Freeze-dried chitosan solubilized in platelet-rich plasma in a sheep model of rotator cuff repair

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INTRODUCTION: Rotator cuff tears are one of the most common shoulder pathologies (1). Surgical reattachment of torn rotator cuff tendons can lead to satisfactory clinical outcome but failures remain common (2, 3). We have developed a method to produce freeze-dried formulations of chitosan (CS), trehalose (as lyoprotectant) and calcium chloride (as clot activator) that are soluble in platelet-rich plasma (PRP) to form injectable implants (4, 5). These CS-PRP implants have previously been shown to improve transosseous rotator cuff repair in a small rabbit model (6) and a feasibility pilot study in sheep revealed that the implants could potentially also improve suture anchor-based rotator cuff repair (7). The purpose of the current pilot study was to determine implant residency, test safety of different implant doses, and assess efficacy over standard of care in a large animal (sheep) model.

METHODS: The study was approved by the Institutional Animal Care and Use Committee. The infraspinatus (ISP) tendon was detached followed by immediate repair in 22 skeletally mature ewes (**Fig 1 a-b**). Repair was done with four suture anchors in a suture bridge configuration (n = 6 standard of care controls). Freeze-dried formulations containing 1% w/v chitosan (number average molar mass 35 kDa and degree of deacetylation 83%) with 1% w/v trehalose and 42.2 mM calcium chloride were solubilized with autologous leukocyte-rich PRP and injected at the tendon-bone interface (**Fig 1c**) and on top of the repaired site (n = 6 with a 1 mL dose and n = 6 with a 2 mL dose). Acute implant residency was assessed histologically at 1 day (n = 2 with a 1 mL dose and n = 2 with a 2 mL dose). Efficacy outcome measures included MRI assessment (1.5 T Siemens Sonata) at baseline, 6 weeks and 12 weeks and histopathology at 12 weeks. MRI images and histological slides were scored by 2 blinded readers (veterinarian and human radiologist, and veterinarian pathologist) and averaged. Safety outcome measures included histopathology of internal organs, hematology parameters, serum chemistry parameters, urine chemistry parameters and synovial fluid cell differential. The Generalized Linear Model task in SAS Enterprise Guide 7.1 and SAS 9.4 was used to compare the different groups with post-hoc analysis to look at pairwise differences.

RESULTS: CS-PRP implants were detected near the enthesis (**Fig 1 d-f**), near the top of the anchors holes (**Fig 1 g-i**) and at the surface of ISP tendon (**Fig 1 j-l**) and muscle (**Fig 1 m-o**) at 1 day post-op. Numerous polymorphonuclear cells (PMNs) were recruited to the implant in the case of ISP tendon and muscle (**Fig 1 k&n**). On MRI, all repair sites were hyperintense compared to normal tendon at 6 weeks and only 1 out 18 repair sites was isointense at 12 weeks (**Fig 2 a-i**). Treatment with the 2 mL dose significantly decreased tendon gap seen on MRI, defined as the length of the hyperintense region between the greater tuberosity and ISP tendon with normal signal intensity, when compared to standard-of-care control at 12 weeks (p = 0.01) (**Fig 2j**). Histologically, none of the repair sites were structurally normal **(Fig 3 a-i)**. A trend of improved structural organization of the tendon (p = 0.06) and improved structural appearance of the enthesis (p = 0.1) with 2 mL dose treatment compared to standard-of-care control was seen at 12 weeks **(Fig 3 j & k)**.

DISCUSSION: CS-PRP implants (2 mL dose) modulated the rotator cuff healing processes in this large animal model, as revealed by a significant decrease in tendon gap on MRI and trends of improved structural organization/appearance of the tendon and enthesis seen histologically at 12 weeks post-op. These promising MRI and histological findings of CS-PRP treated ISP tendons may translate into improved mechanical performance, which will be assessed in a future study with a larger number of animals. Animals exhibited no visible signs of pain and experienced mostly mild transient lameness post-surgery which was similar for all treatment groups. In addition, there was no treatment-specific effect on all standard safety outcome measures, which suggests high safety.

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

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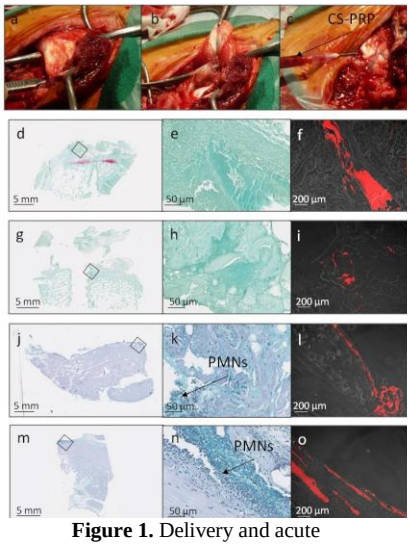

Figure 1. Delivery and acute residency of implants. Chitosan is imaged in red in f, i, l & o.

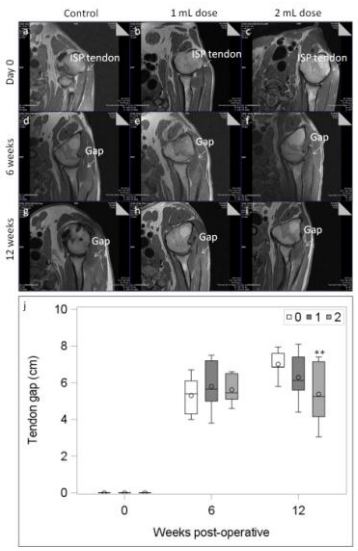

Figure 2. MRI assessment.

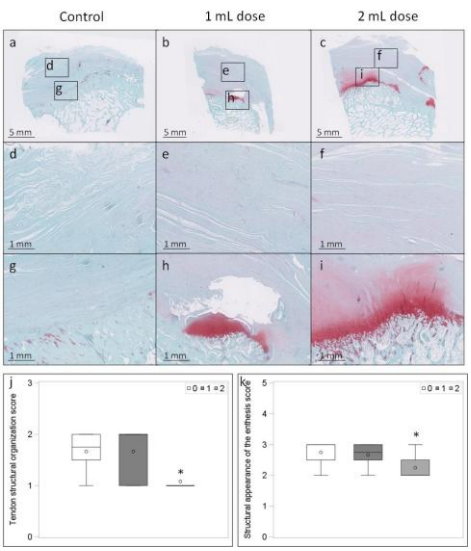

Figure 3. Histological assessment.