Characterizing Strain in an Embryonic Development Inspired Method for Engineered Tendon

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***Abstract*—We have developed a scaffold-free, cell-based, single fiber approach toward tissue engineered tendon. This method provides a culture system in which key aspects of embryonic tenogenesis may be replicated, such as high initial cell density, cell-cell adhesion, and dynamic mechanical cues. Through the application of embryonic development inspired growth factors and mechanical stimulation, we ultimately hope to create a mechanically and structurally matched tendon replacement. We demonstrate here, the ability to apply a well characterized cyclic tensile strain to cell-based, single fibers during development.**

I. Introduction

Current scaffold-dependent approaches to tendon tissue engineering have yet to gain clinical acceptance. Scaffold-free, cell-based approaches to engineered tendon may allow for the use of autologous cells to reduce complications related to immune rejection and scaffold-breakdown byproducts. We have developed a scaffold-free, cell-based approach to tissue engineered tendon in which cells are guided to form single-fibers using growth channels [1]. Recently, we have demonstrated a method to apply cyclic tensile strain to scaffold-free fibers during development [2]. However, the amount of strain applied to the growth channels had not been fully quantified. The objective of this study was to quantify the amount of tensile strain applied to the growth channels to demonstrate how this method may be used to replicate key aspects of embryonic tendon development.

II. Materials and Methods

Growth Channel Fabrication:

A 2 wt% concentration of agarose gel was prepared with 50 ml of Dulbecco's Modified Eagle's Medium (DMEM). After heating to ~60 ^{o}C, the liquid agarose was pipetted (6 ml) into 47-mm diameter Petri dishes, and left to cool in a biosafety cabinet until gelation. The agarose-filled Petri dishes were then mounted on a x-y motorized computer-controlled stage, and a pulsed excimer laser (λ=193 nm, TeoSys, Crofton, MD) with CAD/CAM control was used to micromachine three-dimensional guidance channels into the agarose gel; each with a length of 1.65-cm, width of ~300 μm, and a depth of ~250 μm.

Growth Channel Assembly:

A Flexcell® Tissue Train® plate (Flexcell International Inc., Hillsborough, NC) was modified to include loading posts [2]. The plate and Flexcell® system can apply user-specified cyclic uniaxial tensile strain via a deformable membrane and vacuum system.

Type I collagen sponges (Kensey Nash, Exton Pa) were soaked in 70% ethanol for 24 hours, then soaked and rinsed 6X with sterile phosphate buffered saline (PBS) and left to dry in a biosafety cabinet. Using a biopsy punch, 4-mm diameter disks were punched out of the collagen sponges and 1-mm centered through-holes were punched out of the center of each collagen disk. A 4-mm diameter biopsy punch was used to create cut-outs on both ends of the agarose growth channel in which a prepared collagen disk was inserted. The entire assembly (growth channel and collagen disks) was mounted onto the loading posts of the modified Tissue Train® plate. Human plasma-derived fibronectin, (BD Biosciences, Bedford, MA) was diluted to 1 mg/ml using sterile tissue culture water, and further reduced to 0.3 mg/ml in PBS. Fibronectin was pipetted (30 μl) into each collagen disk, allowed to flow into the growth channels, and dry for one hour in a biosafety cabinet.

Cell Culture:

Human dermal fibroblasts (ATCC, Manassas VA) were grown in T-75 cm^2 tissue culture flasks, trypsinized, and resuspended in culture media (89.5% DMEM, supplemented with 10% fetal bovine serum, 0.5% penicillin-streptomycin, 50 g/ml ascorbic acid). A cell suspension (1-ml) containing approximately 5 x 10^5 cells/ml, as measured by hemacytometer, was pipetted into the fibronectin-coated growth channels in the agarose gel. The seeded channels were placed in a standard cell culture incubator (37 ˚C, 5% CO_2, 95% RH) for 5 minutes at which time, 5 ml of fresh culture media was added. After allowing 24 hrs for fiber formation, dynamically strained fibers were subjected to 0.75% strain at 0.5 Hz for 8 hrs, 8 hrs of static tension, and 1.0% strain at 0.5 Hz for the final 8 hrs while controls were subjected solely to self-generated static tension. Fibers were then removed from the channels and imaged using polarized light microscopy.

Cyclic Tensile Strain Measurement:

Growth channels were prepared as described and soaked in DMEM warmed to 37 ^{o}C for 10 minutes. The top surface of the channel was blotted dry, coated with a random speckle pattern, and mounted into the modified Tissue Train® plate. Cyclic tensile strain was applied via the Flexcell® system at a

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rate of 0.5 Hz. The strain magnitude was specified in the Flexcell® software to be, 0.7%, 0.75%, and 1.0% strain, with each magnitude cycled 5 times. Video of the strained growth channel was collected at 10 frames per second using a Correlated Solutions (Columbia, SC) two-camera system, and strain analysis was completed using Vic-3D 2010 software and virtual extensometer placed in-line with the growth channel. Five separate growth channel assemblies (n=5) were tested and peak strain magnitudes were found for every cycle. Data are reported as average ± standard deviation.

