Dunn Labortechnik

Overview of Applications of Flexcell® Products

Applying Mechanical Load to Cells in Monolayer Culture (Page 2 - 3)

- FX-5000™ Tension System
- Streamer® Fluid Shear Stress Device
- Osci-Flow® Flow Controller
- BioFlex® Culture Plate
- UniFlex® Culture Plate
- Loading Stations™
- StageFlexer®
- FlexFlow™ Shear Stress Device
- StageFlexer® Membranes
- Culture Slips®

Applying Mechanical Load to Cells in 3D Culture (Page 4 - 5)

- FX-5000™ Tension System
- Tissue Train® Culture System
- FX-5000™ Compression System
- BioPress™ Compression Culture Plate
- Flex Jr.™ Tension System
- StageFlexer® Jr.
- StagePresser™
- StagePresser™ Membranes

Tissue Engineering (Page 6 - 8)

- Tissue Train® Culture System
- FX-5000™ Tension System
- ScanFlex™ with XyFlex™
- Tissue Train® Culture Plate (Linear)
- Trapezoidal Tissue Train® Culture Plate
- Tissue Train® Circular Foam Culture Plate
- Trough Loaders™

Cell Signaling (Microscope Measurements) (Page 9 - 10)

- FX-5000™ Tension System
- FX-5000™ Compression System
- Flex Jr.™ Tension System
- StageFlexer®
- StageFlexer® Jr.
- StagePresser™
- FlexFlow™ Shear Stress Device
- StageFlexer® Membranes
- Culture Slips®
- StagePresser™ Membranes

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Applying Mechanical Load to Cells in Monolayer Culture

Equipment, Devices, & Consumables for this Application

- FX-5000™ Tension System
- Streamer® Fluid Shear Stress Device
- Osci-Flow® Flow Controller
- BioFlex® Culture Plate
- UniFlex® Culture Plate
- Loading Stations™
- StageFlexer®
- FlexFlow™ Shear Stress Device
- StageFlexer® Membranes
- Culture Slips®

Introduction

Cells are subjected to compression, tension, and shear in the body and undergo acute and adaptive biochemical changes in response to deformation. Stressing cells in culture simulates the *in vivo* environment causing dramatic morphologic and biochemical responses. There are both short and long term changes that occur when cells are loaded in culture, such as alterations in signaling, the rate and amount of protein synthesis, secretion, or degradation, the rate of cell division, changes in energy metabolism, and other changes in biochemistry and bioenergetics.

For these reasons, Flexcell® introduced tension and fluid shear systems for applying mechanical load to cells in monolayer culture. These systems have broad applications in the biomedical research field since strain, compression, or fluid flow have been found to induce biochemical changes in cells derived from a variety of tissues including cardiac, skeletal and smooth muscle, lung, vascular endothelium, skin, tendon, ligament, cartilage, and bone (see our Publication Database for a more complete list of research in these areas).

Tension System

The FX-5000™ Tension System is a patented, computerized, pressure-operated instrument that applies a defined controlled, static or variable duration cyclic tension, to cells growing *in vitro*. This system utilizes regulated vacuum pressure to deform flexible-bottomed culture plates (Figure 1). When used with BioFlex® culture plates and cylindrical-shaped loading posts, this system applies an equibiaxial strain to the cells cultured in monolayer on the flexible-bottomed membrane of the BioFlex® culture well.
Similarly, when used with UniFlex® culture plates and an Arctangle® loading post (Figure 2), this system applies a uniaxial strain. This system can apply a gradient biaxial strain of up to 30 % in the absence of loading posts.

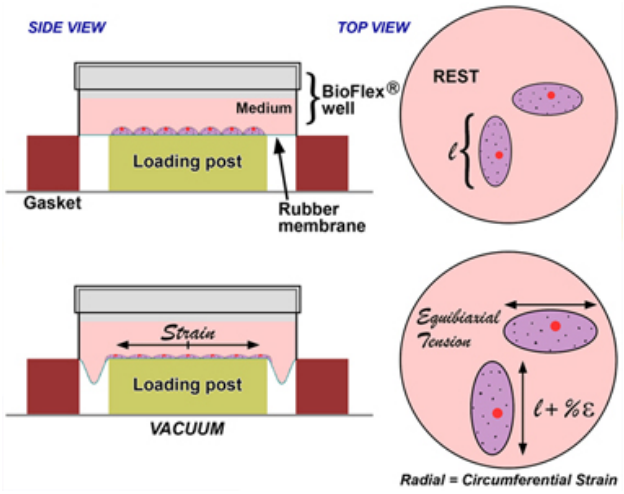

Figure 1: Equibiaxial strain application to cells plated in a BioFlex® well.

