Development of a Tissue Engineered Neoligament for Intra-Articular Ligament Regeneration

Joshua A Parry, Yan Su, Eric Wagner, Mahrokh Dadsetan, Steven C. Chase, Scott Riester, Michael Yaszemski, Andre Van Wijnen, Sanjeev Kakar
Mayo Clinic, Rochester, MN

Statement of Purpose: Intra-articular ligament injuries often lead to degenerative joint disease whether they are treated or not. Current methods of intra-articular ligament reconstruction do not restore the stability of the native ligament increasing the risk of osteoarthritis development.[1] We propose a novel method of combining a poly ε-caprolactone fumarate (PCLF) porous scaffold with the functional strength of suture and seeded with adipose derived mesenchymal stem cells (AMSCs) to restore native ligamentous tissue and its bony insertion. We have previously demonstrated AMSCs are able to attach, grow, proliferate, and remain viable on PCLF for over two weeks in cell culture dishes. With similar biomechanical properties to ligaments, this scaffold-suture complex can act as a foundation for cells to proliferate and regenerate native ligamentous tissue along with the bone-soft tissue interface.

Methods: PCLF was synthesized using the method previously described by our lab.[2] Scaffolds were created by injecting liquid-state PCLF over three dimensional (3D) molds designed with Solidworks CAD software and printed using the SolidScape 3D printer. Before injection two strands of 0-Ethibond suture were threaded through the center of the mold allowing for its incorporation with the polymer. After the scaffolds were cured with ultraviolet crosslinking the molds were degraded in a mixture of acetone and methanol. The scaffolds were seeded with AMSCs in a rotating bioreactor for 3 days followed by static culture. At day 7 and 14 of culture live/dead staining and MTS cell proliferation assays were performed.

The scaffold-suture complex was used to reconstruct the anterior cruciate ligament (ACL) in cadaveric rabbit knees for biomechanical testing. Before undergoing load to failure all ligaments except the ACL were removed. Tested groups included the intact ACL, semitendinosus autograft, and scaffold-suture reconstruction. Load to failure testing was performed with a MTS testing machine. To determine the effect of fatigue on the scaffold after reconstruction one group was subjected to 5,000 cycles of knee flexion before load to failure testing.

Results: PCLF scaffolds with 750 μm pores were produced as shown in figure 1. Theoretic porosity was 42%. At day 7 and 12 of culture cells remained viable and continued to proliferate on the scaffolds as measured by live/dead staining (figure 2) and MTS assay.

Biomechanical testing of the scaffolds, shown in figure 3, demonstrates that the scaffold-suture complex is weaker than native ligament but stronger than the semitendinosus autograft reconstruction. After 5,000 cycles of knee flexion the scaffold remained intact with similar biomechanical properties. Both the scaffold-suture complex and the tendon graft demonstrated a greater amount of stretch at failure compared to native ACL.

Figure 1. PCLF scaffold-suture complex. (Left) ACL reconstruction before load to failure testing. (Upper Right) Microscopic cross sectional view. (Lower Right) Gross view with suture buttons used in fixation.

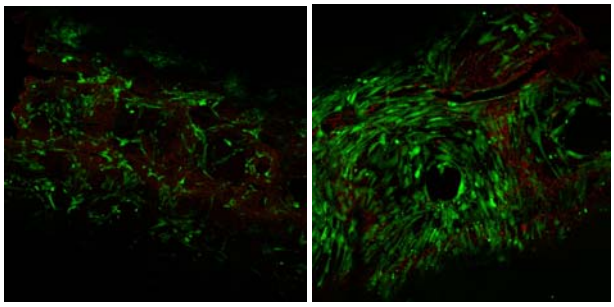

Figure 2. Live dead staining. (A) Day 7 (B) Day 12

	Load to Failure (N)	Stretch at Failure (mm)
ACL 1	329	2.2
ACL 2	376	3.3
Tendon Autograft	35	10
Scaffold 1	80	7.6
Scaffold 2	96	9.3
Scaffold 3	62	10.3
Scaffold After Fatigue Testing	49	9.9

Figure 3. Mechanical testing of native ligament, tendon autograft, and PCLF scaffold-suture complex

Conclusions: AMSCs remain viable and proliferate on the PCLF scaffold-suture complex for up to 2 weeks. When compared to tendon autograft this construct represents a promising scaffold for ligament regeneration. Future studies will demonstrate its performance in a small animal model.

References:

1. Kannus. Clin Rheum, 1989. 8(2):251-60
2. Jabbari, et al. Biomacro. 2005. 6(5):2503-11