# Comparison of transport properties between human and tissue-engineered vertebral endplates

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**INTRODUCTION:** The vertebral endplates are the primary routes through which nutrients and metabolites enter and exit the intervertebral disc.[1] Changes in cartilaginous endplate (CEP) composition, including CEP fibrosis and calcification, can impair nutrient and metabolite transport[2,3] and associate with more severe disc degeneration.[4,5] As an alternative to spinal fusion surgery for the treatment of end-stage disc degeneration, our group has developed a composite tissue-engineered total disc replacement (eDAPS), which includes a porous poly(caprolactone) (PCL) scaffold as an endplate analog that provides an interface between the native vertebral bone and engineered disc. Our prior work has been focused on biomaterial modifications to this scaffold to promote integration *in vivo*; however, the transport properties of these engineered endplates in comparison to human endplates remains unknown.[6] Recapitulating the transport properties of healthy endplates will be essential to the long-term *in vivo* success of the eDAPS. We hypothesized that transport across engineered endplates would decrease with extracellular matrix deposition within the scaffold to reach equivalence with transport across healthy endplates.

**METHODS**: *Engineered endplate fabrication:* PCL endplate scaffolds (10 mm diameter x 1.5 mm in height) were fabricated according to a salt-leaching protocol and coated with hydroxyapatite (HA) according to our established protocols.[6,7] microCT and SEM imaging were used to characterize the formation of HA. HA-coated PCL scaffolds (n=6) were then seeded with goat mesenchymal cells and cultured in chemically defined media with TGF-β3 for 5 and 10 weeks to promote collagen and proteoglycan formation within the scaffold. *Human endplate acquisition:* Eight lumbar spines (L12-L5S1, 5 male, 3 female, 25-70yo) were obtained from human cadavers (Science Care and NDRI). T2-weighted MRIs were obtained for disc Pfirrmann grading, and T2 mapping was used to quantify nucleus pulposus (NP) T2 relaxation times.[8] Spinal motions segments (n=35) were dissected. From these segments, two cylindrical cores (10 mm diameter x 2.50 mm, n=66) were obtained that included the cartilage endplate and adjacent trabecular bone. *Passive diffusion experiments*: Human endplates (n=32), acellular HA PCL scaffold (n=6), and 5-week cell-seeded HA PCL scaffolds (n=4) were used for passive diffusion experiments using a custom diffusion chamber. The first passive diffusion experiment had an upstream chamber of 3.33 mg/mL of glucose (MW=180.16), following a second passive diffusion experiment of 1.1 mg/mL of sodium fluorescein (MW=367.27). From the experiments, triplicates of the downstream chamber were collected every hour for 6 hours. Fluorescence and absorbance were read via a microplate reader, and the concentration of the downstream chamber was calculated based on specific standard curves for each. Total diffusion was quantified by calculating the area under the curve (AUC). *Convection experiments*: A custom loading apparatus (**Figure 1A**) was used to control the driving hydraulic pressure against the native and engineered CEPs (4 mm diameter). The native human CEPs used in these tests were harvested from discs with a range of 93 < NP T2 < 236 ms. We applied a cyclic pressure (0.28–0.55 MPa) and sinusoidal frequency (0.5 Hz). The upstream solute reservoir had 0.1 mg/mL of sodium fluorescein; fluid and solute that passed through the CEPs were collected in a downstream reservoir containing PBS.[2] Permeation experiments were up to 80 minutes in duration. Outcomes included the net fluorescein transport and the hydraulic permeability.[2]

**RESULTS:** Passive diffusion experiments demonstrated that transport through tissue-engineered CEPs was variable but trended lower compared to native human CEPs. Cell-seeded HA PCL had significantly less glucose passive diffusion compared to endplates from degenerative discs, but not statistically different from endplates adjacent to healthy discs (**Figure 2B**). Sodium fluorescein passive diffusion was not statistically different across groups, but demonstrated that sodium fluorescein diffusion is variable within the 5-week cell-seeded HA PCL and that passive diffusion across cell-seeded HA PCL endplates trended higher than for acellular HA PCL (**Figure 2C**). Acellular HA PCL and 10-week cell-seeded HA PCL had significantly higher hydraulic permeability compared to native human CEPs (**Figure 1B**). For CEP porosity, acellular HA PCL had significantly lower porosity compared to native human CEPs (**Figure 1C**).