Figure 2: UniFlex® culture plate on Arctangle® Loading Stations™.

The StageFlexer® allows users to visualize real-time cellular responses to tension. It is a single-well embodiment of a BioFlex® well that fits on a standard upright microscope stage. A StageFlexer® Membrane can be deformed 1) freely in the open chamber producing gradient biaxial strain or 2) across a cylindrical loading post producing equibiaxial strain.

Fluid Shear System

Fluid-induced shear stress occurs in every tissue in the body as a result of interstitial fluid movement. Tissue deformation by compression, tension or shear forces results in the movement of interstitial fluid around cells. Fluid movement acts as a transport vehicle for ions, proteins, carbohydrates and other molecules capable of movement within the matrix. As the fluid moves past cell membranes, a shear stress, τ, is generated. If one assumes that laminar flow occurs through a parallel-plate flow chamber, fluid-induced shear stress values can be determined with the following formula: $\tau = 6\mu Q/bh^2$, where τ is the shear stress in dyne/cm^2, μ is the viscosity of the fluid in dynes/cm^2, Q is the flow rate in ml/s, b is the width of the flow channel in cm, and h is the height of the flow channel in cm.

The Streamer® is a parallel-plate flow system that is used to apply fluid-induced shear stress to cells grown in a monolayer. The system includes a six-chamber laminar flow device designed to hold 75 x 25 x 1 mm Culture Slips®. The Streamer® system can be used to apply laminar, pulsatile*, or oscillating* flow to cell in monolayer culture (Figure 3) and uses a computer-controlled peristaltic pump to regulate shear stress from 0 - 35 dynes/cm^2.

**When used in conjunction with the Osci-Flow® Flow Controller.*

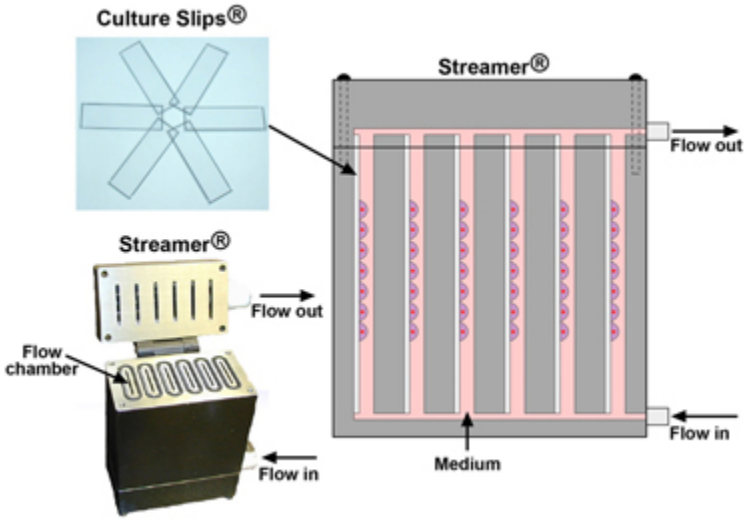

Figure 3. Fluid shear applied to cells in Streamer® device.

The FlexFlow™ is a parallel plate laminar flow device designed to apply fluid shear stress and/or cyclic strain to cells in culture while providing a means for viewing cell activity under a microscope in real-time. Like the StageFlexer®, the FlexFlow™ fits on the stage of a standard upright microscope.

