

Nucleus Pulposus Permeability Dictates Intradiscal Injection Efficacy

Zachary Appel, Arthur J Michalek
Clarkson University, Potsdam, NY
ajmichal@clarkson.edu

Disclosures: Zachary Appel (N), Arthur J Michalek (N)

INTRODUCTION: Due to the avascular nature of the intervertebral disc (IVD), needle injection has emerged as the most practical method for delivering therapeutic agents, such as stem cells. However, retraction of the needle after injection leaves a track in the annulus fibrosus (AF), which provides a ready pathway for the leakage of pressurized injectate.[1] Both low permeability and high swelling pressure of the nucleus pulposus (NP) have been implicated as factors limiting injectate retention. We hypothesized that both permeability and swelling play important roles in dictating fluid pressurization and dispersion during injection as well as leakage risk upon needle retraction. This hypothesis was tested by performing instrumented injections on bovine caudal intervertebral discs following enzymatic digestion of the NP using either collagenase (to increase permeability) or trypsin (to reduce swelling pressure).

METHODS: Three adult bovine tails were obtained from a local abattoir. Following the removal of skin and muscle tissue, vertebral bodies c1 through c6 were transected at mid-height with a saw yielding a total of fifteen motion segments, each consisting of a disc and two flanking hemi-vertebrae. Motion segments were systematically assigned to Control, Collagenase, and Trypsin groups such that tails and anatomical levels were evenly represented. All discs were injected, using a 26G needle, with an average of 30 μ L of 50mmol/L Tris buffer with 1 mmol/L CaCl₂ with the addition of no enzyme (Control), 125mg/mL collagenase (Collagenase), or 125 mg/mL trypsin (Trypsin).[2] The discs were then incubated for eight hours at 40°C, then frozen at -20°C until testing.

Prior to testing, the discs were thawed and warmed to room temperature. A 21G needle was pushed through the disc from the anterolateral aspect then retracted halfway, leaving a needle track defect in the posterolateral aspect of the AF and the tip of the needle in the center of the NP. An instrumented injector [3] equipped with a transducer for measuring fluid pressure and a linear potentiometer for measuring injected volume was used to inject 0.135M phosphate buffered saline with 0.05mg/mL 5-DTAF. Injections were performed in a stepwise manner with 5 μ L steps followed by 3s dwells while both pressure and volume were sampled at 20Hz. Following injection, the motion segments were frozen, transected in the mid-sagittal plane using a hand saw, and photographed under 450nm illumination using a digital camera with a 512nm lens filter to visualize 5-DTAF distribution. Pressure-volume traces were analyzed using Matlab [1] to identify events (deviation of pressure from monotonic with increasing volume) consistent with injectate leakage through the needle track defect. These events were used to record maximum pressure and volume prior to leakage (P_{max} and V_{max} , respectively). Comparisons between groups were performed using a Wilcoxon rank-sum test.

RESULTS: Qualitatively, both enzymes had visible effects on injectate distribution. In all control discs, fluorescent injectate was highly localized at the injection point (Figure 1A). In collagenase treated discs, injectate was broadly dispersed throughout the NP and out into the AF (Figure 1B), and in trypsin treated discs injectate was semi-dispersed through large, but still clearly demarcated, areas of the NP (Figure 1C).

Leakage events were recorded in pressure-volume traces of all specimens (Figure 1D). Control discs leaked with a median P_{max} of 8kPa and V_{max} of 109 μ L. Collagenase treatment significantly decreased P_{max} relative to control. Median V_{max} was increased, but not significantly. Trypsin treatment had no significant effects on P_{max} or V_{max} .

DISCUSSION: The results of this study suggest that permeability, rather than swelling, is the primary driver of the mechanics of intradiscal injection. While cleavage of proteoglycans via trypsin treatment resulted in some increase in injectate dispersion, it did not affect the volume of fluid which may be injected without leakage upon needle retraction. Increasing NP permeability via collagenase treatment resulted in a drastic decrease in injection pressure and increase in injectate dispersion. While median V_{max} was not significantly changed by collagenase treatment, the 5th percentile was increased by approximately 3x, indicating a large increase in the volume which may be injected with a 95% probability of retention. The fact that the 95th percentile of V_{max} was constant across all groups suggests that stiffness of the AF may place an upper limit on injection volume regardless of NP properties.

The results of this study also suggest that care should be taken when using enzymatic digestion to initiate degeneration in pre-clinical models, as changes in tissue permeability may bias the effects of subsequent injected treatments. Furthermore, it should be noted that the present study was performed in the absence of axial compressive load, which has previously [3] been shown to increase maximum injection volume. However, axial compression is also expected to decrease disc height, and subsequently NP volume, the enzyme treated discs, potentially negating this effect.

SIGNIFICANCE: Increased permeability, but not decreased swelling pressure, of the NP results in both a wider dispersion of injectate and an increase in the volume of fluid that may be safely injected without leakage upon needle retraction.

REFERENCES: [1] Varden LJ +, *JOR Spine*, 2019. [2] Antoniou J+, *Spine*, 2006. [3] Varden LJ+, *Eur Spine J*, 2022.

ACKNOWLEDGEMENTS: Specimens were provided by Tri-Town Packing, Brasher Falls, NY. This work was funded by the Clarkson University Honors Program and NSF Award #2138342.

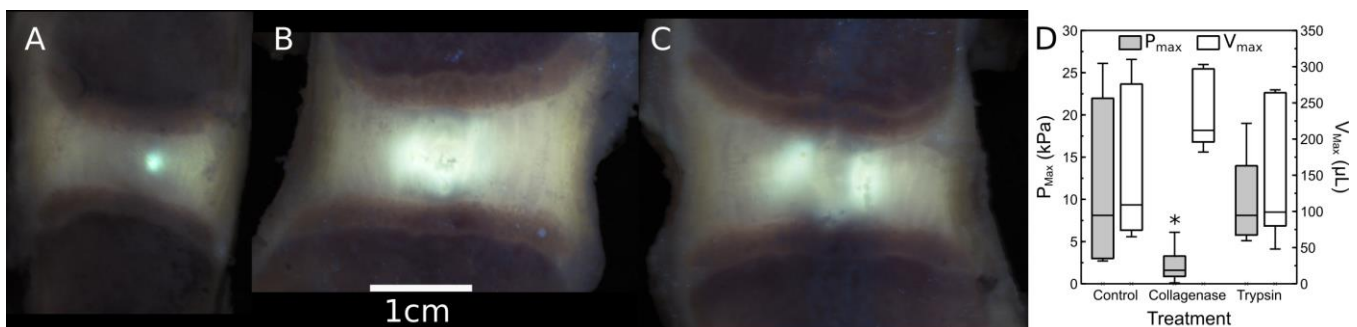


Figure 1: Representative images of midsagittally transected discs showing fluorescent saline injected after Control (A), Collagenase (B), or Trypsin (C) treatment. Comparison of leakage pressure (P_{max}) and leakage volume (V_{max}) (D). * indicate significant ($p < 0.05$) difference relative to control.