TECH REPORT

114:

Collagel® and ThermaCol®

Collagen I Hydrogel Kits for 3D Cell Culture

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Culturing Cells in a Mechanically Active Environment™
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INTRODUCTION

Collagen is widely used for culturing cells in 3D. However, there is large variability due to user error in creating collagen I gel solutions. Kits are known to improve quality control, reproducibility, and reliability. Thus, Flexcell® created two type I collagen hydrogel kits, Collagel® and Thermacol®.

Collagel® kits (Fig. 1) provide a highly reproducible and simple method to produce highly homogeneous collagen gels. Collagel® kits consists of type I collagen solution (Collagel®), 5X MEM (Reagent A), fetal bovine serum (Reagent B), 1 M Hepes (Reagent C), and 0.1 M NaOH in 5X MEM (Reagent D).

Figure 1. *Collagel® Kit consisting of type I collagen solution (Collagel®), 5X MEM (Reagent A), fetal bovine serum (Reagent B), 1 M Hepes (Reagent C) and 0.1 M NaOH in 5X MEM (Reagent D).*

Thermacol® kits (Fig. 2) are distinct from Collagel® kits in that they contain a percentage of telopeptide-containing collagen, which provides the hydrogel with greater stability at low temperature (4^0C), different physical properties upon gelation, and rapid and controllable gelation kinetics (Fig. 3) without effecting cell growth kinetics (Fig. 4).

Figure 2. *Thermacol® Kit consisting of type I collagen solution (Collagel®), telopeptide-containing collagen (Thermacol®), 5X MEM (Reagent A), fetal bovine serum (Reagent B), 1 M Hepes (Reagent C) and 0.1 M NaOH in 5X MEM (Reagent D).*

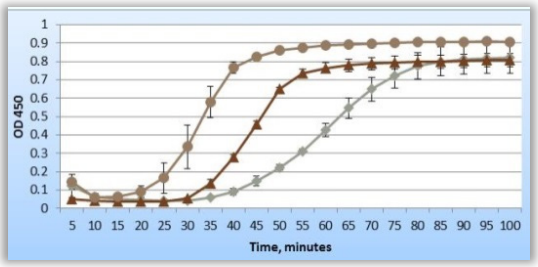

Figure 3. *Kinetics of gelation of Collagel® (0% telopepiptide-containing collagen) and Thermacol® (10% and 20% telopepiptide-containing type I collagen) at 37 °C. n=6 for each time point. Gelation kinetics (optical density 450 nm vs. time). Diamonds = 0% telopepiptide-containing collagen; triangles = 10% telopepiptide-containing type I collagen; circles, = 20% telopepiptide-containing type I collagen.*

In order to analyze the reproducibility of creating bioartificial tissues (BATs) with these kits in conjunction with the Tissue Train® System (Fig. 5), we looked at the compaction kinetics and survivorship of cell-seeded Collagel® constructs using MG63 osteoblasts, equine tendon fibroblasts (TIF), and human flexor carpi radialis tendon fibroblasts (FCR) in both linear Tissue Train® culture plates with nylon mesh anchor tabs and open cell urethane polyester foam

tabs both untreated and coated with Pronectin F.

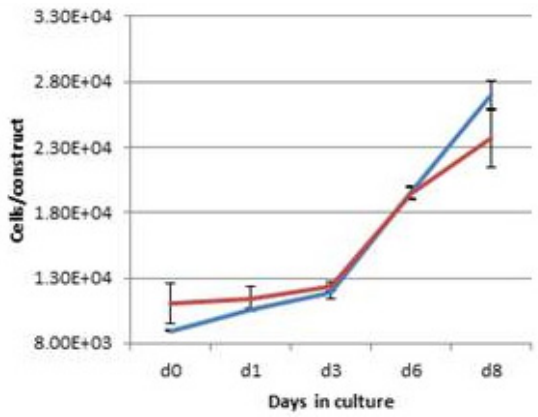

Figure 4. *Growth of HeLa cells seeded in Collagel® (blue) or Thermacol® (red) constructs.*

