

# Evaluating an Interleukin-1 $\beta$ Injection to Induce Degeneration in the Rat Lumbar Spine

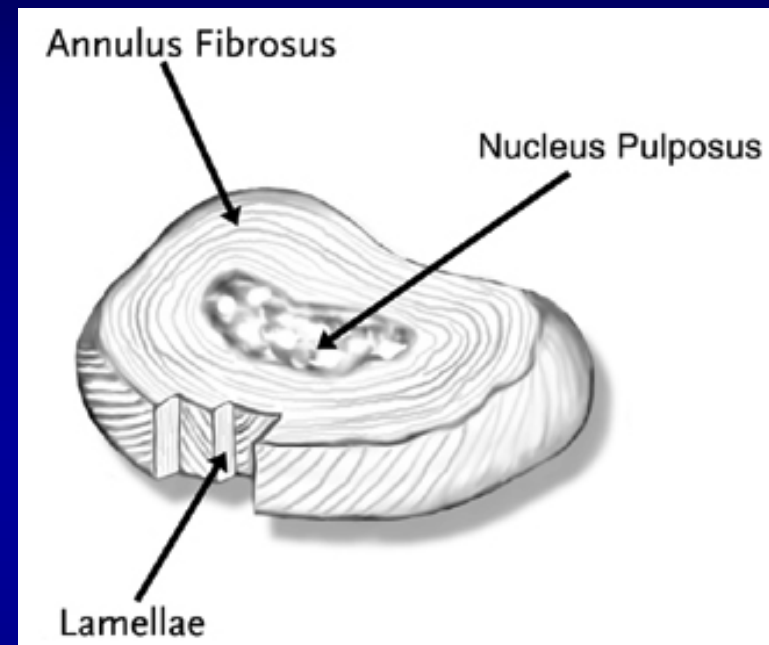
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SUNFEST Symposium  
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# The Intervertebral Disc

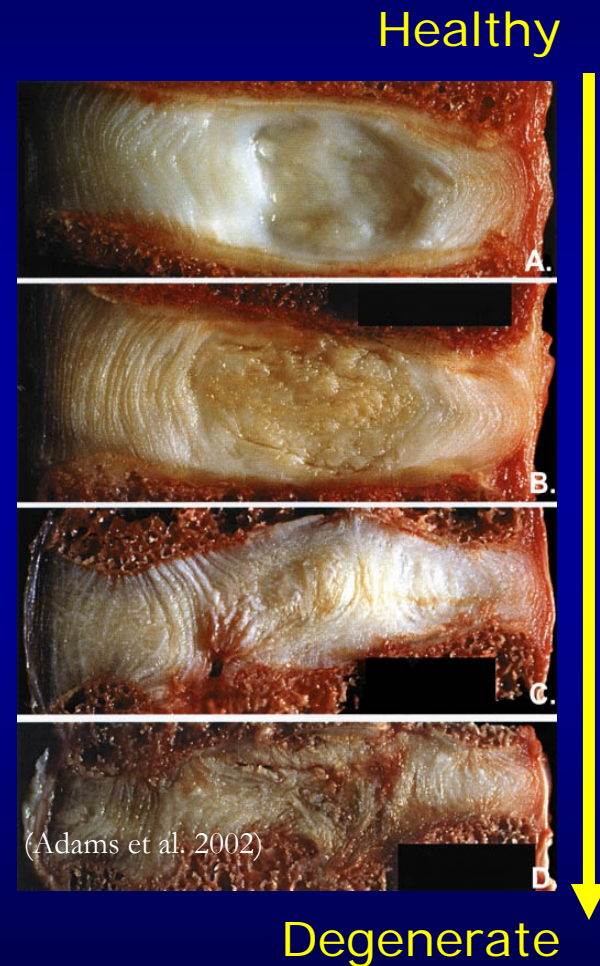
- **Composition:**
  - **Nucleus Pulposus**
    - Gelatinous center, provides osmotic pressure for absorption of force
  - **Annulus Fibrosus**
    - Highly organized fibers the encircle the NP



<http://indyspinemd.com/Images/normalAnat/IntervertebralDisc.jpg>

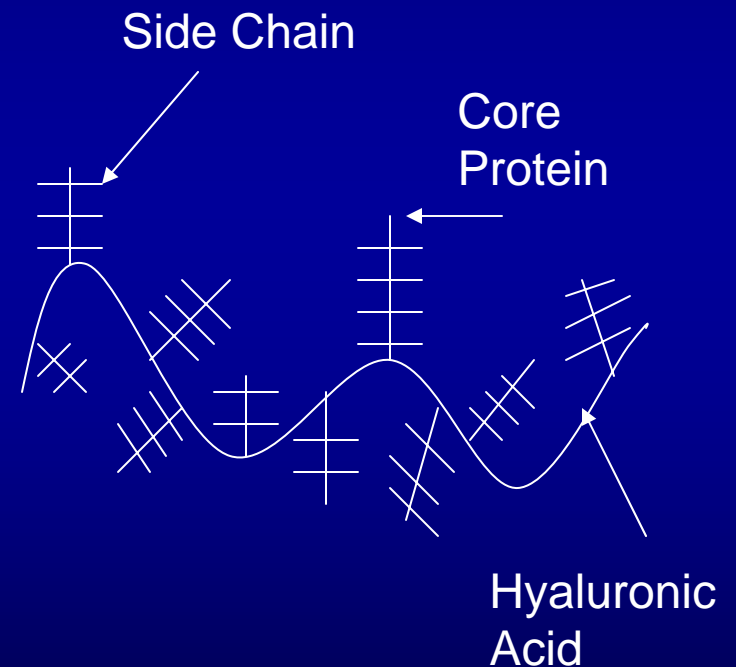
# Disc Degeneration

- Begins in the NP
  - ↓ in water content
  - ↓ in GAG content
- Spreads to AF
  - Disorganization
  - Tears



# Previous Studies

- ChABC used to decrease GAG content
- Corresponded to loss of mechanical function



# Interleukin-1

- Naturally occurring cytokine linked to cartilage degradation
- Shown to be produced by both degenerate and non-degenerate discs
- Linked to matrix degrading enzymes, decrease in production of proteoglycans

## Summer Goal

- Create *in-vivo* rat model of intervertebral disc degeneration brought on by IL-1
- Hypothesis:  
IL-1 will cause decrease in GAG content and a loss of mechanical function



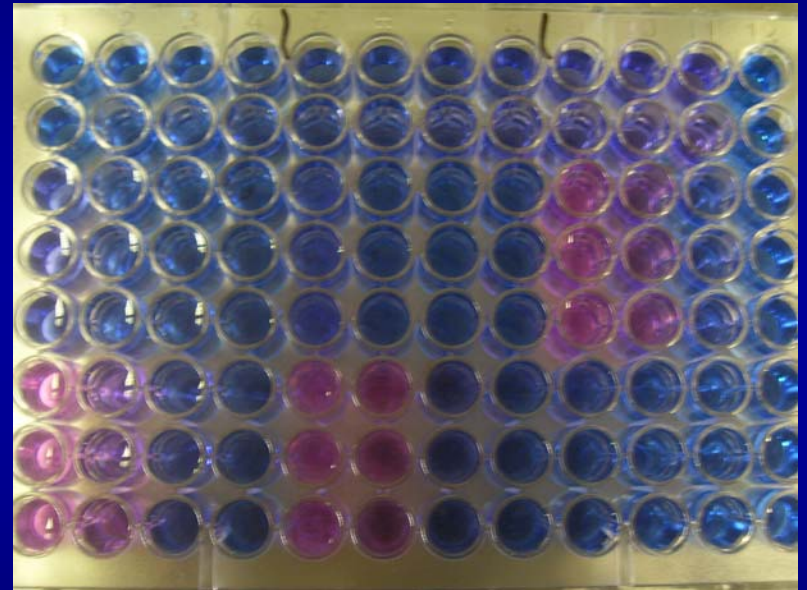
<http://www.paulnoll.com/China/Zodiac/zodiac-rat-pic.gif>

