Abstract 51278 Freeze-dried chitosan solubilised 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 and may eventually lead to irreversible changes in the shoulder causing significant pain and morbidity (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 CS-PRP implants that coagulate rapidly *in situ,* retract much less than PRP-only controls, and exhibit significant bioactivity *in vivo* (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 study was to determine implant residency, test safety of different implant doses, and assess efficacy over standard of care in a large sheep model.

Materials and Methods

Unilateral tears were created in the infraspinatus (ISP) tendons of 22 skeletally mature ewes and repaired 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 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 and safety outcome measures included MRI assessment (Siemens 1.5T) and clinical pathology at baseline, 6 weeks and 12 weeks and histopathology at 12 weeks. MRI images and histological slides were scored by 2 blinded readers and averaged. 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.

1. Delivery and acute residency of implants

- Full-thickness unilateral tears were created in the infraspinatus tendons (**Fig 1 a&b**) and immediately repaired with four suture anchors in a suture bridge configuration.

- Freeze-dried chitosan (CS) formulations were solubilized in autologous platelet-rich plasma (PRP) and injected at the bone-tendon interface and on top of the repaired site (**Fig 1 c**).

- CS-PRP implant was detected near the enthesis, near the top of the anchors holes and at the surface of ISP tendon and muscle at 1 day post-operative (**Fig 1 d-o**).

- Numerous polymorphonuclear cells (PMNs) were recruited to the CS-PRP implant in the case of ISP tendon and ISP muscle (**Fig 1 k&n**).

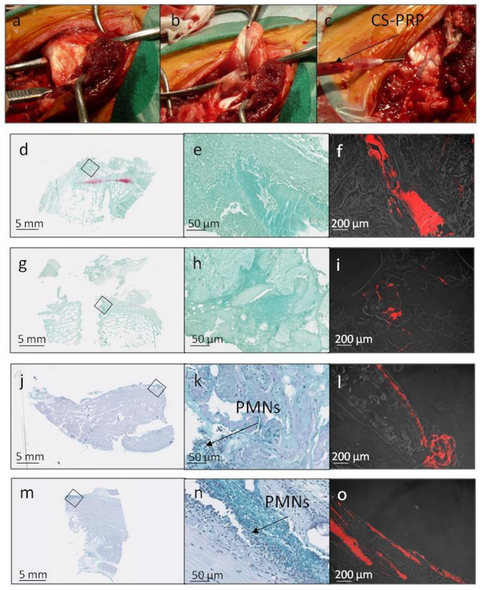

Fig 1. Unilateral tears were created in the infraspinatus tendons (a & b) and repaired with suture anchors in a suture bridge configuration (c). CS-PRP formulations were injected under (c) and above the repaired site. To assess acute implant residency at 1 day post-surgery, paraffin sections were collected at the ISP enthesis (d-f), at the site of anchor insertion (g-i), from ISP tendon (j-l) and ISP muscle (m-o) and stained with Safranin O/Fast Green or imaged with epifluorescent microscopy (formulations contained a rhodamine-chitosan fluorescent tracer for this purpose). Insets in low magnification images show where higher magnification images were acquired.

2. MRI assessment

- 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 of CS-PRP significantly decreased tendon gap (defined as the length of the hyperintense region between the greater tuberosity and ISP tendon that has normal signal intensity) when compared to standard-of-care control at 12 weeks (p = 0.01) (**Fig 2j**).

- There was no other difference between the treatment groups for the other MRI parameters assessed (signal intensity of the repaired area relative to normal tendon, signal intensity of cancellous bone in the repair site, tendon thickness, tissue volume, presence of bursitis, synovial reaction, heterotopic bone formation, erosion along the anchors).

