In Vitro Investigation of Cytokine-Mediated Nucleus Pulposus Degeneration

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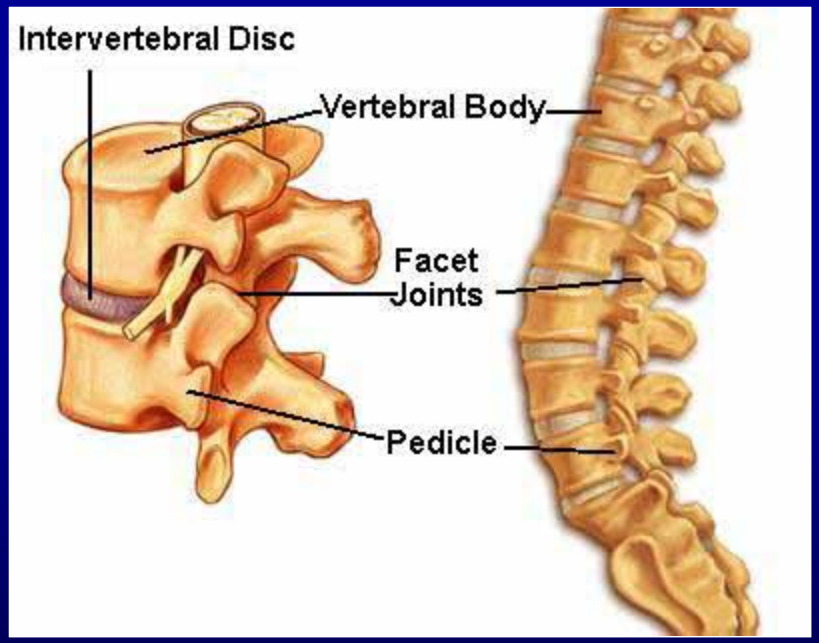
Motivation

- Low back pain
  - High prevalence: 25% of US population
  - Physically and financially detrimental
- Limited treatment
  - Aimed at alleviating painful symptoms
  - Incomplete understanding of biological mechanisms involved

DEGENERATION OF LUMBAR INTERVERTEBRAL DISCS IS STRONGLY IMPLICATED AS A CAUSE OF LOW BACK PAIN.

Intervertebral Discs

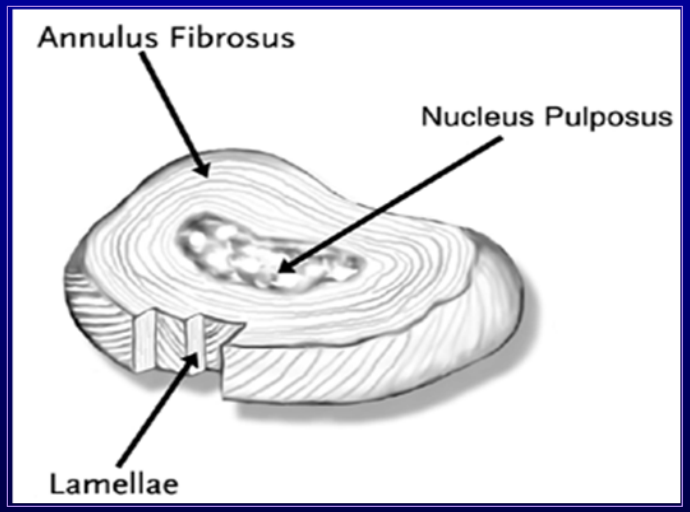
- Spine consists of alternating:
  - Vertebrae
  - Intervertebral discs (IVDs)
- Function of IVDs
  - transfer and distribute compressive loads
  - Permit spinal movement