## Method

- 3 groups: IL-1, PBS Sham, Control (1  $\mu$ L injections)
- 3 discs injected in each rat
- Euthanized 1 or 4 weeks post injection
- Motion segments harvested, kept at -20°C until testing

# Biochemistry

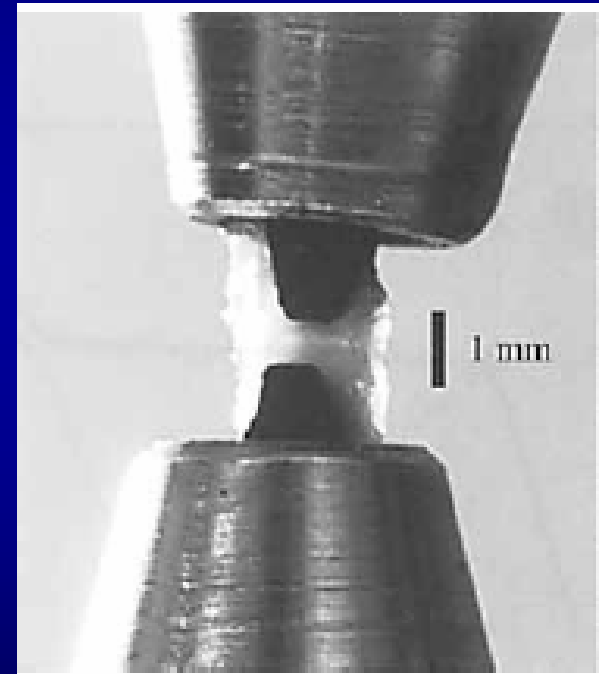
- Discs isolated from motion segment
- Customized punches used to separate NP, IAF, and OAF
- Spectrophotometer used to determine GAG content based on the color metric scale from DMMB assay