III. Results

The agarose acts as a differentially adherent substrate, directing cellular adhesion to within the channel, thus encouraging cell-cell contact with high initial cell density (**Fig. 1a**). Cells form fibers that became well integrated into the collagen disks, allowing for both static and dynamic mechanical stimulation during development (**Fig. 1b**).

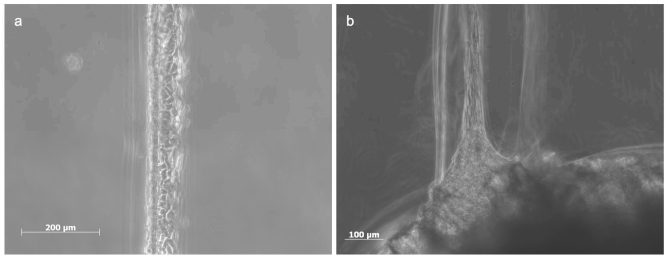

Fig. 1. Phase contrast images (10X) of scaffold-free, cell-based fibers during development. a) 4-hours following seeding, cells are closely packed, forcing cell-cell adhesion within the growth channel. b) 24-hours post-seeding, a cell-based fiber has formed and become well integrated into the collage sponge disk generating static tension within the fiber.

Non-contacting, digital image correlation demonstrated that the modified Tissue Train® plate and Flexcell® system can be used to apply controlled cyclic tensile strain to the growth channels and fibers during development (**Fig. 2**). When prescribed in the Flexcell® system software to apply 0.7%, 0.75%, and 1.0% strain the average strain on the surface of the growth channel between the loading posts was 0.86±0.15%, 0.89±0.14%, and 1.06±0.20%, respectively (n=5).

Cyclic loading increased polarized light intensity (**Fig. 3**) of cyclically strained fibers compared to static tension controls, a strong indication of aligned collagen fibrils.

IV. Discussion

We have developed a scaffold-free, cell-based method that allows us to mimic some key aspects of embryonic tendon development. The ability to encourage cell-cell contact, cell growth into single-fibers, and alignment without the influence of scaffold materials is an important first step to mimic tenogenesis, *in vitro*. However, static and dynamic mechanical cues are also an essential component of embryonic tendon development and maturation. Application of mechanical cues in a scaffold-free approach is a unique challenge as no initial structure is present. Using a modified

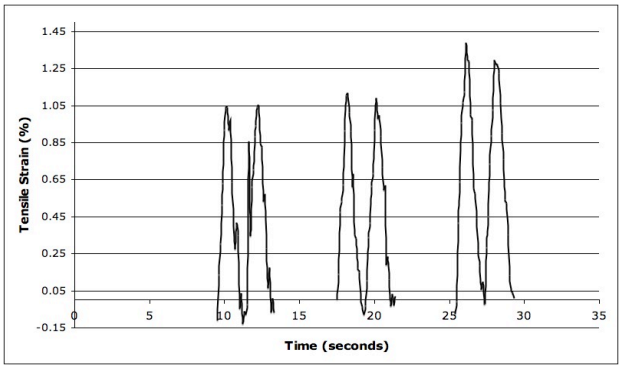

Fig. 2. Raw, unfiltered strain applied directly to the growth channel using a modified Tissue Train® plate, as measured with non-contacting digital image correlation. Two cycles of each Flexcell® specified magnitude are shown.

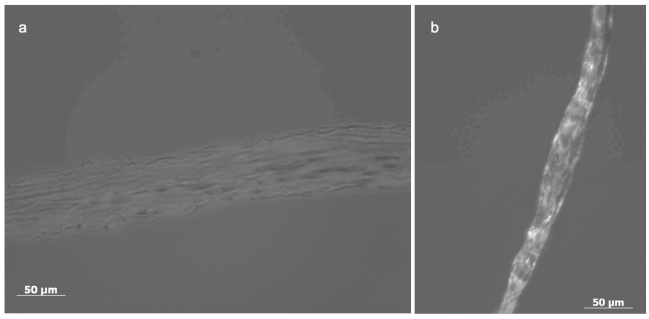

Fig. 3. Polarized light microscopy (20X) of fibers after a) 48 hrs of static tension, and b) 24 hrs of static tension followed by 0.75% strain for 8 hrs, 8 hrs of static tension, and 1.0% strain for the final 8 hours.

Flexcell® Tissue Train® system, we have overcome this limitation and can apply cyclic tensile strain to developing cell-based fibers. Although, the influence of dynamic mechanical loading was evidenced in the fiber structure as cyclically strained fibers showing a dramatic increase in polarized light intensity (indicative of aligned collagen fibrils), the amount of applied strain during fiber development had not been full quantified. The results of this study provide a method to determine the correlation between the strain prescribed in the Flexcell® system and the true strain translated to the growth channel through the modified Tissue Train® plate. Through application of embryonic development inspired growth factors and mechanical stimulation we hope to develop fibers that have tendon-like mechanical properties and structure.

REFERENCES

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