Chitosan-PRP Implants Improve Surgical Repair of Rotator Cuff Tears in a Sheep Model

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INTRODUCTION: Surgical repair of rotator cuff tears can yield satisfactory clinical outcomes but re-tears remain common. Basic studies have shown that a fibrovascular scar tissue forms at the tendon-bone interface following surgical repair of torn rotator cuff tendons. This tissue does not possess the mechanical properties of the native stratified tendon insertion and that is one potential reason for failure. Repair augmentation techniques that restore the stratified structure of the insertion would be advantageous. In a previous pilot study in sheep (1), we showed that using chitosan-platelet-rich plasma (CS-PRP) implants in conjunction with suture anchors improved MRI and histopathology repair outcomes at 3 months post-surgery. The purpose of the current study was to assess efficacy and safety of CS-PRP implants at 6 months in the sheep model, with a larger number of sheep per group than in the pilot study.

METHODS: The study was approved by the Institutional Animal Care and Use Committee. In 48 sheep, the infraspinatus tendon (ISP) was detached and immediately repaired with four suture anchors in a suture bridge configuration. In the treated groups, freeze-dried chitosan matrix containing 1% w/v chitosan (number average molar mass 37 kDa and degree of deacetylation 85%) with 1% w/v trehalose and 42 mM calcium chloride was solubilized with autologous leukocyte-rich PRP and injected at the tendon-bone interface and on top of the repaired site (n = 12 with a 2 mL volume and n = 12 with a 3 mL volume). As an additional safety control group, the chitosan matrix was solubilized in water and applied to the repaired sites (n = 12 with a 3 mL volume). MRI images (acquired with a 1.5 T Siemens Sonata) were measured/scored by 2 blinded readers (veterinarian and human radiologists). Histological sections were scored by a blinded veterinarian pathologist and peer-reviewed by a second veterinarian pathologist. MRI and histopathology outcome measures are presented in **Table 1**. Standard safety outcome measures were also collected. Statistical analyses were performed by independent biostatisticians using SAS Version 9.4 TS1M2. A mixed effect model was used for continuous data and Wilcoxon Exact tests were used for categorical scores.

RESULTS: There were no statistically significant differences between the anchors + 2 mL or the anchors + 3 mL CS-PRP treated groups and so results were pooled for those groups. Tendon gap was decreased by treatment with anchors + CS-PRP compared to anchors only at 3 months ($p = 0.060$) (**Figure 1**). None of the ISP tendons and entheses were identical to normal tissues at 6 months and none had reformed the stratified insertion. However, treatment with anchors + CS-PRP improved several histological features of the tendons and entheses (**Figure 2**). All tendons treated with anchors + CS-PRP had mild cellularity, similar to normal tendon, while cellularity was increased in the tendons treated with anchors only ($p = 0.031$). Inflammatory cells were abundant in the tendons treated with anchors only, but absent in all tendons treated with anchors + CS-PRP ($p = 0.002$). More entheses treated with anchors + CS-PRP exhibited no change/typical appearance of glycosaminoglycan (GAG) staining compared to entheses treated with anchors only ($p = 0.071$). Finally, treatment with anchors + CS-PRP led to more complete healing with a smaller degree of remodeling compared to anchors only ($p = 0.019$). Treatment with the chitosan matrix solubilized in water did not improve any of the MRI or histological outcomes. There was no treatment-specific effect on any of the safety outcome measures (clinical signs, hematology, serum biochemistry, urinalysis, organ histopathology), which suggests high safety.

DISCUSSION: The combination of CS and PRP was essential for repair improvement, as the chitosan matrix by itself had no effect. As in the pilot study, treatment with anchors + CS-PRP decreased the tendon gap at 3 months compared to anchors only. Since tendon gap is the length measurement of abnormally hyperintense tissue adjacent to the humeral head, this decrease is indicative of a faster restoration of tissue with normal MRI signal. As in the pilot study, several histopathological features were improved by anchors + CS-PRP treatment compared to anchors only. These promising findings of surgical treatment augmented with CS-PRP implants may translate into improved mechanical performance, but this was not assessed in the current study.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides further evidence on the efficacy and safety of CS-PRP implants in a large animal model of rotator cuff repair that could potentially be translated to a clinical setting.

REFERENCES: (1) Chevrier et al, Freeze-dried chitosan solubilized in platelet-rich plasma in a sheep model of rotator cuff repair, Transactions ORS 2019.

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MRI	Histopathology
Continuous measurements	**ISP tendon scores**
Tendon gap (mm)	Cellularity
Tendon thickness (mm)	Tenocytes
Tissue volume (cc)	Inflammatory cells
Cancellous to cortical signal intensity (FSE)	Vascularity
Cancellous to cortical signal intensity (STIR)	Structural organization
Categorical scores	GAG expression
Presence of bursitis	**ISP enthesis scores**
	Structural appearance
Synovial reaction	GAG expression
	Bone remodeling
Heterotopic bone formation	**Pan-enthesis scores**
	Healing/remodeling
Erosion	Length of insertion site
	Quality/consistency of GAG staining of cartilage/fibrocartilage

Table 1. MRI and histopathological outcome measures.

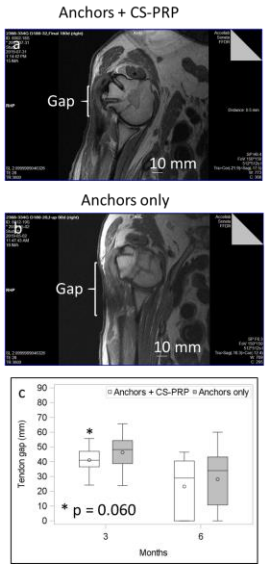

Fig 1. MRI tendon gap measurements.

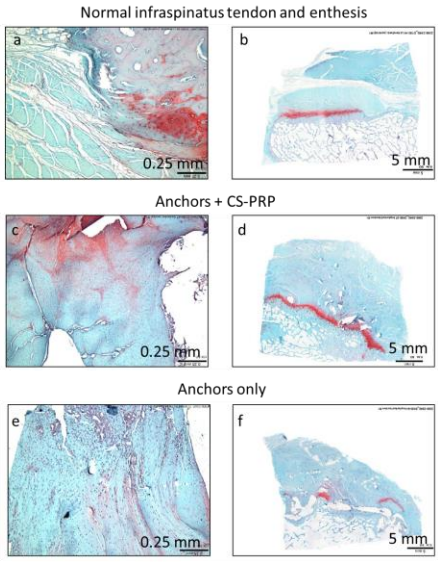

Fig. 2. Safranin O/Fast Green-stained sections.