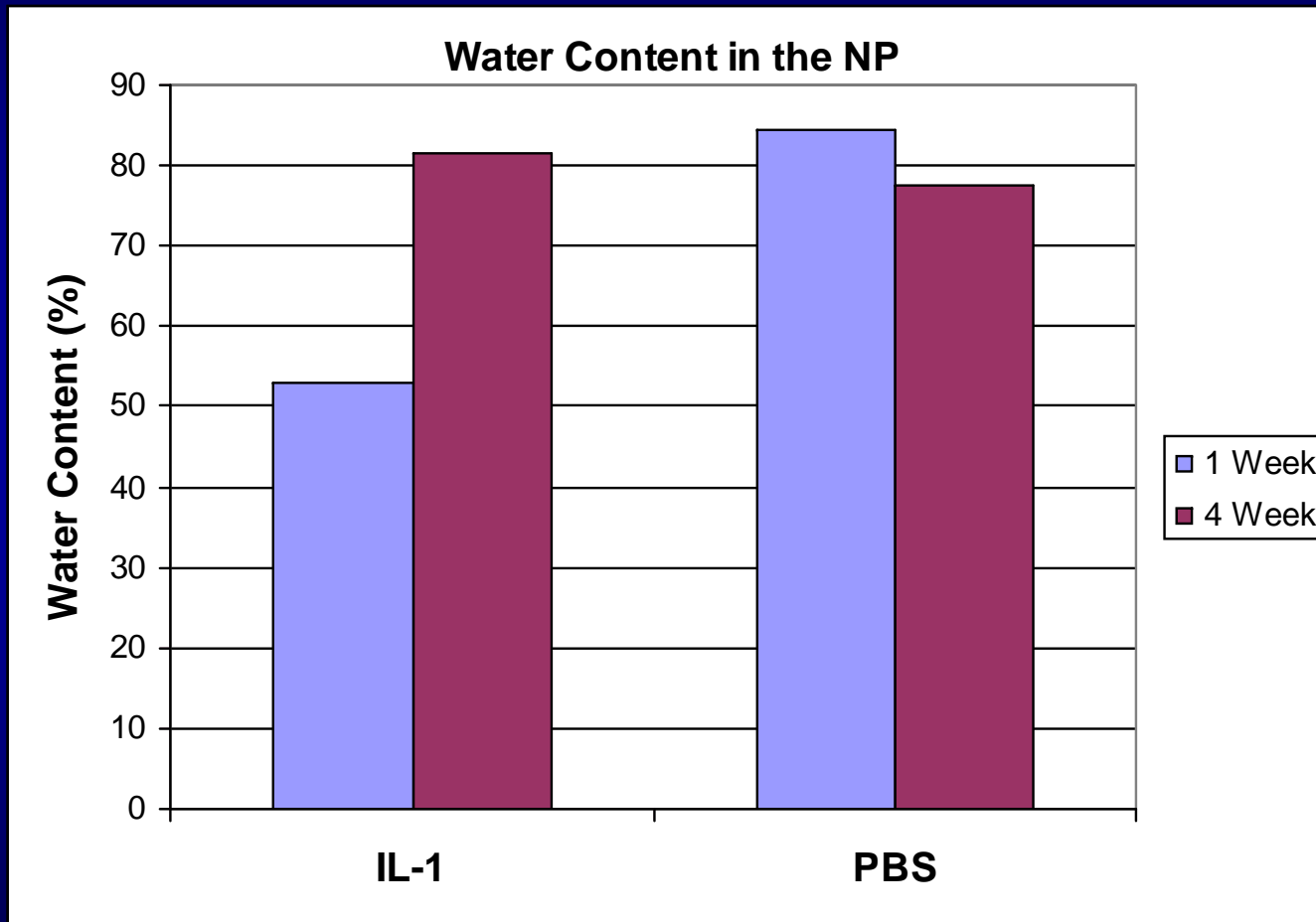


# Mechanical Testing

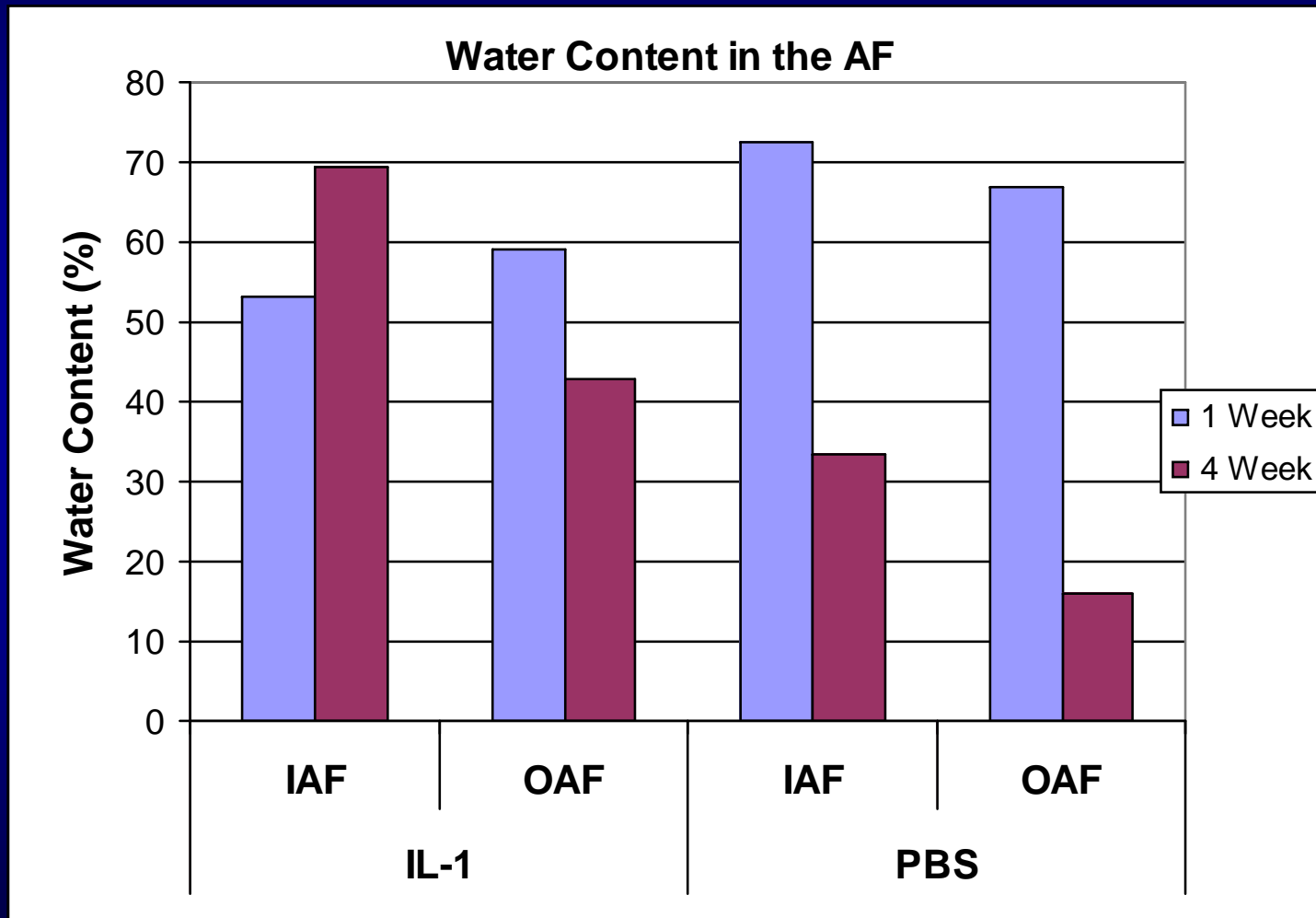
- Axial Compression-Tension  
cyclic testing: 20 cycles of 4.5 N compression to 3 N Tension
- 45 minute Creep test
- Data from final cycle analyzed using trilinear fit model through MATLAB



