



# TECH REPORT

108:

## 24-Well HT BioFlex® Culture Plates

Cell growth on silicone membranes compared  
to standard plastic culture plates

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## INTRODUCTION

The HT BioFlex® plate is a 24-well flexible silicone elastomer bottomed culture plate with total growth surface area of 37.47 cm<sup>2</sup> (1.56 cm<sup>2</sup>/well; Fig. 1). The membrane has a thickness of 0.254 mm with similar properties to Flexcell's standard 6-well BioFlex® plate. In this study, we compared the growth of cells in a 24-well HT BioFlex® plate with that of cells grown in a standard 24-well plastic culture plate.



**Figure 1.** 24-well HT BioFlex® plates.

## CELL CULTURE

Porcine tendon fibroblast cells (tenocytes) were isolated from porcine Achilles tendon according to the method described by (Banes 1988) Porcine tenocytes were maintained in DMEM high glucose medium supplied with 10% fetal bovine serum (FBS), 15 mM glutamine, 20mM sodium pyruvate, 20 mM HEPES pH 7.2, 100 µM 2-phosphoascorbate, antibiotics (100 µg/ml sodium penicillin G, 100 µg/ml streptomycin sulfate, 5 µg/ml Fungisone). Passage 1 cells were used in this report.

## GROWTH CURVE ASSAY

Fibroblasts were plated at 10,000 cells per cm<sup>2</sup> at day 0. Cells (six wells per time point) were collected daily, stained with trypan blue, and live cells were counted using a Countess™ cell counter daily.

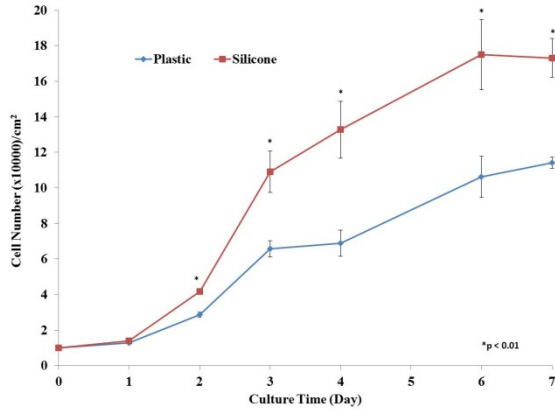
Fibroblasts were also fixed and stained with 0.05% Crystal Violet (three wells per time point). Cell images were recorded daily at 10X with an Olympus microscope.

## PROTEIN ASSAY TO CELL ATTACHMENT AND GROWTH IN 24-WELL HT BIOFLEX® PLATES

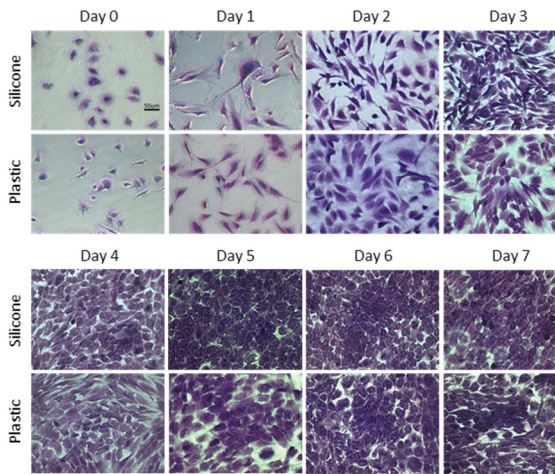
Porcine tenocytes were plated in 24-well HT BioFlex® plates and, as a control, 24-well plastic culture plates at 10,000 cells/cm<sup>2</sup>. Each day, cells in six wells were fixed and stained with SRB (Sulforhodamine B). After rinsing off unincorporated dye, protein-bound dye was dissolved, and SRB specific absorbance as well as fluorescence was measured with a plate reader (for fluorescence measurements, black HT BioFlex® plates were used to reduce internal reflectance).

## RESULTS AND DISCUSSION

Fibroblasts grown on collagen-coated, silicone membranes had an increased growth rate compared to those cells grown on plastic culture plates (Fig. 2 & Table 1). Morphologically, cells on silicone membranes and plastic were similar in appearance (Fig. 3).



