Mechanobiology of the intervertebral disc

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Abstract

Intervertebral disc degeneration has been linked in humans to extreme spinal loading regimens. However, mechanisms by which spinal force influences disc cellularity, morphology and consequently biomechanical function are unclear. To gain insight into mechanobiological interactions within the disc, we developed an *in vivo* murine tail-compression model. Results from this model demonstrate how deviations in spinal stress induce a cycle of altered cell function and morphology as the disc remodels to a new homoeostatic configuration.

Introduction

The intervertebral disc is a pliant, fibrocartilagenous joint that separates spinal vertebrae. It has a number of biophysical features (architectural, cellular and mechanical) that make it unique among bodily organs. Firstly, it is composed of three tissues: an outer-layer annulus fibrosus, a central proteoglycan-rich nucleus pulposus and two hyaline cartilage endplates. Secondly, these tissues contain multiple cell types that include fibroblasts within the outer annulus fibrosus, fibrochondrocytes within the inner annulus, notochordal cells and chondrocytes within the nucleus pulposus, and chondrocytes within the cartilage endplates. Since the disc is avascular, these cells depend largely on diffusion for nutrition and metabolic product removal. Finally, the disc's response to load varies with magnitude (non-linear) and time (viscoelastic), and it is the only joint that allows a full 6° of freedom in movement.

Age-related changes in disc biophysical characteristics occur in all individuals. Common among these are annular disorganization with fibrocartilage production, loss of anatomic distinction between the nucleus and annulus, and decreases in cellularity. These anatomic and cellular changes are associated with fluctuations in disc mechanical stiffness, which decreases during early degeneration and increases again with more advanced stages. Although the mechanisms for these changes are obscure and controversial, most agree that they reflect a combination of mechanically and biologically mediated events.

To clarify mechanobiological interactions, our group has developed an *in vivo* disc-loading model, where controlled force regimens are placed on intervertebral discs and the biological and mechanical consequences monitored. These experiments have shed light on the following important interactions among force, tissue stress/strain, cell function and matrix synthesis/degradation.

Static compression produces disc degeneration in a dose-dependent fashion

For the *in vivo* loading model, the tenth coccygeal disc of 12 week-old Swiss Webster mice is subjected to static compression via stainless steel pins (0.4 mm diameter) inserted percutaneously through adjacent vertebral bodies. Using calibrated elastics, a range of compressive stresses (0.4, 0.8 and 1.3 MPa) are applied for various times (3 h–7 days). Biological and biomechanical responses to compression are assessed either immediately after load removal, or after various recovery periods without load (3 days–4 weeks).

Histologically, normal murine tail discs exhibit morphology and cellularity comparable to young human discs (Figure 1 left-hand panel) [1]. The annulus consists of highly organized fibrous collagenous lamellae populated by elongated fibroblastic cells. In contrast, the nucleus contains physaliphorous notochordal cells in a contiguous cluster surrounded by a thin Alcian Blue-staining proteoglycan-rich zone. A thin cartilage endplate separates the nucleus from adjacent vertebral trabecular bone. *In situ* hybridization analyses demonstrate that transcripts of type II collagen localized primarily to the cells of the inner and middle annulus, cartilage endplate and occasionally to some peripheral nucleus cells. Widespread expression of aggrecan was characteristic of nucleus cells. Cells in the inner annulus and cartilage endplate also exhibited aggrecan but to a lesser extent than type II collagen.

Key words: apoptosis, biomechanics, remodelling, spine.
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Static loading results in distortion of nucleus cell aggregates and lamellae of the inner annulus with a concurrent down-regulation of type II collagen expression within 6 h of loading. At 24 h, type II collagen expression is absent and TUNEL (terminal dUTP nickend labelling)-positive cells began to appear in the inner annulus/peripheral nucleus region. By 7 days, extensive apoptosis is apparent within the inner annulus and nucleus. Overall, cell death is correlated with the magnitude and duration of static loading; a traditional dose-response model [2] demonstrated that there was a linear correlation between cell death and the logarithmic transformation of the stress dose (magnitude and time):

$$Y = \alpha + \beta_1 \log \sigma + \beta_2 \log t \qquad (1)$$

where Y is the probit transformation of the percentage of cells undergoing apoptosis, σ is the applied stress (MPa), t is the stress duration (h), and α, β_1 and β_2 are constants ($\alpha = 1.01, \beta_1 = 1.545$ and $\beta_2 = 1.472$; $R^2 = 0.92$) [3]. More recently, it has become apparent that after 3 h of compression (approximate time for steady state in the displacement response to load), the influence of subsequent time was comparable whether the load was maintained or removed [4]. This observation suggests that apoptosis is mainly triggered by peak matrix deformations associated with transient disc load, rather than other mechanisms associated with prolonged matrix compaction, such as reduced cell nutrition.