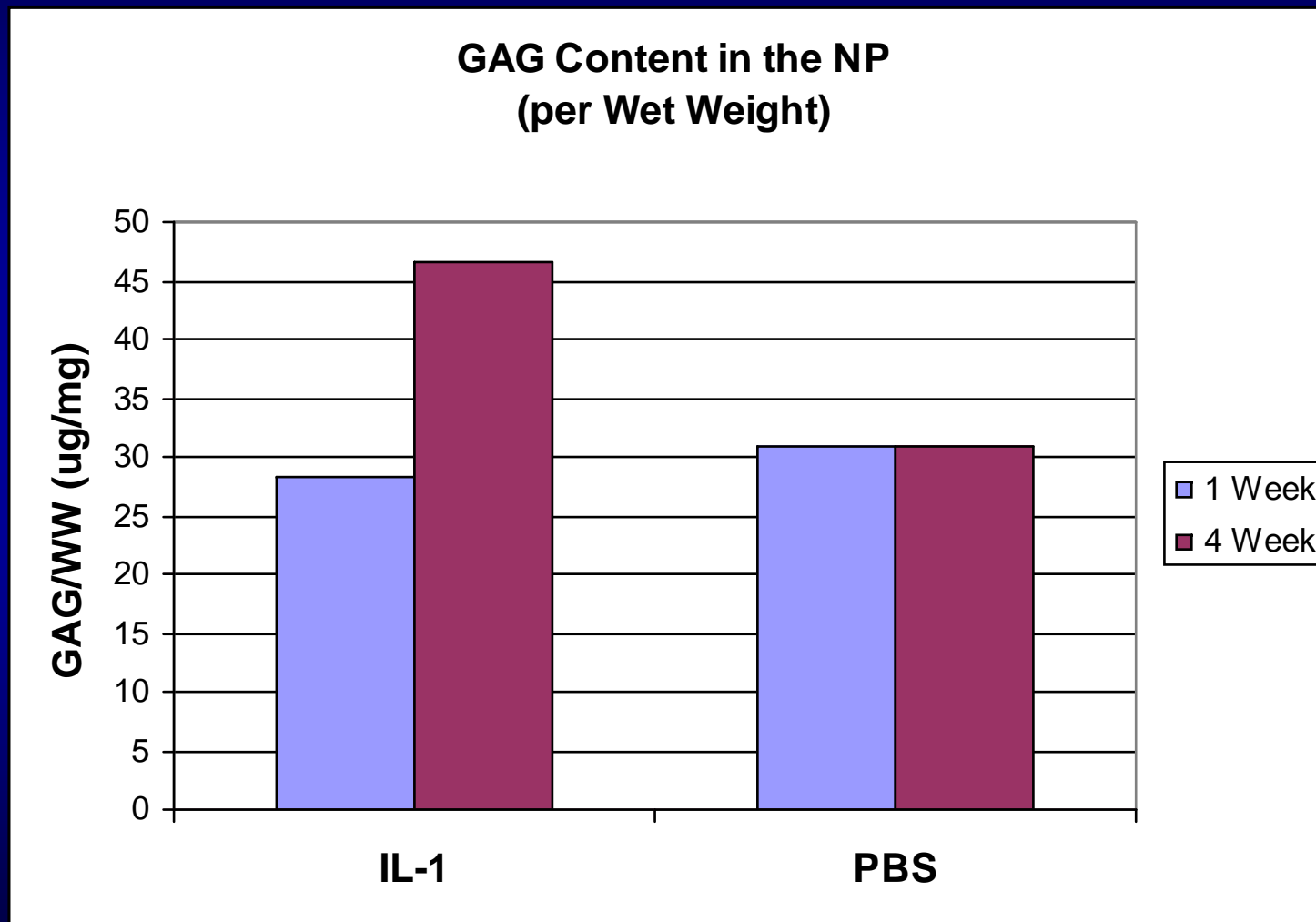
# Results - Water Content



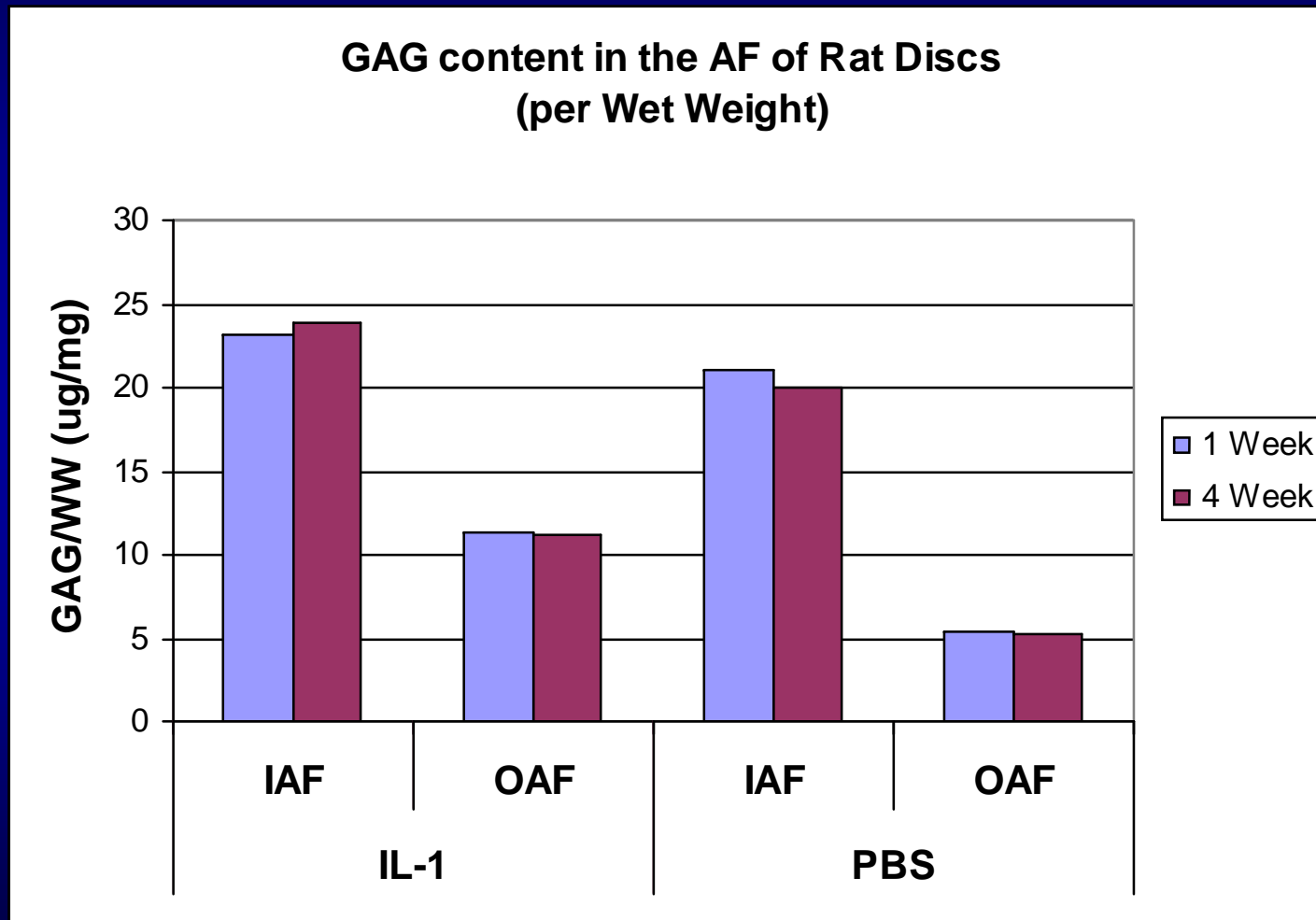
# Results - Water Content



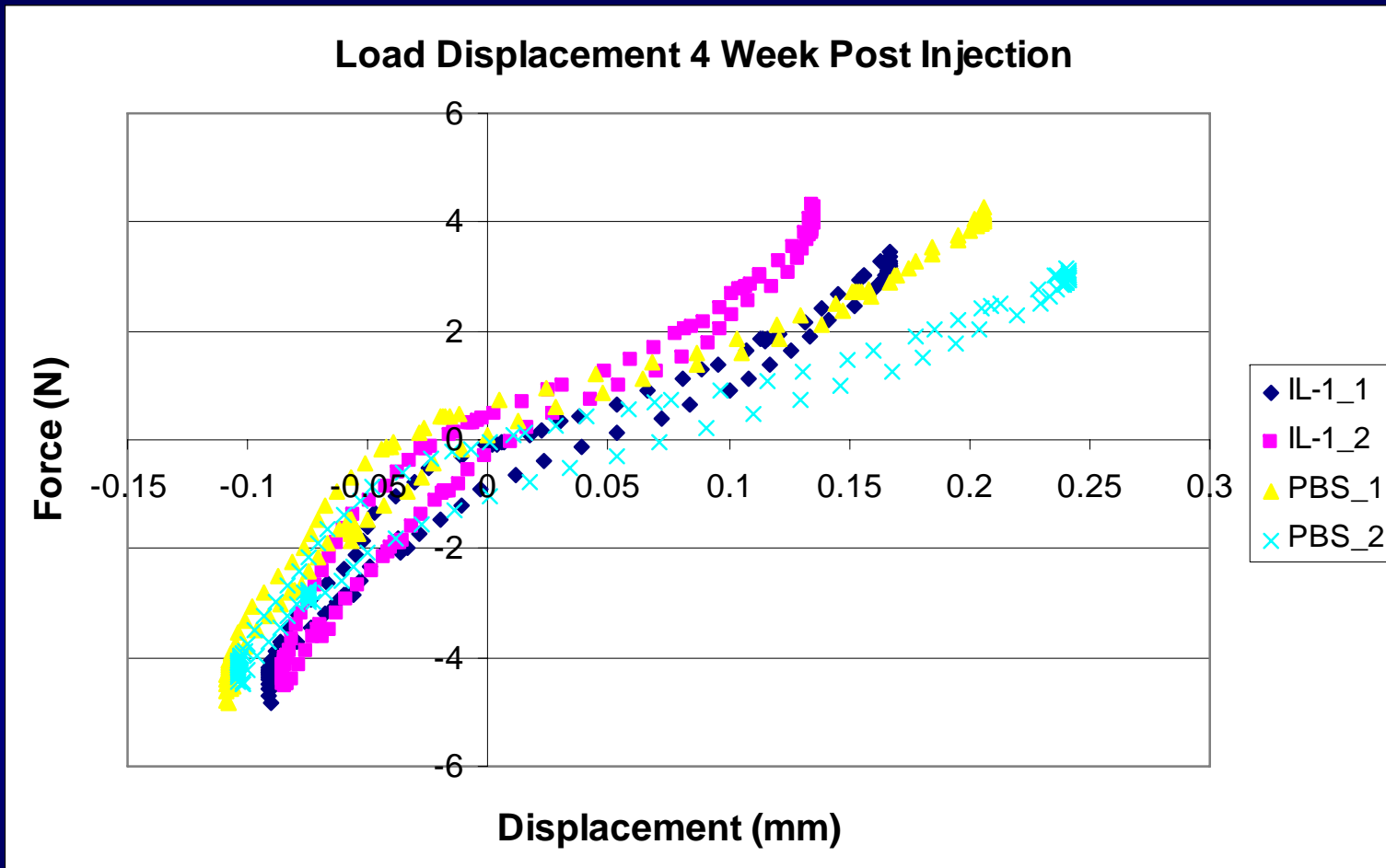
# Results - GAG content



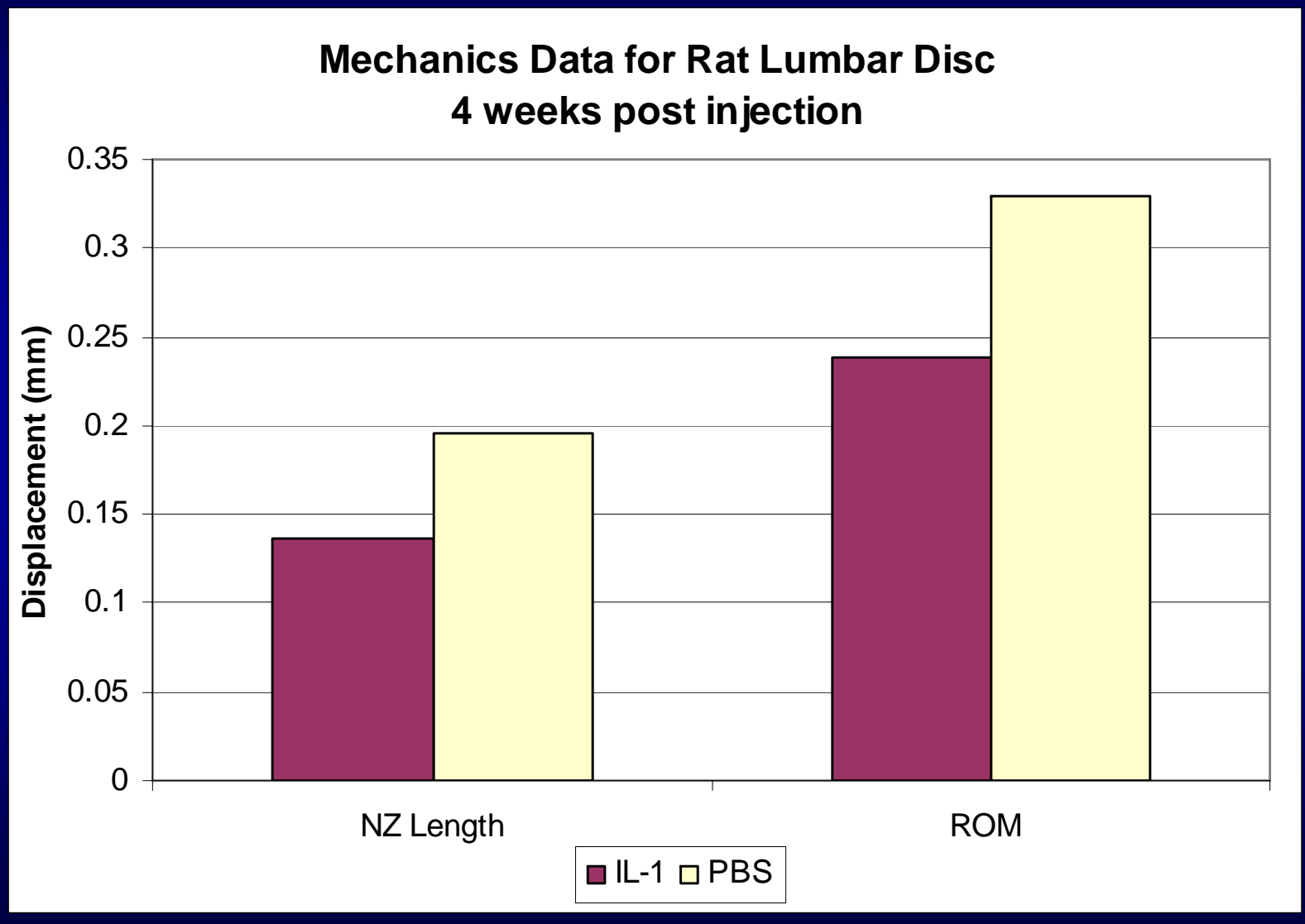
# Results - GAG Content



# Results - Mechanics

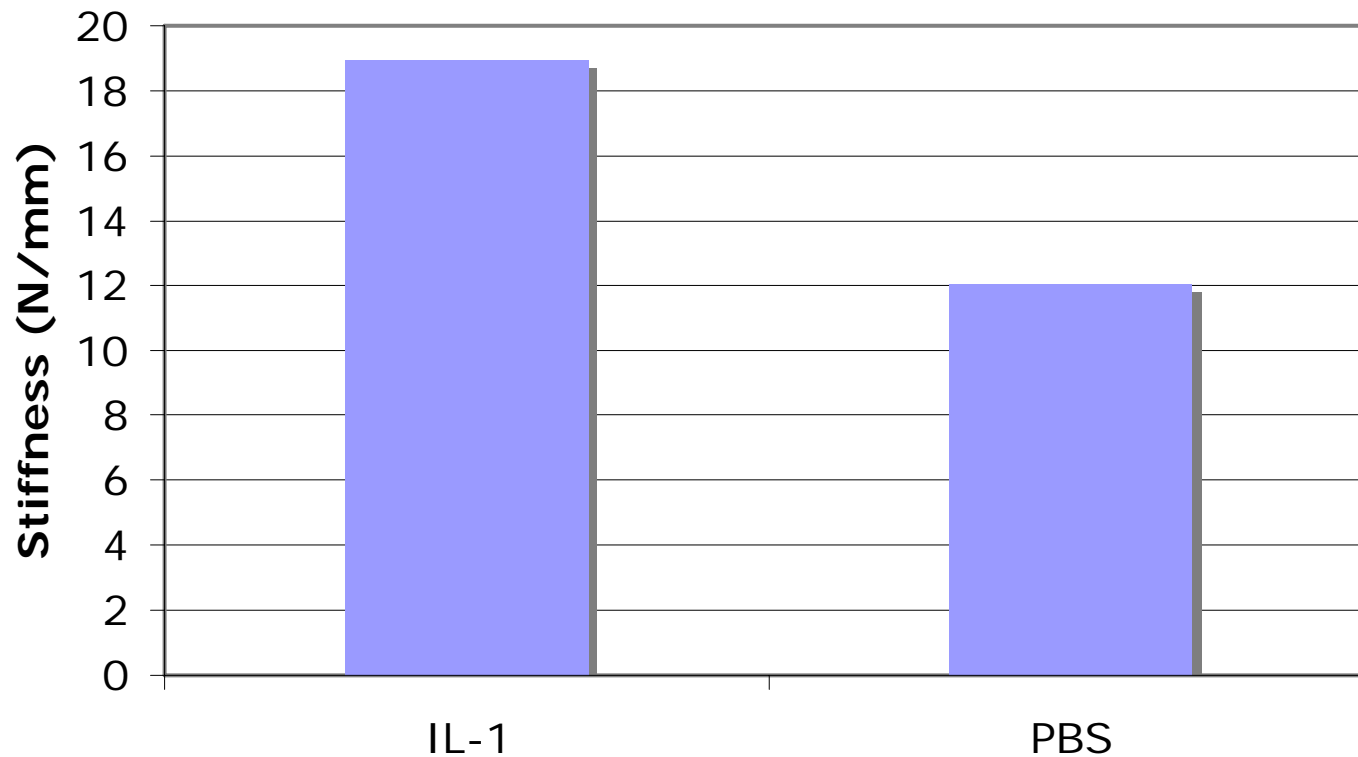


# Results - Mechanics



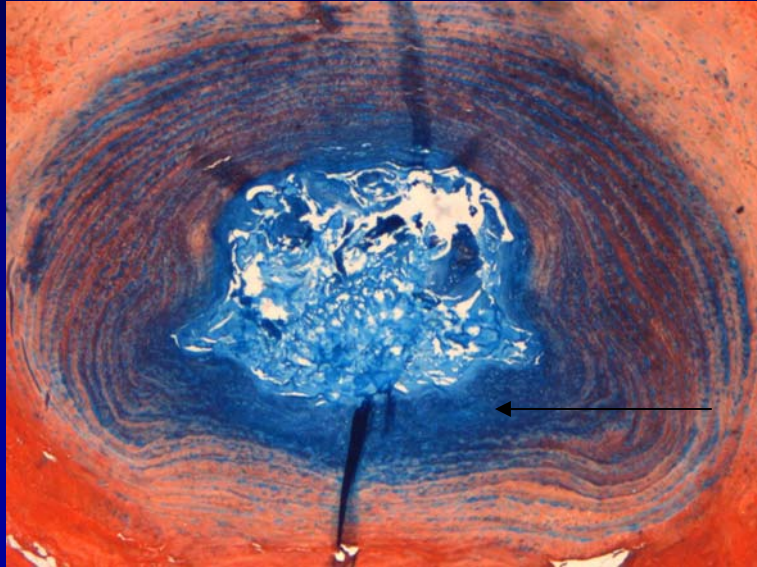
