# The Development of a Xenograft-Derived Scaffold for Tendon and Ligament Reconstruction Using a Decellularization and Oxidation Protocol

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**Purpose:** To evaluate the biological, immunological, and biomechanical properties of a scaffold derived by architectural modification of a fresh-frozen porcine patella tendon using a decellularization protocol that combines physical, chemical, and enzymatic modalities. **Methods:** Porcine patellar tendons were processed using a decellularization and oxidation protocol that combines physical, chemical, and enzymatic modalities. Scaffolds (n = 88) were compared with native tendons (n = 70) using histologic, structural (scanning electron microscopy, porosimetry, and tensile testing), biochemical (mass spectrometry, peracetic acid reduction, DNA quantification, alpha-galactosidase [α-gal] content), as well as in vitro immunologic (cytocompatibility, cytokine induction) and in vivo immunologic nonhuman primate analyses. **Results:** A decrease in cellularity based on histology and a significant decrease in DNA content were observed in the scaffolds compared with the native tendon ($P < .001$). Porosity and pore size were increased significantly ($P < .001$). Scaffolds were cytocompatible in vitro. There was no difference between native tendons and scaffolds when comparing ultimate tensile load, stiffness, and elastic modulus. The α-gal xenoantigen level was significantly lower in the decellularized scaffold group compared with fresh-frozen, nondecellularized tissue ($P < .001$). The in vivo immunological response to implanted scaffolds measured by tumor necrosis factor-α and interleukin-6 levels was significantly ($P < .001$) reduced compared with untreated controls in vitro. These results were confirmed by an attenuated response to scaffolds in vivo after implantation in a nonhuman primate model. **Conclusions:** Porcine tendon was processed via a method of decellularization and oxidation to produce a scaffold that possessed significantly less inflammatory potential than a native tendon, was biocompatible in vitro, of increased porosity, and with significantly reduced amounts of α-gal epitope while retaining tensile properties. **Clinical Relevance:** Porcine-derived scaffolds may provide a readily available source of material for musculoskeletal reconstruction and repair while eliminating concerns regarding disease transmission and the morbidity of autologous harvest.

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Allograft tissue is commonly used in musculoskeletal surgical procedures, and its popularity has grown rapidly, exceeding the use of autograft tissue in many countries. Despite improvements in safety standards for allograft tissue donation, procurement, testing, processing, preservation, storage, and distribution, the risk of disease and malignancy transmission via transplantation of allograft tissues cannot be completely excluded and such an event may cause a potentially life-threatening complication.[1-3]

One approach to eliminate the risk of disease transmission, autograft donor morbidity, and allograft availability is xenotransplantation, the transplantation of nonhuman animal tissues into a human recipient.[4,5] The potential advantages of xenograft tissue include a readily available source of tissue free of donor site morbidity, decreased disease transmission, increased homogeneity in tissue material and physical properties, and decreased surgical time. Recently, the potentially infinite availability of xenografts from the agricultural industry has been the basis for the rapidly developing xenograft market with porcine and bovine-derived grafts being the most relevant xenograft tissues for orthopaedic procedures due to similarities in size and biomechanical properties.[6] However, many studies have shown that xenogeneic tissues express superficial epitopes such as the alpha-galactosidase (α-gal) epitope (Galα1-3Galβ1-4GlcNAc-R). The α-gal epitope interacts with the natural human anti-Gal antibody and causes a complement-mediated as well as a direct T-cell-mediated immune response, resulting in inflammation and rejection.[7,8] Careful breeding of animals free of specific pathogens may make xenotransplantation safer in some respects than human allotransplant donors; however, the potential for transmission of pathogens (viral infection, prion-mediated infection, and bacterial infection) from the xenograft donor to the human recipient exists.[4,5]

Issues of safe and readily available graft material are a particular challenge for traumatic injuries to the musculoskeletal system and complex reconstructions in musculoskeletal oncology.[9,10] A readily available animal source for xenotransplantation may represent a more effective approach to the treatment of these injuries than traditional methods. Thus, approaches to produce xenografts free of infectious and inflammatory material have potential benefits both now and in the future.

We have previously described the processing of allogeneic tissue to produce a scaffold potentially useful for anterior cruciate ligament reconstruction using a process of chemical and detergent oxidation and decellularization that was cytocompatible in vitro, resulted in the removal of viral pathogens, improved porosity was associated with improved integration of the scaffold in vivo, and resulted in the maintenance of necessary tensile properties.[11] Although the process was largely successful, there remains an opportunity for optimization of the source material, minimization of the loss and inherent variability of necessary tensile properties, and improvements in the removal of donor cellular material.

The purpose of this study was to evaluate the biological, immunological, and biomechanical properties of a scaffold derived by architectural modification of a fresh-frozen porcine patella tendon using a decellularization protocol that combines physical, chemical, and enzymatic modalities. We hypothesized that the porcine xenograft tendon could be processed to produce a decellularized, cytocompatible, xenoantigen-depleted, and architecturally modified scaffold that would be suitable for tendon and ligament reconstruction.

## Methods

### Preparation of Xenograft-Derived Scaffolds

Fresh-frozen native porcine tendons (n = 160) were obtained through a single vendor (Animal Technologies, Tyler, TX). Porcine xenograft-derived scaffolds were prepared using a decellularization protocol that was previously developed in our laboratory.[11,12] Scaffolds were produced in groups of 4 at a time. The product, referred to as "scaffold," was freeze-dried (Labconco, Freeze Dry System, Kansas City, MO) for 24 hours before being returned to the freezer and stored at −80°C until further use. Tendons were processed into scaffolds (n = 88) for experimental analysis, whereas others were retained as native tendons (n = 70) for comparison testing.

### Histologic Analysis

Midsubstance portions of the fresh-frozen porcine patella tendons and scaffolds were prepared for histologic analysis and analyzed as previously described and stained using H&E (Sigma Aldrich, St. Louis, MO), 3-color trichrome (Masson's trichrome stain, Sigma Aldrich), and 4,6-diamidino-2-phenylindole (DAPI; Vector, Burlingame, CA) to identify cellular and nuclear components, respectively.[11,12]

### Scanning Electron Microscopy Analysis

Scanning electron microscopy (SEM) was performed on native porcine patella tendons and decellularized and oxidized scaffolds as previously described.[11,12]

### Mercury Intrusion Porosimetry

Sections of both native porcine patella tendon (n = 11) and scaffold (n = 12) were analyzed as previously described.[11,12]

### Detection and Identification of Proteins With Mass Spectrometry

The identities of proteins within the tendon scaffolds were analyzed by using mass spectrometry (MS). Freezer-milled scaffold samples (n = 3) were reduced