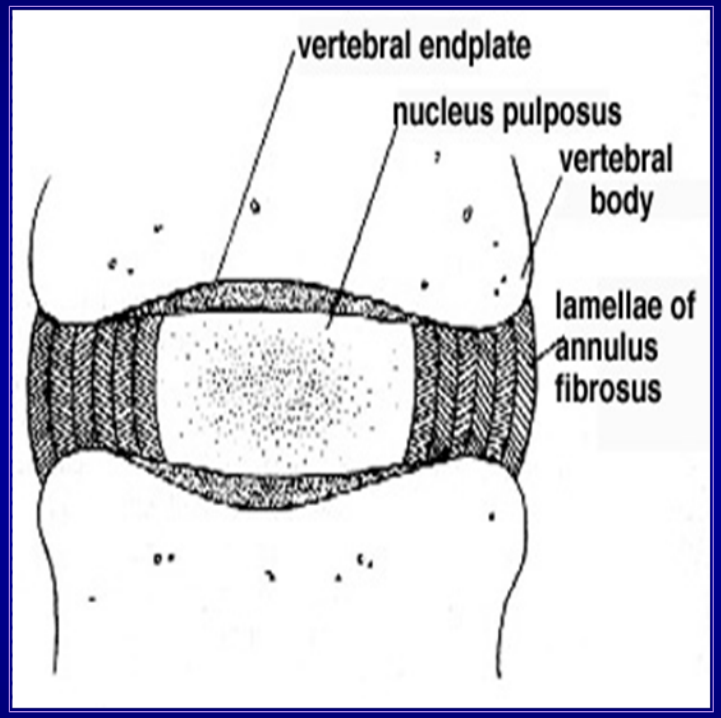
http://www.spineuniverse.com/conditions/back-pain/anatomy-back-pain

Disc Anatomy

- Annulus Fibrosus (AF)
- Cartilaginous End Plates
- Nucleus Pulposus (NP)



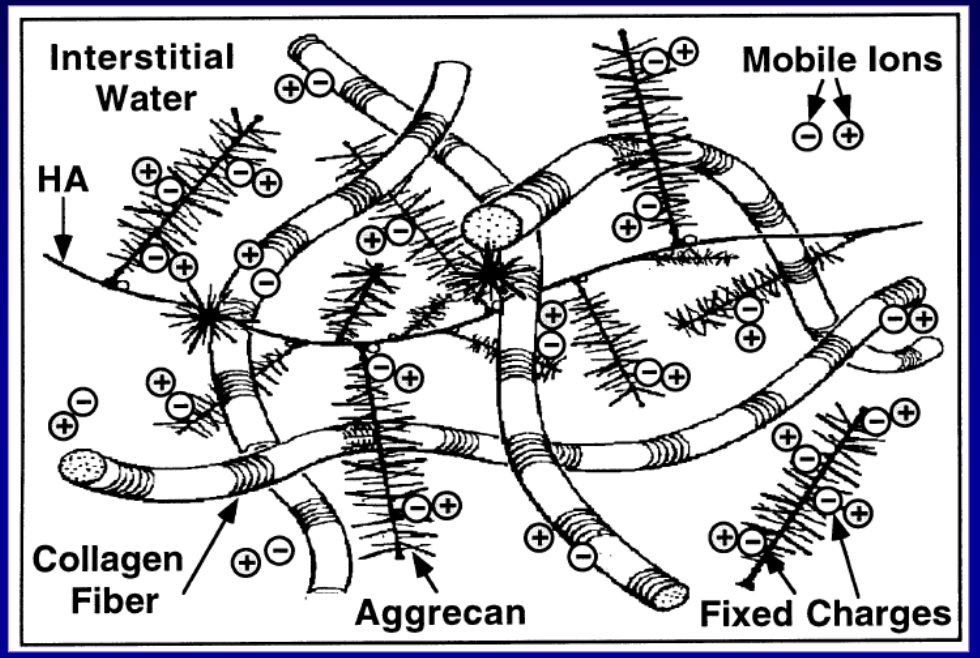
http://www.medscape.com/viewarticle/405642_2



http://www.chiropractic-help.com/L4-Lumbar-Spine.html

Nucleus Pulposus

- Pressurized gel
  - Randomly distributed network of collagen II
  - High hydrated extracellular matrix rich in proteoglycans