# Results - Mechanics

**Neutral Zone Stiffness for Rat  
Lumbar Disc 4 Weeks Post Injection**

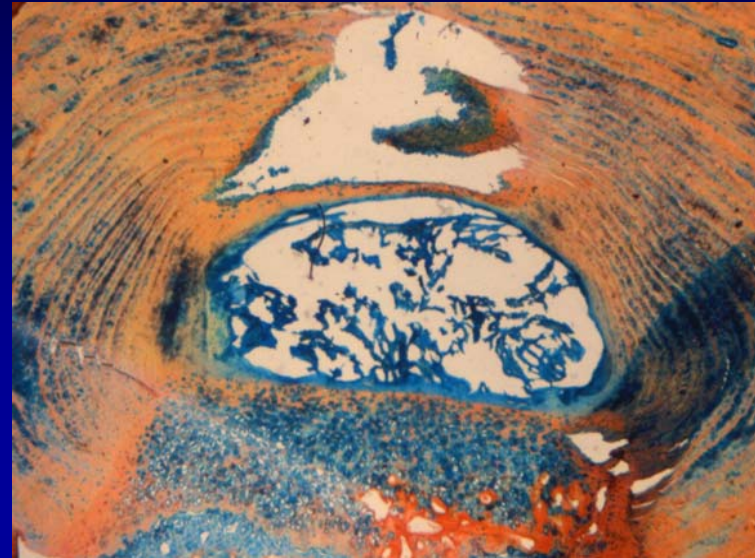




# Results - 1 week Histology

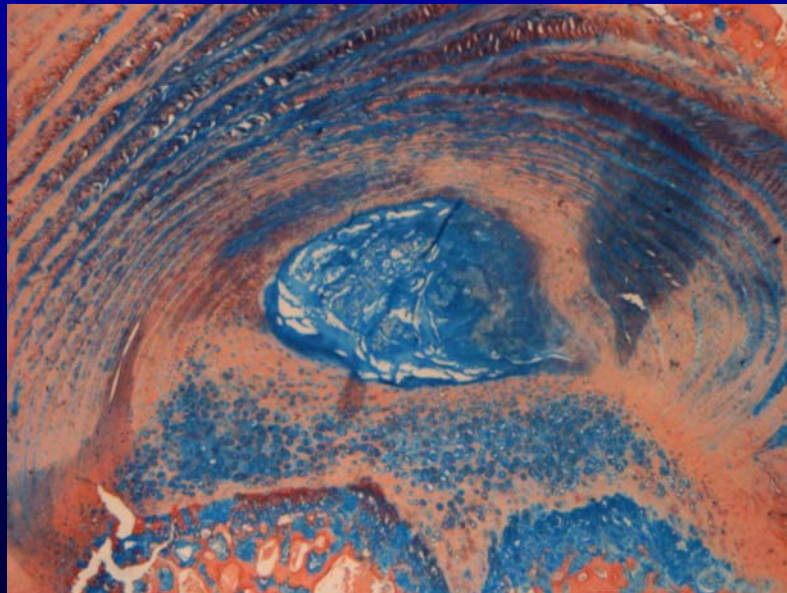


IL-1



PBS

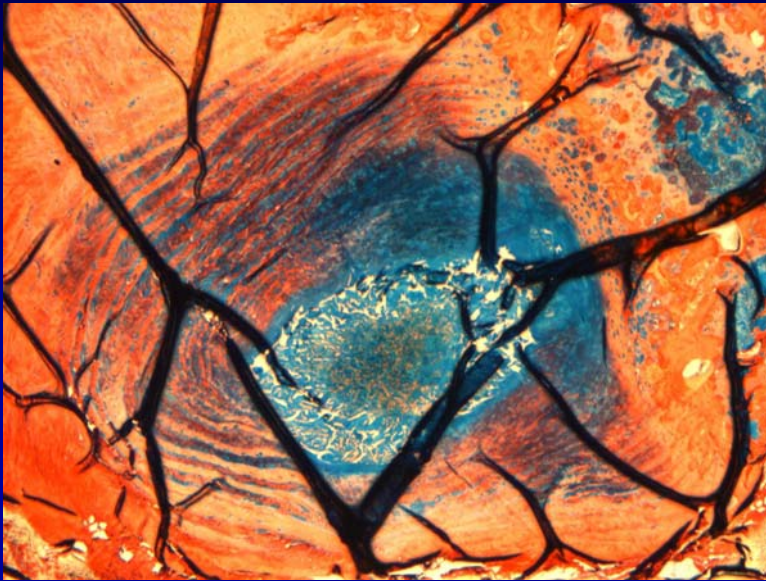
Control



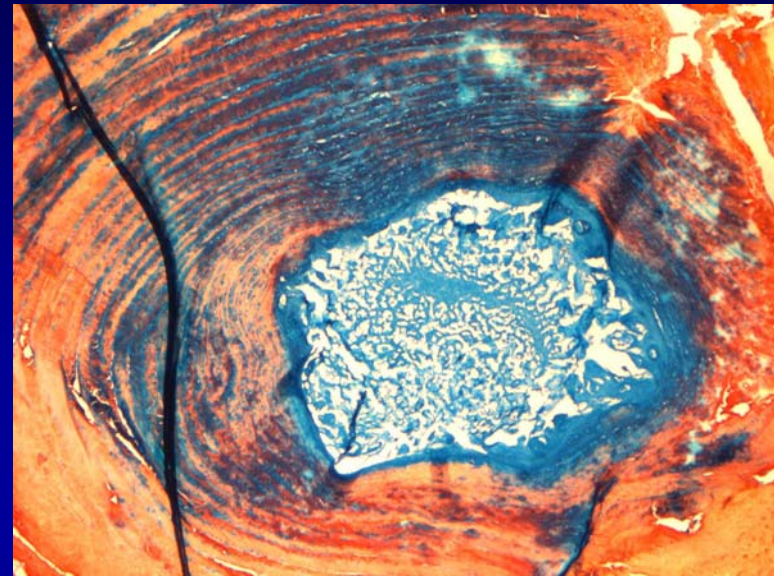
Penn



