Cell-free Biodegradable Synthetic Artificial Ligament for the Reconstruction of Anterior Cruciate Ligament (ACL) in a Rat Model

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Introduction: Traditional ACL reconstruction is usually performed using an allograft or autograft, which has numerous limitations including donor site morbidity, decreased range of motion, and potential infection, which often leads to an extended period of rehabilitation (1). Moreover, the increasing demand for allograft tissues could result in a shortage of grafts available from the donor pool. Although artificial grafts have become an attractive alternative to biological grafts over the last two decades; the use of non-degradable artificial ligaments have not been popular because they have many limitations including the development of synovitis and chronic inflammatory reactions that ultimately lead to poor ligamentization and a high failure rate (2, 3). Therefore, the development of a new biodegradable synthetic graft could represent an important alternative for ACL reconstruction. We report here on the generation of a biodegradable scaffold for ACL reconstruction using a wet electrospinning technique in which fibers of a biodegradable elastomer, poly (ester urethane) urea (PEUU)(4), were concurrently deposited with electrosprayed serum-based culture medium. We hypothesized that this new artificial ligament could eliminate the need for an auto/allograft and improve functional outcomes after ACL surgery by cellular infiltration and good ligamentization.

Methods: All animal procedures were approved by the University of Pittsburgh’s Institutional Animal Care and Use Committee.
1: Synthetic tissue engineered graft fabrication:
PEUU was synthesized from polycaprolactone diol (PCL), 1, 4-diisocyanatobutane (BDI) and putrescine at a molar ratio of PCL:BDI:putrescine 1:2:1 according to methods previously reported (4). For the current study, a wet electrospun PEUU was fabricated by a combination of electrospinning and electrospraying(4). Briefly, cell culture medium (DMEM with 10%FBS, and 1%penicillin/streptomycin) was charged at 7 kV and suspended 4.5 cm above the target mandrel. Concurrently, PEUU in hexafluoroisopropanol solution (12%, w/v) was charged at 10 kV and perpendicularly located 20 cm from the target mandrel. The mandrel was charged at -4 kV and rotated at 250 rpm. (Fig. 1). As controls, a dry electrospun PEUU sheet and a dry electrospun PCL dissolved in HFIP (12% w/v)) sheet were prepared using only polymer electrospinning using the same parameters described above.
2: Animal model of ACL reconstruction and experimental groups:
A reproducible model of ACL reconstruction was created in female Sprague-Dawley rats according to a previous report (5). We established four groups: 1) Wet PEUU artificial ligament (wet PEUU), 2) Dry PEUU artificial ligament (dry PEUU), 3) Dry PCL artificial ligament (dry PCL), and 4) autologous flexor digitorum longus tendon (Tendon). The PCL prosthesis was chosen as a control in the study because PCL is widely used in commercially and PEUU is made from PCL (6). (n=20 in each group)

Results: In vitro material characteristics:
The wet PEUU fibers qualitatively exhibited a greater degree of looping and more tortuosity. In vitro biaxial mechanical property measurements demonstrated that the PCL material was largely isotropic, and very stiff. In contrast, dry and wet PEUU grafts were significantly more compliant in both circumferential and longitudinal axes. (Fig. 2)

In vivo biomechanical evaluation:
The biomechanical evaluation by failure load of tensile test demonstrated that biomechanical strength was significantly higher in the artificial ligament groups than in the control tendon group at 4 weeks and was significantly greater in the wet PEUU group than in all the other groups at 8 weeks. (Fig. 3)

Histological evaluation:
Under light microscopy with Masson’s trichrome staining, the oblique tendon fibers binding to the bone were readily observed in the autologous tendon group. On the other hand, all of the prosthetic materials showed layered fibrous tissue surrounding the materials. Almost no tissue ingrowth was observed in the PCL and dry PEUU, while for wet PEUU polymer degradation was accompanied by collagenous fiber deposition at week 4.

Immunohistochemical staining:
Immunostaining for rat isolectin B4 and DAPI for each of the implanted synthetic grafts and tendon at week 4 revealed cellular and blood vessel infiltration had occurred in the wet PEUU. On the other hand, no cellular infiltration was noted in the dry PEUU and PCL groups. (Fig. 4) The number of COL3A1 and αSMA positive cells at week 4 and 8 was significantly greater in the tendon group compared to the artificial ligament group. A cellular infiltrate possessing contractile smooth muscle cell markers was observed in the wet PEUU group. Control implants of dry PEUU and PCL did not experience substantial cellular infiltration.

Inflammatory response:
To investigate the distribution of inflammatory cells around the tendon-bone sites, immunohistochemistry for polyclonal CD68, known as a marker for macrophages, was performed. The density of CD68+ cells and rat-specific granulocytes were significantly greater in the PCL and dry PEUU groups than the wet PEUU and tendon groups.

Bone tunnel evaluation:
Micro-computed tomography (μCT) performed at 4 and 8 weeks revealed that bone tunnel healing was significantly greater (i.e. smaller tunnels) in the tendon and wet PEUU groups. On the other hand, the other two artificial ligament groups showed slight bone tunnel enlargement.

Discussion: We demonstrated that the wet PEUU grafts exhibited a striking therapeutic effect for rat ACL reconstruction by promoting cellular and blood vessel infiltration, alleviating inflammation and increasing biomechanical strength. Control implants of dry PEUU and PCL did not experience substantial cellular infiltration and did not exhibit native mechanical properties. The role of biomaterial mechanical properties in the ACL ligament is well recognized in a general sense, with weak materials being
associated with a risk for re-rupture and materials that are too stiff being associated with patient discomfort and a limitation in range of motion. The degree to which these factors come into consideration varies with the extent of the repair being considered (7). Wet PEUU is designed to adequately function throughout a period of tissue ingrowth and scaffold remodeling and resulted in the creation of tissues that closely resembled native tissue (8) and represents a regenerative approach likely to reduce complications seen with current replacement materials used for ACL reconstruction.

**Significance:** Our wet electrospinning technique with artificial ligament could readily be exploited for ACL reconstruction without using allograft or autograft, leading to cellular infiltration and enhanced biomechanical strength.

**Figure 2**

**Electron microscopic observation**

![Electron microscopic observation](image)

**Biomechanical stress-stretch curves**

![Biomechanical stress-stretch curves](image)
Figure 1

A

12% PEUU in HFIP 1.5mL/hr

Cell culture medium (0.2mL/min)

B

C

Figure 3

4 Week

Load to failure (N)

35

30

25

20

wet PEUU
dry PEUU
PCL
Tendon

8 Week

Load to failure (N)

35

30

25

20

wet PEUU
dry PEUU
PCL
Tendon

Figure 4

wet PEUU

dry PEUU

PCL

Tendon

Green: Isolectin B4, Blue: DAPI, Bar: 100μm

**: artificial ligament and graft side

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