Influence of Cartilage Endplate Permeability on Intervertebral Disc Nutrition

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Introduction: Low back pain is the leading cause of disability worldwide and is significantly associated with intervertebral disc degeneration. Prior studies suggest that poor disc nutrition is an important reason why disc cells fail to remodel their matrix. Since disc cells require nutrients to survive and function [1], and since nutrients must diffuse across the cartilage endplates (CEP) to reach the avascular nucleus pulposus [2,3], determining how CEP permeability influences disc cell viability is an important step in understanding the mechanisms of disc degeneration and could additionally be useful for predicting the response to new cell-based treatments [4], which place increased demands on endplate nutrition routes. Towards that end, our own work in this area shows that disc health is worse adjacent to a less hydrated CEP. However, mechanistic studies that link improper CEP composition (including low hydration) with reduced permeability and impaired disc cell viability don't exist. Thus, the goals of this study were: 1) to determine the influence of CEP permeability on disc cell viability; and 2) to relate alterations in CEP permeability with depletion-induced changes in hydration. **We hypothesized that less hydrated and less permeable CEPs would impair disc cell viability by limiting nutrient diffusion.**

Methods: *Tissues:* Intact human CEPs bordering the nucleus pulposus were harvested from cadaveric discs (51 year-old: L5, Pfirrmann grade 2; 65 year-old: L2, Pfirrmann grade 3). *Disc cell viability:* To isolate the effect of CEP permeability on disc cell viability, we used diffusion chambers, which mimic the diffusion-limited nutrient environment of the disc *in vivo* [1]. Briefly, the chambers consist of two parallel glass plates separated by 170 µm-tall spacers; disc cells cultured in the center of the chambers are separated from their nutrient source (media with glucose) at the open sides of the chambers by CEP samples with different permeabilities (**Fig 1**). In the present study, nucleus pulposus cells from 28 month-old bovine coccygeal discs were suspended in 1% agarose gel (4 million cells/ml) and loaded into chambers containing sectioned and sterilized CEP samples (2 samples/chamber; $n = 3$ chambers/disc). The chambers were immersed in media (low-glucose DMEM with 6% FCS) and then incubated for 48 hr under 21%/5% O_2/CO_2 at 37°C. After incubation, the chambers were removed from the media, the gels were stained with a Live/Dead assay, and the viable distance was measured using a fluorescence microscope. *Solute transport:* To elucidate the mechanisms affecting CEP permeability, fluorescence recovery after photobleaching (FRAP) was used to measure the transport kinetics of a small fluorescent tracer (sodium fluorescein; 376 Da). Full-thickness CEP samples were immersed in tracer solution (0.1 mg/ml) for 48 hr at 4°C. FRAP was performed with a Leica SP5 confocal microscope at a minimum of 3 spots per sample (20X; 50 µm bleach diameter; 100% laser power; 4% power pre-/post-bleach). Fluorescence intensity in the bleached spot was determined for each image acquired during recovery, and tracer diffusivity was calculated using the Axelrod method (**Fig 2**). We measured solute diffusivity in native CEP samples ($n = 3$ samples/disc) and in matrix-depleted CEP samples ($n = 5$ samples) that were digested in collagenase enzyme (1 mg/ml) for 5 hr at 37°C. *Biochemistry:* Site-matched CEP samples were assayed for their water contents by lyophilization and for their sulfated GAG contents by DMMB assay. *Statistics:* Results (mean ± SD) were compared using *t*-tests.

Results: CEP permeability had a significant effect on disc cell viability. Specifically, the viable distance was 26% shorter in the chambers containing CEP samples from the older and more degenerated disc; those CEP samples had 44% lower solute diffusivity (indicating reduced permeability) compared to the CEP samples from the younger and healthier disc (119.4 ± 24.9 *vs.* 212.4 ± 16.5 µm^2/s; $p < 0.001$). The CEP samples that caused a shorter viable distance also had 14% less water (58.1 ± 4.1 *vs.* 67.3 ± 4.2%; $p < 0.05$) and 32% less GAG (63.8 ± 7.2 *vs.* 94.5 ± 17.6 µg/mg dry wt; $p < 0.05$). In the matrix-depleted samples, solute diffusivity was significantly correlated with CEP hydration ($p < 0.001$, **Fig. 3**).