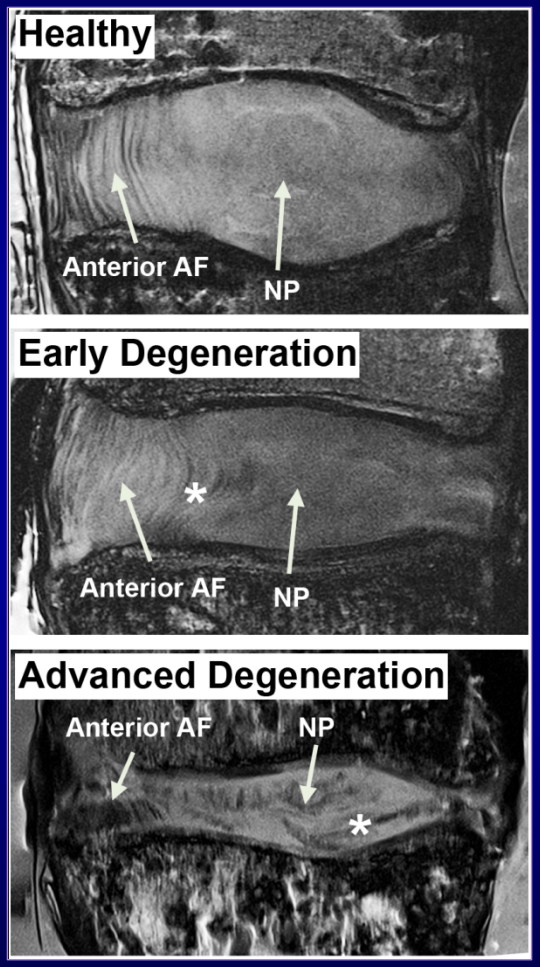


Mow et al, 1998, Int. J. Solids Structures

- Mechanical Function
  - In compression confined peripherally by AF
  - Generating a region of hydrostatic pressure

Human Disc Degeneration

- Degeneration starts in the NP
- Compositional Changes
  - Loss of glycosaminoglycans (GAG)
  - Loss of water
- Impaired mechanical function
  - Reduced NP pressure
  - Altered motion segment stiffness
- NP changes initiate a cascade expanding to other structures
  - Loss of disc height
  - Inward bulging of AF
  - Formation of tears



Smith et al, 2010, Dis. Model. Mech.

Cytokine-Mediated Matrix Degradation

- Pro-inflammatory cytokines
  - Interleukin-1 beta (IL1β)
  - Tumor Necrosis Factor alpha (TNFα)
- Naturally occurring inhibitors of cytokines
  - Interleukin-1 receptor antagonist (IL1ra)
  - Soluble TNF receptor 1 (sTNFR1)
- In IVD degeneration, up-regulation of cytokines, no matched increase of inhibitors
  - Increases in catabolic enzymes: MMP3, MMP13, ADAMTS4
  - Decreases in NP proteins: aggrecan and collagen II

Previous Studies

- Association of IL1β with IVD
  - 1988 Shinmei et al, 1997 Kang et al, 2008 Hoyland et al
- Association of TNFα with IVD
  - 2003 Cooper et al, 2005 Seguin et al, 2008 Hoyland et al

Debate: roles of these cytokines in initiating NP matrix changes

Gap: what is the functional significance of these matrix changes?

Objective

Use an in-vitro NP model to investigate:

1. Effects of IL1β and TNFα on composition and mechanical function

2. Capacity of IL1ra and sTNFR1 to mitigate cytokine-mediated changes

Methods

- Cell Isolation
  - Mature NP cells isolated from bovine caudal discs
- NP Constructs and Treatment
  - NP cells seeded at $20X10^6$cells/ml in agarose gels (4mm diameter X 2.254mm thick)
  - Precultured 6 weeks with transforming growth factor beta 3 (TGF-β3) before treatment

