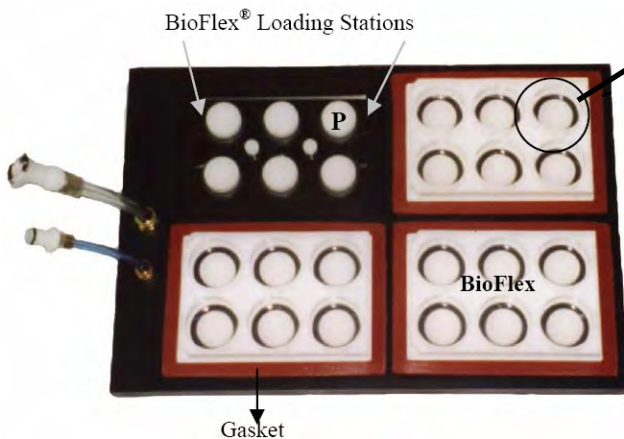


# BIOFLEX CULTURE SYSTEM

## Introduction

BioFlex Loading Stations are designed to provide uniform radial and circumferential strains (equibiaxial strain) to cells cultured on flexible membranes. Similarly, the Arctangle Loading Stations are designed to provide uniaxial strain to cells in monolayer or 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. 15) 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. 16).



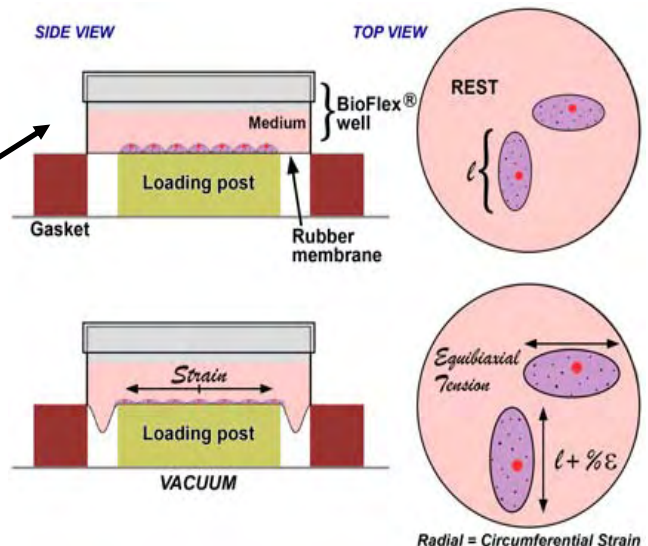
**Figure 15.** BioFlex Baseplate showing the Loading Stations with six Loading Posts (P) and BioFlex culture plates in red rubber gaskets.

When vacuum is applied to a BioFlex culture plate, the membrane deforms across the planar face of the post creating uniform biaxial strain (Fig. 16). 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. 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 Robinair vacuum pump (model #15101-B; 141.6 l/min).



**Figure 16.** Diagram of the side and top view of a membrane deformed across a cylindrical Loading Post creating equibiaxial tension.

Distances were measured between dots using the following method:

A black and white video camera was suspended and leveled directly over a membrane. The camera was adjusted so that each pair of dots filled the maximum horizontal distance across the video screen, allowing for the maximum number of pixels between dots and maximizing measurement accuracy. The image was captured using a Flashpoint video card. Image analysis software program, Sigma Scan Pro (v. 4.0), was used to measure the distances between dots.