# Results - 4 week Histology

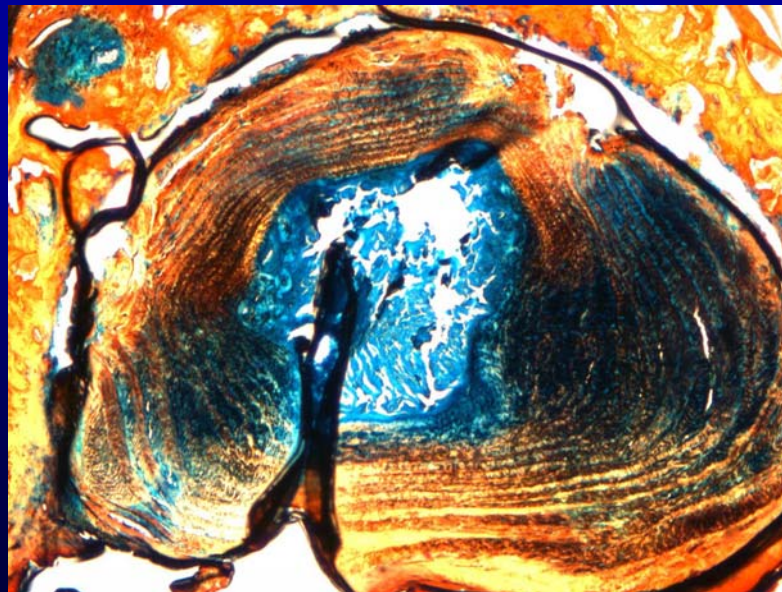


IL-1



PBS

Control



Penn

# Results

- Biochemical results correspond to mechanics
- Recovery of GAG
- % difference of GAG content in IL-1 vs. PBS discs per wet weight
  - 1 week: 8.6265%
  - 4 week: 51.2315%

## **Increase in GAG**

- Possible that the presence of IL-1 caused an increase in IL-1 RA
  - Further regulation
