APOPTOSIS CORRESPONDS WITH DISC STRAIN ENVIRONMENT DURING DYNAMIC COMPRESSION

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INTRODUCTION: There is an extensive body of research that demonstrates the role of tissue-specific mechanical loading regimens for maintaining tissue health and preserving physiologic matrix architecture. Likewise, in the intervertebral disc, a number of studies have shown that mechanical loading has a profound influence on gene expression by disc cells in the short-term which is manifested as changes in disc architecture in the long-term. In our research group, studies of prolonged static compression using an *in vivo* mouse model of compression-induced disc degeneration suggest that excessive hydrostatic stress coupled with loss of annular tensile strain may be contributing factors to degeneration [1, 2]. Recent developments in mechanobiology have suggested that specific regimens of dynamic mechanical loading act as a positive stimulus. Similarly, a previous study in our laboratory [3] has suggested that there may exist a metabolic window within which certain combinations of magnitude and frequency of dynamic loading may have less deleterious consequences compared with static compression. In this present study, finite element analysis was used to investigate the basis for differential effects of dynamic compression observed in our *in vivo* studies. Understanding the relationships among disc loading, tissue stress/strain, and disc cell and matrix biology will be critical for identifying possible mechanical factors that may contribute to disc degeneration.

METHODS: A finite element model was generated based on the geometry of the murine tail disc (Fig 1). Axisymmetric porous media elements were used to simulate fluid-matrix interaction. As previously validated in our lab for human annulus [4], the annular matrix was modeled as isotropic, compressible porohyperelastic ground substance embedded with viscoelastic rebars to simulate collagen fibers. The nucleus was modeled as a porohyperelastic material [1], and also included a strain-dependent swelling pressure based on swelling pressure-porosity relationships elucidated by Urban et al. [5]. Cartilaginous endplates were composed of an isotropic material having poroelastic properties reported in the literature. Bone was modeled as transversely isotropic. Validation of the model was achieved by demonstrating good agreement with the mechanical behavior of mouse motion segments through both compressive creep experiments and quasi-static tension-compression of denucleated discs. Analyses were carried out using Abaqus v6.3 (Abaqus Inc., Pawtucket, RI, USA). One static and four dynamic compression conditions encompassing two magnitudes and two frequencies of loading were simulated according to previous *in vivo* experiments (Table 1). To mimic the experimental conditions, stresses were applied in a ramp-wise fashion over the span of 1 second and then held constant for the remainder of the loading duration. During dynamic compression, unloading was also defined as a ramp over 1 second. Disc mechanical exposure was characterized using peak values of strain energy density (SED) and octahedral shear (distortional) strain at the disc midheight. Region-specific values of SED and octahedral shear strain were compared with the percentages of total cells in the nucleus and annulus that were undergoing apoptosis, as assessed by the TUNEL method.

Table 1 – *Frequency and magnitudes of loading simulated by FEA*

	Dynamic: Low load High freq	Dynamic: Low load Low freq	Dynamic: High load High freq	Dynamic: High load Low freq	Static: High load
Frequency	0.1 Hz	0.01 Hz	0.1 Hz	0.01 Hz	Static
Magnitude	0.8 MPa	0.8 MPa	1.3 MPa	1.3 MPa	1.3 MPa

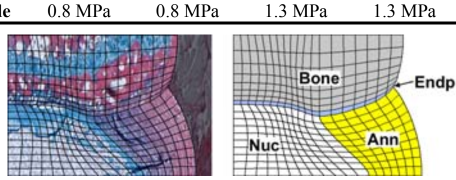

Figure 1 – *FE mesh of the mouse disc superimposed on histology (left) and color-coded to indicate disc structures (right).*

RESULTS: For both SED and octahedral shear strain, finite element simulations predicted distinct mechanical exposures that depended on combinations of magnitude and frequency of compression. This was not unexpected as the viscoelastic properties of the disc likely contribute to the dependence on both stress and time. Peak SED was approximately one order of magnitude greater in the inner annulus compared with the nucleus and outer annulus and exhibited a graded response depending on the frequency and magnitude of the dynamic compression applied (Fig 2). Highest levels were induced, as expected, for the high load static compression case. Despite the graded variation in SED predicted by the model, apoptosis dramatically increased after a certain level of SED was reached. Furthermore, levels of apoptosis were statistically equivalent after this level of mechanical exposure suggesting a metabolic threshold or tolerance limit to strain. Peak octahedral shear strain, or distortion, in the nucleus also exhibited a graded response across loading magnitudes and frequencies, but the static loading possessed slightly less distortion than the 0.01 Hz, 1.3 MPa case (Fig 3). Apoptosis measured in the nucleus exhibited trends strikingly similar to those of octahedral shear strain predicted by the model.

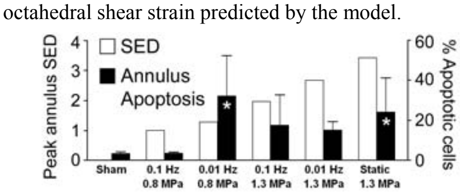

Figure 2 – *Comparison of annular apoptosis with peak annulus SED at the disc midheight normalized to the 0.1 Hz 0.8 MPa case.*

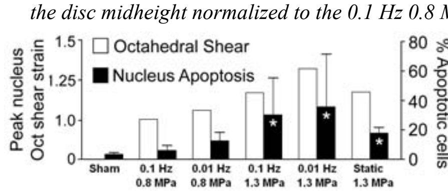

Figure 3 – *Comparison of nuclear apoptosis with normalized peak octahedral shear strain at the disc midheight.*

DISCUSSION: One of the challenges in elucidating the relationships between mechanical loading and pathophysiology for hydrated soft tissues lies in determining the in situ mechanical environment. Finite element models having sufficient detail allow analyses of complex loading simulations without the difficulties of performing and interpreting direct measurements. While mechanical criteria previously proposed for governing tissue remodeling have not been validated for all tissues, existing theories provide starting points for investigation. As a first step, one of the measures we examined was the strain energy density, which provides a simplified measure of the strain state at the region of interest and which has been theorized to govern bone remodeling [6, 7]. Interestingly, for all loading regimens including static compression, the highest levels of SED occurred at the inner annulus, the region of the annulus where we have historically observed the greatest alterations in matrix architecture in response to injurious levels of mechanical load. Similarly, distortional strain, thought to be important for fibrous tissue development [7], may be important in the nucleus since it is subjected predominantly to hydrostatic pressure under normal circumstances. Our analyses indicated that octahedral shear strain, indicative of distortion, increases in a graded fashion as well, but the static loading possessed slightly less distortion than the 0.01 Hz, 1.3 MPa case. Experimentally, it is clear that apoptosis was minimal at loading regimens having the least mechanical exposure. However, although SED and octahedral shear strain exhibited graded increases that depended on combinations of magnitude and frequency of compression, only apoptosis in the nucleus followed these trends. In the annulus, apoptosis was statistically equivalent after a certain level of mechanical exposure suggesting a metabolic threshold or tolerance limit to strain. This validated model may be useful in further identifying and clarifying important mechanobiologic factors associated with disc loading.

REFERENCES: [1] Lotz JC+, Spine, 1998; [2] Lotz JC+, Spine, 2000; [3] Walsh AJL+, ORS, 2002; [4] Duncan NA+, in: Comp Meth Biomech Biomed Eng, 1998; [5] Urban JP+, Conn Tiss Res, 1981; [6] Huiskes R+, J Biomech, 1987; [7] Carter DR+, J Biomech, 1987.

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