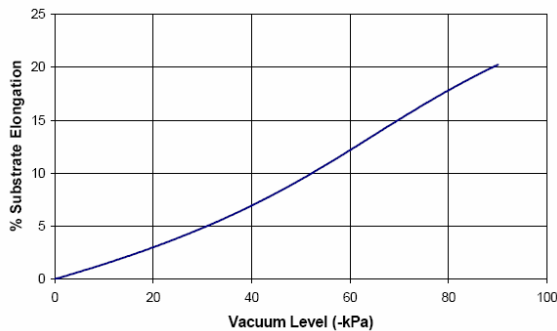


# BIOFLEX CULTURE SYSTEM

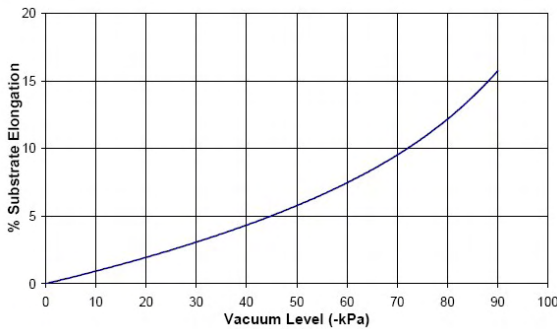
This procedure was repeated for each well and Loading Post station. A similar protocol was used to determine the strains on Tissue Train and UniFlex culture plates.

## Results and Discussion

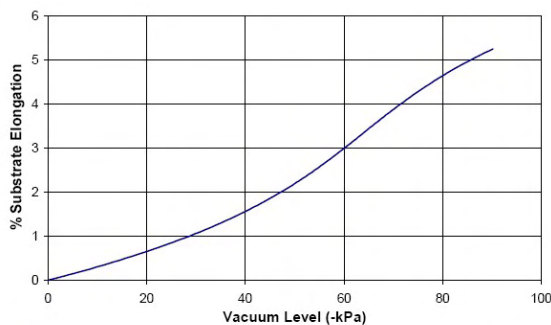
The following figures show the experimental results for the average membrane % elongation relative to the vacuum pressure level for a BioFlex plate and each Loading Post diameter (25 mm, 28 mm, and 31 mm) as well as for UniFlex culture and Tissue Train plates and the Arctriangle Loading Post.



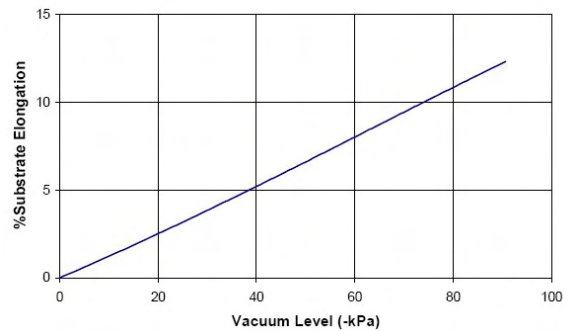
**Figure 17.** Average radial strain vs. vacuum level for the 25 mm post of the BioFlex Loading Post.



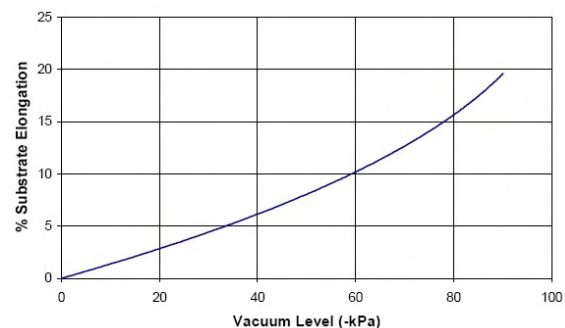
**Figure 18.** Average radial strains vs. vacuum level for the 28 mm post of the BioFlex Loading Station.



**Figure 19.** Average radial strains vs. vacuum level for the 31 mm post of the BioFlex Loading Station.



**Figure 20.** Average strain vs. vacuum level for a UniFlex culture plate atop a 24 mm Arctriangle Loading Post.



**Figure 21.** Average strain vs. vacuum level for a Tissue Train culture plate atop a 24 mm Arctriangle Loading Post.

## 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-4000T can yield up to 30 % substrate elongation when used with BioFlex Loading Stations. The Arctriangle Loading Stations provide uniaxial strain to the central regions of the UniFlex and Tissue Train membranes. The localized strain can vary  $\pm 1.5\%$  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 17% elongation can be achieved for gel constructs strained with the Tissue Train Culture System.

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