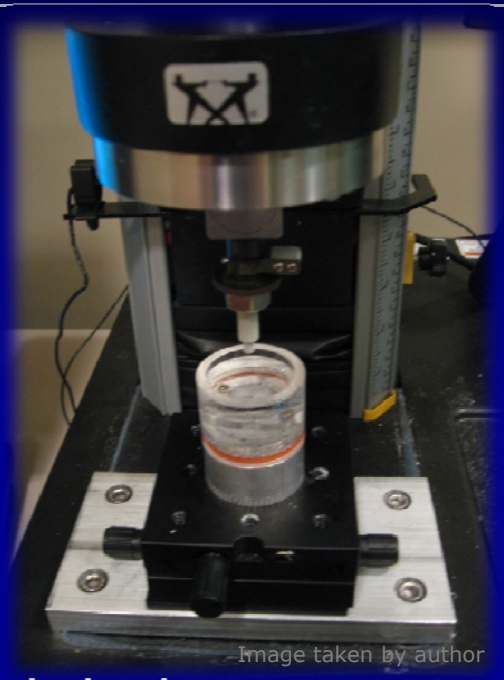
IL1 Treatment Groups (n=7)	TNF Treatment Groups (n=7)
IL1β, 10ng/ml	TNFα, 10ng/ml
IL1β, 10ng/ml + IL1ra, 100ng/ml	TNFα, 10ng/ml + TNFR1,100ng/ml
IL1ra, 100ng/ml	TNFR1, 100ng/ml
Control (no treatment)	Control (no treatment)

Histology

- Embedding
  - Samples fixed in 4% paraformaldehyde and dehydrated in graded series of ethanol
  - Embedded in paraffin
  - Sectioned at 7 μm-thickness from middle
- Staining
  - Alcian Blue (AB): GAG
  - Picrosirius Red (PR): collagen

Image taken by author

Mechanical Testing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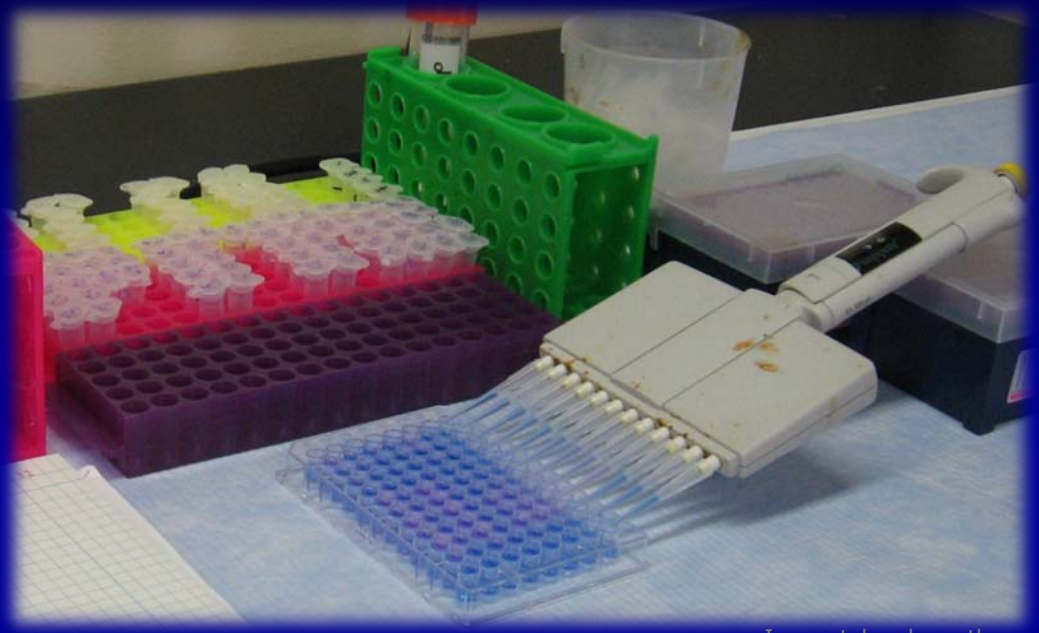
- Confined compression
  - to replicate physiological conditions of NP
- Device
  - Acrylic chamber fixed above porous platen
  - Impermeable ceramic indenter for applying compression
- Tests
  - Static preload (0.02N for 500s): equilibrated thickness
  - Stress relaxation test: 10% strain applied at 0.05%/s, then relaxation to equilibrium for 10min
- Calculations
  - Aggregate modulus (H_A): final stress/applied strain
  - Hydraulic permeability (k_0): linear biphasic theory