After load removal, architectural remodelling progresses from modest disruption and disorganization of the inner annulus at 6 days, to significant loss of nuclear cellularity, dissolution of inner annular layers and phenotypic metaplasia of inner annular fibroblasts at 4 weeks (Figure 1, right-hand panel). The loss of nuclear volume due to notochordal cell death is partially offset by a 30% increase in proteoglycan content. This new matrix is likely synthesized by remaining, viable notochordal cells and inner annular fibrochondrocytes, as these cells demonstrate punctate aggrecan gene expression. Overall, these loaded/recovered discs exhibit a number of features comparable with degenerating human discs, including loss of disc height, loss of distinction between the inner annulus and nucleus, fibrocartilage production within the annulus and biomechanical hypermobility.

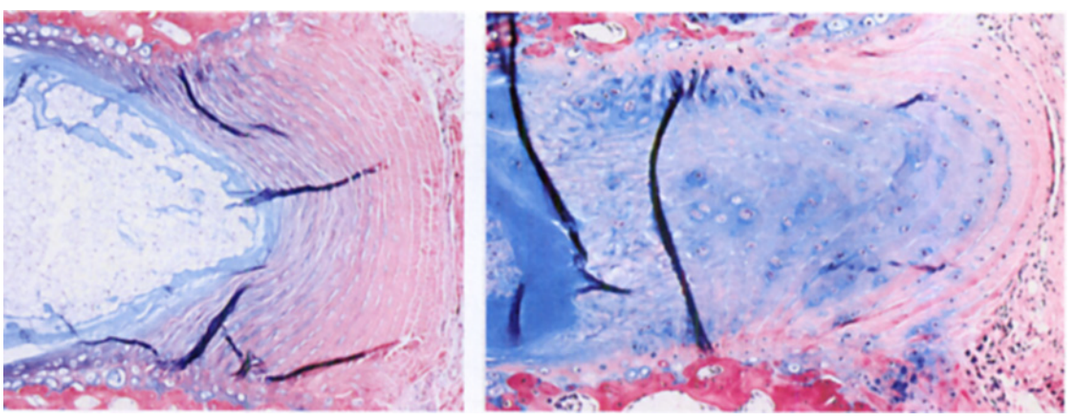
Mechanical response of the disc changes with degeneration

The structural organization and distinct material properties of individual disc tissues cause the disc's mechanical response to be non-linear and time-dependent. Healthy discs are largely water (approx. 80%) in a charged, permeable (0.013 mm^4Ns, where Ns is Newton seconds [5]), pliant matrix. Water helps define tissue volume, creates spaces for molecular transport and offers resistance to compression (since water is essentially incompressible). In the extracellular matrix, water is generally extrafibrillar (in relation to collagen) and defines a continuum of pores in

Figure 1

Histology of the normal and degenerated murine tail intervertebral disc

Normal murine tail disc architecture (left-hand panel). The nucleus consists of notochordal cells surrounded by proteoglycan matrix. Distinct annular layers are populated by elongated fibroblastic cells. After compressive loading and recovery, notochordal cell death is accompanied by proteoglycan production (right-hand panel). Inner annular architecture is lost and fibroblastic metaplasia to chondrocytes is apparent. Magnification, × 20.



the charged, collagen/proteoglycan network. At rest, Donnan ionic swelling causes the tissues to attract water until there is an equilibrium among fluid pressure, matrix stress and externally applied loads. Rapidly applied forces are initially resisted by increased interstitial fluid pressure and elastic matrix deformation. With increasing time, fluid movement allows matrix compaction that, in turn, leads to increased fixed charge density, decreased porosity and hence enhanced resistance to further deformation.

