TECH REPORT

113:

Tissue Train® Anchor Options

Comparison between the non-woven nylon and the urethane polyester foam anchors

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Culturing Cells in a Mechanically Active Environment™
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INTRODUCTION

The Flexcell® Tissue Train® Culture System (Fig. 1) utilizes a novel method to culture and mechanically load cells in a three-dimensional (3D) hydrogel or cell-assembled matrix thereby subjecting them to more native growth conditions. The system consists of a flexible bottom Tissue Train® culture plate with mesh-like (Fig. 2) or foam (Fig. 3) anchors at the culture well periphery for cell and matrix attachment.

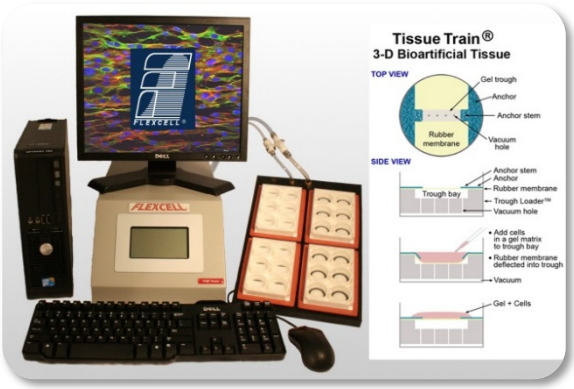


Figure 1. *Tissue Train® Culture System used to create 3D bioartificial constructs and apply strain to these constructs in vitro.*

Figure 2*. Linear Flexcell® Tissue Train® culture plate with non-woven nylon mesh anchors at north and south ends for gel-construct attachment.*

Figure 3. *Linear Flexcell® Tissue Train® culture plate with open cell foam anchors at north and south ends for gel-construct attachment.*

A cell-populated matrix gel may be cast by placing the Tissue Train® culture plate on a gasketed baseplate with a planar faced cylinder bearing a central trough, or Trough Loader™, beneath each flexible well (Fig. 4). Each cylinder face has a trough with evacuation holes in its base. A vacuum applied to the trough bottom will deform the membrane into the conformation of the trough, providing a space for delivery of cells and hydrogel. The baseplate and culture plates are transferred to a CO_2 incubator at 37 °C where the construct is held in position in the trough until polymerization is complete. When vacuum is released, the cell-gel construct rises into the plane of the culture well.

Mechanical load in the form of equibiaxial or uniaxial tension may be applied using the Flexcell® FX-5000™ Tension System. Cell-gel constructs or cell-assembled matrices may be mechanically loaded uniaxially or equibiaxially using special Loading Stations™ (Arctangle® or cylindrical loading posts).

