Freeze-dried chitosan-PRP implants improve meniscus repair in an ovine model

I. Purpose
Menisci are structurally complex and play an essential weight-bearing role in the knee joint. Although there has been a recent increase in the number of meniscus repairs performed yearly in the US [1], only a small number of all meniscal tears are repairable so that current surgical treatment of meniscal tears often involves partial meniscectomy which increases the risk of developing osteoarthritis (OA) [2]. Trephination and meniscus wrapping techniques have had some success in pre-clinical models [3] and in clinical studies [4] to augment the rate of healing for complex tears [5]. We have developed a freeze-dried chitosan polymer that can be resuspended in PRP to produce a homogenous mixture that coagulates once implanted and sustains recruitment of host cells to the site. The purpose of this study was to investigate whether healing of ovine meniscus tears can be repaired by applying chitosan-PRP implants and/or wrapping the meniscus with a collagen membrane.

II. Methods
- Formulations containing 1% (w/v) chitosan (80% DDA and Mr, 40 kDa), 1% (w/v) trehalose (lyoprotectant) and 42.2 mM calcium chloride ( clot activator) were freeze-dried and solubilized in autologous PRP prior to application.
- Sodium citrate anti-coagulated whole blood was collected from sheep and a two-step centrifugation process was used to extract leukocyte-platelet-rich plasma (L-PRP).
- Chitosan-PRP implants were injected into the defects via 2 trephination channels and the tears were sutured. A portion of a Chondro-Gide collagen membrane was wrapped around the meniscus and sutured. 0.5 mL chitosan-PRP mixture was injected under the membrane. Tissue repair was assessed at 6 weeks post-surgery.

III. Meniscus repair study design
- A surgical-induced 10 mm longitudinal tear with a horizontal incision was created unilaterally at the anterior portion of the medial meniscus in 6 sheep (Fig. 1).
- Experimental groups include: chitosan-PRP only (n = 2 knees), chitosan-PRP + wrap (n = 2 knees) or wrap only (n = 2 knees). The contralateral knees were left intact (n = 6 knees) (Fig. 2).
- The repair tissue was highly cellular and well integrated to surrounding host meniscus (Fig. 4 a to d).
- Structural integrity was different in matching areas in intact meniscus (Fig. 4 e & h).
- There was no healing in the meniscus treated with wrapping alone (Fig. 4 e & f).
- Significant cell infiltration was observed at the outer portion of all torn and treated menisci (Fig. 4, compare a, c, e, vs g).
- More suture tracks were present in menisci treated with the wrapping technique (arrows, Fig. 4 c & e versus a).

IV. Macroscopic appearance of meniscal tears
- The tears were macroscopically visible at 6 weeks.
- The edges of the tears were well approximated.
- Sutures were apparent in all treatment groups.
- A reddish repair tissue and signs of neo-vascularization were visible in one CS-PRP treated meniscus at 6 weeks (Fig. 3 a).

V. Microscopic assessment of repair tissues
- Complete healing and seamless integration were observed in one chitosan-PRP treated tear (Fig. 4 a & b), while there was partial healing in one tear treated with chitosan-PRP and wrapping (Fig. 4 c & d).
- The repair tissue was highly cellular and well integrated to surrounding host meniscus (Fig. 4 a to d).
- Structural integrity was different in matching areas in intact meniscus (Fig. 4 e & h).
- There was no healing in the meniscus treated with wrapping alone (Fig. 4 e & f).
- Significant cell infiltration was observed at the outer portion of all torn and treated menisci (Fig. 4, compare a, c, e, vs g).
- More suture tracks were present in menisci treated with the wrapping technique (arrows, Fig. 4 c & e versus a).

VI. Freeze-dried chitosan-PRP implants did not induce deleterious effects in the adjacent joint tissues
- There was a mild to moderate synovitis in most treated knees, when compared to contralateral menisci at 6 weeks. Changes include intimal hyperplasia and an increase in synovial vascularization (Fig. 5 a to c).
- Mild changes to the articular surfaces were visible in all treated and intact knees at 6 weeks. Histological changes included a loss of glycosaminoglycan (GAG) and some structural abnormalities (Fig. 5 d to f).
- Electromechanical properties of the tibial plateau and the distal femurs were mapped across the articular surfaces using the Arthro-BST device. The average quantitative parameters (QP) calculated for the medial femoral condyle and for the tibial plateau were similar in all treatment groups and in the contralateral intact menisci, suggesting the surgically induced meniscus tear does not cause severe cartilage degeneration within 6 weeks (Fig. 5 g & h).

VII. Conclusion
Chitosan-PRP implants showed evidence of a superior regenerative effect compared to wrapping the meniscus with a collagen membrane. Using the wrap in conjunction with chitosan-PRP implants did not further improve repair and the additional sutures needed to secure the wrap created significant damage to the menisci. This suggests that chitosan-PRP implants by themselves could be sufficient in overcoming the current limitations of meniscus repair. Further study is certainly required before we would recommend using this clinically. One limitation in the current study is that no biomechanical testing of strength or function of the repaired meniscus was done.

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References