Discussion: In this study of the human CEP, we sought to determine the influence of CEP permeability on disc cell viability and to establish a composition-permeability relationship. **Our results demonstrated that low CEP permeability impairs disc cell viability by limiting nutrient diffusion, and that reduced CEP hydration plays a central role.** Horner and Urban previously reported that disc cell density affects viability [1]. Here we extend those prior findings by showing that CEP permeability also influences disc cell viability, independent of cell density. Taken together, these data suggest that the viable distance from the nutrient source is a balance between CEP permeability ("nutrient supply") and disc cell density ("nutrient demand").

Since the CEP is an order of magnitude *less* permeable than the bony endplate [5], we focused on solute diffusivity in the CEP. In the matrix-depleted samples, solute diffusivity (and thus permeability) was positively correlated with the amount of fluid through which the solute could diffuse. This is consistent with previous results [6], and may explain why the viable distance was so much shorter in the CEP samples with lower hydration. One implication of these findings is that poor disc health may be directly tied to diminished CEP composition — rather than just an incidental finding [7]. A second implication is that cell-based therapies for restoring a moderately degenerated disc [4] may have limited efficacy if CEP permeability isn't also improved.

Significance: Back pain associated with a degenerated intervertebral disc causes substantial disability. Our findings are significant because they demonstrate that CEP permeability directly influences disc cell nutrition and may therefore be a key target for new prevention strategies, diagnostics and therapeutics.

References: 1. Horner and Urban, *Spine* 2001; 2. Maroudas+, *J Anat* 1975; 3. Urban+, *Clin Orthop Related Res*, 1977; 4. Oehme+, *Stem Cells Int* 2015; 5. Rodriguez+, *Spine* 2011; 6. Roberts+, *Spine* 1996; 7. Antoniou+, *Spine* 1996.

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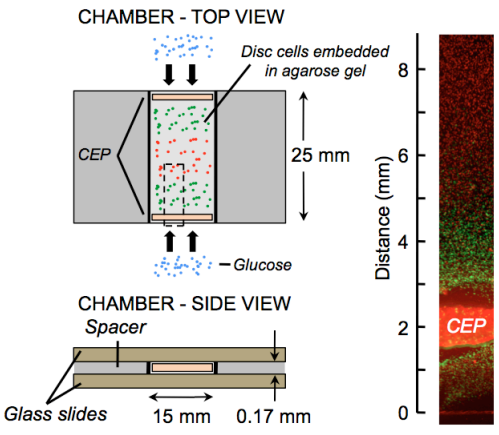

Fig 1. Diffusion chambers were used to isolate the effect of CEP permeability on disc cell viability. During incubation, disc cells in the chambers obtain glucose via diffusion across CEP samples at the open sides of the chamber. CEP permeability, which controls nutrient supply, and cell density, which determines nutrient demand, affected viable distance. See live/dead transition in the micrograph from boxed region.

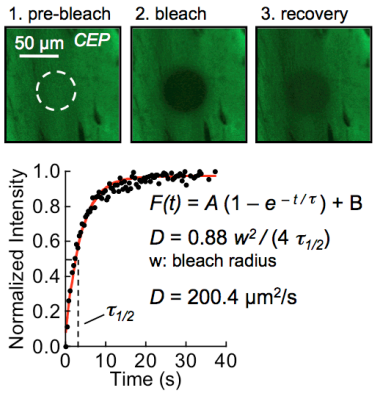

Fig 2. Tracer diffusivity, *D*, was measured with FRAP. After the tracer permeates the CEP sample (pre-bleach), a laser bleaches the fluorophores within a spot (bleach). This creates a dark spot, which recovers its fluorescence as fluorescent tracers diffuse into the bleached spot and bleached tracers diffuse out (recovery). *D* is calculated by curve-fitting the fluorescence intensity during recovery.

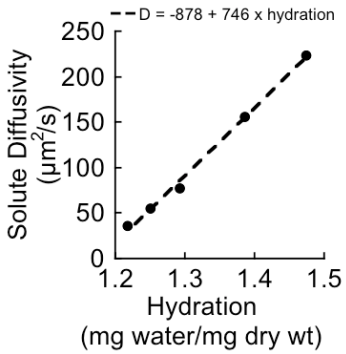

Fig 3. In matrix-depleted CEP samples, reductions in CEP hydration caused significant decreases in solute diffusivity ($p < 0.001$). Solute size: 376 Da.