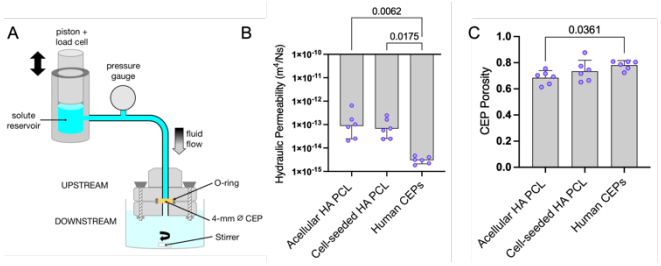


Figure 1. (A) Schematic of custom loading apparatus. (B) Hydraulic Permeability and (C) CEP Porosity for each experimental group.

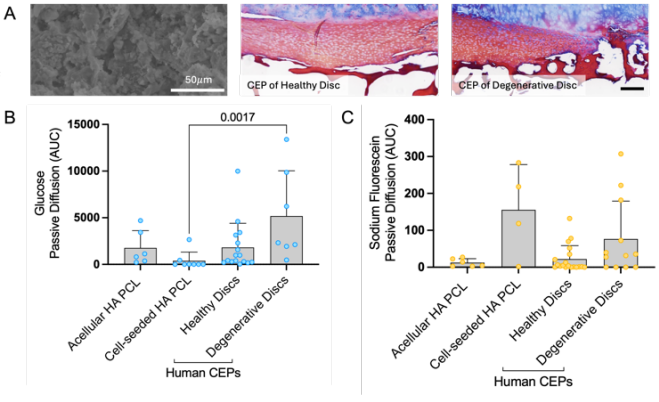


Figure 2. (A) SEM image of HA PCL scaffold and CEP histology images (Mallory Heidenhain). (B) Glucose and (C) Sodium Fluorescein passive diffusion for experimental groups. Endplate scale = 555 μm.

**DISCUSSION:** Passive diffusion experiments demonstrated trending reductions in glucose diffusion and increases in sodium fluorescein diffusion following chondrogenic pre-culture of MSC seeded endplates. Future work will quantify water, proteoglycan and collagen content to help explain some of these differences. In addition, we plan to study the effects of longer durations of endplate pre-culture (10 weeks) to determine how matrix maturation within the endplates affects diffusion. Endplates adjacent to degenerative discs also exhibited trending increases in diffusion compared to endplates adjacent to healthy discs, yet importantly, diffusion across engineered endplates was more similar to diffusion across endplates adjacent to healthy discs versus degenerative discs. The low hydraulic permeability of healthy human CEPs is consistent with the dense, negatively charged extracellular matrix, which resists fluid flow. With higher hydraulic permeability, the engineered CEPs had less resistance to fluid flow compared to native human CEPs, which may lead to greater convective transport. This may improve nutrient transport into tissue-engineered total disc replacements, but may come at the expense of increased creep deformation under physiologic loading. CEP porosity is an important factor that influences hydraulic permeability and solute transport[2]. Despite engineered CEPs having higher permeability, they have low porosity, which indicates that other factors affect permeability. Potential factors affecting permeability can include matrix maturity and cross-linking/fiber anisotropy. Future studies will focus on tuning factors that control porosity and permeability for engineered CEPs while also taking into consideration the contribution to biomechanical creep.

**SIGNIFICANCE:** Overall, the results from this study can inform design improvements to the endplate interface of composite, tissue engineered disc replacements to enhance their long-term in vivo performance. Ultimately if successful, composite engineered disc replacements such as the eDAPS offer a promising alternative to fusion surgery for patients with end-stage disc degeneration and back pain.

**REFERENCES:** [1] Maroudas+ *J. Anat*., 1975 [2] Sampson+ *J. of Biomechanics*, 2018 [3] Benneker+ *Spine*, 1976 [4] Antoniou+ Spine 1996 [5] Bonnheim+ Eur Spine J 2022 [6] Fainor+ *Cells Tissues Organs*, 2023 [7] Kim+ *Acta Biomater, 2020* [8] Ashinsky+ *J. Anat*., 2020

**ACKNOWLEDGEMENTS:** This study was supported by the Department of Veterans Affairs and the ORS Spine Section Travel Fellowship.