections from each explant were analyzed for each stain. Student's t-test was used to evaluate statistical significance.

RESULTS AND DISCUSSION

Chondrocyte viability in the superficial and transitional zones of cartilage explants declined from 89% in low shear stress to 62% in high shear stress. Apoptosis increased from 2.4% in low shear stress to 16.7% in high shear stress, while p53 expression increased from 23% to 48% (Figure 1). Differences in viability, apoptosis and p53 expression were significant ($p < 0.05$). No significant changes were seen in the deep zone (data not shown) staining results.

Exposure to high shear stress induced chondrocyte death *via* apoptosis. High shear stress also induced p53 expression, indicating that cell death was imminent. This suggests that high shear stress causes chondrocyte depopulation that could contribute to cartilage degeneration in post-traumatic OA.

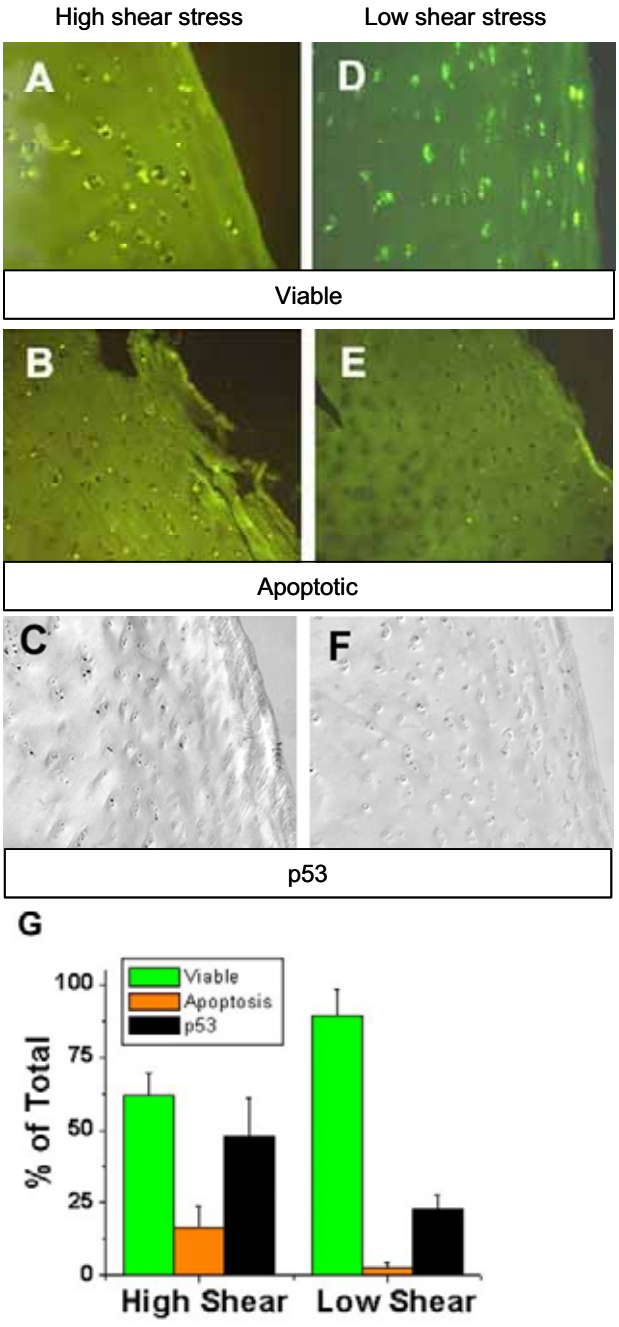

Figure 1: Effects of high and low shear stress on cartilage explants. (A,D) Calcein AM staining for viable cells (green). (B,E) TUNEL reaction for apoptosis (red-orange). (C,F) Immunostaining for p53 (black). (G) High shear stress decreases cell viability, increases apoptosis, and increases p53 expression, as compared to low shear stress.

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