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Methods for Immunochemical Staining of Cells in Three Dimensional Bioartificial Tissues (BATS)

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Culturing Cells in a Mechanically Active Environment[™]
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INTRODUCTION

Flexcell®'s Tissue Train® three dimensional bioreactor allows for the culture of cell-populated hydrogels in linear, circular and other geometric shapes with and without applied mechanical load. The principle involves casting a hydrogel in a shape created by vacuum-deforming each rubber bottomed well of a 6-well Tissue Train® culture plate into a trough created below the membrane. Each trough has a specified shape (i.e., linear, circular, or trapezoidal) For linear troughs, the resulting constructs are 25 mm x 3 mm x 3 mm upon formation, then are reduced in dimensions as cells compact and remodel the matrix (*Fig. 1*; Garvin et al., 2003; Qi et al., 2009, 2007, 2006; Triantafillopoulos et al., 2004; Sumanasinghe et al., 2009).

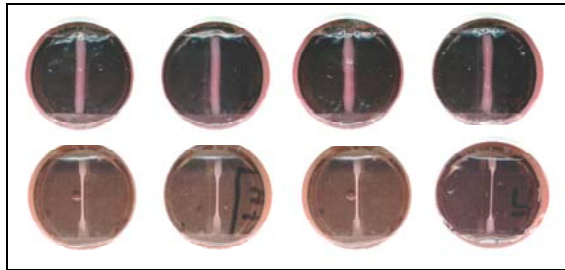


Figure 1. Linear cell-seeded hydrogels after initial formation (upper row) and a week in culture (lower row).

FIXING THE BIOARTIFICIAL CONSTRUCT

Cell-populated hydrogels can be fixed *in situ* in 70% methanol or 10% neutral-buffered formalin (NBF) for 4 hours, then stored at 4 °C with a ziplock bag around the plate to prevent evaporation. BATs can be stained with routine histologic stains such as hematoxylin and eosin, Mallory's Triple stain or Giemsa stain or with reagents for fluorescence microscopy.

HISTOLOGIC PREPARATION

BATs can be paraffin embedded, sectioned and stained or embedded in medium for frozen sectioning. BATs can be cut from the connecting nylon tab ends or held in place with the rubber and nylon tabs connected. The latter helps maintain the geometry of the BAT in its linear form, dimension and orientation. BATs can be stained with hematoxylin and eosin as per protocol (*Fig. 2*).

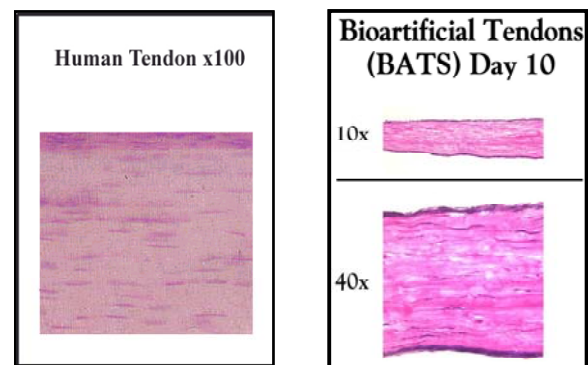


Figure 2. Longitudinal cross-section of a human tendon (left) and a linear BAT construct (right) stained with Hematoxylin and eosin (Garvin et al., 2003).

IMMUNOFLUORESCENCE STAINING

BATs can be fixed as above then washed 3X in buffer and immunostained with primary antibody for overnight at 4°C or for 1 hour at room temperature, *in situ* or removed from the well and stained in a 1 ml poly tube. BATs are then washed 3X in buffer and stained with secondary antibody for 30 minutes at room temperature, washed 3X in buffer then wet mounted on a slide and prepared for fluorescence observation.

FLUORESCENCE MICROSCOPY OF BATs

The collagen hydrogel has low fluorescence but will autofluoresce in the fluorocein



range. Rhodamine and DAPI stains are excellent for cells in BATs.

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