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

Gabrielle Deprés-Tremblay<sup>1</sup>, Anik Chevrier<sup>2</sup> and Michael D Buschmann<sup>1, 2<sup>-</sup></sup> <sup>1</sup> Biomedical Engineering Institute and <sup>2</sup>Chemical Engineering Department, Polytechnique Montreal, Montreal, QC, Canada

**Purpose:** The purpose of the study was to examine the feasibility of applying chitosan-PRP implants in conjunction with transosseous suturing to improve rotator cuff repair compared to suturing only at 1 day, 2 weeks and 8 weeks post-surgery in a rabbit model.

**Materials and Methods:** Bilateral full-thickness tears were created in the supraspinatus tendons (SSP) of the rotator cuff of NZW rabbits (n = 4 female retired breeders). The tears were immediately repaired via a transosseous suturing technique. On the treated side, a chitosan-PRP hybrid mixture was additionally injected at the repair site. Freezedried chitosan cakes were prepared using 1% w/v chitosan (80% DDA and  $M_n$  40kDa), 1% w/v trehalose as lyoprotectant and 42.2mM calcium chloride (CaCl<sub>2</sub>) as clot activator, and solubilised with autologous PRP immediately prior to injection.

**Results:** Chitosan-PRP implants induced recruitment of polymorphonuclear cells (PMNs) to the tendon and to the muscle endomysial space from 1 day to 2 weeks post-surgery (**Figure 1**). Endochondral ossification and new bone formation were apparent at 2 weeks in the control shoulder only, close to the insertion site (**Figure 1**). At 8 weeks post-surgery, the SSP tendon insertion site in the control shoulder showed abnormal integration, with significant bone overgrowth into the tendon itself (**Figure 2**). In contrast, the superior part of the SSP enthesis in the treated shoulder had near-normal structure with a calcified interface between the tendon and the bone (**Figure 2**). Both control and treated shoulders showed hypertrophy and altered structure of the SSP tendons at 2 and 8 weeks post-surgery (**Figures 1&2**). The humeral head articular cartilage had normal appearance and showed no signs of degeneration in both control and treated shoulders (**Figure 2**).

**Conclusion**: Chitosan-PRP implants in conjunction with transosseous suturing showed promising histological findings at the SSP insertion site compared to suturing alone in this pilot feasibility study.

Keywords: chitosan, platelet-rich plasma, supraspinatus, rotator cuff repair



**Figure 1.** Safranin O/Fast Green-stained paraffin sections of rabbits treated with transosseous suturing + chitosan-PRP and sacrificed at 1 day (A-D) or 2 weeks (E-H) post-surgery and paraffin sections from a shoulder treated with suturing only at 2 weeks post-surgery (I-L). Chitosan-PRP implants induced recruitment of polymorphonuclear cells (PMNs) to the tendon and to the muscle endomysial space from 1 day to 2 weeks post-surgery (B, C, D, F, G & H), while fibroblasts were apparent in the control shoulder at 2 weeks post-surgery (K&L). Endochondral ossification and new bone formation were apparent at 2 weeks in the control shoulder only, close to the insertion site (J). Supraspinatus tendon tissue was hypertrophic and structurally abnormal in all groups (A, E & L). Outlines in A, E & L show where higher magnification images to the right were acquired.



**Figure 2.** Safranin O/Fast Green-stained paraffin sections of both shoulders from a rabbit sacrificed at 8 weeks post-surgery and treated with transosseous suturing + chitosan-PRP (A-D) or suturing only as control (E-H), and an unoperated normal shoulder (I-L). The superior part of the supraspinatus enthesis on the suturing + chitosan-PRP treated side had a calcified interface between the tendon and the bone (B), that was structurally similar to that of the unoperated normal shoulder (J). The tendon insertion site on the suturing-only control site was abnormal with significant bone overgrowth into the tendon tissue itself (F). The supraspinatus tendons on both treated and control sides were hypertrophic, hypercellular and more vascularized (C&G) than the unoperated normal shoulder (K). Humeral head articular cartilage on both sides showed no signs of degeneration (D&H) and appeared normal (L). Outlines in A, E & L show where higher magnification images to the right were acquired.