Applying Mechanical Load to Cells in 3D Culture

Equipment, Devices, & Consumables for this Application

- FX-5000™ Tension System
- Tissue Train® Culture System
- FX-5000™ Compression System
- BioPress™ Compression Culture Plate
- Flex Jr.™ Tension System
- StageFlexer® Jr.
- StagePresser™
- StagePresser™ Membranes

Introduction

Formation of tissues *in vitro* that are structurally and functionally viable requires several basic conditions, such as 1) cells 2) matrix 3) media and growth factors and 4) mechanical stimulation. These conditions are linked to each other and act in conjunction to form a structurally robust tissue that can withstand biomechanical forces. As a tissue develops, its cells fabricate an extracellular matrix in a given geometry according to developmental pathway cues. Several signal transduction pathways may be involved in generating the composition of the extracellular matrix. Some of these pathways are regulated by mechanical deformation of cell matrix and transmitted into the cell via membrane bound proteins such as integrins, focal adhesion complexes (mechanosensory complex), cell adhesion molecules and ion channels. Cells can also respond to ligands, such as cytokines, hormones or growth factors that are released as a result of matrix deformation.

In order to maintain the integrity and strength of musculoskeletal tissues, the cells may require maintaining a certain level of intrinsic strain. In the absence of this intrinsic strain, the tissue will lose its strength leading to failures or fractures. It is well accepted that immobilization of limbs, bed rest or a reduction in the intrinsic strain level in a tissue leads to bone mineral loss, tissue atrophy, weakness and in general, a reduction in anabolic activity and an increase in catabolic activity. Physical activity, on the other hand, results in anabolic effects including an increase in biomechanical strength and an increase in the intrinsic strain in a tissue.

To generate a tissue *in vitro* that is more or less equivalent to the native tissues, it is of utmost importance to create an environment that would mimic the *in vivo* conditions. Culturing cells in a mechanically active environment increases cell metabolism and alters cell shape and other properties. Therefore, it is vital to create and maintain a mechanically active environment (i.e., tension, shear stress or compression) for the cells during the formation of tissues in vitro. In addition to the dynamic environment, culturing cells in 3D environment more closely simulates the native environment than a static 2D culture method.

Tension System

Three-dimensional bioartificial tissues (BAT), which have been created with the Tissue Train® Culture System, can be mechanically loaded with the FX-5000™ Tension System. This system is a patented, computerized, pressure-operated instrument that applies a defined controlled, static or variable duration cyclic tension, to cells growing *in vitro*. This system utilizes regulated vacuum pressure to deform flexible-bottomed culture plates. When used with Tissue Train® culture plates and an Arctangle® loading post, this system can apply up to 20% uniaxial strain to a BAT (Figure 4).

The StageFlexer® Jr. is designed to allow users to remove a membrane from a Tissue Train® culture plate well and continue observing cellular responses to strain under a microscope in real-time. Strain can be controlled with the FX-5000™ Tension System or the Flex Jr.™ Tension System.

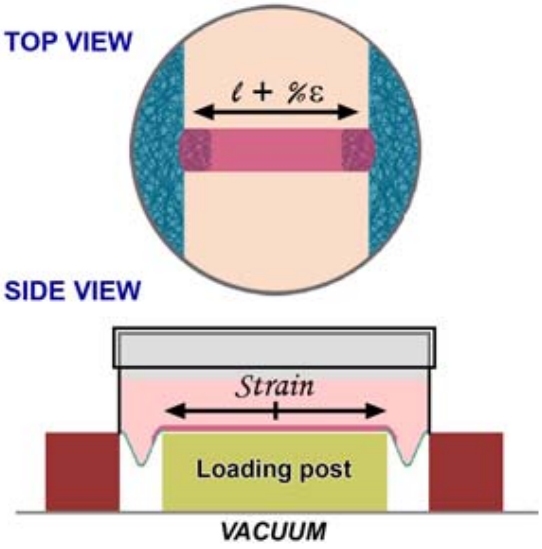

Figure 4: Uniaxial strain application to a bioartificial tissue construct.

Compression System

The FX-5000™ Compression System is a patented, computerized, pressure-operated instrument that applies a defined controlled, static or variable duration cyclic compression, to cells growing *in vitro*. This system utilizes regulated air pressure to deflect the flexible-bottomed BioPress™ culture plates. A tissue sample or a 3D cell culture is compressed between a piston (attached to the flexible-bottomed membrane) and a stationary platen (Figure 5). This system can apply an unconfined compressed of up to 14 pounds of applied force.