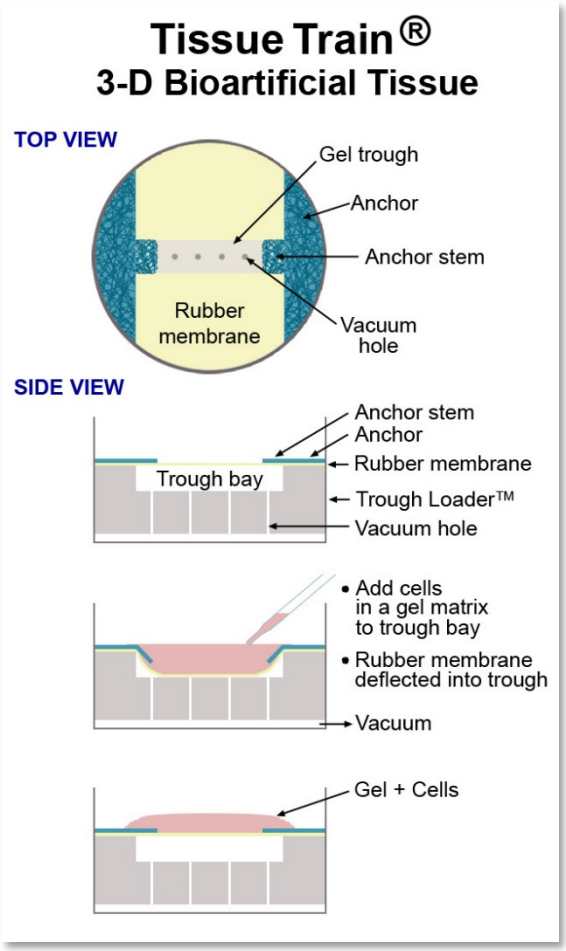


Figure 4. *Schematic of how bioartificial tissues are created with a Flexcell® Tissue Train® Culture System. A well of a Tissue Train® plate with anchors at east and west ends is centered atop a Trough Loader™. When vacuum is applied, the rubber membrane and anchor stems are pulled into the trough bay creating a linear mold. Cells in a hydrogel matrix are dispensed into the mold with a pipette. After polymerization of the hydrogel, the vacuum is released, leaving a cell-seeded hydrogel attached to the anchor stems of the Tissue Train® plate.*

Bioartificial tissues (BATs) prepared with this system undergo significant morphological changes including integration into the anchoring material, alignment with the axis of attachment/strain, and compaction of the matrix. Furthermore, construct survival (time to failure or breaking of the construct), is related to cell type, cell density, and composition as well as architecture of the anchoring material.

We analyzed the effects of the anchor material (Fig. 5) by looking at the compaction kinetics and construct survival of cell-seeded Collagel® constructs using MG63 osteoblasts, equine tendon internal fibroblasts (TIF), and human flexor carpi radials tenocytes (FCR) in both linear Tissue Train® culture plates with nylon mesh anchor tabs (Fig. 5A) and open cell urethane polyester foam tabs with various pore size (Fig. 5B&C) both untreated and coated with Pronectin F.

METHODS

BATs were cast as linear collagen hydrogels using a Flexcell® Collagel® kit in Tissue Train® culture plates (150-200 µL cell-seeded collagen solution/well). Linear Trough Loaders™ were placed beneath the silicone elastomer-bottomed wells of the culture plate that were subjected to static vacuum tension (FX-5000™ Tension System) during the dispensing of the cell-seeded collagen solution and polymerization at 37 °C. BATs were imaged every hour for the first 24 hours and every 6 hours thereafter using the ScanFlex™ system and serial kinetic images analyzed (XyFlex™ software) to determine changes in surface area over time and/or construct survival (i.e., when the BAT broke *in vitro*).

To determine the ability of the Tissue Train® system to produce well-differentiated tissue constructs, MC3T3E1 and MG63 osteoblast-like cell lines were used to generate linear BATs. BATs were subjected to cyclic strain (10%) for four hours per day in an osteoblast differentiation media (50 µM phosphoascorbate, 0.1 µM dexamethasone, 10mM β-glycerophosphate, and 5% serum in MEM). Of 12 constructs of each type tested, all survived a 21 day course of cyclic strain (i.e., the BAT did not break *in vivo*).

In addition, we compared two urethane open cell foams of varying pore size (Fig. 5).