We utilized a one-dimensional fluid transport model to parameterize these time- and deformation-dependent features of disc compression [6]. The time-dependent axial strain in response to a constant force (creep) can be described by the following equation:

$$\varepsilon(t) = \varepsilon_0 + \left(\frac{\sigma_0 - P_{osm}}{D} - \frac{h_i G}{2kD^2} \right) \times \left[1 - \exp\left(-\frac{2kDt}{h_i} \right) \right] + \frac{G}{D} t \quad (2)$$

where t is the time, $\varepsilon(t)$ is the axial strain, ε_0 is the axial strain at $t = 0$, σ_0 is the external compressive stress, P_{osm} is the initial nuclear swelling pressure, h_i is the initial disc height, k corresponds to the endplate permeability, D represents the strain dependence of the swelling pressure and G represents the time dependence of annular deformation. These three parameters (k, D, G) provide insight into those features that dominate the disc's response to load, and the manner by which they change with degeneration.

The above formulation was fitted to experimental creep data collected from bone/disc/bone specimens previously subjected to either sham treatment (percutaneous pin placement with no load application, $n = 28$) or 1 week of 1.3 MPa compression followed by 4 weeks of recovery ($n = 30$). Specimens were subjected to five successive creep cycles (either 0.4 or 0.8 MPa applied stress), with the duration of creep being 20 min followed by a 40 min recovery period.

After *in vivo* compression and recovery, disc height decreased by 19% ($P < 0.0001$) and the elastic compressive modulus was reduced by 44% ($P < 0.053$). The parameter D, which represents strain dependence due to swelling of the nucleus, decreased by approx. 40% in the degenerated discs ($P < 0.03$). The parameter G, representing the time dependence of the collagen fibres in the annulus, tended to be smaller in the degenerated discs, but the differences were not statistically significant. Similarly, whereas there was a trend of increased permeability (k) in the degenerated discs, this difference was not statistically significant.

These degeneration-related trends compare with measurements made in humans [7] and, together with histological observations, support similarities between this murine model and human disc degeneration. Moreover, parameterization using the model provides insight into possible mechanisms behind mechanically induced degeneration. A decrease in D suggests that, with degeneration, the nucleus is less effective at resisting compression, perhaps due to the shift in composition of the nucleus from being primarily cellular to consisting of mostly proteoglycan matrix. The trends of decreasing G and increasing k are consistent with our recent finding that loading activates latent metalloproteinases (A. H. Hsieh and J. C. Lotz, unpublished work), which may contribute to compromised matrix integrity.

Patterns of matrix deformation correlate with alterations of cell function and matrix structure

To understand the relationships between global disc deformation and spatial patterns of matrix deformation, we developed a porohyperelastic finite element model for the murine tail motion segment using commercial software (ABAQUS version 6.2; Hibbitt, Karlsson & Sorensen, Inc., Pawtucket, RI, U.S.A.) and custom material property definitions. Material properties of the annulus, nucleus, endplate and bone were obtained from the literature for similar tissues. The annulus was modelled as an isotropic, compressible Mooney–Rivlin material with values for the hyperelastic coefficients obtained from previous experiments in our laboratory [5,8,9]. Collagen fibres in the annular matrix were represented as non-linear reinforcing elements (REBARS), whose orientation with respect to the transverse plane varied with radial position to simulate the lamellae [8]. The nucleus pulposus was also represented as a porohyperelastic material whose properties were based on previously validated results [10]. In addition, a novel strain-dependent swelling pressure was incorporated into the material definition of the nucleus with swelling properties based on extensive work by Urban and colleagues [11–13]. As the nucleus is compressed and the tissue porosity decreases, the fixed charge

density increases, resulting in a greater propensity for swelling:

$$\Pi = 2.1185 - 2.5425 \cdot P \qquad (3)$$

where Π is the swelling pressure and P is the tissue porosity (defined as volume of fluid/total volume). Permeability in the endplate adjacent to the nucleus was enhanced to permit fluid exchange between the disc and the adjacent vertebrae. Trabecular bone was modelled as transversely isotropic with properties obtained from the literature [14,15]. The model was used to predict spatial patterns of collagen fibre strain, dilatational strain (matrix volume change), shear strain (matrix distortion), hydrostatic stress, fluid pressure and strain energy density.

For static loading, the model predicts a creep response comparable to that measured experimentally. Further, the analysis predicts location-dependent changes in matrix deformation with compression and time. After a period of loading, creep deformation results in loss of collagen fibre strains within the inner annulus along with an increase in hydrostatic stress (Figure 2). When comparing with the *in vivo* compression response, these results suggest that alterations in matrix pressure, stress and distortion contribute to changes in cell function and matrix architecture. Consistent with observations in other tissues, areas of elevated hydrostatic stress and diminished distortion coincide with regions of fibroblastic metaplasia to chondrocytes, and disorganization of lamellar collagen. These predictions and their association with histological observations suggest that morphological changes attributed to aging and degeneration represent tissue adaptation to chronic mechanical loading.

