# TECH REPORT

# 112:

# Cell Seeder™

## Cell growth in 24-well Flexcell® culture plates using Cell Seeders™

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*Culturing Cells in a Mechanically Active Environment*™
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## INTRODUCTION

Cell Seeders™ (Fig. 1) are designed to keep cells in the central region of the HT BioFlex® culture wells during plating to help prevent the cells from being subjected to undefined strains when using the cylindrical Loading Stations™. This report discusses the amount of strain applied to the membrane during seeding as well as the growth of cells in the HT BioFlex® culture plates when cells are plated using Cell Seeders™.

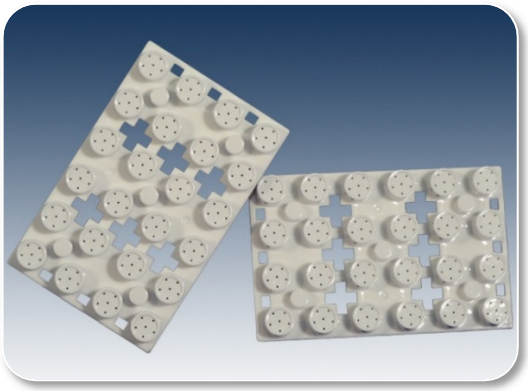

***Figure 1.*** *24-well Cell Seeder™.*

## STRAIN QUANTIFICATION

A 24-well Cell Seeders™ was placed in a HT BioFlex® baseplate. The radial and circumferential strains were experimentally determined by imprinting the HT BioFlex® membrane with a dot pattern. Strain was determined at different locations on the membrane by measuring the change in distance between a pair of dots under various vacuum pressure levels. All vacuum pressure measurements were made using a digital manometer. Vacuum was applied with a Leybold Trivac D8B vacuum pump.

Distances were measured between points using the following method:

A Canon Compact EOS Digital Rebel XTI® camera equipped with a macro lens was leveled and fixed directly above the membrane. The resolution of the image was adjusted to ensure each pair of dots filled the maximum horizontal distance across the digital image, maximizing the number of pixels and measurement accuracy. Regimens were designed to look at pressures from 0-90 kPa. At each static step, the image was captured using a Lexar™ memory card. Adobe Photoshop® CS2 image analysis software was used to measure the distances between the dots. This procedure was repeated for 3 random wells in 3 plates.

## CELL CULTURE

HeLa and REVC cells obtained through ATCC. HeLa cells were maintained in MEM with 10% fetal bovine serum (FBS) with antibiotics (100 µg/ml sodium penicillin G, 100 µg/ml streptomycin sulfate, 5 µg/ml Fungisone). REVC cells were maintained in High-Glucose DMEM with 10% FBS, 20mM HEPES, non-essential amino acids, and antibiotics (100 µg/ml sodium penicillin G, 100 µg/ml streptomycin sulfate, 5 µg/ml Fungisone).

Cells were plated using the HT Cell Seeders™ per the method in the *HT Cell Seeder User Manual*. Cells were seeded at 30,000 cells/cm$^2$ and allowed to adhere for 2 hours prior to removing non-adherent cells and adding growth media.

Cells were stained with crystal violet on days 1, 3, 5 and 7 post-plating.

## RESULTS AND DISCUSSION

### *Strain Quantification*

The following figure shows the experimental results for the average membrane % elongation relative to the vacuum pressure level for a HT BioFlex® plate and a 24-well

cell seeder (Fig. 2). During plating, cells may be exposed to low levels of static strain since the membrane is being pulled into the seeder mold.

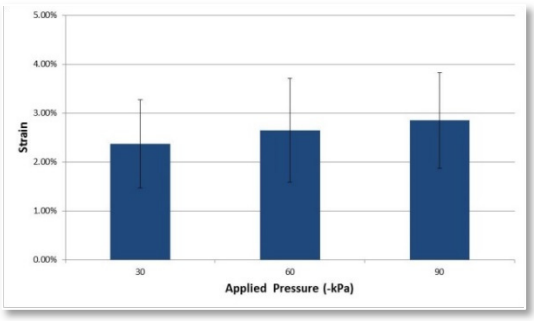


***Figure 2.*** *Average substrate strain in a HT BioFlex® well when using a 24-well Cell Seeder™.*
***NOTE: Seeders are only used during plating and not during application of uniaxial strain.***

### *Cell Growth*

Cells remained in the central portion of the well after plating using the 24-well Cell Seeders™ for up to seven days (Fig 3).

Taken together, these data indicate that the Cell Seeders™ can be used when culturing cells to keep them isolated in the central portion of the well and away from the well periphery, where undefined strains occur during mechanical loading with the FX-5000™ Tension System.

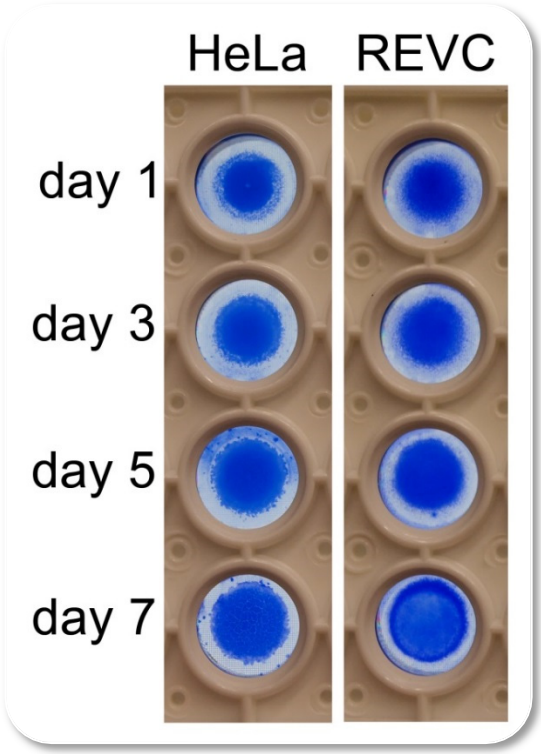


***Figure 3.*** *HeLa and REVC cells stained with crystal violet on days 1, 3, 5, and 7 post-plating.*