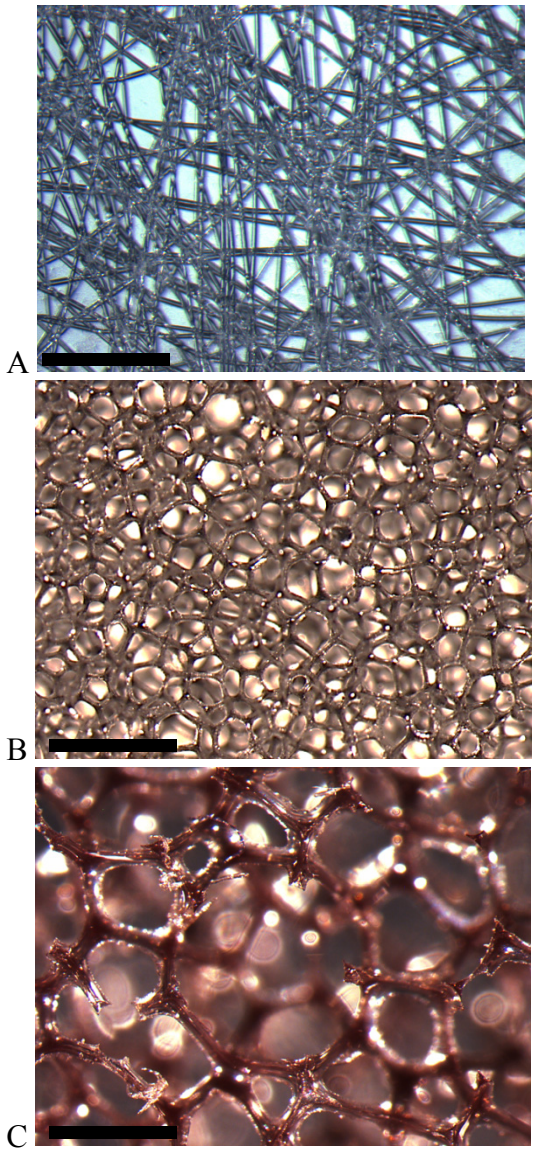


Figure 5. *A) Non-woven nylon mesh B) Small-cell open cell urethane foam. C) Large-cell open cell urethane foam. Bar = 1 mm.*

RESULTS AND DISCUSSION

Differences in the architecture/composition of the material used to facilitate anchoring of the 3D linear construct (non-woven nylon mesh vs. open cell urethane polyester foam) were observed to have an impact on construct survival (Fig. 6) but not on the compaction kinetics (Fig. 7). BATs comprised of primary human tenocytes anchored to a 3D open cell foam exhibited significantly increased construct survival as compared to identical BATs adhered to linear nonwoven mesh anchor tabs (Fig. 6).

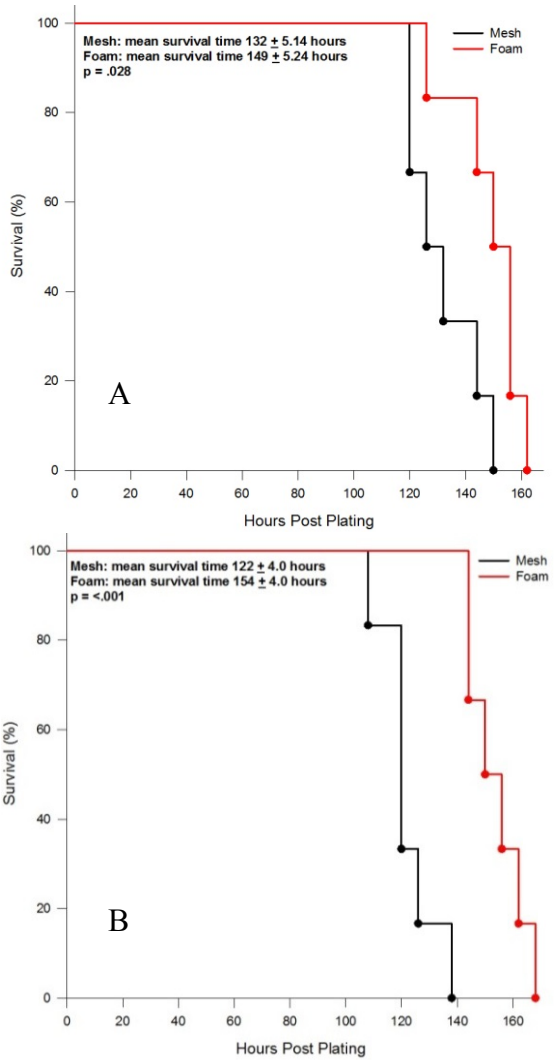


Figure 6. *Construct survival (n=6 BATs per group) of (A) human surface tendon fibroblasts and (B) human internal tendon fibroblasts plated on nonwoven nylon mesh (black) or open cell urethane polyester foam (red) anchor tabs.*

Coating of open cell urethane polyester foam with a positively-charged protein polymer that incorporates multiple copies of the RGD cell attachment epitope (pronectin) resulted in a significant increase in equine TIF construct survival (Fig. 8).

