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

An Nguyen

Wade Johannessen, Dawn M. Elliott
McKay Orthopaedic Research Laboratory
University of Pennsylvania

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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

**Diagram:**

<table>
<thead>
<tr>
<th>Intramolecular</th>
<th>Intermolecular</th>
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*Penn*
**Study Hypothesis**

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

- Non-degenerate sheep IVD
- Model degeneration with Chondroitinase ABC (ChABC)
- Degenerate IVD
- Apply genipin treatment
- IVD with restored mechanical function
ChABC Dose Finding Results

Concentration of ChABC (U)

Swelling Pressure (MPa)

- 0 U
- 0.1 U
- 0.5 U
- 1.0 U
- 2.5 U

Target (using human data)
Genipin Cross-Linking Results

![Bar chart showing swelling pressure comparison between Control and Genipin treatments. The Genipin treatment shows a higher swelling pressure compared to the Control.]
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
More cross-linking

Preliminary Ninhydrin Data

![Graph showing absorbance and concentration of free amino acid groups for Genipin and Control samples.](graph.png)

- Genipin
- Control
- Standard Curve

Absorbance

Concentration of free amino acid groups

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.