Synovial membrane responses to CTGF/TGFβ3-polymeric meniscal scaffold transplantation in an ovine model

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Introduction: Due to the well-established risk of osteoarthritis (OA) following meniscectomy, scaffolds are being developed for meniscus replacement. In a previous study, we demonstrated that a 3D-printed meniscal scaffold loaded with recombinant human connective tissue growth factor (CTGF) and transforming growth factor-β3 (TGFβ3) achieved meniscal regeneration.[1] The synovial membrane may play a key role in this regeneration, as the host cells that populate the meniscal allograft likely originate in the synovium. Additionally, synovial blood vessels may accommodate metabolic needs of the regenerating meniscus. However, the effects of the meniscal scaffold material and growth factors on synovial membrane homeostasis are unknown. Thus, the purpose of this study was to evaluate synovial membrane homeostasis following CTGF/TGFβ3-containing meniscus scaffold transplantation in sheep.

Methods: All study procedures were approved by Cornell Institutional Animal Care and Use Committee. Study design: Anatomically shaped ovine meniscus scaffolds were fabricated with poly-ε-caprolactone (PCL) (100 µm fibers; 100~200 µm channels). Skeletally mature sheep (n=23; 2-4 yrs of age) were randomly allocated to three groups: 1) scaffold with CTGF (5µg) + TGFβ3 (5µg) (n=10); 2) scaffold with CTGF (5µg) + TGFβ3 (10µg) (n=7); 3) scaffold without growth factor (n=6). Unoperated contralateral limbs served as controls. Surgical procedure: Sheep were operated unilaterally. A medial parapatellar arthrotomy and medial femoral condylectomy were used to expose the medial meniscus. The medial meniscus was removed, and replaced with a scaffold secured to the surrounding joint capsule with 2-0 ethibond sutures. The condylectomy was repaired with 2 lag screws and the joint was closed. Sheep were confined to pen exercise until radiographic healing of the condylectomy (approximately 2 months) and then allowed free paddock exercise. Animals were euthanized at 6 or 12 months post-op. Histology: The knee joints were dissected and synovial tissue was removed, processed for histology, and stained with hematoxylin and eosin. Two blinded observers performed histological scoring (TU, YN) using the OARSI histopathologic synovial scoring system[2] **(Table 1)**. Each sample was scored in four different locations using both 10x and 20x magnification and the average total score was recorded. Statistical analysis: An independent samples Kruskal-Wallis test was used to compare the four groups, followed by post-hoc Dunn's multiple comparison tests with Bonferroni correction. Intraclass correlation coefficients (ICC) were used to assess inter-rater reliability of histological scores. P-values less than 0.05 were considered significant.

Results: Synovial samples from each treatment group demonstrated occasional lymphocytes in the absence of plasma cells or neutrophils. Numerous peripheral vascular structures were present in the treatment groups, along with increased collagen compared to control samples (**Fig 1**). There was a significant difference between the four groups (5µg TGFβ3, 10µg TGFβ3, 0 growth factor, and control; $p<0.001$). There was a higher (more abnormal) histological score for each of the three treatment groups compared to the control group (0 growth factor vs. control, $p=0.022$; 5µg TGFβ3 vs. control, $p=0.005$; 10µg TGFβ3 vs. control, $p<0.001$) (**Figs 2 & 3**). There was no significant difference in average histological score between the three scaffold groups. There was near-perfect inter-rater reliability for each histological score component and total histological score according to the Landis & Koch (1977)[3] criteria (**Table 2**).

Discussion: The lack of difference in histological score between synovial membranes of the three treatment groups suggests that the addition of CTGF and TGFβ3 to the scaffold does not incite an inflammatory or fibrous response compared to the 0 growth factor meniscal scaffold. Rather, the presence of occasional lymphocytes is consistent with a response to surgical intervention as opposed to what would be seen with chronic synovitis. Chronic synovitis would appear as accumulation of lymphoid follicles, synovial tissue proliferation and perivascular cuffing of cells (**Fig 4**). Study limitations of this large animal model study include relatively small sample size and lack of sham surgery controls.

