Effects of pH and Inflammatory Interactions on Mechanical Nociception in the Degenerative IVD

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Disc Degeneration and Cell Therapy

- Lower back pain is the largest contributor to global disability rates and ranks 6th in terms of overall disease burden [1]. Degenerative disc disease (DDD) is the breakdown of IVDs and is a major contributor to back pain [2].
- Intervertebral Discs (IVDs) help hold the vertebrae together, provide flexibility, and act as shock absorbers for the spine [3].
- IVDs consist of three main parts: the fibrous annulus fibrosis (AF), the gelatinous nucleus pulposus (NP), and cartilaginous end plates (EPs) on the inferior and superior ends of the IVD [3].
- The acidic, hypoxic, and inflammatory environment produced within the degenerating IVD is highly unfavorable for cells, often inducing their apoptosis or senescence, creating a positive feedback loop which furthers degeneration (Figure 1)

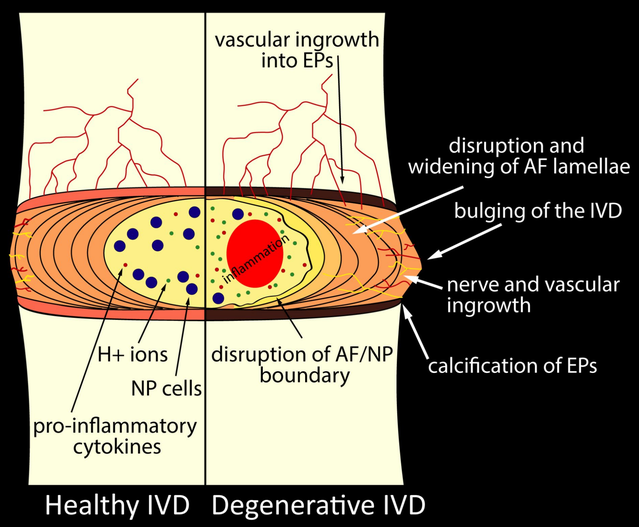
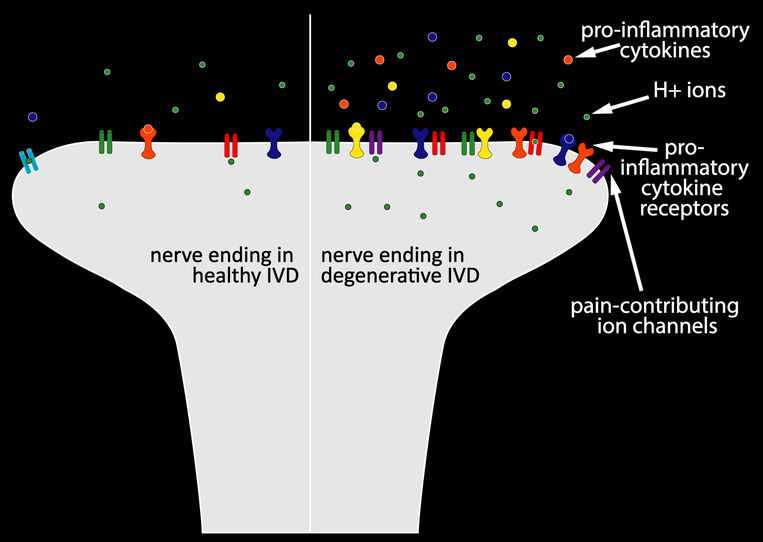


Figure 1: Intervertebral discs act as shock absorbers and give flexibility to the spine. Degenerative Disc Disease is the breakdown of the intervertebral disc. The degenerating disc is a harsh environment for cell growth, which lowers efficacy of potential cell therapies.

- Pro-inflammatory cytokines and low pH present with DDD are believed to cause neoinnervation and other degenerative changes, such as IVD structure change also lead to changes in mechanical signaling. All of these changes can lead to hyperalgesia [2].



- A more thorough understanding of these mechanisms is necessary for developing better treatment strategies targeting these mechanisms.

CRISPR-Guided Gene Modulation

- CRISPR-guided epigenome editing systems are capable of robustly modulating gene expression [4].
- When a Krüppel associated box (KRAB) effector domain is tethered to deactivated Cas9 (dCas9), this system results in tri-methylation of the histones at or near the promoter of the target gene which leads to gene repression (Figure 2) [4].