Biochemical Analysis

- Preparation
  - Wet and dry weights of samples
  - Papain digestion for dried samples



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- Assays
  - DMMB (1,9-dimethylmethylene blue dye-binding) assay: GAG
  - OHP (orthohydroxyproline) assay: collagen
    - First acid hydrolysis of sample digests

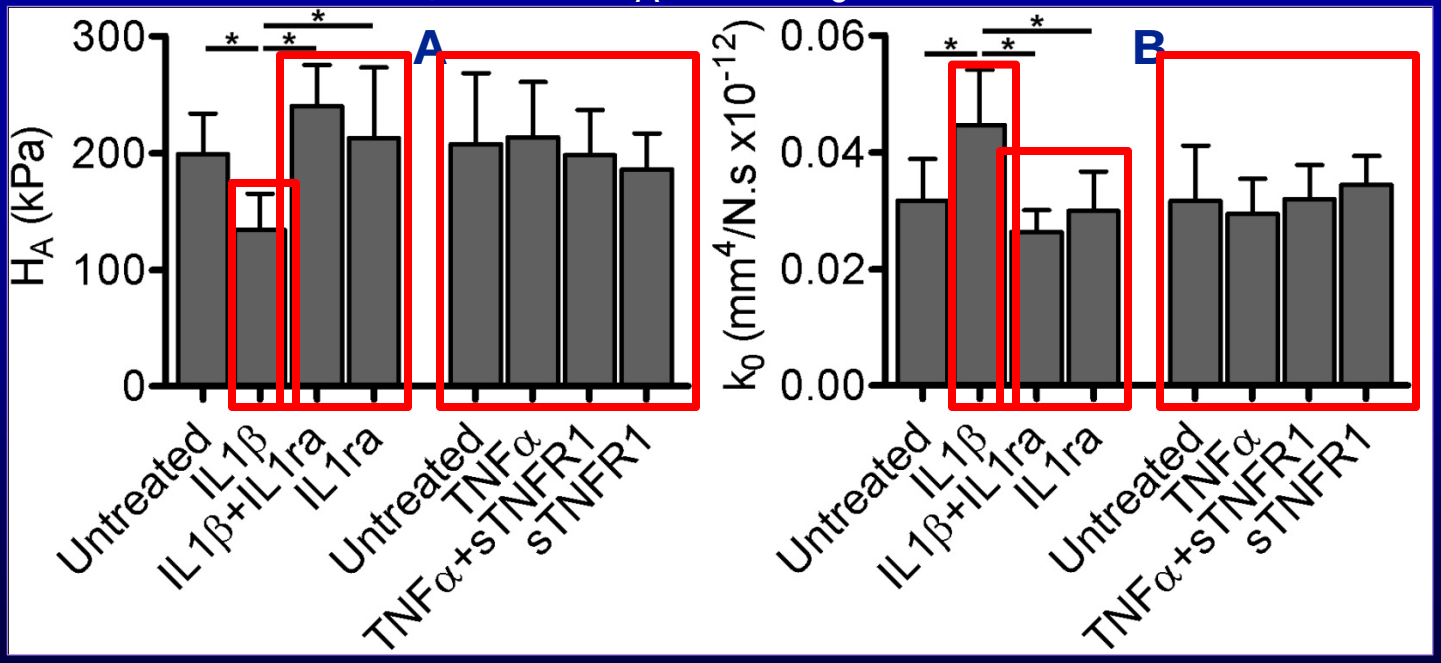
Results- Histology

- GAG (AB):
  - Uniformly distributed and intense
- Collagen (PR):
  - More diffuse, pericellular and intercellular

Histology staining for functionally mature constructs.

