# ENGINEERING OF FUNCTIONAL TENDON

**Sarah Calve (1,2), Robert G. Dennis (2,3,4), Paul E. Kosnik II (5), Karl Grosh (3,4), Ellen M. Arruda (1,3)**

(1) Macromolecular Science and Engineering Center
University of Michigan
Ann Arbor, MI

(2) Institute of Gerontology
University of Michigan
Ann Arbor, MI

(3) Department of Mechanical Engineering
University of Michigan
Ann Arbor, MI

(4) Department of Biomedical Engineering
University of Michigan
Ann Arbor, MI

(5) Vice President of Engineering
Tissue Genesis Incorporated
Honolulu, HI

## INTRODUCTION

Tendons are densely packed connective tissues that transmit the force generated by muscle to bone. They are stiff in tension yet flexible enough to conform to their physiological environment. These material properties are attributed to the parallel fibrils of collagen which make up about 75 % of the dry weight of tendon.[1]

Fifty percent of the approximately 33 million musculoskeletal injuries each year in the United States involve tendons and ligaments.[2] When the tendon is damaged beyond repair, partial or whole replacement is necessary. The ideal replacement is autologous tendon, but transplantation is limited by the availability of viable tissue and clinical practice has turned to artificial prostheses. Current synthetic replacements include Dacron grafts, carbon fibers and Silastic sheets, but these are unable to restore function for the long term due to their tendency to degrade and mechanical incompatibilies.[3]

Because of its relatively avascular nature, tendon is a prime candidate for engineered tissue replacement. Under appropriate conditions *in vitro*, tendon-like structures will self-assemble from a confluent layer of fibroblasts.

## METHODS

Primary rat tail tendon fibroblasts are plated on a laminin coated SYLGARD substrate and maintained in a growth medium containing 20% fetal bovine serum (FBS) and 100 μg/ml ascorbic acid. When the cell layer becomes confluent, the amount of FBS in the media is dropped to 7%, slowing down the rate of proliferation and inducing the cells to delaminate. The laminin concentration on the substrate is carefully determined in order for the confluent cell layer to progressively delaminate from the SYLGARD substrate, beginning at the perimeter. Two anchor points are provided from which the monolayer does not separate. After full delamination the monolayer self-assembles into a cylindrical construct suspended between the anchor points.

## RESULTS AND DISCUSSION

The stress-strain response of the constructs resembles the non-linear behavior of immature tendons. (Figure 1) Between 0 - 5% extension, there is a region of low stiffness after which the slope starts to increase until the construct starts exhibiting linear behavior at a strain of 0.11. The construct failed at a strain of 0.2. The ultimate tensile strength is within the same order of magnitude as embryonic chick tendon, ~ 2 MPa.[4] The tangent moduli of the chick tendon and the construct, measured at the linear portion of curve, are 27 MPa and 20 MPa respectively. We find that the longer the constructs are maintained in culture, the stiffer they become, a trend which is also seen over the course of development of tendons in utero.[4]

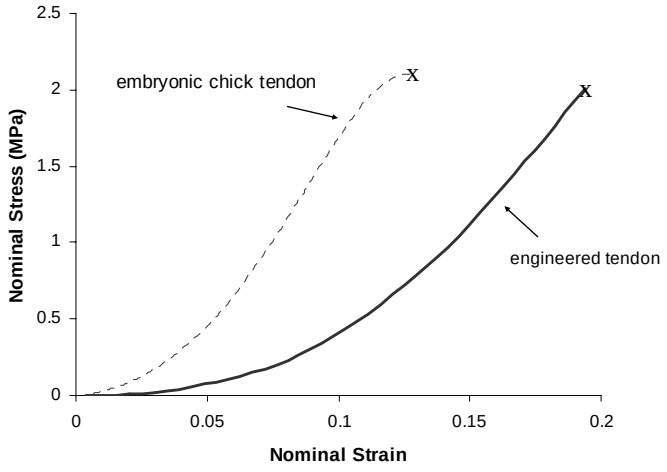


**Figure 1. Comparison of stress-strain response between embryonic chick tendon (E14) and engineered construct.**

Ultrastructural analysis shows that the constructs also display a morphology similar to immature tendons. The collagen fibrils secreted by the fibroblasts are ~ 60 nm in diameter and there is an increased cellularity and disorganization that is characteristic of the early stages of tendon development.[5] During maturation, the number of cells decreases and the collagen fibrils increase up to an order of magnitude in diameter. This growth has been correlated with the increase in mechanical loading from in utero movements to locomotion after birth.[6]

We are currently in the process of developing bioreactors that will apply cyclic mechanical stimuli to the constructs while in culture over extended (months) periods of time. We hypothesize that the morphological and mechanical response of the constructs will change when loaded while in culture, in a manner similar to developing tendons *in vivo*.

Reference List


1. Nordin,M., Lorenz,T. & Campello,M. Basic Biomechanics of the Musculoskeletal System. Nordin,M. & Frankel,V.H. (eds.), pp. 102-125 (Lippincott Williams & Wilkins, New York,2001).

2. Butler,D.L. & Awad,H.A. Perspectives on cell and collagen composites for tendon repair. *Clinical Orthopaedics and Related Research* S324-S332 (1999).

3. Iannace,S., Sabatini,G., Ambrosio,L. & Nicolais,L. Mechanical-Behavior of Composite Artificial Tendons and Ligaments. *Biomaterials* **16**, 675-680 (1995).

4. McBride,D.J., Hahn,R.A. & Silver,F.H. Morphological Characterization of Tendon Development During Chick Embryogenesis - Measurement of Birefringence Retardation. *International Journal of Biological Macromolecules* **7**, 71-76 (1985).

5. Parry,D.A.D. & Craig,A.S. Ultrastructure of the Connective Tissue Matrix. Ruggeri,A. & Motta,P.M. (eds.), pp. 34-64 (M. Nijhoff Publishers, Boston,1984).

6. Germiller,J.A., Lerner,A.L., Pacifico,R.J., Loder,R.T. & Hensinger,R.N. Muscle and tendon size relationships in a paralyzed chick embryo model of clubfoot. *Journal of Pediatric Orthopaedics* **18**, 314-318 (1998).