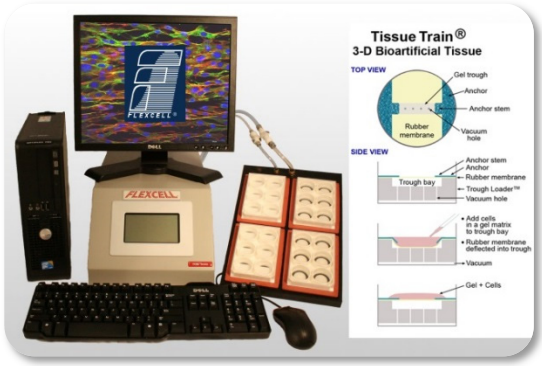

Figure 5. *Tissue Train® Culture System used to create 3D bioartificial constructs and apply strain to these constructs in vitro.*

METHODS

The relative gross morphology, compaction kinetics and survivorship of various cell types including tenocytes (isolated from primary human and equine explants) and osteoblasts (human bone MG63 cells) were evaluated and compared. Briefly, BATs were cast as linear collagen hydrogels using a Flexcell® Collagel® kit in Tissue Train® culture plates (150-200 µL of 1 x 10^6 cells/mL collagen solution/well; Fig. 6).

Linear Trough Loaders™ (Fig. 6, inset) 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 developed into bioartificial tendons as the matrix was compacted by cell-driven collagen fiber and cell alignment. BATs were imaged every hour for the first 24 hours and every 6 hours thereafter using the ScanFlex™ system. Serial kinetic images were analyzed (XyFlex™ software) to determine changes in surface area over time and/or time to failure (i.e., when the BAT broke *in vitro*).

Figure 6. *Linear Flexcell® Tissue Train® culture plate with non-woven nylon anchor stems at north and south ends for gel-construct attachment and a linear Trough Loader™ (inset) that is used as a mold to form the linear construct during gel polymerization.*

RESULTS AND DISCUSSION

Matrix compaction rates varied in accordance with tissue origin (Fig. 7). Cells derived from fibrous connective tissue demonstrated the greatest compaction but decreased construct survivorship as compared to cells derived from mineralized connective tissue (bone; Fig.8).

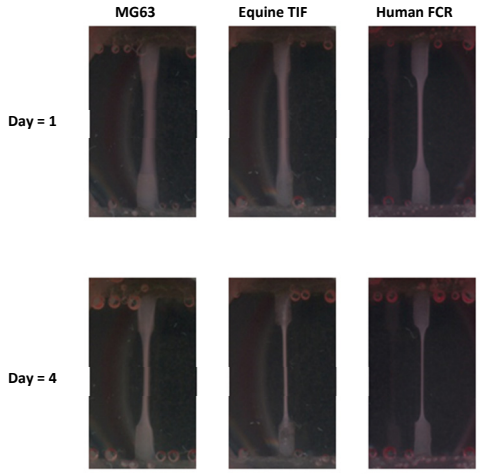

Figure 7. *ScanFlex™ images of representative MG63 osteoblasts and equine and human tendon fibroblast-seeded BATs at 1 and 4 days post plating.*

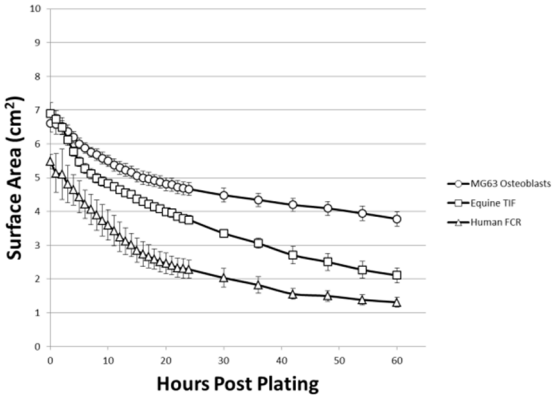

Figure 8. *Matrix compaction kinetics of MG63 osteoblasts (○), equine tendon fibroblasts (TIF; □), and human tendon fibroblasts (FCR; Δ) on standard nonwoven mesh anchor tabs. n=6 BATs per cell type.*

Differences in the architecture/composition of the material used to facilitate anchoring of the 3D linear construct did not impact compaction kinetics (Fig. 9).