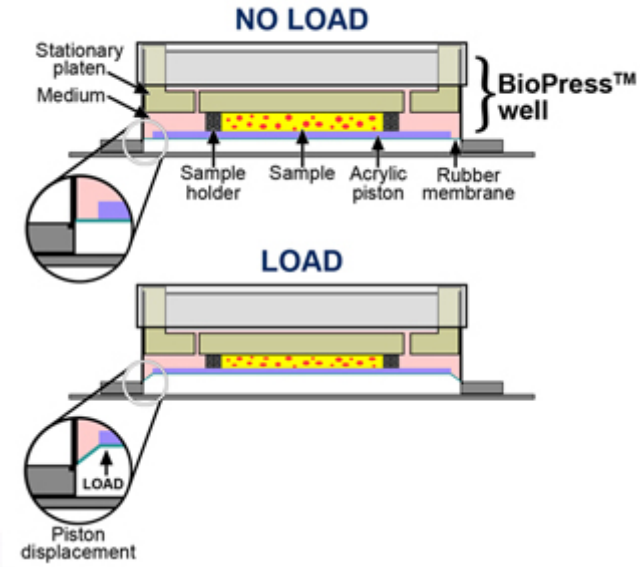

Figure 5: Schematic of how compression is applied to tissue samples in a BioPress™ well.

The StagePresser™ is designed to compress a single tissue sample or cells in a gel while viewing the cellular activity under a microscope. The StagePresser™ uses a piston adhered to a rubber membrane to apply force to a sample in culture. The piston is moved upward by positive air pressure applied to the silicone membrane. A FX-5000™ Compression System controls the compression frequency, amplitude, waveform, and cycles (or time period).

Tissue Engineering

Equipment, Devices, & Consumables for this Application

- Tissue Train® Culture System
- FX-5000™ Tension System
- ScanFlex™ with XyFlex™
- Tissue Train® Culture Plate (Linear)
- Trapezoidal Tissue Train® Culture Plate
- Tissue Train® Circular Foam Culture Plate
- Trough Loaders™

Introduction

Formation of tissues in vitro that are structurally and functionally viable requires several basic conditions, such as 1) cells 2) matrix 3) media and growth factors and 4) mechanical stimulation. These conditions are linked to each other and act in conjunction to form a structurally robust tissue that can withstand biomechanical forces. As a tissue develops, its cells fabricate an extracellular matrix in a given geometry according to developmental pathway cues. Several signal transduction pathways may be involved in generating the composition of the extracellular matrix. Some of these pathways are regulated by mechanical deformation of cell matrix and transmitted into the cell via membrane bound proteins such as integrins, focal adhesion complexes (mechanosensory complex), cell adhesion molecules and ion channels. Cells can also respond to ligands, such as cytokines, hormones or growth factors that are released as a result of matrix deformation.

In order to maintain the integrity and strength of musculoskeletal tissues, the cells may require maintaining a certain level of intrinsic strain. In the absence of this intrinsic strain, the tissue will lose its strength leading to failures or fractures. It is well accepted that immobilization of limbs, bed rest or a reduction in the intrinsic strain level in a tissue leads to bone mineral loss, tissue atrophy, weakness and in general, a reduction in anabolic activity and an increase in catabolic activity. Physical activity, on the other hand, results in anabolic effects including an increase in biomechanical strength and an increase in the intrinsic strain in a tissue.

To generate a tissue *in vitro* that is more or less equivalent to the native tissues, it is of utmost importance to create an environment that would mimic the *in vivo* conditions. Culturing cells in a mechanically active environment increases cell metabolism and alters cell shape and other properties. Therefore, it is vital to create and maintain a mechanically active environment (i.e., tension, shear stress or compression) for the cells during the formation of tissues *in vitro*. In addition to the dynamic environment, culturing cells in 3D environment more closely simulates the native environment than a static 2D culture method.

