Biomechanical Comparison of Supercritical CO2 Treated and Gamma Irradiated Tendon Allografts with Autograft

INTRODUCTION
Supercritical carbon dioxide (ScCO2) has been proposed as an alternative to gamma irradiation for the terminal sterilization of musculoskeletal allografts. Gamma irradiation is perceived as having a deleterious effect on the biomechanical properties of allograft tissue, while limited evidence is available on the biomechanical impact of ScCO2. The objective of this in vivo cranial cruciate ligament (CCL; analogous to human ACL) replacement study was to compare autografts to gamma irradiated and ScCO2-treated allografts in a sheep model. We hypothesized that the biomechanical properties of the ScCO2 treated allografts would exceed that of the gamma irradiated allografts and perform similarly to autografts.

METHODS
Thirty-six skeletally mature ewes underwent CCL replacement with either a ScCO2-treated allograft (n=18), gamma irradiated allograft (n=9), or autograft control (n=9). Animals were assigned to one of three post-operative time groups (6, 12 and 24 wks). Lateral digital extensors (LDEs) were procured for both allograft and autograft use. Allograft tendons were sterilized using either the ScCO2 process for four hours (Nova2200; NovaSterilis, Inc.) or gamma irradiation (2.0-2.5 Mrad dosage). All tendons were frozen until implantation. Once the CCL was removed, a double-bundle LDE (auto/allo)-graft was inserted into femoral and tibial tunnels oriented along the CCL’s footprints and secured with an Endobutton and interference screw (Arthrex), respectively. Animals were allowed unrestricted movement post-op. At 6, 12 or 24 wks, animals were euthanized and intact knees were retrieved and prepared for either biomechanical testing or histology.

Knees were frozen in full extension for sectioning. The visible graft fixation were used as a guide to cut the knee on a band saw, resulting in a section that contained a femur bone block, tibia bone block, and the graft all in a planar orientation (Fig 1). Sections were cut to a 15-18mm thickness. Samples were stored at -70°C until testing.

Prior to testing, samples were thawed in ambient saline for at least twenty minutes. Extrinsic soft tissues were removed until only the bone-ligament graft complex remained. The bone blocks were potted in fiberglass resin and installed in the testing system (E3000; Instron) so that the graft was aligned with the loading axis of the machine. Specimens were preloaded to 5N and then anterior-posterior and medial-lateral graft dimensions were measured using calipers. Displacement and strain were set to zero prior to beginning the test. The specimen was subjected to 12000 cycles at 2 Hz of a predetermined subfailure level displacement, then held at the maximum displacement for 10 min, and finally stretched to failure at a rate of 300 mm/min. Specimens were kept moist throughout testing with loosely draped saline soaked gauze.

The subfailure displacement target for each specimen type was determined from the literature [2-4]. Average failure loads and stiffnesses from similar studies were combined, and it was assumed that the CCL is subjected to 20% of the failure load during activities of daily living [5]. This yielded a corresponding displacement target for each time-group of specimens. The resulting target displacement (6 wks=0.7 mm, 12 wks=1.0 mm, 24 wks=1.4 mm) was used as the control parameter for the cyclic portion of the test. Fatigue resistance is the ratio of the mean load of the final cycle to the mean load of the first cycle. Failure load is the maximum load measured during the failure portion of the test.

The graft testing order was randomized and the graft treatment was blinded; only healing time was known for each graft so that it could be subjected to the appropriate displacement. A one-way ANOVA was performed on the measured data, and Student’s t-tests were performed on the measures that showed a significant difference.

RESULTS
All specimens failed at the tissue midsubstance. Fatigue resistance was reduced with time, but was not significantly different between treatments within any of the time groups (p>0.05). ScCO2-treated graft failure loads were significantly lower than autograft at 6 and 24 weeks (p=0.002). ScCO2 and gamma irradiated grafts were not significantly different from one another in any measure (failure extension, initial stiffness, failure stiffness, and relaxation load decrease).

DISCUSSION
While failure properties are traditionally the sole measure used to evaluate auto/allo- graft performance, subfailure cyclic behaviors are likely more characteristic of tissue function during activities of daily living. Our results show that there are no biomechanical differences between ScCO2-sterilized allografts, gamma irradiated allografts, and autografts subjected to a physiologic-based subfailure regimen. Both allografts illustrated biomechanical improvements over time that were comparable to those of autograft, suggesting that the allografts were similarly healed and remodeled (histological analyses are currently underway). However, our failure data shows that autograft ultimate load is superior to that of ScCO2-sterilized allograft after prolonged healing and suggests that autograft load capacity is restored more rapidly than either allograft. Still, ScCO2-sterilized allografts were shown to be biomechanically equivalent to gamma irradiated allografts throughout the functional range tested, which encourages further exploration of this new technology as an alternative method for allograft sterilization.

REFERENCES

Figure 1: Schematic of knee sectioning along graft plane [1].

Figure 2: Fatigue resistance (mean ± SD). Columns represent n=2 for Auto and Gamma and n=4 for ScCO2.

Figure 3: Failure load (mean ± SD; *p=0.002). Columns represent n=2 for Auto and Gamma and n=4 for ScCO2.