Effect of Cross-Linking on Mechanical Function in the Degenerate Nucleus Pulposus

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Motivation

• Low back pain
  – #2 reason Americans see their doctor
  – #2 reason for missing a workday
  – #1 cause of disability

• Intervertebral disc (IVD) degeneration
Intervertebral Disc

- Nucleus Pulposus (NP)
  - High proteoglycan content
  - Hydrated gel

- Annulus Fibrosus (AF)
  - High collagen content
  - Organized fibers
Intervertebral Disc Degeneration

• Complex process

• Proteoglycan content
  - Decreases in NP with degeneration
  - Changes in mechanical properties
    - Significant decrease in swelling pressure

Proteoglycan
(Alberts, *Molecular Biology of the Cell*)
Cross-Linking

- Provide mechanical loading support
- Formed by reactions between amino side groups
- Cross-linking agent: Genipin

Intramolecular

Intermolecular
Hypothesis:
Cross-linking will restore mechanical function in the degenerate nucleus pulposus

Model degeneration with Chondroitinase ABC (ChABC)

Apply genipin treatment

Non-degenerate sheep IVD

Degenerate IVD

IVD with restored mechanical function
ChABC Dose Finding Results

Swelling Pressure (MPa)

Concentration of ChABC (U)

0 U 0.1 U 0.5 U 1.0 U 2.5 U Target (using human data)

0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40
Genipin Cross-Linking Results

![Bar graph showing swelling pressure (MPa) for control and genipin treatments.](image)
Next Step: Quantifying Cross-Linking

- Spectrophotometry
  - Measures amount of light absorbed by a substance
  - Beer’s Law: \([\text{Absorbance}] = k[\text{Concentration}]\)
  - Standards of known concentrations are used to make a standard curve
Ninhydrin Assay

• Spectrophotometry protocol for free amino acid group determination
• More cross-linking
  – Lower concentration of free amino acid groups
  – Lower absorbance measured
Preliminary Ninhydrin Data

More cross-linking

Concentration of free amino acid groups

Absorbance

Genipin

Control

Standard Curve

Genipin
Control

More cross-linking
Future Work

- Finalize ChABC dosage
- Test ChABC and genipin together
- Quantify genipin cross-linking

By understanding the effects of cross-linking, an alternative treatment for disc degeneration and low back pain may be developed.