Biochemical and Physiological Dynamics in Ligament Injury & Healing

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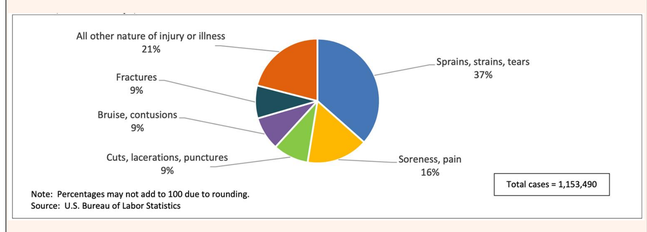
Introduction

In agriculture, musculoskeletal injuries pose the most common safety risk for workers and interfere persistently with the business economics. AgSafe estimated that approximately 40% of the reported sprains and strains injuries involve the back [1]. Because agriculture relies heavily on manual labor, the economic health of agriculture industry depends greatly upon the health of its workforce. Understanding the underlying biochemical mechanisms of injury and healing provides an informed scientific basis to improve worker safety and to promote efficacious healing therapies.



Percent distribution for occupational injuries and illnesses with days away from work by selected nature of injury or illness, all ownerships, 2015

Purpose

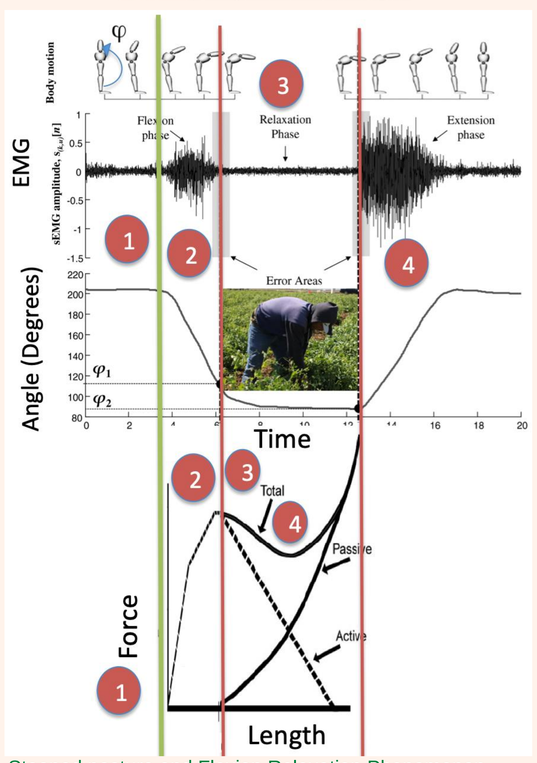
The aims of this study are to 1) develop an animal model 2) characterize the biochemical response to ligament injury and healing 3) establish a ligament transection injury healing protocol 4) impose a mild ligament injury and 5) investigate the efficacy of traditional treatment methods. The mild injury model will mirror the common injury experienced by workers, who are constantly stooped while performing agricultural tasks such as harvesting strawberries and hand weeding.

Economic Incentives

Substantial financial losses are associated with work related musculoskeletal disorders (WMSDs). The annual cost of Low Back Disorders (LBDs) exceeds $100 billion. In the US alone, 13 million people will develop LBDs annually due to their occupation making it the most prevalent musculoskeletal problem in the workplace [2]. Therefore, addressing the musculoskeletal disorders in agriculture will not only improve worker health and well being, but will also increase productivity, lower cost, and preserve a solid agricultural industry.

Stooped Work Posture

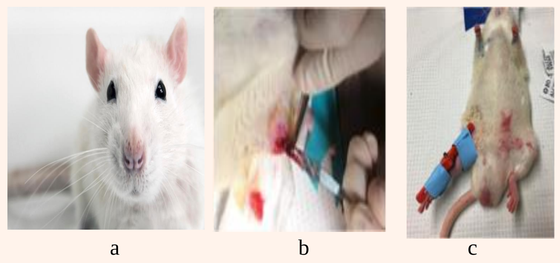
Stooped Posture is seen in many agricultural tasks when workers are bent forward and down at the waist and/or mid-back while maintaining straight legs. Sustained stoop exposes spinal ligaments and vertebral desks to high forces that may induce injury.



