Tendon Tissue Engineering Using Mesenchymal Stem Cells, Natural Biomaterial, and Mechanical Stimulation

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Statement of Purpose: Tendon injuries affect several individuals yearly, in particular athletes, and in many cases prevent participation in daily activities and work capabilities. Lacking a satisfactory clinical treatment for tendon pathologies, research has been directed toward exploring alternative approaches. Several attempts have been made to develop collagenous tissue engineered models that have physical and mechanical properties comparable to native tendons[1, 2]. It has been shown that dynamic mechanical loading *in-vitro* gives collagenous constructs a tendon-like appearance and increases their mechanical strength[3, 4]. Bone marrow mesenchymal stem cells (MSC's) have been widely employed to treat tendon defects *in-vivo*[5, 1] and in tendon tissue engineering *in-vitro*[1].

In our study, we attempt to develop a tissue engineered tendon model using umbilical cord decellularized veins as scaffolds. We hypothesize that culturing MSC seeded constructs under cyclic mechanical loading conditions would enhance tendogenesis *in-vitro*.

Methods: Fresh human umbilical cords from full term placentas were procured from the Women's Delivery Center at the Norman Regional Hospital, Norman, OK. The human umbilical vein (HUV) was dissected automatically as described by Daniel et. al.[6]. HUV sections were decellularized in 1% sodium dodecyl sulfate (obtained from ®Baker) and then dehydrated in 75% ethanol. Decellulerized vein scaffolds were stored in autoclaved phosphate buffere saline (Atlanta Biologicals) at 4°C for a maximum of two weeks before being used.

Bone marrow stromal cells were harvested from the femur and tibia of 6-8 week old male Wistar® rats (Harland Laboratories)[7]. Cells were cultured, expanded *in-vitro*, and used for experimental purposes between the third and fifth passage. Decellulerized scaffolds were seeded with type I collagen (2mg/ml final concentration) and MSC's (1 million cells/ml) under sterile conditions and cultured for periods of one and two weeks in α-media (Gibco®) supplemented with 10% fetal bovine serum (Atlanta Biologicals), 0.1 mM nonessential amino acids, and 10% antibiotic solution (Gibco®). A bioreactor (Figure 1) was designed to mechanically stimulate the seeded constructs. The bioreactor is capable of stimulating three constructs simultaneously. A peristaltic pump is employed to recirculate the flow media around the constructs. Samples were loaded for a period of 1 hour/day at a frequency of 0.0167 Hz. A 150gm load (5% strain) was applied per construct. Samples cultured statically in petri dishes were used as controls. Samples were tested for cellularity (using pico green dye method[7]), morphology (paraffin embedding and H&E staining), and tensile strength (United Testing Systems, Inc., Model SSTM-2K, Flint, Mich.).

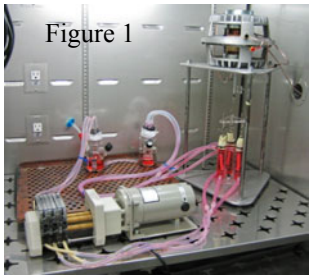
Figure 1

Results/Discussion: Mechanical stimulation of the constructs increased cellularity significantly. Samples cultured in the bioreactor for a week contained 70% more cells than those cultured under static conditions. Two weeks of stimulation increased cellularity 20 folds over the controls. This trend of increased cellularity in the stimulated constructs over the static controls was evident microscopically for samples stained with H&E. Cells were evident throughout the construct and by two weeks of stimulation cells migrated from the center of the HUV 75% towards the outer end of the scaffold. Contrarily, constructs that were cultured statically showed higher density of cells on the inner part of the HUV. Few cells migrated half way into the thickness of the treated HUV. Moreover, mechanically stimulated samples showed tendon-like parallel orientation of collagen fibers as opposed to a random orientation for static constructs. After one week only, mechanicallly stimulated samples had almost 90% higher tensile strength than the static controls (Figure 2). Samples cultured in the bioreactor for two weeks gained 222% tensile strength over the decellulerized constructs. It is evident that mechanical stimulation increased the tensile strength of constructs considerably. This is probably due to the realignment of collagen fibers due to stretching in a parallel manner that resembles those in native tendons.

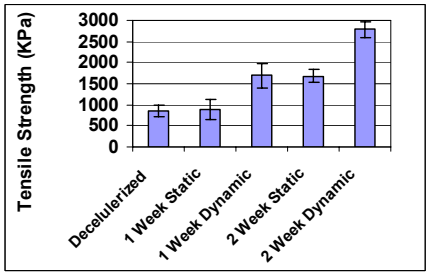

Figure 2. Ultimate Tensile Strength of Constructs

Conclusions: Mechanically stimulating the constructs using the novel bioreactor caused a significant increase in cellularity and tensile strength and realigned the fibers into a parallel tendon-like structure. Optimization of seeding densities, stretching durations, and loading levels for extended culture periods should further enhance the mechanical properties of tissue engineered tendon constructs.

References:

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