

# BIOARTIFICIAL TENDONS: DYNAMIC 3D TENOCYTE CULTURE AS A MODEL FOR TENDON DEVELOPMENT, TENDON INJURY, TISSUE ENGINEERING & EVALUATION OF RESPONSE TO DRUGS AND GROWTH FACTORS

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## INTRODUCTION

The biology of non-weight-bearing connective tissues has been under-studied due to the lack of a satisfactory *in vitro* model. An experimental system has been developed that permits 3D culture and load application to linear or circular cell-populated matrices. In this system, uniaxial or equibiaxial strain can be applied to the construct by means of a pressure-operated Flexercell™ Strain Unit and Tissue Train™ culture plates.

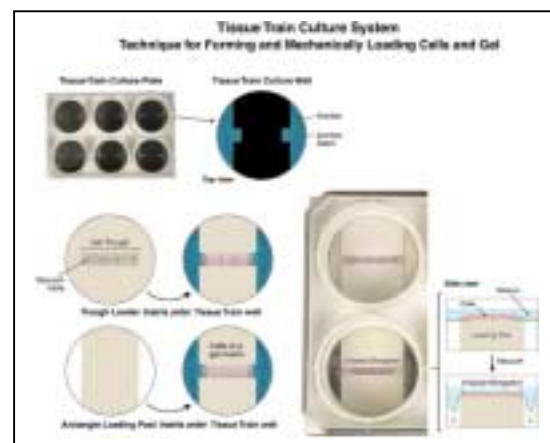
## METHODS

Internal tendon fibroblasts were isolated from the tensile load-bearing matrix of human or chicken flexor digitorum profundus tendons (Banes et al., 1988; Banes et al., 1995). Cells were cultured in DMEM-H with 10% fetal calf serum (FCS), insulin, transferrin and selenium, 0.1 mM ascorbate-2-phosphate, 20 mM HEPES, pH 7.2, with penicillin and streptomycin.

Passage 3 cells were trypsinized and mixed at a concentration of  $10^3$ - $10^4$  cells/ $\mu$ l in base-neutralized Vitrogen™, containing 10% FCS in DMEM. For each construct, 200  $\mu$ l of the cell-matrix mixture was dispensed into the 25 x 3 x 3 mm trough present in the 6 wells of a flexible bottom, Tissue Train™ culture plate (Figure 1). A trough in the membrane was formed by placing the Tissue Train™ culture plate atop a loading jig with conduits allowing vacuum to deform the rubber

membrane into a trough of the jig's dimensions, beneath each culture well. Flexible but inelastic nylon anchors were bonded at north and south poles of each well with free anchors to which the cells and matrix bonded. Once the cell-populated matrix had gelled, the vacuum was released and the linear 3D cell-matrix construct was left connected to the polar attachments.

Uniaxial force application was achieved by placing an arctangle loading post (rectangle with curved short ends) beneath each well and applying vacuum regulated for frequency, amplitude and duration by a Flexercell™ Strain Unit.

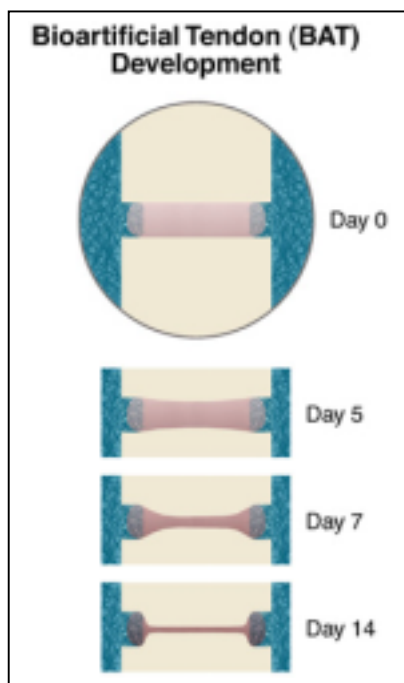


**Figure 1:** Tissue Train culture system

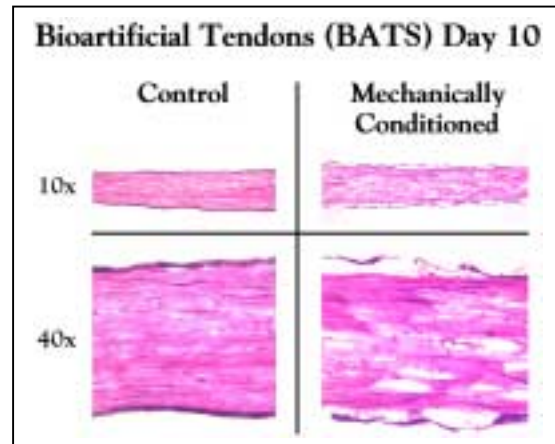
## RESULTS

Tendon cells in a linear collagen gel attached to matrix-bonded anchor ends to form a 3D construct (Bioartificial tendon or BAT). These BATs were cultured for up to 30 days. The BATs initially assumed an hourglass shape, but then the cells reorganized the matrix within 2-3 days, leading to a radial contraction of the construct (Figure 2). This contraction process was inhibited by cytochalasin D, suggesting that the actin cytoskeleton is a necessary component to this event.

H&E stained control cultures showed that cells had elongated and spread evenly throughout the matrix but were densely packed on the surface of the BAT, similar to an epitendon (Figure 3). BATs subjected to 1% uniaxial elongation at 1 Hz for 8 hours per day for up to 30 days, had the same overall architecture as controls but more elongated cells and collagen matrix separation.



**Figure 2:** Bioartificial tendon reorganization



**Figure 3:** Bioartificial tendon after 10 days of culture, with and without mechanical conditioning.

## DISCUSSION

The 3D tenocyte-populated linear BAT matrix is a unique model to study tendon cell interactions and responses to mechanical load. BATs can be maintained for up to 1 month with a tendon-like architecture and viable cells. Once biochemical, biomechanical and functional validation studies are performed, this model could be used to study tendon overuse injuries and response to growth factors and pharmaceuticals.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Banes et al., *J. Orthop. Res.*, 6:83, 1988
- Banes et al., *J. Biomech.*, 28: 1505, 1995