Stooped posture and Flexion Relaxation Phenomenon. Passive soft tissue (e.g. spinal ligaments and desks) support high forces during the muscle's silence period at deep flexion angles (i.e. stooped posture).

Animal Models

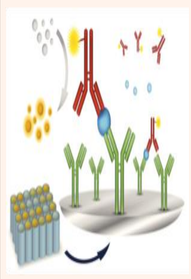
Animal models were established to induce an acute Medial Collateral Ligament (MCL) injury with a transection and then with a sustained submaximal static load simulating stooped posture. Two treatment interventions were used: A Nonsteroidal Antiinflammatory Drug (NSAID) and a Platelet Rich Fibrin (PRF). Biomechanics, biochemistry, and functional recovery were assessed.



a b c

(a) Wistar rat model. **(b)** Surgical transection injury. **(c)** Sustained submaximal static injury

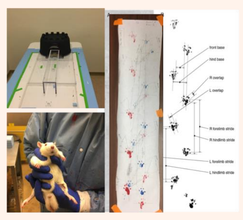
Evaluation Methods



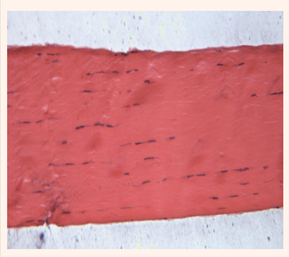
Sandwich ELISA depiction

ELISA: Quantitative method measured Van Willibrand Factor (VWF), Vascular Endothelial Growth Factor (VEGF), P-Selectin, and L-Selectin after injury injury using a sandwich Enzyme Linked Immunosorbent Assay (ELISA) [4]. These factors provide insight into platelet plug formation and recruitment of immune cells at different stages of repair.

Gait: A gait test observed the rat's locomotion during a walk along a straight path. It measured eight parameters: stride lengths, front and back paws stride widths, paws overlap, mean stride length, toe spread, and inner toe spread.



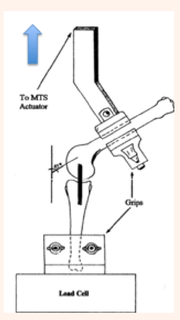
Gait testing apparatus



Hematoxylin and Eosin stained section of a healthy ligament. 20x

Histology: Histological analysis performed at specified time points to characterize collagen organization, vascularization, and cellularity. Hematoxylin & Eosin (H&E) stains helped to visualize tissue samples. Modified Bonar Scoring technique quantified tendinopathy.

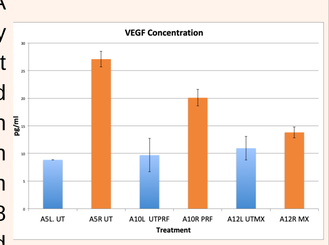
Material Properties: Material testing measured the mechanical performance of the Femur-MCL-Tibia segments with a force and displacement resolutions of 0.01 N and 0.001 mm, respectively. The segments were pulled to tensile failure at a strain rate of 10%/s (.0.6-0.8 mm/s) [5]. Four parameters were assessed: load to failure, displacement, stiffness, and energy to failure [6].



MTS testing apparatus

Initial Results

ELISA: VEGF ELISA results showed that on Day 5 after injury, animals that received no treatment had a significant increase in VEGF expression in comparison to sham controls (27.1 and 8.8 pg/ml). In the PRF treated animals VEGF expression increased two folds (20.1 and 9.7 pg/ml). Meloxicam (MX) treated animals showed no difference (10.9 and 13.8 pg/ml).



ELISA results for VEGF

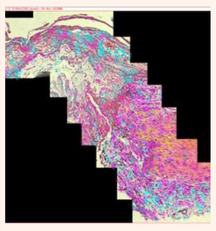
Histology: Initial findings show that cellularity, collagen organization, and vascularity scores (summarized as the total histology index) were higher in healing ligament (21.47) than in sham control (3.25) at all regions of analysis (Tables 1 and 2). Outer regions (skin and joint sides) appeared to show higher combined total histology indices (12.3) than the same regions in sham controls (02.63).

