# Development of an *in vitro* tendon healing model for the assessment and development of therapeutic strategies

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## Introduction

Recently, there has been a dramatic increase in the use of platelet-rich plasma (PRP) for the treatment of orthopaedic injuries including Achilles, patellar, and rotator cuff tendon tears. Platelets play important roles in tissue repair and remodeling. Once platelets reach a site of injury, they release bioactive proteins that aid in nearly all facets of tissue healing. Platelet-rich plasma appears to improve the rate and quality of healing. However, many questions remain regarding its use for tendon injuries, including optimal formulation and mechanisms of action. Progress towards addressing these critical questions for clinical use of PRP is currently limited by the lack of a valid *in vitro* model to assess the efficacy of various therapeutic applications of this orthobiologic.

## Objectives

1) To develop an effective *in vitro* model to assess potential therapeutic strategies for augmenting tendon healing
2) To compare the healing potential of platelet-rich plasma (PRP) for the treatment of tendon injuries

## Methods

Tissue Harvest: With IACUC approval, common calcaneal tendons were harvested from both hind limbs of 12 dogs euthanatized for reasons unrelated to this study. Each tendon was aseptically divided into 5 cm long sections for a total of 72 tendon explants. A 3 mm diameter defect was created in the center of 60 explants using a dermal punch.

Treatment Groups: Explants were randomly assigned to 1 of 6 treatment groups (n=12/group): 7% PRP, 2% PRP, platelet-poor plasma (PPP), whole blood, no treatment, no injury. For the defect treatment groups, 100 µL of 7% PRP, 2% PRP, PPP, or whole blood obtained and processed from canine donors was delivered into the defect and allowed to clot at room temperature for 10 minutes. Cultured canine fibroblasts (2.2 million cells in 1 mL) were seeded onto each explant and allowed to adhere to the defect at room temperature for 10 minutes.

Tissue Culture: Explants were maintained in 10 mL of standard tissue culture for 14 days. Media was changed every 3 to 4 days. At day 14 of culture, tendon explants were assessed for biomechanical properties (load at 1 mm, 2 mm, 3 mm and stiffness), cell viability, and histological appearance.

Statistical Analysis: Data were analyzed with SigmaPlot® using t-tests with significance set at $p<0.05$.

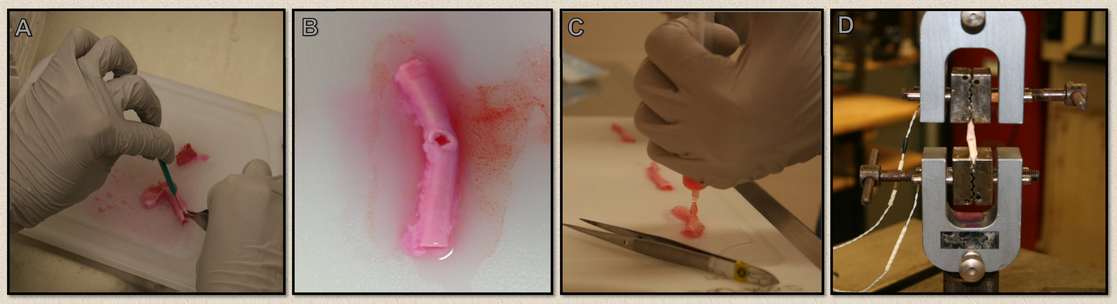


Figure 1: A) Dividing tendon into 5 cm long sections. B) Tendon with 3 mm diameter defect. C) Delivering treatment into tendon defect. D) Testing tendon for biomechanical properties.

## Results

All tendons were successfully harvested and sectioned into explants. The 3 mm dermal punch was effective in consistently creating full-thickness "core" defects in each explant. However, fungal infection was noted in 46 of 72 explants between day 4 and day 14 in culture. Contaminated tendons were discarded, and complete analyses were completed on the remaining 26 explants: 7% PRP (n=5), 2% PRP (n=4), PPP (n=4), whole blood (n=4), no treatment (n=5), no injury (n=4). For the tendon explants analyzed, there were no statistically significant differences among treatment groups compared to controls for biomechanical properties, cell viability, or histological grading. During processing, tendons were sliced transversely through the center of the defect. As a result, the biological material in the defect was displaced, hampering objective and subjective evaluations of the repair. A more effective processing method would involve sectioning the tendons longitudinally in a coronal plane so that the biological material in the defect would be less likely to be displaced.

