Freeze-dried chitosan-PRP in a rabbit model of rotator cuff repair

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Introduction:

Rotator cuff injury is the second most common musculoskeletal pathology after lower back pain and the most common shoulder condition for which patients seek therapy. Rotator cuff tears result in shoulder pain, stiffness, weakness and loss of motion. Degenerative changes in the structure and composition of the tendons make healing very difficult. Our laboratory has developed a method to produce lyophilized 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* (1). The purpose of this study was to apply CS-PRP implants in conjunction with transosseous suturing in a rabbit model of rotator cuff tear and to assess healing histologically. A pilot study in 4 rabbits was first performed to assess feasibility, followed by a pivotal study in 13 rabbits to assess efficacy.

Methods:

Bilateral full-thickness tears were created in the supraspinatus tendons (SSP) of the rotator cuff of New Zealand White rabbits (Pilot study; n = 4 female retired breeders and Pivotal study; n = 13 females aged 8 months). The tears were immediately repaired via a transosseous suturing technique (2). On the treated side, a chitosan-PRP hybrid mixture was additionally injected into the bony trough and at the repaired site. Freeze-dried chitosan cakes were prepared using 1% w/v chitosan (80% DDA and M_n 36.4 kDa), 1% w/v trehalose as lyoprotectant and 42.2 mM calcium chloride as clot activator, and solubilized with autologous leukocyte-rich PRP immediately prior to injection.

1. Surgical model

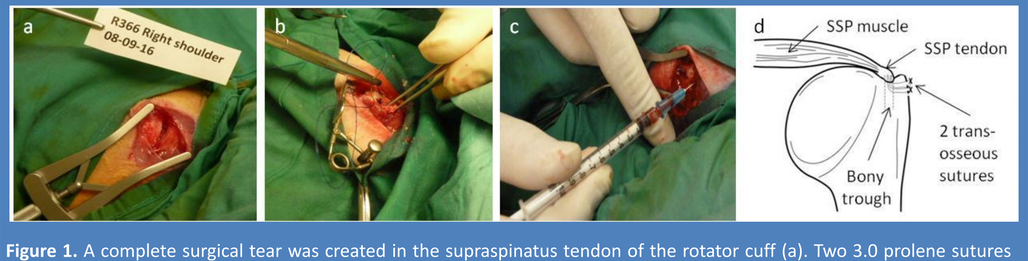

Figure 1. A complete surgical tear was created in the supraspinatus tendon of the rotator cuff (a). Two 3.0 prolene sutures were pre-placed through the lateral holes, the bony trough and the tendon itself in a modified Mason-Allen pattern (b). The CS-PRP mixture (150 µL) was injected into the bone tunnel prior to suturing the tendon (c). Additional CS-PRP mixture (150 µL) was then injected at the repaired insertion site and in the tendon. Schematic representation of the surgical model (d).

2. CS-PRP implant residency at 1 day

-CS-PRP implants was found adhering to the SSP tendon surface, within the bony trough and in some areas of the SSP tendon/muscle unit at 1 day (n=6 shoulders).
-The SSP tendon was fully transected in all cases.

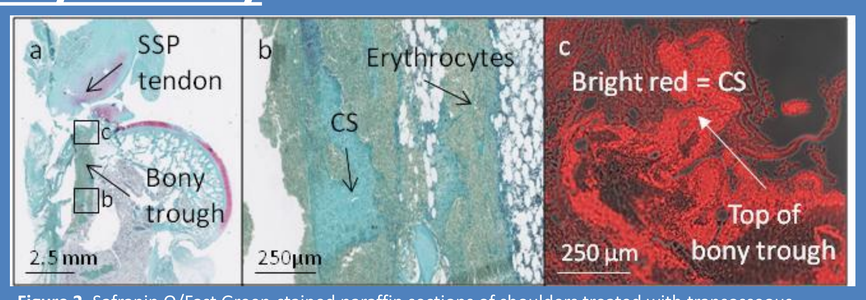

Figure 2. Safranin O/Fast Green-stained paraffin sections of shoulders treated with transosseous suturing + CS-PRP after 1 day (a to c) . A rhodamine-chitosan tracer was used to image CS with epifluorescence in red in c.

3. CS-PRP induced recruitment of PMNs at 7 and 14 days

-CS-PRP induced recruitment of polymorphonuclear cells (PMNs) to the tendon and to the muscle endomysial space at 7 and 14 days (n=2 shoulders).
-Chondrogenesis in the tendon and endochondral ossification were apparent in the control suturing only group (n=2 shoulders).