Furthermore, coating of open cell urethane polyester foam with a positively-charged protein polymer that incorporates multiple copies of the RGD cell attachment epitope (pronectin) did not significantly change compaction kinetics (Fig. 10).

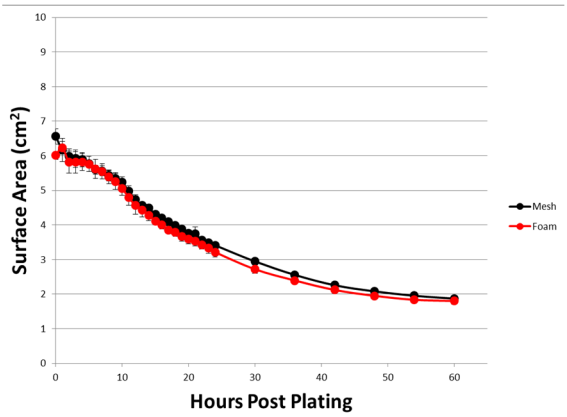

Figure 9. *Compaction kinetics (n=6 BATs per group) of human tendon fibroblasts plated on linear Tissue Train® anchor tabs comprised of nonwoven nylon mesh (black) or open cell urethane polyester foam (red).*

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 cell type.

NOTE: *The anchor material (foam vs. mesh) does effect construct survival as measured by time to construct failure/detachment from the anchor stems. Please see Tech Report 113: Tissue Train® Anchor Options for more information.*

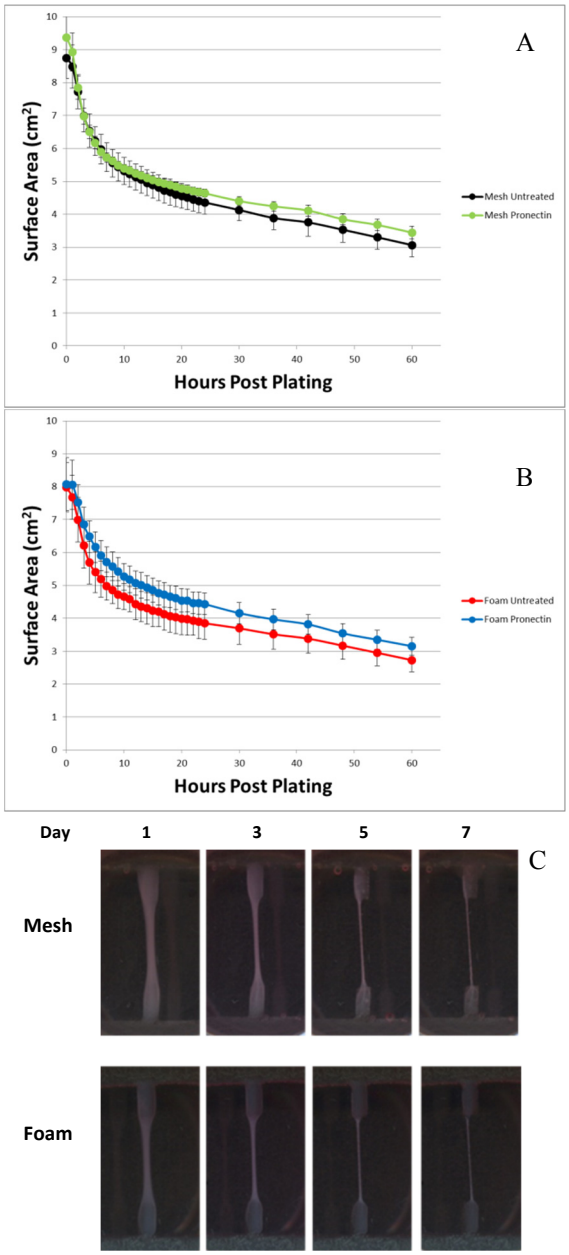

Figure 10. *Compaction kinetics (n=6 BATs per group) of equine tendon fibroblasts plated on (A) nonwoven nylon mesh either untreated (black) or pronectin coated (green) or (B) open cell urethane polyester foam either untreated (red) or pronectin coated (blue). C) ScanFlex™ images of representative BATs at 1, 3, 5, and 7 days post plating.*