Collagen Crosslinking Reagent Utilized to Stiffen Soft Palate in Equine Snoring and Apnea

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Snoring and OSA

• **Snoring**
  - Vibration of the tissues in the airway of the nose and throat, especially soft palate
  - 19-48% of men, 14-24% of women
  - Leads to sleep cycle disruption of snorer and family, sleep deprivation, daytime lethargy, drowsiness, and OSA

• **Obstructive Sleep Apnea (OSA)**
  - Interrupted breathing caused by obstruction of airway
  - 4.2% of Americans age 16 & older, ~10 million adults
  - Leads to serious respiratory and cardiovascular complications

• **Treatments:** Lifestyle changes, OTC medications, CPAP therapy, surgical procedures
  - 40-80% success rate
Objectives

- Crosslinking
  - Direct crosslinking of polypeptide chains
  - Formation of amide cross-linked bonds by carbodiimides
- Genipin Benefits From Previous Studies:
  - Modify mechanical properties
  - Increase resistance to mechanical degradation
  - Stabilize tissue
  - Preserve microstructural and compositional integrity
  - Increase strength, stiffness, fatigue resistance, and energy to permanently deform tissue

Genipin Chemical Structure
Hypothesis

• The hypothesis was to inject a non-toxic protein crosslinking reagent into the soft palate to augment the mechanical properties and reduce deformation and vibration of the palate, thus increasing the tissue’s resistance to mechanical degradation.
Animal Model

- **DDSP: Dorsal displacement of the soft palate**
  - Caused by displacement of caudal edge of the soft palate while exercising
  - ‘Snoring’ or ‘choking’ noise occurs 70 to 80% of the time
  - Can be self-corrected by swallowing or moving head around
- **Prevalence: 10 -20% of competitive horses**
- **Current Treatments:**
  - Tongue Tie
  - Tie-forward procedure
  - Laser staphylectomy
  - Myectomy or Tenectomy
  - Cornell Collar
Wind Tunnel Study

- **Methods:**
  - Transient and Steady State (cyclic) Flow at 14 m/s
  - Laser micrometer on XY system to measure ΔZ in 9 locations
  - Analyzed deformation and vibration frequency characteristics
    - Controls, 0.33% soaked, 50 mM, 100 mM, 150 mM
- **Results for Injections:**
  - Transient Displacement:
    - avg. 51% reduction
  - Steady State Displacement:
    - avg. 34% reduction
  - Cross linked blue area: avg. 47%

### Free End Maximum Deflections

![Graph showing %Reduction after Treatment for DMSO, Soaked, 50 mM, 100 mM, 150 mM. The graph includes bars for transient and steady state reduction.](chart.png)
Mechanical Testing

- 12 palates; 4 sections/palate
- 3 Groups:
  - Control – untreated
  - Buffer – 0.5 mL of 50 mM phosphate saline buffer
  - Genipin – 0.5 mL of 50 mM buffered genipin
- 3 Mechanical Tests
  - Cyclical Loading – +/- 3 mm for 20 cycles
  - Stress Relaxation – Constant 10 N for 5 mins
  - Tensile Load to Failure – Load at 1 mm/sec
Mechanical Testing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Change from Buffer</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Ultimate Tensile Stress</td>
<td>50.27% ↑</td>
<td>0.034</td>
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<tr>
<td>Ultimate Tensile Strain</td>
<td>36.26% ↓</td>
<td>0.092</td>
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<tr>
<td>Young’s Modulus</td>
<td>60.38% ↑</td>
<td>0.044</td>
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<tr>
<td>Toe Region Slope</td>
<td>117.84% ↑</td>
<td>0.124</td>
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<tr>
<td>Yield Point Stress</td>
<td>50.23% ↑</td>
<td>0.031</td>
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<tr>
<td>Yield Point Strain</td>
<td>38.01% ↓</td>
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<tr>
<td>Resilience</td>
<td>12.78% ↓</td>
<td>0.752</td>
</tr>
<tr>
<td>Toughness</td>
<td>58.11% ↓</td>
<td>0.166</td>
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</tbody>
</table>

Stress-Strain Curve - Genipin Sample

- Stress-Strain Curve
- Toughness
- Resilience
- Offset Strain

Stress (MPa) vs. Strain
Pilot *in vivo* Study

- **2 Phases**
  - **Efficacy Phase:** 3 DDSP Horses
    - Returned to owner’s post treatment
  - **Safety Phase:** 3 Control Horses
    - Euthanized and palates harvested

- **Efficacy Phase Examinations**
  - Dynamic Endoscopes – confirm DDSP
  - Audio Recordings – quantify changes in snoring loudness and frequency

- **Reagent**
  - Genipin: 100 mM concentration
  - Buffer: 10% DMSO EPPS-P
    - 10% Dimethyl sulfoxide (DMSO), 3-[4-(2-Hydroxyethyl)-1-piperazinyl]propane sulfonic acid Phosphate (EPPS-P)
Pilot *in vivo* Study

- 1-meter long, 9 mm – outer diameter fiberoptic endoscope with a transendoscopic injection needle
- Injected with two 1 mL injections of 100 mM buffered genipin reagent
  - Injected at 5:00 and 7:00 positions with the epiglottis occupying the 6:00 position
- Difficult Procedure
  - Tough outer membrane – needle slippage
  - Soft palate pillowing – excessive injections
- 1 horse had hyper salivation and decreased appetite for 24 hours
**In vivo Study**

**Pre Treatment Spectrogram**

**Post Treatment Spectrogram**

**Comparison of Percent Area of Red Squares on Spectrogram Graphs**

- **Pre Treatment Area**
- **Post Treatment Area**

<table>
<thead>
<tr>
<th>DDSP Horse ID</th>
<th>Horse #1</th>
<th>Horse #2</th>
<th>Horse #3</th>
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<tr>
<td></td>
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<td>30.00</td>
<td>32.00</td>
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<tr>
<td></td>
<td>0.00</td>
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**University of Kentucky**

College of Engineering
Department of Biomedical Engineering

**UK**

*see blue.*
In vivo Study

- No specific trends in severity of inflammatory infiltrates
- All specimens had a region of tearing/lesions in the middle
  - Treated and untreated tissue
  - Surrounded by viable tissue
  - Mild to moderate fibroplastic response
  - ‘Pillowing’ of palate during injections
- 2 palates had gray necrotic tissue on mucosal surface
  - Mild to moderate fibroplastic response
  - Colonized by bacteria
  - Errant delivery of reagent

Necrotic tissue with neutrophils

Fibroplasia with few neutrophils
Conclusions

<table>
<thead>
<tr>
<th>Wind Tunnel</th>
<th>Mech. Testing</th>
<th>Pilot Equine in vivo Study</th>
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</table>
| • Large reductions in soft palate deformations  
  • Reduction in vibration amplitude of soft palate  
  • Crosslinking treatment covered half of the palate | • %Difference > 30% in 7 categories  
  • Statistically significant results in 3 categories  
  • Increase in strength and stiffness of soft palate | • At least 1 horse cured of DDSP  
  • All horses had reduction in snoring loudness  
  • No specific inflammatory infiltrate trends in histologic analysis  
  • Evidence of adverse responses due to errant injections  
    • Necrotic tissue on mucosal surface  
    • Tearing and lesions due to ‘pillowing’ during injections |
Acknowledgments

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