Significance: The lack of synovial inflammation or chronic synovitis present in the synovial membrane samples from these joints indicates that meniscal transplantation using PCL, CTGF, and TGF-B3 does not incite an excessive inflammatory or fibrotic response in the synovium.

References: 1. Chang LH *et al*. Science Med Translational, 2014; 2. Little CB *et al*. Osteoarthritis Cartilage, 2010; 3. Landis JR & Koch GG. Biometrics, 1977.

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Figures and Tables:

Figure 1. Representative histological appearance of the three scaffold + treatment groups. Black arrows=vascular structures; blue arrows=fibroblasts; yellow arrows=lymphocytes. H&E stain.

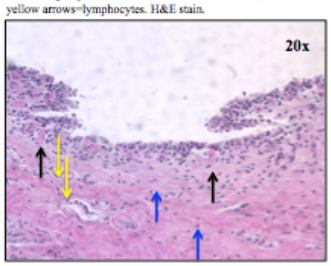

Figure 2. All three treatment groups (5µg TGFβ3, 10µg TGFβ3 and 0 growth factor) demonstrated significantly higher average histological scores compared to the control group. There was no difference between the three treatment groups.

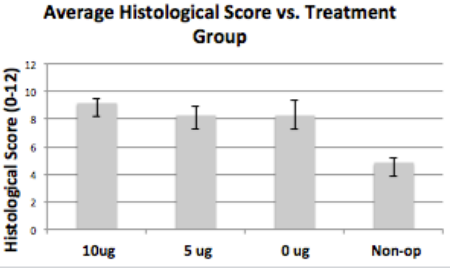

Figure 3. Synovial membrane samples demonstrating similar morphology and cellular architecture between the three scaffold + treatment groups. H&E stain.

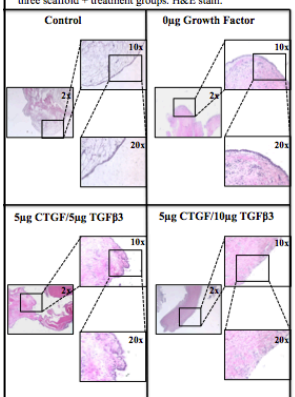

Figure 4. Synovial membrane with chronic synovitis. There is formation of lymphoid follicles, synovial tissue proliferation and perivascular cuffing. H&E stain.

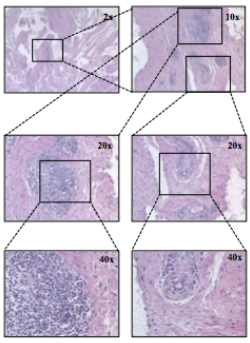

Table 1. Microscopic scoring system.

Criteria	Score	Observation
Intimal hyperplasia	0	Normal (one cell deep only)
	1	Mild, focal (2- 4 cells deep, and <20% area)
	2	Mild diffuse (2- 4 cells deep, and >20% area) or Moderate focal (five or more cells deep, and <20% area)
	3	Moderate diffuse (five or more cells deep, and >20% area)
Inflammatory cell (lymphocytic/plasmocytic) infiltration	0	Normal (occasional cell)
	1	Mild or focal infiltration, no lymphoid aggregates
	2	Moderate diffuse infiltration, no lymphoid aggregates
	3	Marked discreet lymphoid aggregates
Sub-intimal fibrosis (loose connective tissue areas only)	0	None
	1	Light, focal (<20% area) collagen staining
	2	Heavy focal (<20% area) or slight diffuse collagen staining
	3	Heavy diffuse collagenous staining
Vascularity	0	0-2 vascular elements per 100× field
	1	3-4 vascular elements per 100× field
	2	5-8 vascular elements per 100× field
	3	More than eight vascular elements per 100× field
Aggregate score (joint)	0-12	Sum of the scores obtained for the four criteria above

Table 2. Inter-rater reliability for histological scores was near-perfect.

Criteria	Intraclass Correlation Coefficient (ICC)	95% CI
Intimal hyperplasia	0.812	0.653-0.898
Inflammatory cell	0.784	0.606-0.882
Sub-intimal fibrosis	0.774	0.584-0.877
Vascularity	0.757	0.541-0.869
Aggregate score (joint)	0.899	0.815-0.945