Table 1: Injured Ligament. Total Histology Index

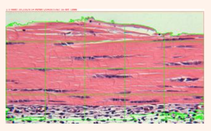
Region	Cellularity Index (0-3)	Collagen Index (0-3)	Vascularity Index (0-3)	Total Histology Index (0-9)
1	1.75	2.00	2.00	**5.75**
2	1.32	2.00	1.00	**4.32**
3	1.02	1.00	3.00	**5.02**
4	1.38	2.00	3.00	**6.38**
Total	**5.47**	**7**	**9**	**21.47**

Table 2: Sham Ligament. Total Histology Index

Region	Cellularity Index (0-3)	Collagen Index (0-3)	Vascularity Index (0-3)	Total Histology Index (0-9)
1	0.50	0.40	0	**0.90**
2	0.43	0	0	**0.43**
3	0.19	0	0	**0.19**
4	0.9	0	0.8	**1.73**
Total	**2.02**	**0.4**	**.8**	**3.25**



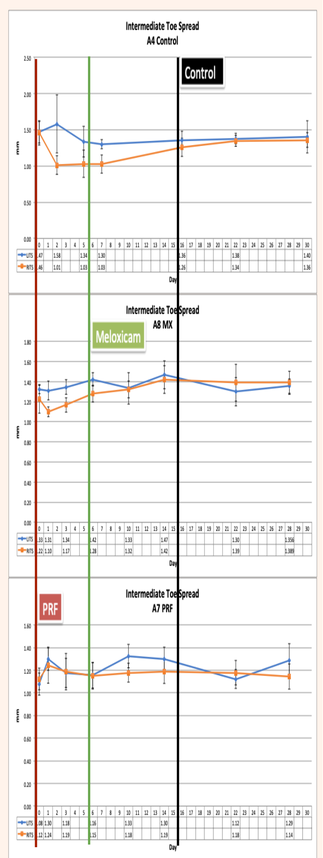
H&E Stained section of injured ligament. 20x



H&E stained section of a sham ligament. 20x

Gait: Initial gait analysis results showed that out of the eight parameters measured, only the Inner Toe Spread (ITS) parameter can detect significant differences between the control and injured limbs.

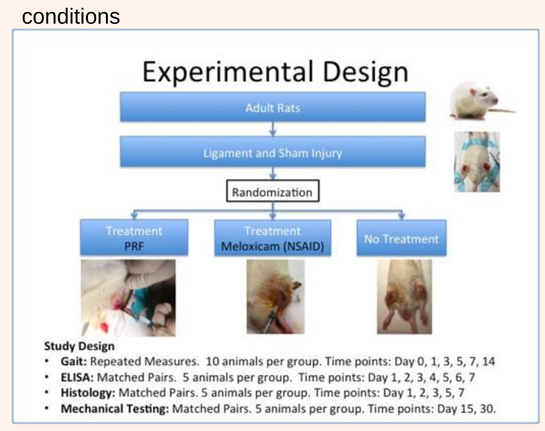
In control animals with no intervention, the ITS decreased sharply on the injured right limb but not on the uninjured left limb. The ITS in the injured limb gradually returned to the left limb level at day 15. With meloxicam treatments, ITS showed a smaller initial decrease on the right injured limb. But the ITS recovered to the uninjured limb level by day 6. In the PRF treated animal, the ITS of both the injured and uninjured limbs show no significant difference.



Gait testing results for control, NSAID, and PRF treated animals

Progress and Next Steps

- Animal models have been established.
- Treatment methods and experiments for ELISA, histology, and gait analysis have been piloted and optimized.
- Initial results show the utility of optimized laboratory methods in evaluating ligament injury.
- Future experiments will focus on; 1) scaling the study to evaluate statistical significance and 2) building and evaluating the mechanical testing apparatus. Submaximal injury will be further optimized and experiments will be repeated under submaximal injury with sustained static loading conditions to mimic occupational exposures under stooped posture conditions



Planned experiments and assessment timepoints

Summary

- ELISA has mapped the change in biochemical factors after injury. VEGF responds very differently with meloxicam or PRF intervention.
- Histology has characterized the course of injury and healing.
- Immediately after injury, Gait analysis (ITS) can track the recovery. Meloxicam seems to mask the pain and may not help healing. PRF treatment may actually help in healing.
- The initial studies with acute MCL transection has set the basis for experiments to characterize injury/response to sustained submaximal ligament injury.
- Findings from this study will help improve treatment methods, reduce stooped work postures exposure to injury, and provide insights into effective injury prevention policies.

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Acknowledgments

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