Tech Report 101:

Loading Stations

Quantification of Strain on the Membrane Surface

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Culturing Cells in a Mechanically Active Environment™
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INTRODUCTION

Cylindrical BioFlex® Loading Stations™ are designed to provide uniform radial and circumferential strains (equibiaxial strain) to cells cultured on flexible membranes. Similarly, Arctangle™ Loading Stations™ are designed to provide uniaxial strain to cells in monolayer or three-dimensional (3-D) gel matrices. Loading Stations™ together with the flexible-bottomed culture plates and the Flexcell® Tension system provide regulated strain to the cells.

Loading Stations™ are comprised of a 3.3" x 5" Lexan® plate and six removable Delrin® planar faced cylindrical (25 mm, 28 mm, or 31 mm diameter; Fig. 1) or Arctangle™ loading posts. The posts are positioned on the Lexan® plate such that each is centered beneath the rubber membrane of the 35 mm well of a BioFlex®, UniFlex® or Tissue Train® culture plate (Fig. 2).

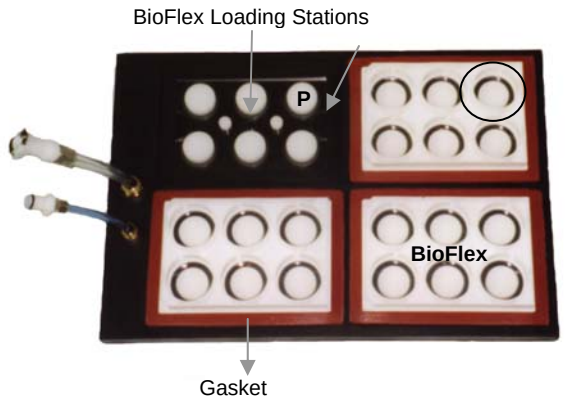

Figure 1. BioFlex® baseplate showing the Loading Stations™ with six loading posts (P) and BioFlex® culture plates in red rubber gaskets.

When vacuum is applied to a BioFlex® culture plate, the membrane deforms across the planar face of the post creating uniform biaxial strain (Fig. 2). Similarly, when vacuum is applied to a Tissue Train® or UniFlex® culture plate, the membrane deforms across the planar face of the post only at the east and west poles creating uniaxial strain. Use of Loading Stations™ provides both constrained distension to the flexible membrane and nominal fluid shear stress.

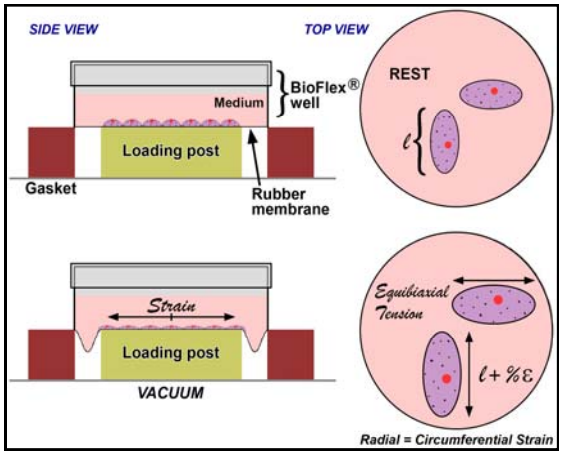

Figure 2. Schematic of the side and top view of a membrane deformed across a cylindrical loading post creating equibiaxial tension.

The purpose of this study was to determine the substrate strain levels for a given vacuum pressure level and loading post geometry.

METHODS

Four Loading Stations™ each with six Delrin® posts were placed in a BioFlex® baseplate. The radial and circumferential strains were experimentally determined by imprinting the 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. Loctite® lubricant was applied to each Delrin® post to enable frictionless movement against the rubber membrane.

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 step through pressures from 0-90 kPa in the desired increments of pressure to maximize adequate sampling. 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 each well and loading post station. A similar protocol was used to determine the strains on Tissue Train® and UniFlex® culture plates.

RESULTS AND DISCUSSION

The following figures show the experimental results for the average membrane % elongation relative to the vacuum pressure level for a BioFlex® plate and each loading post diameter (25, 28, and 31 mm; Fig. 3-5) as well as for the UniFlex® and Tissue Train® plates and the Arctangle™ loading post (Fig. 6-7).