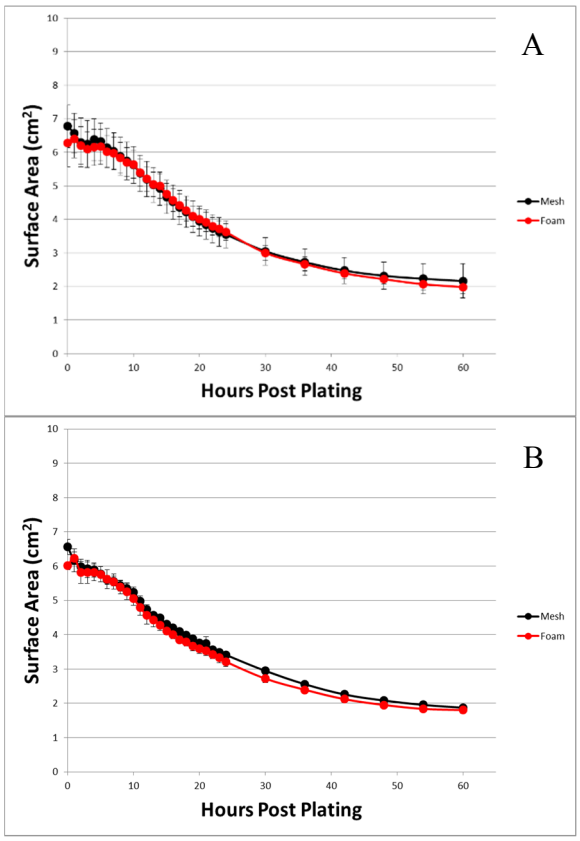
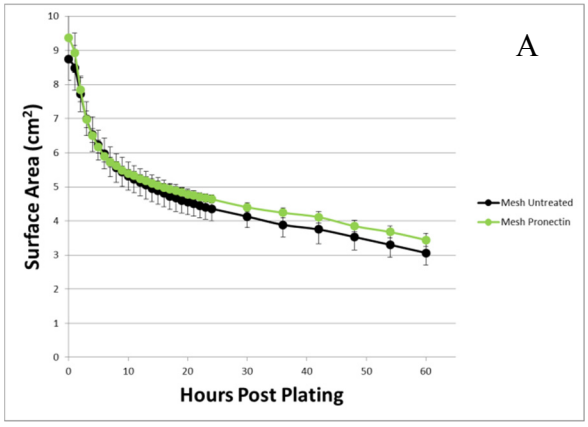
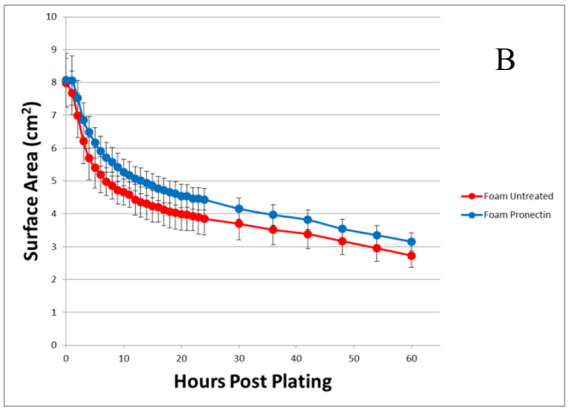
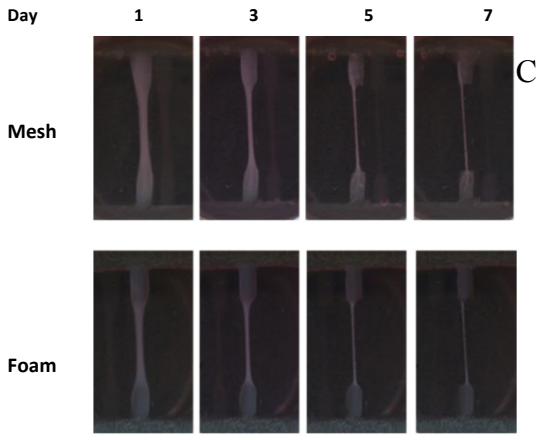