CRISPR-Guided Gene Modulation Continued

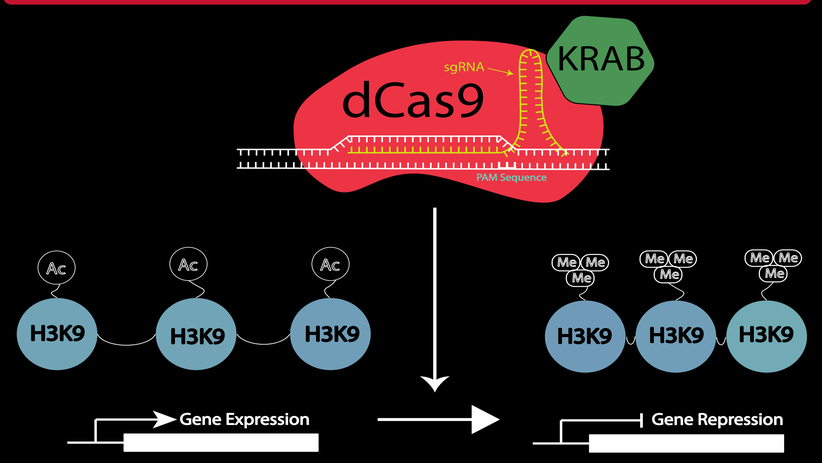


Figure 2: The dCas9-KRAB complex is able to efficiently tri-methylate the histones (H3K9) of the transfected host cells at the location on the DNA that is complementary to the single-guide RNA (sgRNA). This tri-methylation leads to repression of the target gene.

Research Hypothesis

Regulation of cell cytokine receptor expression profiles, utilizing CRISPR epigenome editing-based vectors, will lessen nociceptive neuronal response in an in vitro model of degenerative disc disease.

Methods – Engineering the CRISPR Vectors

- The genes that we have selected to target with our CRISPR-dCas9-KRAB vectors have been shown to play a role in inflammation and pain signaling from degenerative discs [3,5]. Our target genes are:
  - IL6ST: cytokine receptor which binds IL-6
  - TNFR1: cytokine receptor which binds TNF-α

Figure 3: Schematic of the CRISPR-dCas9-KRAB vector.

1. A CRISPR-dCas9-KRAB backbone vector was built (Figure 3).
2. 3-6 gRNAs were designed for each target gene (TNFR1 & IL6ST) – these gRNAs each gene promoter in a slightly different place.
3. The gRNAs were cloned into the vector backbone (two individual gene targeting vectors and one that targets both).
4. The vectors were each packaged in a lentivirus envelope to allow for transfection of the host cells.
5. Vectors were screened *in vitro* by optimizing transduction and knockdown efficiency. In the end, one vector was chosen for each target gene.

Methods – Degenerative IVD Model

1. Rat DRG neurons are seeded onto either healthy or degenerative human IVD tissue and are given time to adhere.
2. Cells are transduced with a CGRP reporter virus to mark nociceptive neurons.
3. Groups are either transduced with a non-target or knockdown vector for the genes of interest.
4. Cells are cultured in either healthy or low pH media.
5. Tissue is attached to clamps on mechanical testing frame and is exposed to single stretches of strains from 1-15% at 1 Hz while imaging.
6. Neuronal response is analyzed to determine significant differences between different groups and strain rates.

Design and Construction of the Mechanical Testing Frame

- Design of mechanical testing frame (Figures 4 & 5) was based off of an existing design from the lab of Dr. Jeffrey Weiss, with modifications made to better suit our needs. Custom code was also built to run our system at 1 Hz from strains from 1-15%.