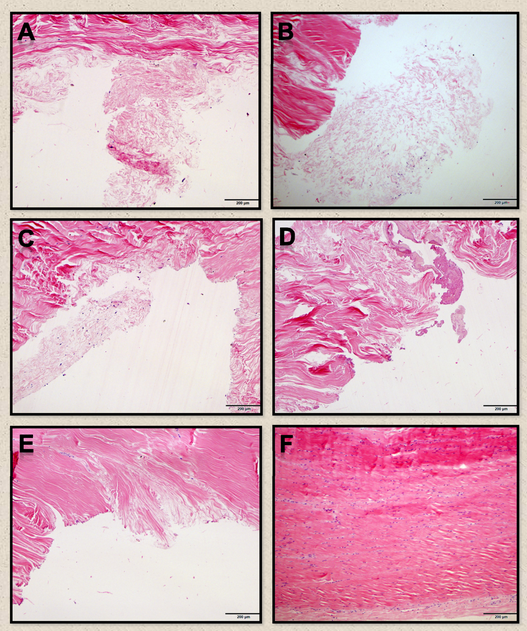


Figure 2: Representative H&E histological images after 14 days of culture. A) 7% PRP B) 2% PRP C) PPP D) whole blood E) no treatment F) no injury. Tissue repair was not observed in any of the treatment groups after 14 days of culture. Further, there were no significant differences between treatment groups in the histological evaluation of the tissue around the tendon defect.

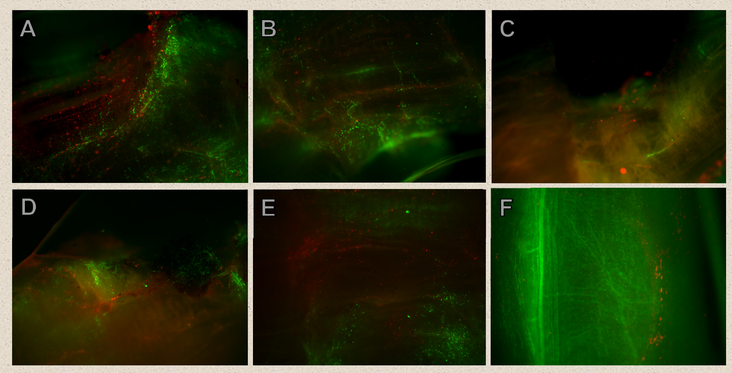


Figure 3: Representative fluorescent tissue viability images after 14 days of culture. A) 7% PRP B) 2% PRP C) PPP D) whole blood E) no treatment F) no injury. A subjective decrease in tissue viability was observed around the margin of the injury in all injured groups.

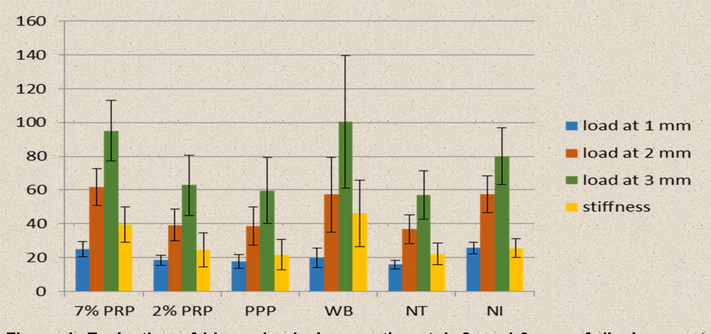


Figure 4: Evaluation of biomechanical properties at 1, 2, and 3 mm of displacement and measured stiffness of the tendon. The data indicates that an injury of this size and location did not significantly decrease the biomechanical properties of the tendon, even without treatment.

## Conclusions

- Based on source and location of the tendons used for this study, modifications in preparation, harvest, and culture techniques are needed to prevent infection during explant culture
- Initial results suggest that this model can provide an effective method for assessing biomechanical and biological aspects of tendon defect healing
- While none of the treatments evaluated were successful in promoting functional repair of tendon defects, results were valuable for optimizing model development and therapeutic techniques
- Altering the method of processing will make future objective and subjective evaluations of the tissue more precise
- Ongoing studies in our laboratory are evaluating the use of PRP on scaffolds for tendon and meniscus healing based on data derived from this study

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