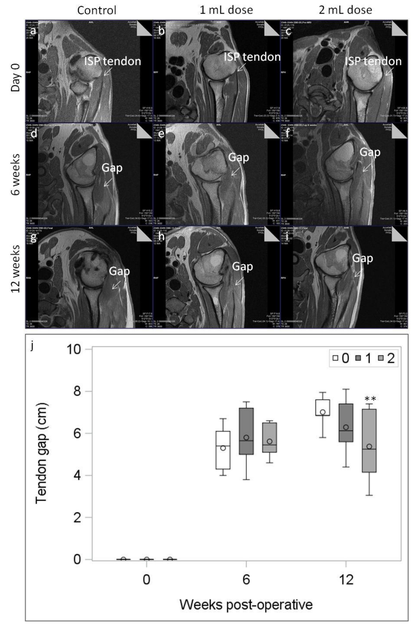

Fig 2. Representative MRI images collected at day 0 (pre-operative) (a-c), 6 weeks (d-f) and 12 weeks (g-i) from the three treatment groups. Tendon gap was significantly lower (**p < 0.05) in the group treated with 2 mL dose CS-PRP at 12 weeks compared to standard-of-care control. Data in j are presented as median (line); Box: 25th and 75th percentile; Whisker: Box to the most extreme point within 1.5 interquartile range (n=6 per treatment group).

3. Histological assessment

- Histologically, none of the repair sites were structurally normal **(Fig 3 a-i)**.

- There was 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 at 12 weeks **(Fig 3 j & k)**.

- There was no other difference between the treatment groups for the other histological parameters assessed (cellularity, tenocytes, vascularity, inflammatory cells, glycosaminoglycan content in tendon and at enthesis, attachment).

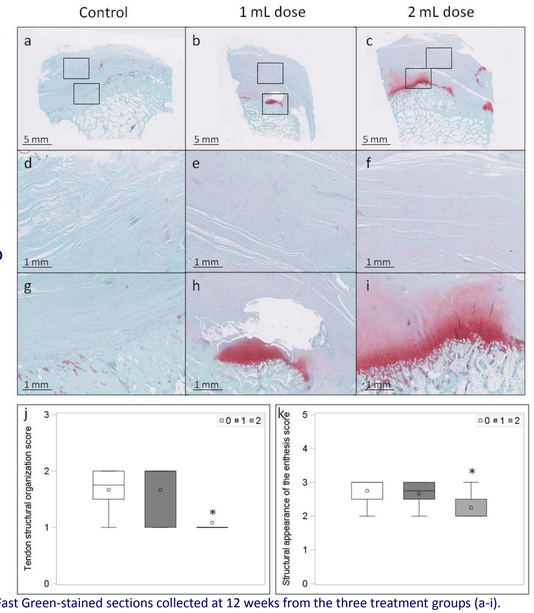

Fig 3. Representative Safranin O/Fast Green-stained sections collected at 12 weeks from the three treatment groups (a-i). There was a trend (*p ≤ 0.1) of improved structural organization of the tendon and improved structural appearance of the enthesis in the group treated with 2 mL dose CS-PRP compared to standard-of-care control. Insets in low magnification images show where higher magnification images were acquired. Data in j & k are presented as median (line); Box: 25th and 75th percentile; Whisker: Box to the most extreme point within 1.5 interquartile range (n=6 per treatment group).

Discussion

CS-PRP implants (2 mL dose) modulated some rotator cuff healing processes in this large animal model, as revealed by a significant decrease in tendon gap and trends of improved structural appearance of the tendon and enthesis at 12 weeks post-operative. The promising MRI and histological findings of CS-PRP treated ISP tendons would be expected to translate into superior mechanical performance, and this will be assessed in a future animal study. 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 histopathology of internal organs, hematology parameters, serum chemistry parameters, urine chemistry parameters and synovial fluid cell differential, which suggests high safety.

Conclusions/Conflict of interest declaration

This study provides evidence on the safety and efficacy of CS-PRP implants in a large animal model that could potentially be translated to a clinical setting. AC, MBH, FXL, ML and MDB hold shares, FXL is an employee and MDB is a Director of Ortho Regenerative Technologies Inc.

References

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