Net Transport Into the Degenerated Intervertebral Disc Is Enhanced via Low Rate Loading Induced Convection In Vivo

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Introduction: The intervertebral disc (IVD) is avascular and relies on transport from the adjacent microvasculature to receive nutrients and expel waste products. For this reason, a loss of nutrition has been widely implicated as a primary contributor to disc degeneration. Intuitively then, enhancing transport into the disc may be a strategy to slow, prevent or reverse the degenerative cascade. In healthy discs, transport is mediated primarily by diffusion, but in a degenerative disc diffusion is often reduced. Convective transport (bulk flow) is a potential strategy for augmenting diffusion into a degenerated disc. Deformation of the disc from mechanical loading can induce forced convection. The IVD is viscoelastic and responds to mechanical loading in a rate-dependant manner. Loading at low rates causes the disc to deform, stimulating bulk fluid flow and convective transport. Forced convection as a strategy to enhance transport into a degenerative disc has never been previously investigated. The purpose of this study was to establish whether forced convection can augment diffusion into a degenerative intervertebral disc in vivo.

Methods: Following IACUC approval, degeneration of the intervertebral disc was induced via needle puncture with a 16G needle at two lumbar levels of the spine in 5 New Zealand white rabbits. Degeneration was allowed to progress for 8 weeks following needle puncture. At the conclusion of the 8 week period, we utilized a custom designed loading apparatus to apply cyclic axial compression and distraction loading to a single degenerated lumbar motion segment in vivo. To quantify the contribution of dynamic loading to disc transport, animals were administered the small molecule MRI contrast agent gadodiamide (0.3 mmols/kg, IV). Animals were then subjected to low rate loading (0.5 cycles/sec, 200 N) for either 2.5 minutes (n=3), 5 minutes (n=1) or 10 minutes (n=1). Immediately at the conclusion of the designated loading period, the animals were euthanized and the lumbar spines harvested. Post-contrast enhanced 7T MRI was used to quantify the transport of gadodiamide into the nucleus pulposus. Transport into the degenerated, loaded level was normalized in each animal to the adjacent, degenerated, unloaded level. T2 relaxation constants in the nucleus for punctured and intact discs were also quantified via MRI. µCT was used to quantify subchondral bone density. Punctured and intact discs were then processed for histology via cryosectioning and the sections stained with safranin-o and fast green to assess structural changes to the disc. Stained sections were analyzed in Image J using the histogram tool to quantify the intensity of safranin-o staining as a measure of disc proteoglycan content. Subchondral bone density, T2 constants and safranin-o staining intensity data were pooled from all animals, and statistical differences between punctured and intact discs were assessed using a two-sample t-test.
**Results:** As shown in Figure 1, by 8 weeks post-needle puncture, substantial degenerative changes were observed on histology, including disc height reduction, disorganization of the annulus fibers and fibrosis of the nucleus. T2 constant mapping illustrated a significant mean 25.5% (p<0.001) decrease in nucleus T2 constant in punctured discs compared to intact discs. This corresponded with a significant mean 22.5% (p=0.004) decrease in safranin-o staining intensity in the nucleus of punctured versus intact discs, indicating a loss of nucleus proteoglycans in the punctured disc. µCT analysis illustrated osteophyte formation in the vertebral endplate region in six out of ten punctured levels; however, there were no detectable differences in subchondral bone density between endplates adjacent to punctured discs and endplates adjacent to intact discs. In degenerated discs, low rate axial compression and distraction loading caused the rapid uptake and clearance of gadodiamide in the nucleus pulposus, as shown in Figure 2. 2.5 minutes of low rate loading significantly enhanced net transport into the loaded, degenerative nucleus as compared to the unloaded, degenerative nucleus by a mean 12.6% (p=0.008).

**Discussion:** At the loaded degenerative levels, transport into the disc is governed by a combination of passive diffusion plus loading-induced forced convection. At the unloaded, adjacent degenerative levels, transport is governed by diffusion alone. With this study, we have shown for the first time that loading induced convection can enhance the uptake and clearance of small molecules in a degenerative intervertebral disc in vivo. The rapid exchange of small molecules with loading observed in this study can be explained by the reduced proteoglycan content of the degenerated disc. A reduction in disc proteoglycan content is associated with a decrease in osmotic pressure in the disc compared to a healthy disc. This reduces the stiffness of the disc, such that substantial deformation of the disc and bulk fluid flow occurs with each applied loading cycle. [1] These results suggest that low rate loading may be beneficial to disc health, as it may not only enhance nutrient uptake into the disc, but also waste product clearance.

**Significance:** Human degenerative discs demonstrate a decrease in diffusion of only 11.5% to 15% relative to healthy discs.[2] In this study, we affected a mean 12.6% increase in transport into a degenerative intervertebral disc in vivo via low rate loading-induced convection. This suggests that forced convection has the potential to restore transport in degenerative discs to the levels of normal healthy discs. This is clinically relevant because forced convection could be achieved via exercise or physical therapy regimens that elicit disc compression and distraction. If transport can be enhanced into the discs of patients with early stage disc degeneration, this could stimulate a regenerative response, culminating in the slowing or reversal of the degenerative cascade.
Figure 1. Safranin-o and fast green staining of control (A) and punctured (B) discs illustrates the structural changes to the disc which occur 8 weeks following needle puncture. Images are 5X.

Figure 2. In a degenerated disc, low rate loading induces the rapid uptake and clearance of gadodiamide in the disc. Values above 100% indicate increased gadodiamide concentration in the loaded disc relative to the unloaded disc.

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