The size and shape of the tissue matrix would also directly affect the type, magnitude, direction and distribution of physiological forces within the tissue matrix. The composition of tissue may also depend on the types of forces that the tissue undergoes. Depending on the anatomical location, some tissues may experience both tensile and compressive forces within the tissue leading to multiple compositions. For example, the midsubstance (where tensile forces exist) of an Achilles tendon is comprised of dense fibrous connective tissue, while the area where tendon presses against calcaneus (where compressive forces exist) is comprised of fibrocartilaginous tissue. The shape of the tissue also plays a major role in the location of its failure. Most failures in Achilles tendons occur at the calcaneal junction where it joins the bone and has the least thickness. Therefore, it is clear that the native shape of the tissue needs to be simulated *in vitro* to facilitate studying the failure mechanism as well as the healing mechanism of tissues. Flexcell®'s Tissue Train® Culture System was developed to address these segments of the culture world, providing both a 3D matrix, dynamic strain to cells and matrix, and multiple geometries for creating bioartificial tissues of different shapes (i.e., linear, trapezoidal, and circular).

Tissue Train® System

Flexcell®'s Tissue Train® Culture System is a stand-alone 3D culture system that allows investigators to create 3D geometries for cell culture in a matrix gel or allow the cells to build a self-assembled matrix that connects to the anchors in a Tissue Train® culture plate. Flexcell® currently has molds and/or plates for creating three different shaped hydrogels: linear, trapezoidal, and circular. The Tissue Train® System can be used to create bioartificial constructs with cells from the cardiac, musculoskeletal, dermal, lung, gastrointestinal, bone marrow, and adipose tissues to name a few. (See our Publication Database to see how researchers are currently using this system).

Figure 6 illustrates how a linear bioartificial tissue (BAT) is created with the Tissue Train® Culture System. In brief, a Tissue Train® culture plate is set atop a Trough Loader™ and a vacuum is applied with the FX-5000™ Tension System pulling the flexible-bottomed rubber membrane of the culture plate downward into the linear trough. A cell and gel matrix suspension is dispensed into the trough between the two anchor stems with a pipette. After polymerization, the vacuum is released and a linear hydrogel, or bioartificial tissue, has been created that is attached to the culture plate via the anchor stems at the east and west poles.

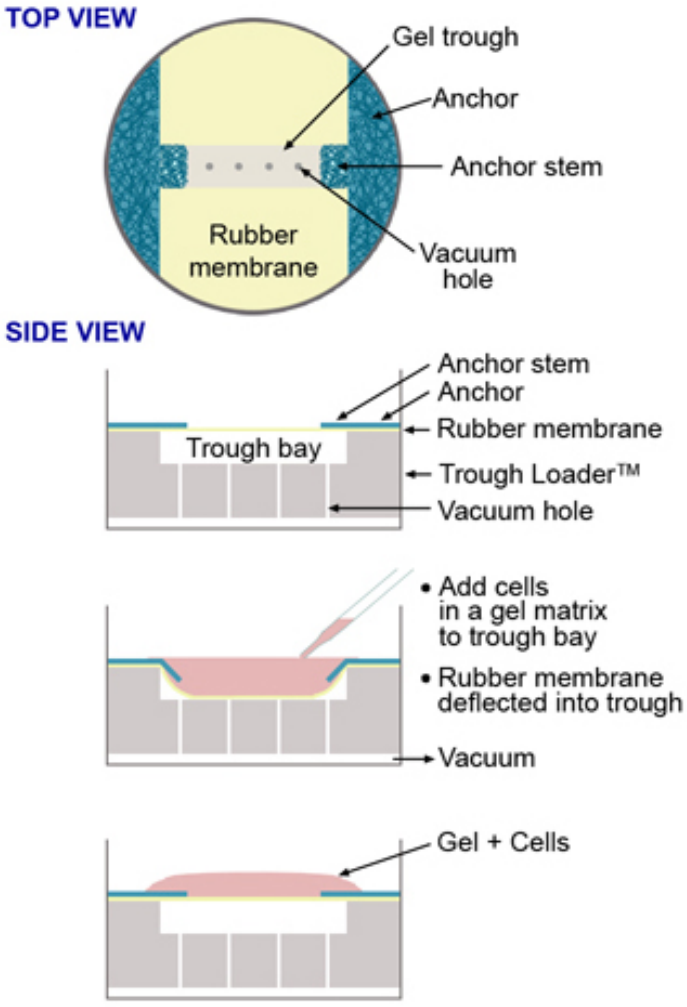

Figure 6: Bioartificial tissue development with the Tissue Train® Culture System.