Results- Mechanics

- IL1 Treatment Groups
  - IL1β: 33% decrease in H_A and 41% increase in k_0
  - IL1β +IL1ra and IL1ra only: no significant difference
- TNF Treatment Groups
  - No changes in H_A and k_0

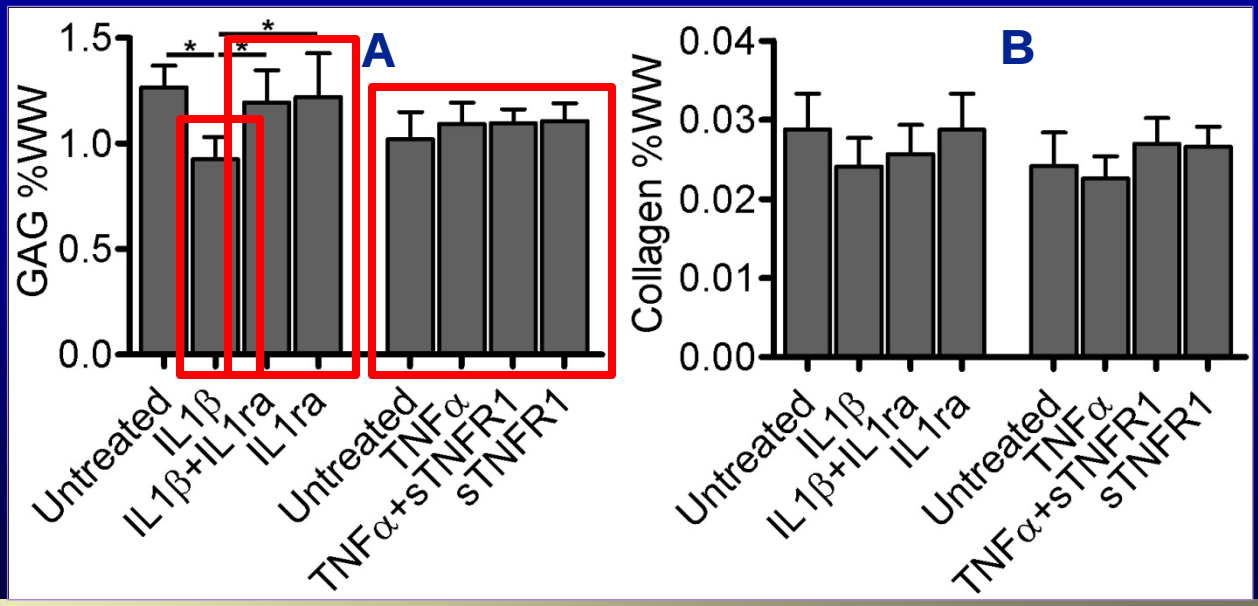


A. aggregate modulus, **B**. hydraulic permeability (*p<0.05)

Results- Biochemical Analysis

- GAG:
  - IL1β: 27% decrease relative to untreated controls
  - IL1β + IL1ra and IL1ra only: no significant difference from controls, significantly greater than IL1β only
  - All TNF groups: no significant difference
- Collagen: similar trends as GAG, no significant differences

A. GAG content as %/water weight, **B**. collagen amount as %/water weight (*p<0.05)



Conclusions

- IL1β plays a more direct role than TNFα
  - Mechanical and biochemical analyses: significant effects for IL1 treatment groups, not TNF groups
  - Short-term exposure to IL1β induced matrix changes that are functionally significant
- Significant inhibitory effects place IL1ra as a key therapeutic agent
  - IL1ra can effectively prevent NP matrix changes and associated functional changes induced by IL1β
- Results are clinically significant for developing novel treatment approaches for IVD degeneration

Future Work

- Investigate gene expression
  - Down-regulated anabolic genes and up-regulated catabolic genes associated with degeneration
  - Quantify changes in catabolic enzyme activity and matrix synthesis following cytokine exposure
- Develop biodegradable polymeric microspheres to deliver therapeutic agents
- Evaluate therapeutic agents in an in-vivo model of disc degeneration

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