



# Tech Report 205:

# "Substrate Shock"

## Cell Growth Problems

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*Culturing Cells in a Mechanically Active Environment*<sup>™</sup>  
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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 Flex I® 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 genetic type I substrate of the Flex I® collagen plates. This extracellular matrix (as are the other ECM used by Flexcell®) is covalently bonded to the silastic membrane. Essentially, a monolayer of the matrix is present. A more uniform substrate is available than if the matrix was simply absorbed onto the membrane. Other substrates such as collagen genetic type IV, elastin, laminin, fibronectin as well as amino and carboxyl surfaces are available from Flexcell®, on both the novel rubber and the traditional plastic-bottomed 25 mm 6-well and 83 mm single well, cell culture plates. 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

### **MICROORGANISM CONTAMINATION**

*Mycoplasma* infections can have deleterious effects on cell cultures. Potential effects of mycoplasmal contamination can include alterations in growth rates, altered cellular transformations, chromosomal abnormalities, and altered nucleic acid and protein synthesis (Stanbridge, 1981). These effects may become more obvious on the Flex I® silastic substrates than on the more usual plastic surface as a result of the additional stress caused by a shift of substrate and simply because of paying more attention to cells when plating on the novel surface. If you are experiencing a slower than usual growth rate, altered morphology, or other unusual reactions of your cell line on the Flex® plates, you should test for the presence of *Mycoplasma* or other microorganism. Often a single check for the organism may not be sufficient depending on the challenge present, so you should plan to make several examinations before ruling out such a contamination.

### **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 rear cells on the rubber substrates in larger dishes and then pass cells to the smaller experimental dishes. Flexcell manufactures Flex III™ 83 mm, single well dishes for this purpose. 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 less trauma when passed or split into the 6-well plates for experimentation.

## REFERENCES

Stanbridge EJ, 1981. Mycoplasma Detection -- an Obligation to Scientific Accuracy. *Israel J Med Sci* 17:563-568.

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

Ryan JA, 1994. Understanding and managing cell culture contamination. Corning, Inc. Technical Publications TC-CI-559.