Chitosan-platelet-rich plasma implants can be injected into meniscus defects to improve repair

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Introduction: Meniscal damage is present in at least one-third of the knees of middle-aged or elderly persons[1] and untreated meniscal damage leads to radiographic signs of osteoarthritis (OA)[2] and increased degeneration over a 2 year period[3]. Since wound healing is triggered by blood clotting, the capacity for natural repair is optimal in the periphery of the meniscus, where it is vascularised, and diminished in the inner margins. Only a small portion of all meniscal tears are thus considered repairable so that current surgical treatment often involves partial removal of torn meniscus which increases the risk for developing OA[4]. Orthopaedic surgeons are currently injecting autologous platelet-rich plasma (PRP) to treat different conditions[5]. Injectable coagulating implants composed of freeze-dried chitosan solubilized in PRP have previously been developed and have been shown to be more biologically potent than PRP alone and to have tissue regeneration capacity *in vivo*[6]. These chitosan-PRP implants are expected to improve meniscus repair by providing high concentrations of platelet-derived growth factors to the implantation site and by inducing cell recruitment and angiogenesis. The purpose of this study was to investigate whether chitosan-PRP implants can improve repair in a sheep meniscus defect model.

Methods: Bilateral 10-mm long full-thickness tears were created in the anterior portion of the medial menisci in 7 skeletally mature (2-6 years) Texel-Cross sheep (Fig 1). A ~ 1.5 cm arthrotomy allowed access enough to create the tears at ~1/3rd the length between the capsular and free borders (schematized in yellow in Fig 1b), in the red-white zone. The tears were then rasped with Kelley forceps. Freeze-dried chitosan cakes (containing 1 mL chitosan formulation, a lyoprotectant and calcium chloride as clot activator, see Fig 1a) were solubilized with 1 mL autologous PRP and 0.5 mL of each solubilized chitosan-PRP formulation was injected into the tears through two trephination channels created with 18-gauge needles (schematized in green in b). The tears were sutured in a horizontal mattress pattern using 3-0 polypropylene sutures 5 minutes after injection (schematized in red in b). Controls were 0.5 mL of autologous PRP recalcified with 42.2 mM calcium chloride. The menisci were macroscopically and histologically assessed.

Results: Chitosan-PRP implants were partly resident in the meniscal tears and trephination channels at 1 day post-surgery, even without any post-operative immobilization. The tears were macroscopically visible at the time of necropsy and the edges of the tears were usually well apposed. A reddish repair tissue and signs of neo-vascularization were visible in one chitosan-PRP treated knee at 3 months. The chitosan-PRP implants induced cell recruitment from the vascularized periphery of the menisci towards the trephination channels (Fig 2a-d). A highly cellular repair tissue was seen in one chitosan-PRP tear at 3 weeks post-surgery (Fig 2e-f). Partial integration between the repair tissue and the original meniscal tissue was achieved in this treated knee. Complete healing with a highly vascularized repair tissue and seamless repair tissue integration was seen in one chitosan-PRP treated tear at 3 months (Fig 2m-n). There was no repair tissue synthesis in the PRP controls (Fig 2 i-j and Fig 2o-p).

Discussion: The meniscus tear model chosen was challenging since it could contain only a limited volume of the implant and was a bilateral model that did not permit the animal to protect the treated knee from weight-bearing post-operatively. The variability that we observed in the healing response could have resulted from implant loss at early time points post-operative. Improving the residency of the chitosan-PRP implants would be expected to induce a more reproducible healing response. Even with these limitations, we found that chitosan-PRP implants induced cell migration and repair tissue synthesis in meniscus while PRP alone did not. Chitosan-PRP implants have several features that reveal a greater potential than PRP alone to improve meniscus repair.

Significance: Surgical techniques to adequately repair tears located in avascular regions of the meniscus are currently lacking. Injectable implants composed of freeze-dried chitosan solubilized in autologous PRP have the potential to improve current surgical repair techniques by inducing cell migration, angiogenesis, tissue synthesis and remodeling/integration.

References: 1) Englund M et al. 2008. New England Journal of Medicine. 359: 1108-1115. 2) Englund M et al. 2009. Arthritis and Rheumatism. 60: 831-839. 3) Zanetti M et al. 2005. Radiology. 237: 993-997. 4) Cicuttini FM et al. 2002. Journal of Rheumatology. 29: 1954-1956. 5) Lopez-Vidriero E et al. 2010. Arthroscopy. 26: 269-278. 6) Chevrier A et al. 2015. Transactions International Cartilage Repair Society.

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a **Meniscus repair study (n = 7 sheep) with freeze-dried chitosan-PRP implants**

CS DDA (%)	CS M_n (kDa)	CS % (w/vol)	HCl (mM)	Trehalose (mM)	$CaCl_2$ (mM)	Number of knees
80	36	1	29	26	42.2	n = 1 at 1 day and n =2 at 3 weeks and 3 months
85	43	1	31	26	42.2	n = 1 at 1 day and n =2 at 3 weeks and 3 months
0	0	0	0	0	42.2	PRP-only controls: n = 2 at 3 weeks and 3 months

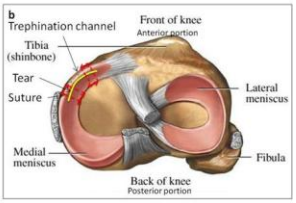

Figure 1. Study design.

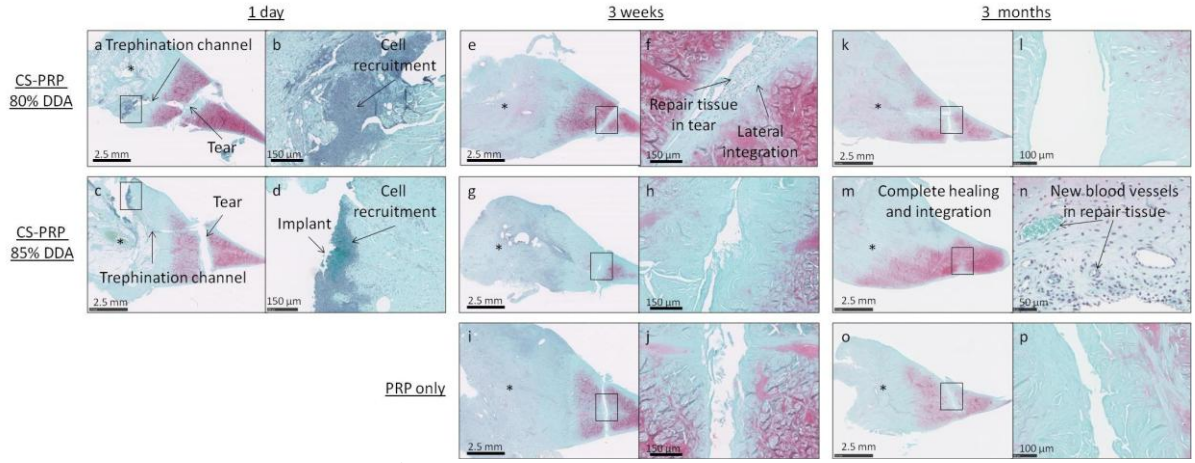

Figure 2. Histological appearance of meniscal tissues 1 day (a-d), 3 weeks (e-j) and 3 months (k-p) post-surgery.