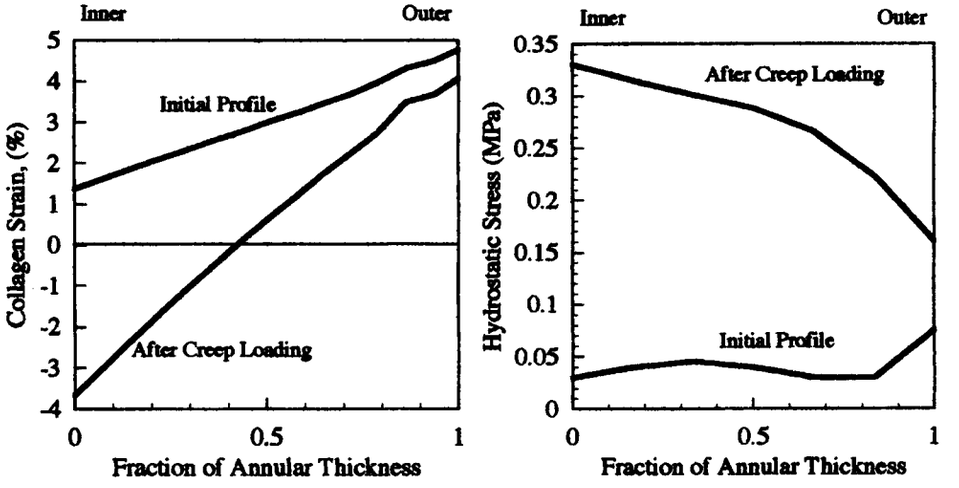
The association with matrix deformation and cell activity has been further clarified using an *in vivo* tail-bending model [16]. In this approach, static bending was applied via percutaneously applied pins into adjacent vertebrae. The exposure consisted of either a fixed angular deformity (18°) or a constant bending moment (4.25 Nmm). The exposure continued for 7 days at which time the animals were killed and the tissues analysed.

In contrast with pure compression, no cell death was observed within the nucleus of these bent discs. Rather, extensive apoptosis was noted on the concave side of the deformity, and was associated with significant differences in annular cell shape. The average shape (aspect ratio) of the apoptotic cell nuclei in the concave annulus (1.66 ± 0.09) was statistically more rounded than non-apoptotic cells in both the convex annulus (2.16 ± 0.04; $P < 0.0001$) and the annulus of sham discs (2.09 ± 0.03; $P < 0.0001$). Finite element simulations of bending demonstrated increased compressive stresses in the concave-side annulus with bending, while the nuclear pressures were only minimally affected. Taken together, results of the compression and bending studies demonstrate how changes in matrix pressure and distortion away from homoeostatic values lead to alterations

Figure 2

Finite element analysis predictions of the collagen fibre strain (left-hand panel) for the lamella of the annulus fibrosus as a function of radial position and time

After creep loading, the fibres of the inner and middle annulus lose tension, whereas tension of the outer annulus in maintained. After creep, an opposite trend in compressive hydrostatic stress (right-hand panel) is predicted for the inner and middle annulus.



in cell function and matrix composition. Compression selectively influences nuclear and inner annular cells, where computational simulations demonstrate the largest deviation in matrix stress and strain. Bending, in contrast, induces significant changes in matrix stress, strain and cell function on the concave annulus, while sparing the nucleus.

Dynamic stimulation can balance anabolic and catabolic influences of load

When loads are applied statically, disc creep can result in excessive matrix deformations that, in turn, can have deleterious biological consequences. During dynamic loading, compression is intermittently diminished, allowing for cessation of creep, and therefore an opportunity for disc recovery via swelling and elastic responses. Because of this, we questioned whether periods of dynamic stimulation could provide a mechanical stimulus for disc matrix synthesis, without the excessive deformation and adverse consequences of static loading.