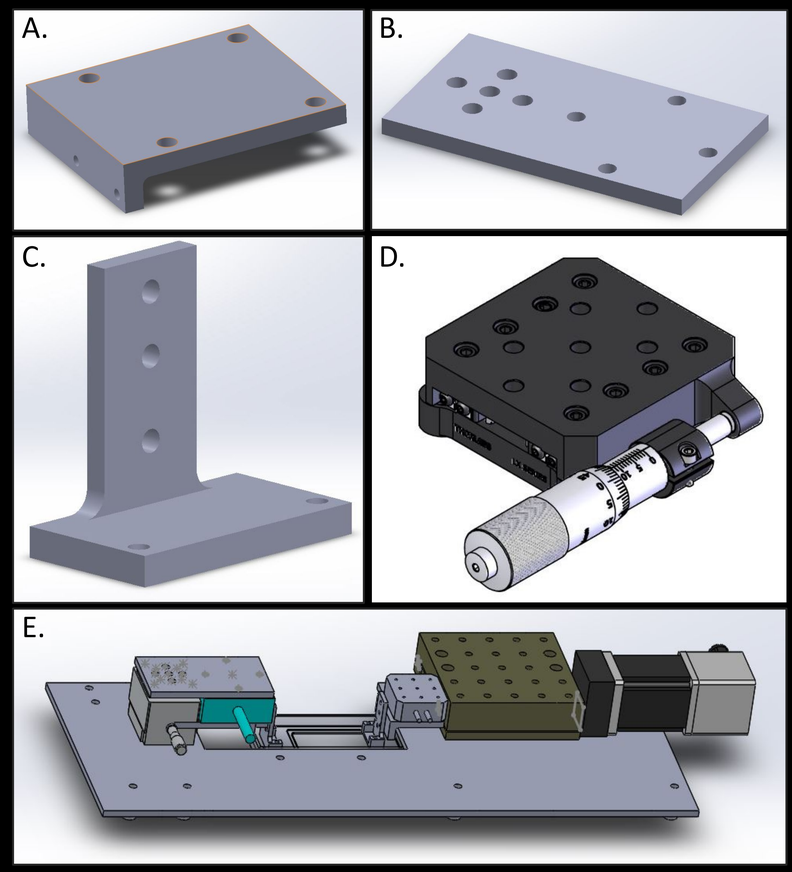


Figure 4: A-D: Computer-aided design parts of various individual parts of the mechanical testing frame. E: An early computer-aided design assembly of the mechanical testing frame.

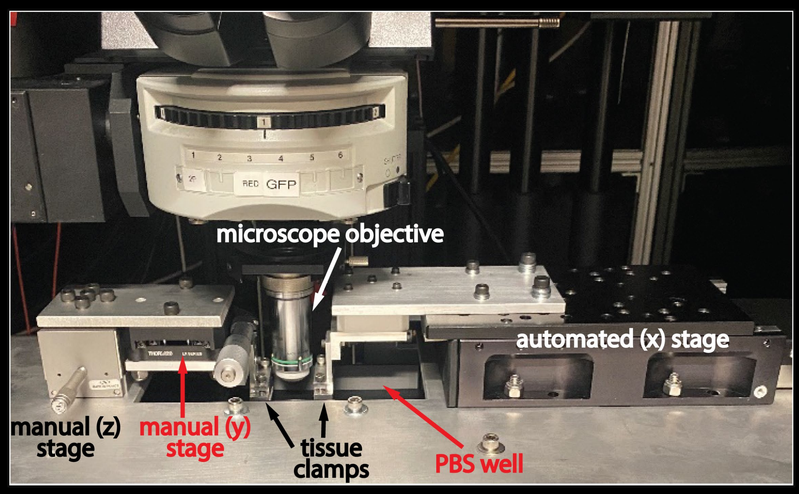


Figure 5: Picture with labels of the mechanical testing frame under the 2-photon microscope.

Discussion

- Understanding the underlying mechanisms of DDD is crucial for our ability to develop better therapeutics.
- This research helps us better understand these underlying mechanisms, specifically how the cytokine receptors IL6ST and TNFR1 contribute to low back pain and how they synergize with low pH to further perpetuate this pain.
- Our *in vitro* model with our custom mechanical testing frame and corresponding code is able to model the complex environment of the degenerative IVD.

References

[1] Hoy et al. 2014 *Annals of the Rheu. Diseases.*
[2] Burke et al. 2002 *J Bone Joint Surg Br.*
[3] Dowdell et al. 2017 *Neurosurgery.*
[4] Parsi et al. 2017 *Methods Mol Biol.*
[5] Stover et al. 2020 *Journal of Ortho. Research.*

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