CELL DEATH IN CYCLICALLY LOADED CARTILAGE EXPLANTS: EFFECTS OF TISSUE MATURATION

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INTRODUCTION
Extensive evidence has suggested that the structural compositions and biochemical properties of articular cartilage evolve during development and maturity of the joint, including levels of protein synthesis [5] and collagen synthesis [4], proteoglycan composition [1], and link protein content [6]. While some studies have indicated that the tensile properties of immature cartilage differ from those of mature cartilage [7], the compressive strengths have shown little changes with tissue maturity [2]. In mechanically insulted cartilage explants on mature specimens, the incidence of cell death was depth-dependent [3]. Considering the decreased thickness of the superficial zone as well as the changes in biochemical and biomechanical properties in cartilage during the tissue maturation, we investigated whether there were differences in chondrocyte survival from mechanical insults over the maturation of articular cartilage.

METHODS

Tissue Harvest. Full-depth articular cartilage from calf (4-8 weeks) and mature bovine (18-24 months) knees were obtained from a local abattoir within 4 hours post-mortem. Explants were then taken using a 7-mm biopsy punch, and were sliced to a uniform thickness of 1mm from superficial to deep zone. The tissue was incubated in serum-free DMEM with 1% antibiotics and 1% Hepes buffer at 37°C in a humidified atmosphere with 95% air and 5% CO₂.

Mechanical Loading. Explants were loaded with 1.0 MPa cyclic confined compression at 0.5 Hz for 1 to 16 hours as previously described [3].

Analysis of Cell Death. Cell death in non-loaded control and loaded cartilage explants was evaluated by uptake of 60 µM propidium iodide, a cell membrane impermeable dye. Viable cells were determined by uptake of 10 µM fluorescein diacetate, a hydrolysis activity dye. The fluorescent images were captured by a color CCD camera (Optronics, Goleta, CA) under a fluorescence microscope (Optiphot-2, Nikon, Japan). The depth of cell death from the articular surface was analyzed [3].

Statistical Analysis. The effects of maturity on cell death in loaded cartilage explants were analyzed by two-way ANOVA. Analysis of the rates of increase in cell death with increased duration of loading was determined by linear regression.

RESULTS
The depth of cell death in the superficial zone of loaded explants from both mature and immature joints (Fig 1), was higher than non-loaded controls (p<0.001), after 1 hour of loading. Increases in the amounts of cell death were seen between 1 and 6 hours of loading, but there were no differences between 6 and 16 hours in both immature (maximum depth = 58.1±51.6 µm) and mature explants (maximum depth =116.0 ± 6.0 µm). The depth of cell death in immature cartilage was significantly higher than that of mature cartilage (p<0.001) (Fig 2). All dead cells in the mature and immature samples were localized in the superficial tangential zone (Fig 1).

The rates of increase in cell death with increased duration of loading from 1 to 6 hours were analyzed using a linear regression model. Over this time course, the rate of increase in cell death in immature explants was 18.6 µm/hr (R²=0.57), as compared to the rate of 8.4 µm/hr (R²=0.52) in mature explants.

DISCUSSION
The objective of this study has been to investigate the cell death in cartilage at different stages of tissue maturity as subjected to mechanical insults. Our study showed that under mechanical insults, the chondrocyte death was localized to the superficial zone in both immature and mature cartilage. This finding supports the hypothesis that the chondrocytes in the superficial tangential zone are more vulnerable to mechanical insults than those in the middle and deep zones [3]. This is due primarily to the depth-dependent biomechanical properties seen in the different levels of maturity [7,8].

The finding that the rates of increase in cell death were greater in immature than mature cartilage, may also be attributed to a thicker superficial zone in the immature cartilage.

These differences may be explained by the structural and biochemical differences that occur during the maturing process [1,4,5,6], or by changes in the mechanical properties of the cartilage matrix that accompany maturity [7].

It should be noted, however, that evidence indicates that the compressive strengths between calf and adult bovine cartilage are similar [2], which may indicate that while the strength is similar, local changes in its ultrastructure, biomechanical properties and cellular adhesion to matrix may alter the ability of the cells to withstand mechanical insults.

REFERENCES

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