Disc Composition and Mechanical Function Transforms with Age

Stem Cells
Aggrekan Promoters
Growth Factors

Exogenous Crosslinking (ECM²)

Stress induced cytokines
Mechanical stimulation of sensitized nerves

From Jeff Lotz, Ph.D.
Are Highly Crosslinked Discs with Lower Cellularity & Water Content Vulnerable or Painful?

- “…age-related changes of the disc begin soon after birth…in some adolescents and young adult discs more than 50% of the nucleus cells were necrotic”

- Majority of discs (57%) in symptomless women over age 50 show a loss of T2 signal (80% by age 70)

- Decreased water content is not correlated with back pain

- Crosslinking doubles nutritional flow to/from center of disc
Introducing Réjuve™

Réjuve is:
• a revolutionary micro-invasive tissue revitalization technique
• injectable protein crosslinking of native tissue
• the better solution for avascular, biologically challenged tissues
• a conservative / biomimetic approach
• immediately effective/ long-lasting with covalent bonds
• Inexpensive to produce

Genipin

• Injectable collagen crosslinking
• Immediate effect
• Long-lasting covalent bonds
Demonstration of Crosslinking Effect (Tendon)

Crosslinks engage all fibrils at low loads, with no loss of flexibility.

0 Hours Post-Injection

2 Hours

4 Hours

6 Hours

24 Hours

24 Hours
Preclinical Studies

Preclinical Testing

- Three-fold reduction of fatigue-induced disc degradation
- 4-fold reduction of joint instability human lumbar joints—
  59% increase in peak tear resistance and 72% increase in
  resilience
- Fluid flow to and from the nucleus doubled with genipin
- Restored all joint stability parameters to within 6% of intact
- Reduced disc bulge under load by 38% –
  70% increased resistance to interlamellar shear of the

>50% increase in GAG retention in human disc specimens–

Yerramalli CS, Chou AI, Miller GJ, Nicoll SB, Chin KR, Elliott DM,
The effect of nucleus pulposus crosslinking and
glycosaminoglycan degradation on disc mechanical function.

Toungate, J, Zhang, Y, Slusarewicz, P, Hedman, T, Effect of
Genipin Crosslinking on the Retention of Proteoglycans on the
Intervertebral Disc, *New Horizons in Intervertebral Disc

Hedman, T, Final Report – Research Accomplishments, Phase
II SBIR Grant – DDD Preclinical Testing, Submitted to the
National Institutes of Arthritis, Musculoskeletal, and Skin
Diseases (NIAMS), September, 2012.

Toungate, J., Slusarewicz, P., Zhang, Y., Hedman, T.P., Effect of
Genipin Crosslinking on the Retention of Proteoglycans on the
Intervertebral Disc.
(Manuscript completed 2013)
Performance as a Disc Therapeutic

- **No effect on genetics**
- **Increases nutritional flow/permeability 100% / GAG retention 50%**
- **Improves mechanical properties/ durability 25%-300%**

- **Reduces disc bulge >25%**
- **Reduces joint instability 4-fold**
- **Increases tear resistance >50%**
- **Provides adhesion of adjacent tissues >50%**

- **Exhibits minimal toxicity (sub-cu; neurotox; large-animal, disc injections 6-month study; total of 9 studies)**
- **Fast-acting / Long-lasting / Inexpensive**
- **Repeatable (2X@40mM ≈ 1X@80mM)**
- **Useful as an adjunct to surgery (discectomy, adjacent disc)**
DDD Degenerative Cascade

Nutritional Deficiency / Mechanical & Biochemical Degeneration

Annulus Overload / Dehydration

Bulges / Fissures / Cracks / Mechanical Insufficiency

Disc Collapse / Herniations / Facet Degeneration

Nerve Root Compression / Facet Joint Pain

Early Stage Disease

Réjuve as a primary treatment

Réjuve as a surgical substitute (flexible stabilization – better than fusion?) or an adjunct to surgery (ASD prophylactic, post-discectomy, spondylolisthesis, etc.)

*Weber ‘94
6-Month Sheep Study – Usability of the Device

- 16 successful fluoroscopic image-guided injections in 8 lumbar discs of 4 sheep
- Standard equipment, similar to standard techniques, challenging animal model: discs 1/3 height of human discs
Successful Delivery

Réjuve
Your Spine Rejuvenated.
**Health, Behavior & Bloodwork**

- **Happy sheep**: No infections, clinically relevant or prolonged inflammation, or adverse neuromuscular effects were observed.
- No concern by veterinary staff regarding bloodwork over course of study.

---

**A**

**White Blood Cell (WBC)**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3)</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

**B**

**Bilirubin (BR)**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR (mg/dL)</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td>3.5</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**C**

**Lymphocytes (LYM)**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYM (%)</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**D**

**Creatine**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine (mg/dL)</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td>3.5</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Mid-Study Imaging

<table>
<thead>
<tr>
<th></th>
<th>0226</th>
<th>0241</th>
<th>0242</th>
<th>0243</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1</strong></td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
</tbody>
</table>
No Inhibition of Growth

- New, untreated annulus tissue growth around entire periphery of treated discs
- Sheep weight increased 100-150%
Mechanical Effects

- Higher compression-bending stiffness without loss of viscoelastic (fluid-like) behavior
- Replicates *in vitro* effects shown to reduce instability (neutral zone) of degenerated human joints
- Effects moderated by presence of untreated outer annular ring of new tissue
- Any damage from needle puncture eliminated or mitigated
Previous *In Vitro* Instability Studies

- Joint instability/neutral zone has been linked to clinical incidence of pain, is thought to be associated with increased strain of imbedded afferent nerves.

![Graph showing Instability Score (IS) and Neutral Zone](image)

**Instability Score (IS)**

- **NZ/(ROM*NZ Slope)**
  - **Calf**
  - **Human**
  - **Controls Treated & Retested**
  - **CT&R Large NZ**
  - **CT&R Small NZ**

![Graph showing Neutral Zone](image)

**Neutral Zone**

- **Caudal**
- **PBS Soaked (Control)**
- **GAG degraded**
- **Collagen degraded**
- **Genipin Soaked**
Histology - Annulus

- No signs of cell depletion, tissue structural changes, infection or inflammation or any adverse reaction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annulus Fibrosus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4X</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>20X</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Histology – Inner Annulus/Nucleus

- Basophilic bodies (below) may explain molecular mechanisms by which crosslinking increases GAG retention and nutritional flow through the disc tissue as indicated in previous *in vitro* studies.

<table>
<thead>
<tr>
<th>Nucleus Pulposus/Inner Annulus Fibrosus</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4X</td>
<td><img src="40X.png" alt="Image" /></td>
<td><img src="40X.png" alt="Image" /></td>
</tr>
<tr>
<td>20X</td>
<td><img src="20X.png" alt="Image" /></td>
<td><img src="20X.png" alt="Image" /></td>
</tr>
</tbody>
</table>