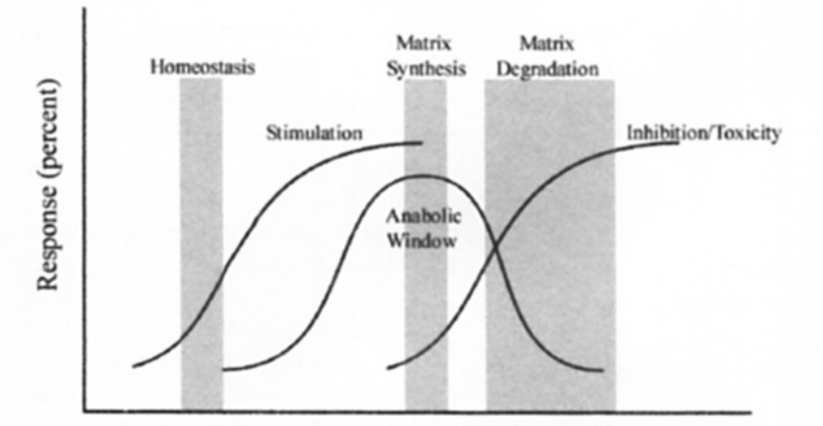
To provide *in vivo* dynamic stimulation of murine tail discs, we designed a pneumatic device that can be worn by mice continuously. Disc compression is produced by inflation of the latex bladder within a plastic housing that surrounds the mouse tail. The bladder is connected via a pneumatic line and swivel to a pressure source external to the animal's cage. An electronically controlled valve regulates pressure and provides a waveform with a pre-defined magnitude, frequency and duration. Using this device, discs were compressed cyclically at one of two frequencies (0.1 Hz or 0.01 Hz) and one of two peak stresses (0.8 MPa or 1.3 MPa). Cyclic compression was applied from 0 MPa to the peak stress for 6 h/day for each of 7 days.

As expected, biological responses were both load- and frequency-dependent. For the four exposures, apoptosis was minimum for the higher frequency and lower stress. Conversely, the lower-frequency, higher-stress loading enhanced aggrecan gene expression within the inner annulus and also elevated total proteoglycan content above sham discs. The strain energy density, which has been theorized as a remodelling stimulus in other tissues [17,18], was used to represent a single measure of overall disc exposure. Finite element simulations demonstrated that combinations of frequency and stress, because of the viscoelastic properties of the disc, produce strain environments corresponding to distinct strain energy density values. The lowest strain energy density occurs for the high-frequency, low-stress case where the biological response was the least, and the strain energy density (and the response) increases when the

Figure 3

Schematic representation of the disc cell population's response to mechanical stimulation

The anabolic window is defined as the difference between the frequency of stimulatory and inhibitory or toxic responses. Ideal loading (a combination of load magnitude, frequency and duration) leads to maximum matrix synthesis. Excessive stimulation (low frequencies and/or high stresses) leads to increasing cell inhibtion or toxicity and matrix degradation.



frequency is lowered and/or the stress level is raised. Static load at the higher stress results in the greatest strain energy density. Similar trends were also predicted for hydrostatic strain and maximum shear strain.

The anabolic window defines a balance between stimulatory and inhibitory response to load

The above results demonstrate that macroscopically, the disc responds to loading in a complex, time-dependent fashion. Due to its architecture, external loads are 'filtered' into spatial patterns of fluid pressure and matrix stress. Under homoeostatic conditions, these pressure and stress patterns coincide with appropriately organized matrix and cellular phenotype. The results of our computational and experimental studies suggest that, with alterations in spinal load, patterns of matrix stress and fluid pressure deviate from ideal levels and lead to cell inhibition and, in extreme cases, cytotoxicity. Consequently, anabolic responses to load represent a balance between stimulation and inhibition, both of which can be related to matrix stress and fluid pressure (Figure 3). Since matrix stress and fluid pressure are related to spinal load in a time- and magnitude-dependent fashion, the complex association between spinal load and beneficial or detrimental outcomes, while apparent, remains to be quantified. Once established, however, disc mechanobiological relations will contribute significantly to efforts on disease prevention and therapy development.

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Received 2 July 2002

The role of the physicochemical environment in determining disc cell behaviour

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Abstract

The cells of the intervertebral disc exist in an unusual environment. They are embedded in a dense matrix containing a high concentration of aggrecan whose fixed negative charges regulate the extracellular ionic composition and osmolarity; both extracellular cation concentrations and osmolarity are considerably higher than those experienced by most cell types. The disc also is avascular. Oxygen levels in the centre of the nucleus, where cells may be 6–8 mm from the blood supply, are very low. Since metabolism is mainly by glycolysis, lactic acid is produced at high rates and hence the pH is acidic. Finally, the disc is subjected to mechanical forces at all times; these vary with

Key words: fixed charge, lactate, mechanotransduction, osmolarity, oxygen.
Abbreviation used: GAG, glycosaminoglycan.
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