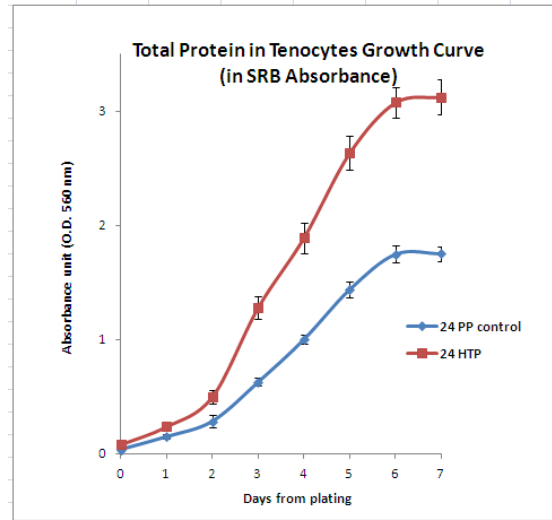
**Figure 2.** Growth curve adjusted for area differences between standard 24-well tissue culture plate (diamonds) and a 24-well HT BioFlex® plate (squares).



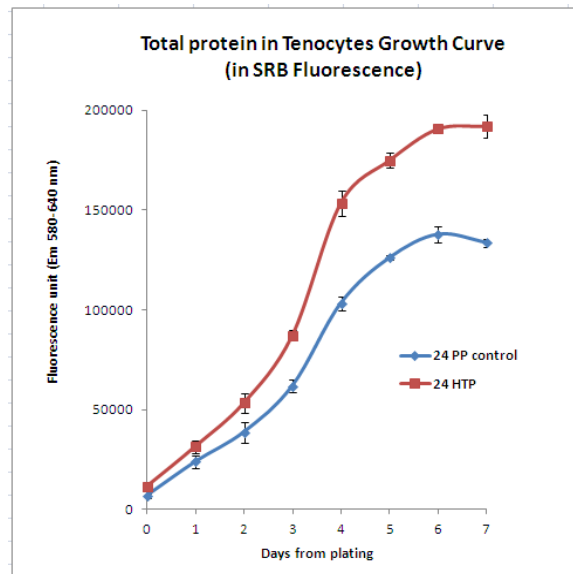
**Figure 3.** Images of tendon fibroblasts stained with crystal violet on days 0-7 of culture on either silicone membranes (HT BioFlex® plates) or 24-well plastic culture plates. Scale bar = 50 μm

Total cellular protein levels were measured as SRB absorbance intensity (O.D.560 nm) (Fig. 4) and fluorescence intensity (Ex525 nm/Em580-640 nm) (Fig. 5) in a plate reader.

Values were normalized to cell number and growth surface area.



**Figure 4.** Total protein in SRB absorbance.



**Figure 5.** Total protein in SRB Fluorescence



**Table 1.** Cell growth on the silicone membrane in a 24-well HT BioFlex® culture plate compared to that of cells grown in standard 24-well plastic tissue culture plates.

Time (days)	Cell Count (averaged)		Cell Density (cells/cm)		Growth Constant (k)		Generations per Hr (g/t)		Hrs per Generation (t/g)	
	Plastic	Silicone	Plastic	Silicone	Plastic	Silicone	Plastic	Silicone	Plastic	Silicone
0	19000	15600	1.00E+04	1.00E+04						
1	24600	22000	1.29E+04	1.41E+04	0.0108	0.0143	0.0155	0.0207	64	48
2	54400	65000	2.86E+04	4.17E+04	0.0219	0.0297	0.0316	0.0429	32	23
3	125000	170000	6.58E+04	1.09E+05	0.0262	0.0332	0.0377	0.0479	26	21
4	131000	207000	6.89E+04	1.33E+05	0.0201	0.0269	0.0290	0.0389	34	26
6	202000	273000	1.06E+05	1.75E+05	0.0164	0.0199	0.0237	0.0287	42	35
7	217000	270000	1.14E+05	1.73E+05	0.0145	0.0170	0.0209	0.0245	48	41

These data indicate the cell growth in 24-well HT BioFlex® plates is comparable if not better than cells grown on 24-well plastic culture plates. Flexcell's HT BioFlex® culture plates can be used in high throughput assays where control of mechanical stimulation and high replicate numbers are needed. These plates can also be used in three dimensional culture in linear and circular Tissue Train® embodiments for tissue engineering.

**REFERENCES**

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