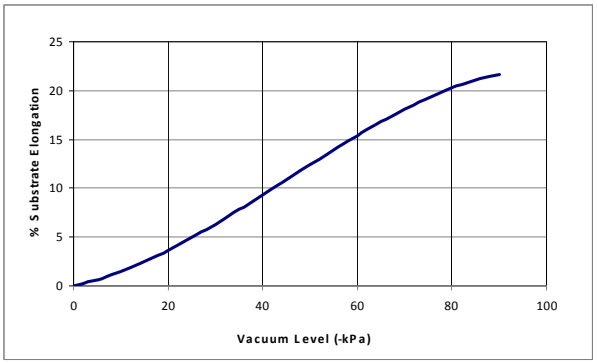

Figure 3. Average radial strain vs. vacuum level for a BioFlex® culture plate atop a 25 mm BioFlex® Loading Station™.

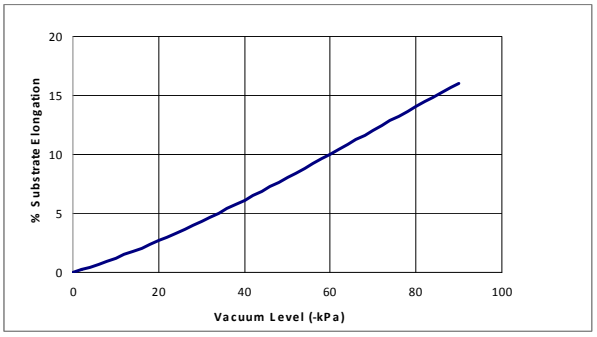

Figure 4. Average radial strain vs. vacuum level for a BioFlex® culture plate atop a 28 mm BioFlex® Loading Station™.

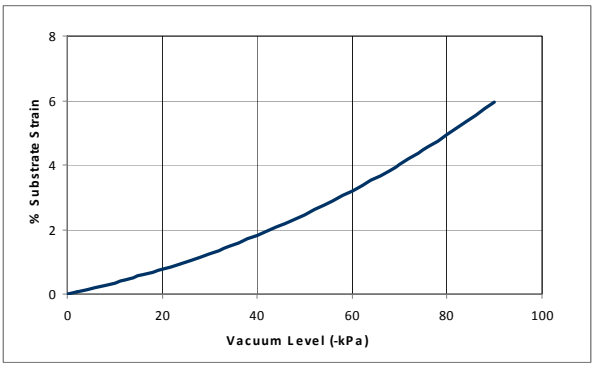

Figure 5. Average radial strain vs. vacuum level for a BioFlex® culture plate atop a 31 mm BioFlex® Loading Station™.

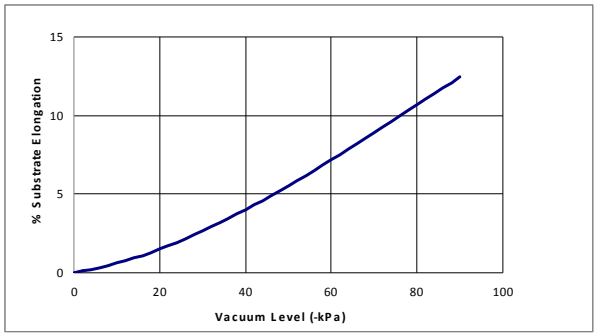

Figure 6. Average strain vs. vacuum level for a UniFlex® culture plate atop a 24 mm Arctangle™ Loading Station™.

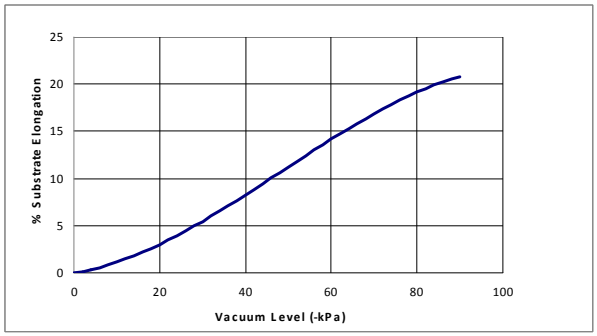

Figure 7. Average strain vs. vacuum level for a Tissue Train® culture plate atop a 24 mm Arctangle™ Loading Station™.

CONCLUSIONS

BioFlex® Loading Stations™ provide uniform radial and circumferential strain to the BioFlex® membrane. This allows cell culture in a uniform biaxial environment with all cells stretched over the loading posts receiving the same amount of strain. The FX-5000™ Tension System can yield up to 21.8% substrate elongation when used with the BioFlex® Loading Stations™. The Arctangle™ Loading Stations™ provide uniaxial strain to the central regions of the UniFlex® and Tissue Train® membranes. The localized strain can vary ± 0.8% of the average strain value over the entire uniaxial strain region on the UniFlex® culture plate, with a maximum average strain of 12.2%. Up to 20.8% elongation can be achieved for gel constructs strained with the Tissue Train® Culture System.

Lexan® is a registered tradename of G.E. Plastics Inc.; Delrin® is a registered tradename of E. I. DuPont Co.