The FX-5000™ Tension System provides the investigator with a tool to apply regulated uniaxial or equibiaxial strain to the growing bioartificial tissues. A user can define a frequency, elongation and duration of strain in a regimen that simulates the strain environment of the native tissue in the body (see *Applying Mechanical Load to Cells in 3D Culture* for further information).

Additionally, the cells will remodel their extracellular matrix over time (Figure 7). A measure of this remodeling is gel compaction over time. ScanFlex™ is an automated image collection system that allows users to periodically scan items placed on a scanner bed. The ScanFlex™ software controls a digital scanner and allows users to program the number of times and the time intervals when digital scans are taken. When used in conjunction with the Tissue Train® culture plates, ScanFlex™ can be used to determine the change in area of a bioartificial tissue. Furthermore, the area of a BAT can be measured using the XyFlex™ image analysis software. XyFlex™ software allows the user to automatically measure the BAT area in a large sequence of images.

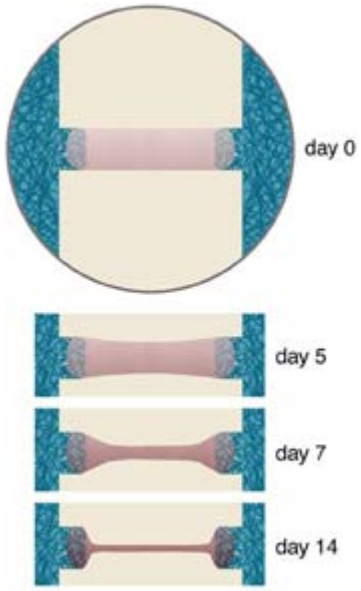

Figure 7: Illustration of gel compaction in a bioartificial tissue.

Cell Signaling (Microscope Measurements)

Equipment, Devices, & Consumables for this Application

- FX-5000™ Tension System
- FX-5000™ Compression System
- Flex Jr.™ Tension System
- StageFlexer®
- StageFlexer® Jr.
- StagePresser™
- FlexFlow™ Shear Stress Device
- StageFlexer® Membranes
- Culture Slips®
- StagePresser™ Membranes

Introduction

Several of the pathways activated in response to mechanical loading (see Figure 7) can be analyzed in real-time. For this reason, Flexcell® developed a line of microscopy devices that can used to visualize cells in both 2D and 3D culture systems that are being to subjected to mechanical load in real-time.

Tension Devices

The StageFlexer® allows users to visualize real-time cellular responses to tension. It is a single-well embodiment of a BioFlex® well that fits on a standard upright microscope stage. A StageFlexer® Membrane can be deformed 1) freely in the open chamber producing gradient biaxial strain or 2) across a cylindrical loading post producing equibiaxial strain (Figure 8).

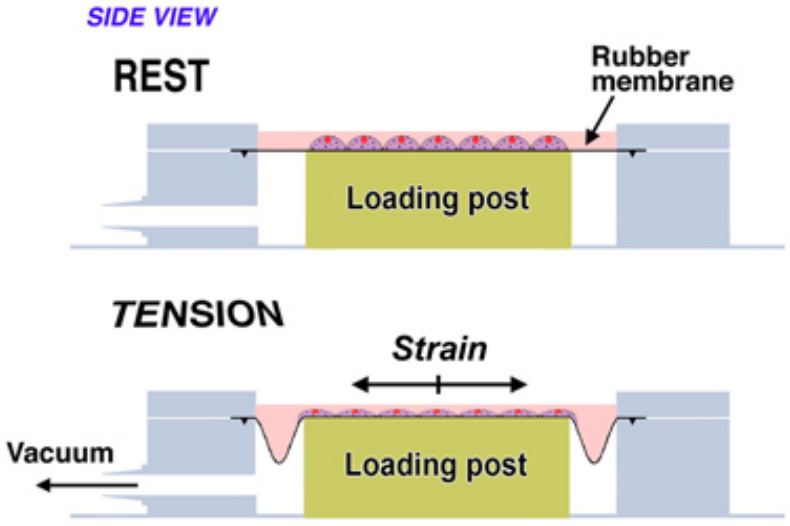

Figure 8: Equibiaxial strain application to cells plated on a StageFlexer® Membrane and clamped in a StageFlexer® Strain Device

The StageFlexer® Jr. is designed to allow users to remove a membrane from a BioFlex®, UniFlex®, or Tissue Train® culture plate well and continue observing cellular responses to strain under a microscope in real-time. Strain can be controlled with the FX-5000™ Tension System or the Flex Jr.™ Tension System.

