Dispersion of Intradiscally Injected Particles is Limited by Size

Austin J Boie, Arthur J Michalek
Clarkson University, Potsdam, NY
ajmichal@clarkson.edu

Disclosures: Austin J Boie(N), Arthur J Michalek (N)

INTRODUCTION: As the intervertebral disc (IVD) is the largest avascular organ in the body, injection via hypodermic needle into the nucleus pulposus (NP) is the most practical means of therapeutic delivery. Leakage of injectate via the track left by the needle through the outer annulus fibrosus (AF) has been suggested as a possible cause of reduced efficacy of intradiscal injection, along with adverse outcomes. While the fluid mechanics of injectate movement through the NP tissue have been previously studied,[1] not much is known about how a biphasic material, consisting of solid particles in a fluid carrier, behaves. Proposed injectates range from protein peptides (with hydrodynamic radius on the order of 1nm),[2] to stem cells (diameter of 15µm), to hydrogel microspheres (with diameters up to 200µm).[3] This study hypothesized that increasing solid phase particle diameter would decrease particle dispersion through the NP.

METHODS: Four adult bovine tails were obtained from a local abattoir, and skin and muscles were removed. Disc levels c1-c2 through c5-c6 from each tail were systematically assigned to a small bead (SB) or large bead (LB) particle group, such that tails and levels were evenly represented in both groups. Each disc was injected with saline containing a small green fluorescent molecule (SM: 0.05mg/mL 5-DTAF) along with 50mg/mL red fluorescent polystyrene beads (Small: 45-53µm, Large: 125-150µm). Injections were performed through the right posterolateral aspect of each disc using a 21g needle inserted to a depth of one half of the disc diameter, as measured with a dial caliper. After inserting the needle, the syringe plunger was pushed down for ten seconds, then pulled back to aspirate any unabsorbed injectate. The syringe was weighed before and after injection to estimate injected volume.

Following injection, each disc was removed from the adjacent vertebrae using a scalpel, wrapped in aluminum foil, and frozen. The frozen discs were then trimmed to mid height using a cryostat and photographed. Photographs were taken under illumination from a blue LED lamp with peak emission at 450nm, using a digital camera equipped with a macro lens and dichroic lens filter with peak transmission from 500-600nm. Digital photographs were analyzed in Matlab to quantify carrier and particle dispersion. SM dispersion area was calculated by thresholding the green channel of the image and recording the size of the resulting contiguous area. As beads were frequently observed in discrete pockets, the outer perimeter of areas in the thresholded red channel was used to define particle dispersion area.

Disc diameters were used with previously reported[4] level-wise aspect ratio and annular thickness measurements to estimate NP volume and NP cross-sectional area for each disc. Injection volume was normalized to NP volume, and particle dispersion areas were normalized to NP cross-section. Normalized dispersion areas were compared using a Wilcoxon rank sum test.

RESULTS:

There was no significant difference in injectate delivery volume between the SB and LB groups, either in absolute value (Median 75µL, IQR 40-130 µL) or when normalized to NP volume (Median 6.6%, IQR 4.4-15%), indicating that the presence of the beads did not affect SM dispersion. Similarly, there was no significant difference in SM dispersion area between SB and LB groups. SM dispersion was visibly limited to the NP (Figure 1 A-C) and had a median dispersion of 26% of NP area, with an interquartile range from 20-34% (Figure 1 D).

In both particle size groups, particle dispersion area was visibly smaller than SM dispersion area (Figure 1 A,B). While there was some dispersion of SB particles into the NP (Figure 1 A), there was very little dispersion of LB particles (Figure 1 B) away from the needle track. In three out of ten discs in the LB particle group and two out of ten discs in the SB particle group, no particles were observed in the NP (Figure 1 C). The dispersion areas, relative to NP area, of SB and LB particles were significantly lower than that of SM, and LB had a significantly lower dispersion area than SB (Figure 1 D). Normalized dispersion area decayed exponentially with increasing particle size (Figure 1 E).

DISCUSSION: While needle injection is an efficient means of delivery for small molecules (5-DTAF has a hydrodynamic radius of approximately 1nm, comparable to proposed therapeutic peptides), dispersion is reduced for larger particles. The results of this study suggest that while particles up to 50µm in diameter may disperse through the NP, movement of larger particles may be restricted by tissue pore size. In addition to reduced dispersion into the NP, the results of this study indicate that leakage risk is also increased following injection of larger particles; despite aspiration of un-delivered injectate prior to needle retraction, several discs in the LB group had particles visible in the AF. This study is limited by the use of bovine caudal IVDs, as these are analogous to healthy human discs. Degeneration of the human NP is associated with decreased primary permeability (via fibrosis) and increased secondary permeability (via fissuring). Particle dispersion in a degenerate disc is thus expected to be both less uniform and highly dependent on whether the injection site was near a fissure.

SIGNIFICANCE: Intervertebral disc nucleus pulposus pore size limits the dispersion of injected particles.

REFERENCES: [1]Varden+, *JOR Spine*, 2019. [2]AlGarni+, *Tissue Eng. Part A*, 2016. [3] Chen+, *J Control Release*, 2025. [4] Michalek+, *JOR Spine*, 2025.

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.

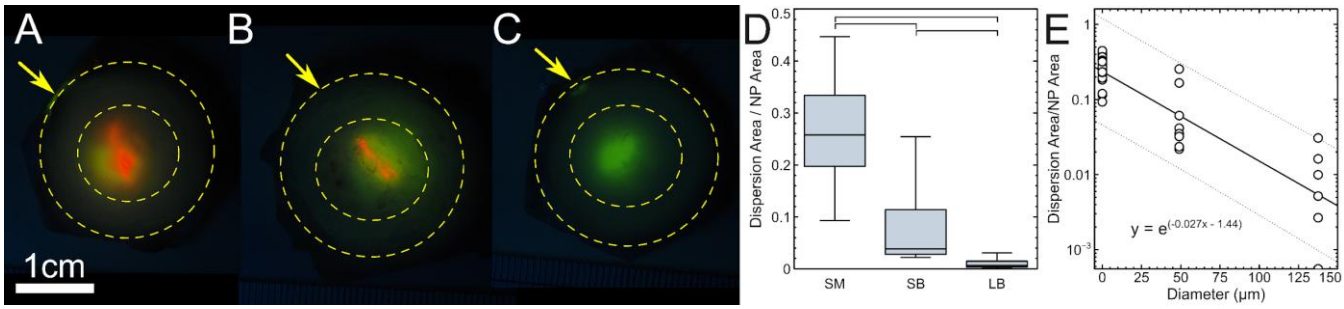

Figure 1: Representative photographs (A-C) showing dispersion of SM particles in green and SB (A) and LB (B) particles in red. In (C), an LB injected disc in which no particles were delivered. Dashed lines indicate inner and outer AF boundaries, and arrows indicate injection site. Median, IQR, and range of normalized dispersion areas (D). Bars indicate significant ($p<0.05$) difference. Normalized dispersion area versus diameter (E).