Effects of Mechanical Stimulation on a Cell-Seeded Scaffold Developed for Tendon and Ligament Regeneration

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Objectives

Tissue-engineered tendons have the potential to significantly improve the treatment of tendon and ligament injuries, especially those associated with tumors, trauma, and congenital deficiencies for which autograft or allograft tissue might not be available in sufficient quantity for reconstruction. The development and characterization of an architecturally-optimized, decellularized and oxidized biocompatible scaffold derived from flexor digitorum profundus tendon was previously described (1,2).

The goal of the current study was to seed the scaffolds with allogeneic tenocytes and determine the effects of subsequent culture in either static, or mechanically-stimulated culture environments. It was hypothesized that mechanical stimulation would result in a seeded scaffold with tensile properties that would be significantly greater than those of seeded scaffolds cultured in a static environment.

Methods

Scaffold Preparation: Scaffolds (40 mm x 5 mm x 1 mm) were prepared and stored as previously described (1,2).

Cell-seeding of Scaffolds: Scaffolds were equilibrated in tenocyte media for 24 hours. 600 µL of a 50 million cell/ml suspension of allogeneic tenocytes in media was seeded by pipette on the tendon scaffolds (n=12). Scaffolds were maintained in culture for 7 days. After 7 days, 6 scaffolds remained in culture and 6 were mounted in a bioreactor. Media was changed in all cultures throughout the experiments every 48 hours.

Mechanical Stimulation of Seeded Scaffolds: 6 scaffolds were mounted in a commercially-available bioreactor (Tissue Growth Technologies, Minnetonka, MN). Mechanical stimulation was applied in a sinusoidal pattern at a maximum strain of 5% of the clamp-to-clamp distance using a frequency of 1 Hz for 60 minutes per day for a total of 7 days.

Histological Analysis: Portions of the mechanically-stimulated and statically cultured seeded scaffolds were fixed and processed for histology. Cross-sectional and longitudinal sections were stained with hematoxylin and eosin (H&E) and Masson trichrome stain, respectively.

Scanning Electron Microscopy (SEM) Analysis: Cross-sectional and longitudinal specimens of mechanically-stimulated and statically cultured seeded scaffolds were fixed and processed for SEM.

Tensile Testing: Mechanically-stimulated and statically cultured seeded scaffolds were cut into 20 mm x 3mm x 1 mm "dogbones" with a custom punch and placed on an Instron 5542 (Canton, MA) uniaxial load frame for load to failure testing. Ultimate tensile stress (MPa) and stiffness (N/mm) were calculated for each sample.

Statistics: All numerical data are presented as mean ± standard error of the mean (SEM). Differences are considered significant at $p<0.05$ using Student's t-test for a two-tailed distribution with samples of unequal variance.

Results

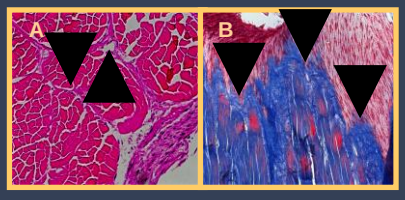

Figure 1. Histology: Cells can be seen infiltrating from the outer portion of the scaffold to the inter-fascicular areas (arrows) of both the statically-cultured and mechanically-stimulated seeded scaffolds (Figure 1A, H&E, 200X, x-sect.). Mechanically-stimulated seeded scaffolds exhibited more extensive infiltration of the scaffold, with an area of disorganized collagen (arrows) at the interface between the scaffold and seeded cells (Figure 1B, Masson, 50X, long.).

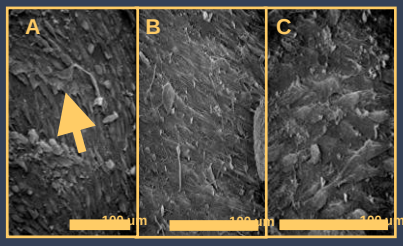

Figure 2. Scanning Electron Microscopy (SEM): Alignment of seeded cells parallel to the long axis of the scaffold and the collagen fascicles (arrow) on the surface of both the mechanically-stimulated and the statically-seeded scaffold was observed at 7 days (Figure 2A, 400X, long). However, cells of the mechanically-stimulated seeded scaffold exhibited more extensive, narrow and elongated cell bodies and processes (Figure 2B, 745X, long) than those of the statically cultured seeded scaffolds at 14 days (Figure 2C, 745X, long).

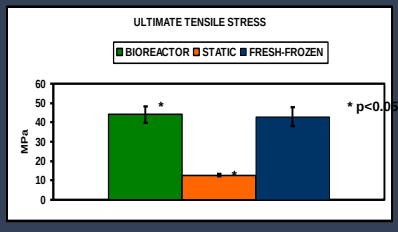

Figure 3. Tensile Testing: Testing of mechanically-stimulated seeded scaffolds determined that the ultimate tensile stress (UTS, MPa) was 347% (44.05 ± 4.23 MPa, n=3) of that observed for the statically cultured seeded scaffolds (12.69 ± 0.65 MPa, n=3), and 102.57% of that observed for fresh-frozen FDP tendons (42.95 ± 5.04 MPa, n=8).

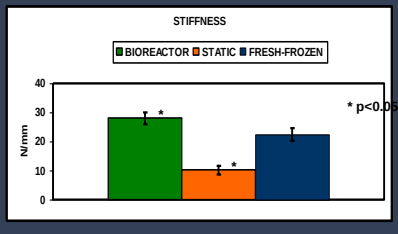

Figure 4. Tensile Testing: Similarly, stiffness determined for the mechanically-stimulated seeded scaffolds was 275.06% (28.07 ± 2.02 N/mm, n=3) of that observed for the statically-cultured seeded scaffolds (10.21 ± 1.54 N/mm, n=3), and 125.48% of that observed for fresh-frozen FDP tendons (22.37 ± 2.13 N/mm, n=8).

Conclusions

A scaffold for tendon and ligament regeneration can be seeded with allogeneic tenocytes in the presence of mechanical stimulation to produce a tissue-engineered tendon that:

1) exhibits tensile properties that are significantly greater than those observed for statically-cultured constructs. More importantly, the tensile properties of the tissue-engineered tendons were not significantly different from those observed for fresh-frozen FDP tendons.

2) promotes more extensive infiltration of seeded cells than was observed in statically-cultured constructs. Similarly, an area of disorganized collagen, suggestive of potential scaffold remodeling, was also observed at the interface between infiltrating seeded cells and the unpopulated portions of the mechanically-stimulated scaffold. This morphology was not observed in statically-cultured seeded scaffolds.

3) demonstrates alignment of seeded cells. This morphology was also observed in statically-cultured constructs. However, the cells of the mechanically-stimulated constructs were more elongated, narrower and exhibited more extensive cellular processes than those of the statically-cultured seeded scaffolds, potentially indicative of stronger cell adhesion and integration in the mechanically-stimulated seeded scaffolds.

Remodeling, potentially involving the synthesis of collagen, and improved cell adhesion and integration may partially explain the increase in tensile properties observed in the mechanically-stimulated seeded scaffolds.

Clinical Significance

Naturally-derived, biocompatible scaffolds have the potential to significantly improve the treatment of tendon and ligament injuries, especially those for which autograft or allograft tissue might not be available in sufficient quantity for reconstruction.

References

1. Whitlock et al., 53rd Annual Meeting ORS, San Diego, 2007
2. Whitlock et al., Biomaterials, July, 2007

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