TECH REPORT

106:

Matrix Bonded Growth Surfaces

Growing cells in a more natural matrix environment

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Culturing Cells in a Mechanically Active Environment™
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INTRODUCTION

Flexcell®'s unique culture plates provide researchers with matrix bonded growth surfaces that promote attachment and growth of a variety of cell types. Matrix coatings, such as type I collagen peptides, elastin, fibronectin (as RGD repeat peptides), and laminin (as YIGSR peptides), enhance attachment of specific cell types (see Table 1). These specialty growth surfaces help to better simulate the *in vivo* environment.

Flexcell® culture plates including BioFlex®, UniFlex®, HT BioFlex® and Tissue Train® series culture plates (Fig. 1), StageFlexer® membranes and Culture Slips® are available with the following treatments:

Genetic type I collagen for improved attachment and adherence of cells including:

- Continuous cell lines
- Primary cells
- Aortic, venous, and capillary endothelial cells
- Chondrocytes
- Ligament fibroblasts
- Lung type II epithelial cells
- Osteoblasts
- Tendon fibroblasts
- Smooth, striated and cardiac muscle cells
- Myoblasts
- Myocytes

Fibronectin, as RGD repeat peptides, and ProNectin F for the improved attachment of cells including:

- Embryonic cells
- Fibroblasts

Laminin, as YIGSR peptides, for the improved attachment of cells including:

- Astrocytes
- Glial cells
- Neurons
- Cells grown on type I collagen or ProNectin F

Positively charged amino hydrophilic for the improved attachment of cells including:

- Endothelial cells
- Smooth muscle cells

Elastin for the improved attachment of cells including:

- Endothelial cells
- Smooth muscle cells

NOTE: *See the integrin table below to match your cell's integrin panel with the appropriate growth surface.*

Figure 1. *Flexcell®'s multi-well rubber-bottomed culture plates.*

Flexcell®'s culture plates are stringently tested to assure the highest quality control and the best cell attachment and growth possible. Attachment factors are covalently bonded to the culture plate rubber membranes or plastics using our proprietary methods that result in optimal cell adherence and clarity for viewing cells. Culture plates are sterilized with gamma radiation and have a shelf life of one year.

Table 1. Vertebrate Integrins Grouped in Subfamilies Sharing a Common β Subunit

Subunits		Ligands Sequenced	Minimal Sequence of Integrin Binding Site*
β_1^+	α_1	Collagen, Laminin	
	α_2	Collagen, Laminin	DGEA
	α_3	Fibronectin, Laminin, Collagen	RGD
	α_4	Fibronectin, VCAM-1	EILDV
	α_5	Fibronectin	RGD
	α_6^+	Laminin	
	α_7	Laminin	
	α_8	?	
	α_V	Vitronectin, Fibronectin	RGD
β_2	α_L	ICAM-1, ICAM-2	
	α_M	C3b component of complement (inactivated), Fibrinogen, Factor X, ICAM-1	
	α_X	Fibrinogen, C3b component of complement	GPRP
β_3^+	α_{IIb}	Fibrinogen, ProNectin F, von Willebrand factor, Vitronectin, Thrombospondin	RGD, KQAGDV
	α_V	Vitronectin, Fibrinogen, von Willebrand factor, Thrombospondin, Fibronectin, Osteopontin, Collagen	RGD
β_4^+	α_8^+	Laminin	
β_5	α_V	Vitronectin	RGD
β_6	α_V	Fibronectin	RGD
β_7	α_4	Fibronectin, VCAM-1	EILDV
	α_{IEL}	?	

CELL GROWTH CONCERNS

Requirements and conditions for cell growth can vary from cell line to cell line as well as among same cell types from species to species. Isolation of monotypic cells from primary culture can be fraught with difficulties. After going through the procedures to isolate cells from primary culture, not having cells adhere and grow on the flexible membranes of the BioFlex® experimental plate is at best frustrating. The following suggestions on ways to improve cell growth on the membranes may help to reduce the frustration level.

Match substrates to cells. Most cells seem to do well on the collagen type I substrate of the BioFlex® plates (Fig. 2). In general, the substrates can be ranked as listed below in terms of substrate suitability. This is not intended to be a universal guide or to replace the necessity of doing the experiment to determine the most suitable substrate for your cells.

Most general ----------> Most specific

Collagen I, Fibronectin, Elastin, Laminin, Collagen IV, Amino, and Carboxyl

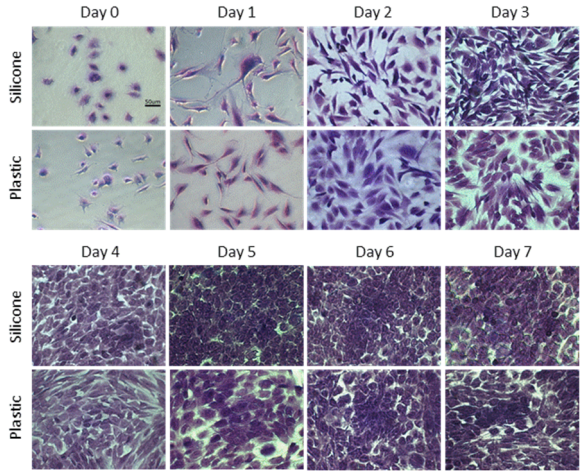

Figure 2. *Images of tendon fibroblasts stained with crystal violet on days 0-7 of culture on either silicone membranes (Collagen I-coated HT BioFlex® plates) or 24-well plastic culture plates. Scale bar = 50 μm*

Substrate Shock. Cells isolated or reared on plastic culture dishes may be subjected to additional insults when passed from the plastic to the silastic membranes. In most cases, cells recover rapidly and are not particularly troubled by the change in substrate. Other cell lines may find the switch difficult and are slow to recover. If you are experiencing problems with attachment, adherence or growth, you may find it beneficial to initially grow your cells on the rubber substrates. Primary isolations of cells from tissues (Freshney, 1987) are done directly onto these plates using the same techniques that are used with plastic dishes. Your cells become conditioned to the substrate and are subjected to fewer traumas when passed or split into the multi-well plates for experimentation.

MECHANICAL LOAD

The Flexcell® Tension System (Fig. 3) provides a strain component for dynamically culturing cells *in vitro*. Researchers use the Flexcell® culture plates together with the tension system to apply a defined, controlled, static or variable duration cyclic tension to cells.

Figure 3. *Flexcell® FX-5000™ Tension System*

The Flexcell® Streamer® (Fig. 4) applies fluid flow to cells in culture. Researchers use Culture Slips® together with the flow system to apply a controlled laminar, oscillatory, or pulsatile flow to cells.

Figure 4. *Flexcell® Streamer® Shear Stress Device*

Flexcell®'s culture plates together with Flexcell®'s systems for applying mechanical load provide the investigator with the ability to grow cells *in vitro* in a manner that better simulates an *in vivo* environment.

REFERENCES

Freshney IR, 1987. Culture of Animals Cells -- A Manual of Basic Technique. 2nd Ed. New York, Wiley-Liss.