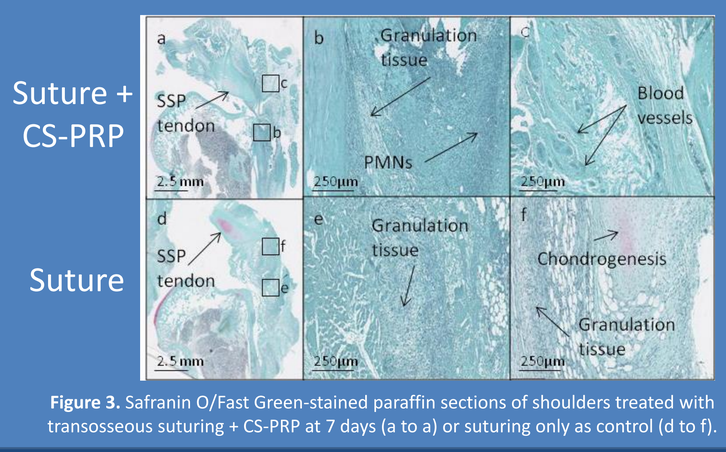

Figure 3. Safranin O/Fast Green-stained paraffin sections of shoulders treated with transosseous suturing + CS-PRP at 7 days (a to a) or suturing only as control (d to f).

4. CS-PRP improved macroscopic attachment of tendon at 2 months

-Two complete gaps and two partial gaps were present between the stump of the tendon and the humeral head surface in the control suturing group at 2 months (n=9 shoulders).
-There were no gaps in the CS-PRP treated group (n=9 shoulders) although the repair tissue was not identical to intact (n=6 shoulders).

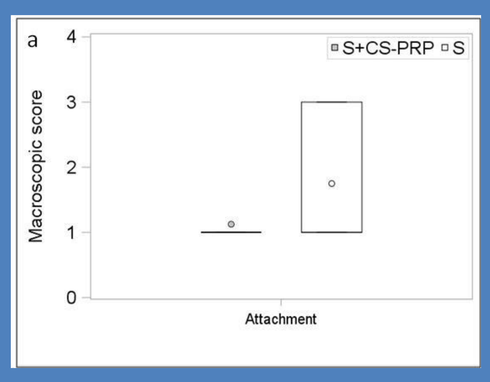

Figure 4: Tendon attachment was scored macroscopically at necropsy where 0 indicates complete attachment with structurally normal tissue and 3 indicates presence of a gap (a).

5. CS-PRP inhibited bone overgrowth into tendon tissue at 2 months

-SSP tendon structure was altered in both treated and control shoulders at 2 months compared to intact shoulders.
-SSP tissues were hypercellular and hypervascular.
-Significant bone overgrowth was observed in the SSP tendon of 4 out of 9 shoulders treated with sutures at 2 months, but was almost completely inhibited by CS-PRP treatment.
-Polymorphonuclear cells were still present in 3 out of 9 shoulders treated with suturing + CS-PRP at 2 months, suggesting that implant was not fully degraded in those animals.

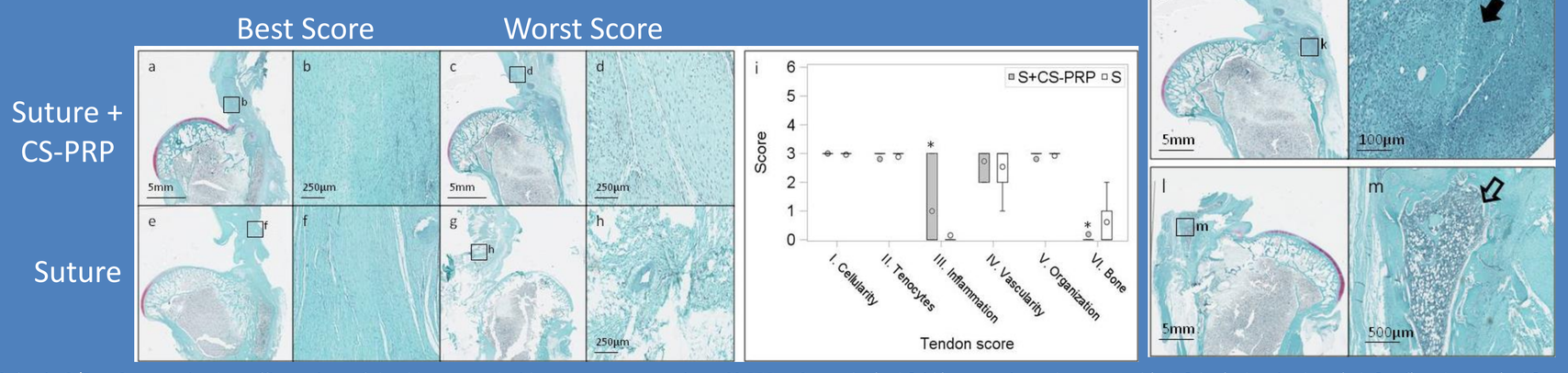

Figure 5. Safranin O/Fast Green-stained paraffin sections of shoulders treated with transosseous suturing + CS-PRP at 2 months (a-d & j-k) or suturing only as control (e-h & l-m), showing best (a-b & e-f) and worst (c-d & g-h) histological scores for tendon repair tissue. Tendon histological appearance was scored with a system based on Watkins et al (3) and Ide et al (4), where 0 represents the best score (i). Significant differences (*) were observed between treatments for presence of inflammatory cells (p=0.003) and bone overgrowth into the tendon (p=0.05). Black arrow in panel k highlights the presence of inflammatory cells in the tendon repair tissue in one CS-PRP treated shoulder at 2 months. Empty arrow in panel m highlights the presence of bone overgrowth in the tendon repair tissue in one control shoulder at 2 months.