Figure 7. *Compaction kinetics (n=6 BATs per group) of (A) human surface tendon fibroblasts and (B) human internal tendon fibroblasts plated on linear Tissue Train® anchor tabs comprised of nonwoven nylon mesh (black) or open cell urethane polyester foam (red).*







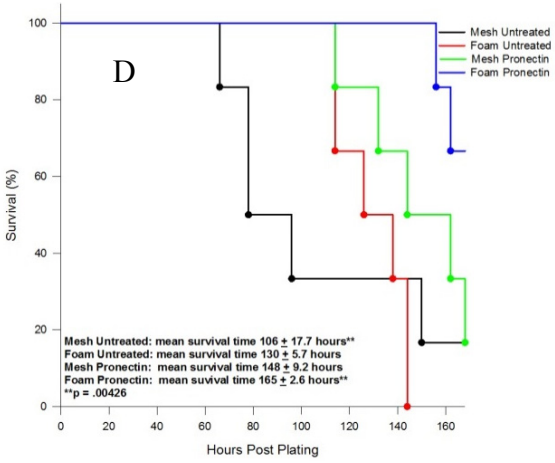


Figure 8. *Compaction kinetics (n=6 BATs per group) of equine tendon internal fibroblasts plated on (A) nonwoven nylon mesh either untreated (black) or pronectin coated (green) or (B) open cell urethane polyester foam either untreated (red) or pronectin coated (blue). C) ScanFlex™ images of representative BATs at 1, 3, 5, and 7 days post plating. D) Construct survival (n=6 BATs per group) of equine tendon internal fibroblasts plated on nonwoven nylon mesh or open cell urethane polyester foam either untreated or pronectin coated.*

MG63 cells seeded on the urethane foam with smaller pores had smoother integration than constructs seeded on the large pore foam (Fig. 9). The gel constructs began to pull off of the large pore foam sooner for both MG63 cells and C2C12 cells (Fig. 10).

Figure 9. MG63 cell-seeded constructs in the well of a Circular Foam Tissue Train® culture plate with large (top) and small (bottom) pore urethane foam anchors. Arrow = where construct is starting to fail or pull off of the foam anchor.

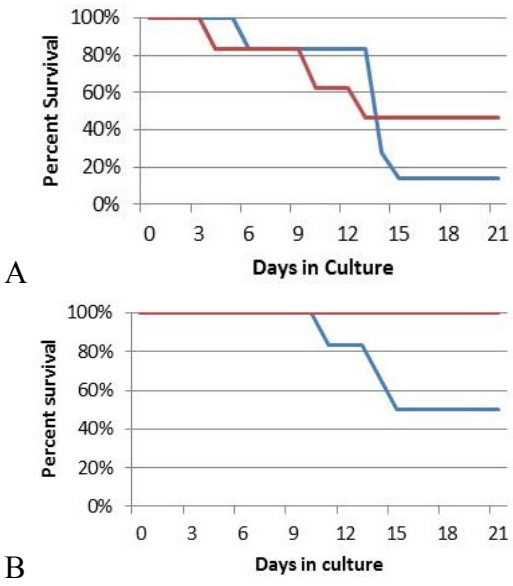


Figure 10. C2C12 (red) MG63 (blue) cell-seeded construct survival in Circular Foam Tissue Train® culture plates with large (A) and small (B) pore urethane foam anchors.

CONCLUSIONS

- Foam anchors have comparable compaction kinetics compared to the non-woven nylon mesh anchors.
- Foam anchors have a significantly increased construct survival time for linear 3D BATs.
- Foam anchors allow for increased surface area for cell-hydrogel anchoring.
- Smoother anchor-hydrogel interphase for circular constructs seeded in the small pore urethane polyester foam anchors.
- Taken together, these data suggest that although various cell types are capable of matrix re-organization and compaction to form linear 3D BATs, the gross biological properties of a given BAT are largely dependent upon both cell type and architecture/composition of the anchoring material.