- If IL-1 acts as foreign substance, cells may produce antibodies against it to prevent further attack
- Future studies: observe levels of IL-1 RA in addition to IL-1 in the disc

# Challenges

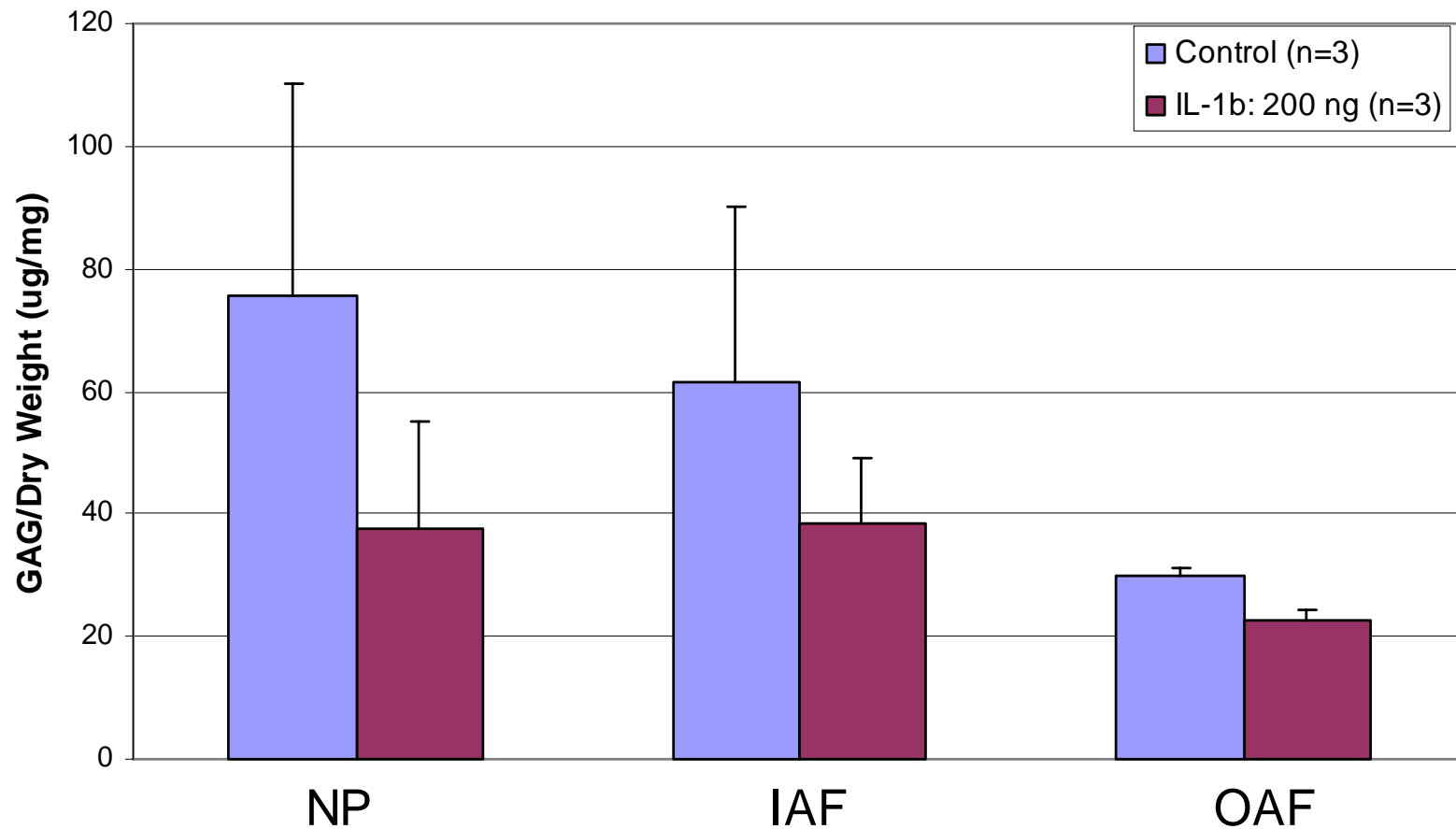
- Difficulties with mechanical testing led to loss of 1 week data
- Small study (n=2)
- Discs susceptible to tears during dissection and sectioning