6. CS-PRP improved microscopic attachment of tendon and structure of enthesis at 2 months

-Microscopic gaps were more frequent in the case of suturing only controls at 2 months.
-Treatment with suturing + CS-PRP improved tendon attachment, less gaps were present and the tendon was more frequently attached at the anatomically correct location.
-Fibrocartilage was present at the insertion site of the CS-PRP treated shoulders and absent in suture-only controls, where the repair tissue was usually more fibrous.
-Polarized light microscopy revealed that the entheses of CS-PRP treated shoulders were structurally similar to intact shoulders but partial restoration of the calcified interface was only achieved in one CS-PRP treated shoulder.

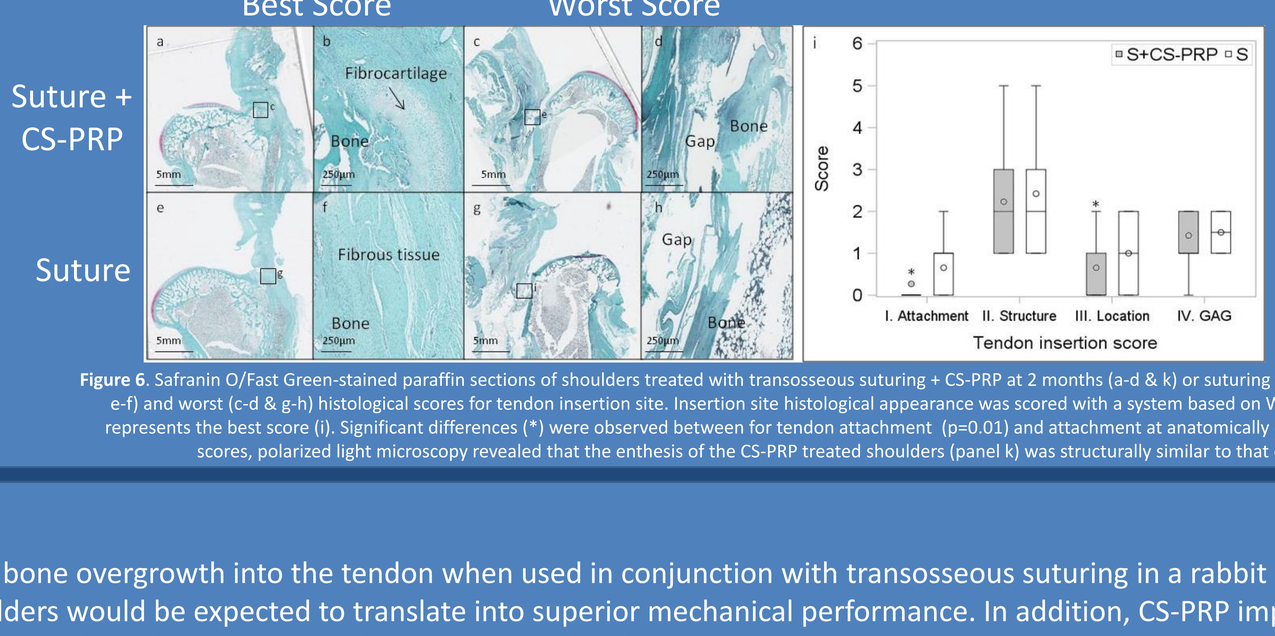

Figure 6. Safranin O/Fast Green-stained paraffin sections of shoulders treated with transosseous suturing + CS-PRP at 2 months (a-d & k) or suturing only as control (e-h & l), showing best (a-b & e-f) and worst (c-d & g-h) histological scores for tendon insertion site. Insertion site histological appearance was scored with a system based on Watkins et al (3) and Ide et al (4), where 0 represents the best score (i). Significant differences (*) were observed between for tendon attachment (p=0.01) and attachment at anatomically correct site (p=0.009). In the case of best scores, polarized light microscopy revealed that the enthesis of the CS-PRP treated shoulders (panel k) was structurally similar to that of an intact shoulder (panel j).

Conclusions:

CS-PRP implant improved SSP tendon attachment and inhibited bone overgrowth into the tendon when used in conjunction with transosseous suturing in a rabbit model. The promising histological findings at the SSP insertion of CS-PRP treated shoulders would be expected to translate into superior mechanical performance. In addition, CS-PRP implants did not induce any deleterious effects in the joint humeral head and glenoid articular surfaces (data not shown), suggesting high safety.

References:

1) Chevrier et al, 2017 J Tissue Eng Regen Med In Press 2) Trudel et al, 2010 JOR 28,6:710-5
3) Watkins et al, 1985, Am J Vet Res 46,10:2091-96 4) Ide et al, 2009 Arthroscopy 25,6:608-16

Acknowledgements:

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