Compression Devices

The StagePresser™ is designed to compress a single tissue sample or cells in a gel while viewing the cellular activity under a microscope. The StagePresser™ uses a piston adhered to a rubber membrane to apply force to a sample in culture. The piston is moved upward by positive air pressure applied to the silicone membrane (Figure 9). A FX-5000™ Compression System controls the compression frequency, amplitude, waveform, and cycles (or time period).

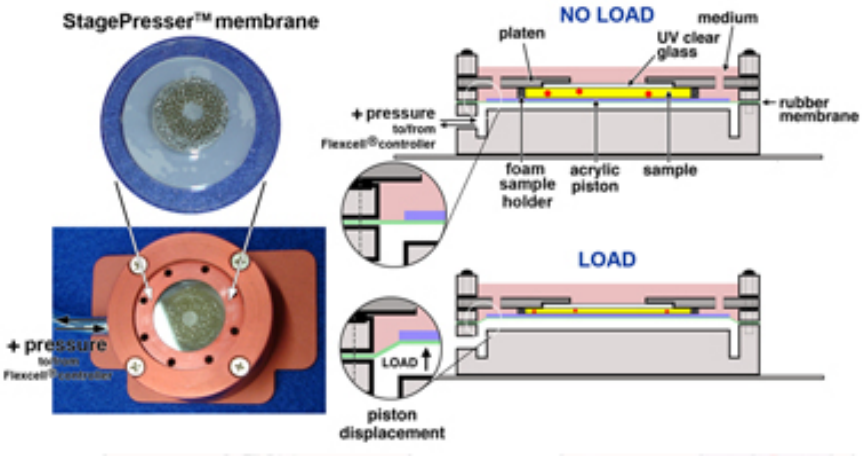

Figure 9: Schematic of how compression is applied to tissue sample or 3D cell cultures in a StagePresser™.

Shear Stress Devices

The FlexFlow™ is a parallel plate laminar flow device designed to apply fluid shear stress and/or cyclic strain to cells in culture while providing a means for viewing cell activity under a microscope in real time (Figure 10). Like the StageFlexer®, the FlexFlow™ fits on the stage of a standard upright microscope.

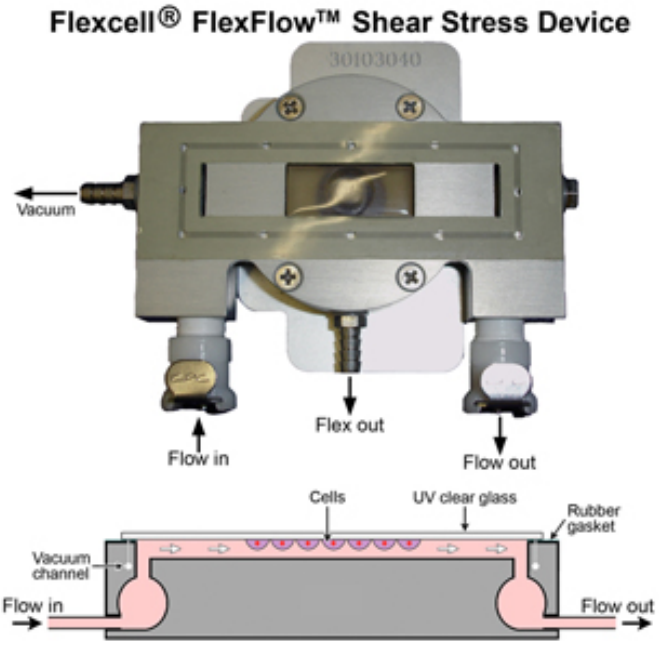

Figure 10: Schematic of fluid flow in a FlexFlow™ Shear Stress Device.