## Future Work

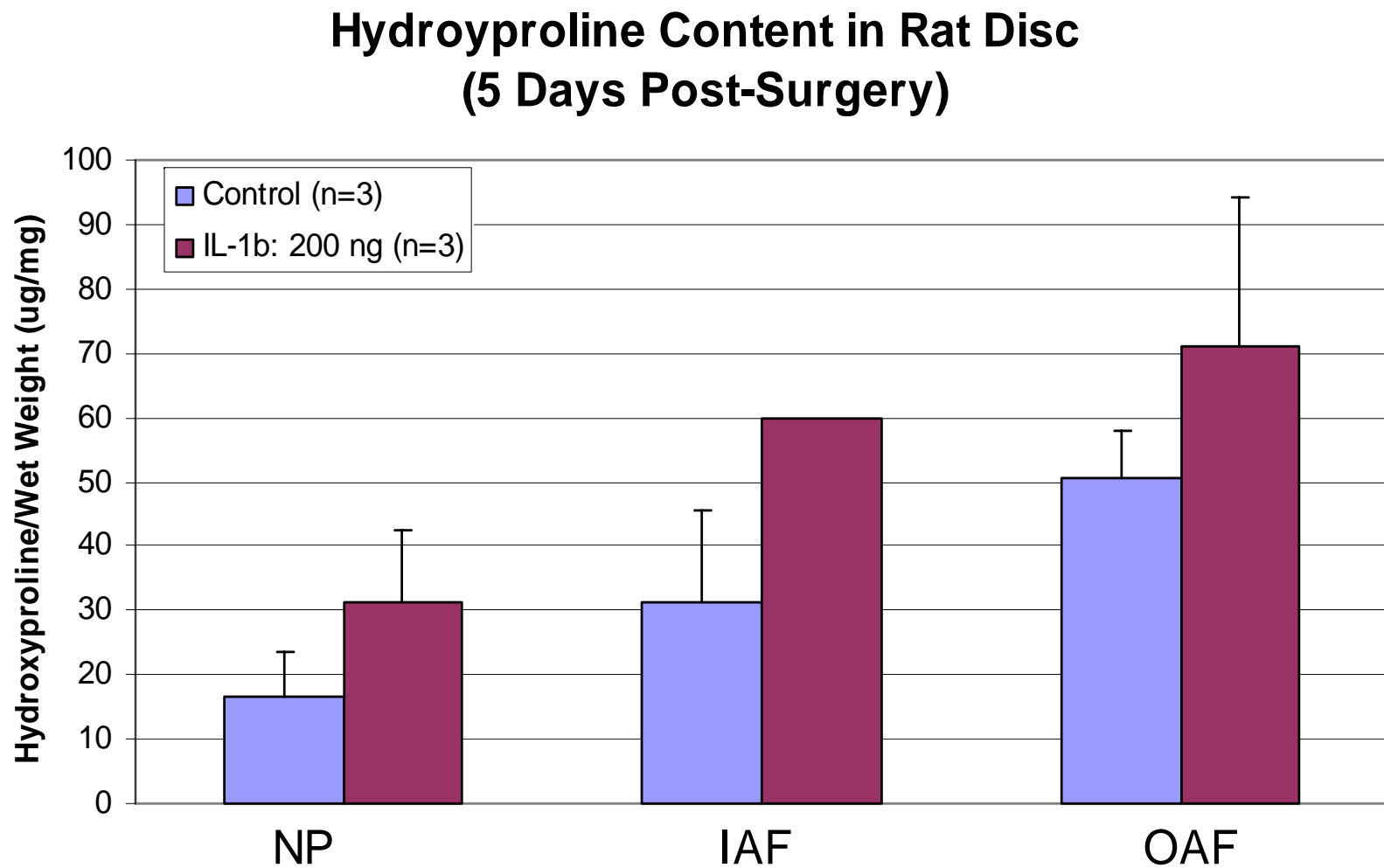
- Develop method to provide lasting effect of IL-1: released over time
- Observe levels of IL-1 RA
- Monitor *activity* of IL-1 in the disc
- Larger study

# Results: Interleukin-1b

## GAG Content in the Rat Disc (5 Days Post Surgery)

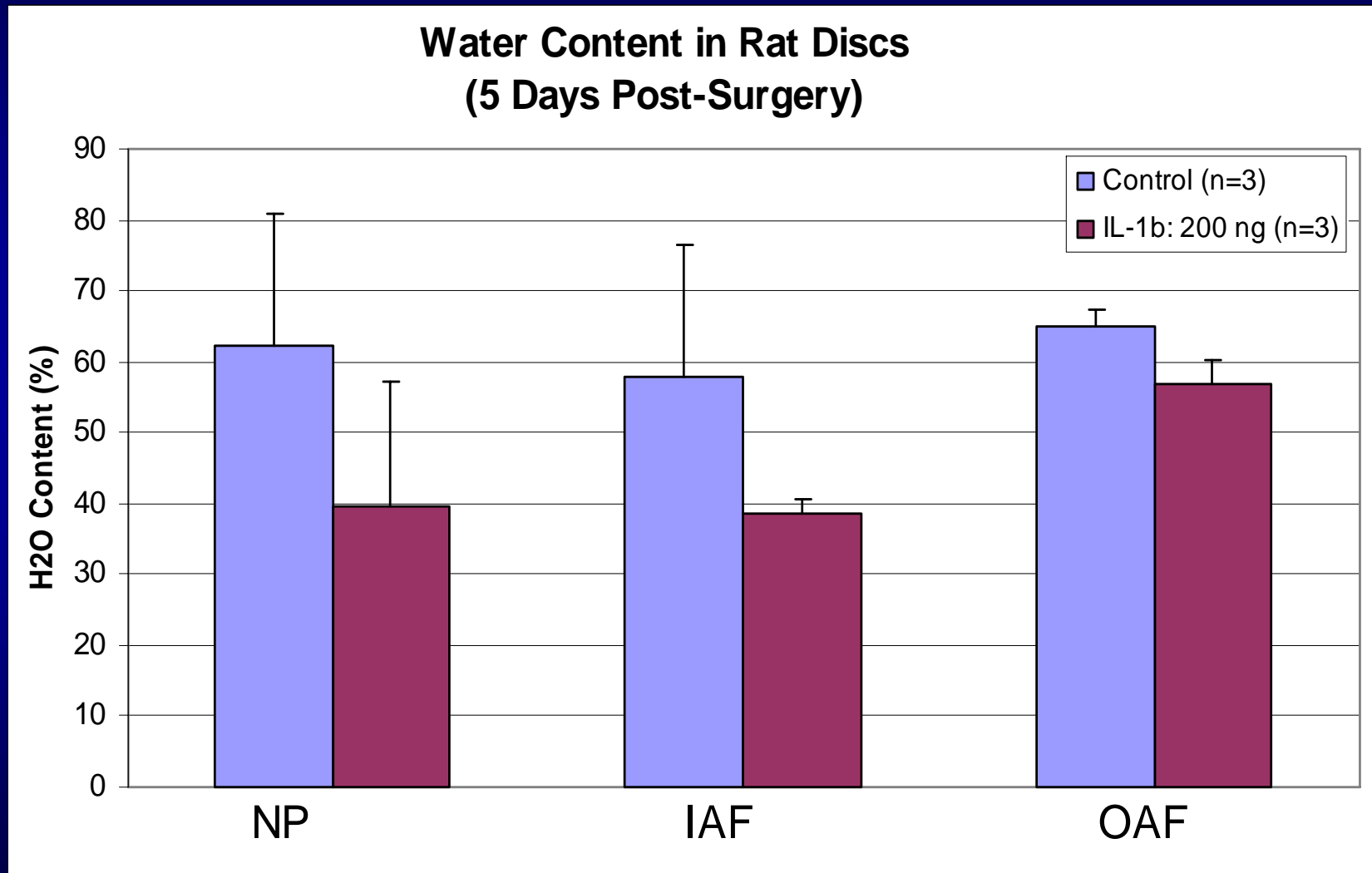


# Results: Interleukin-1b





# Results: Interleukin-1b



## Future Work

- Increase sample size to look at impact of IL-1 on the disc at short, medium, and long time points
- Establish temporal inter-relationships between mechanics, biochemistry, molecular and cellular events as a results of IL-1beta in the rat disc

# Thank You

Supported by the

**National  
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McKay Orthopaedic Research Laboratory