

# TECH REPORT

## 111:

# Sample Preparation and Setup of BioPress™ Culture Plates and Compression Baseplate Assembly

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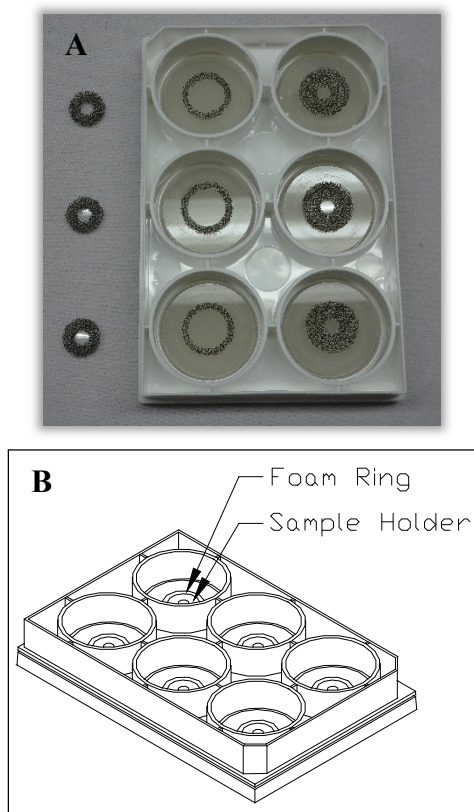
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The BioPress™ compression culture plate contains a foam ring and sample holder in each well (Fig. 1). The foam ring is used to contain the removable sample holder. In preparing a 3D culture or tissue explant sample, be sure that the sample thickness is no less than 1000 µm and no greater than 3 mm (0.118"). The thickness of the compressed foam is 350 µm; therefore, thinner samples will not be compressed. A sample thickness of 1000 µm or more will ensure compressibility. The maximum chamber height is 3.1 mm (0.122"); therefore samples with a thickness greater than 3 mm (0.118") will be preloaded when the platens are inserted into the wells of the culture plates.



**Figure 1.** A) BioPress™ culture plate with sample holders in the wells on the left and both a foam ring and sample holder in the wells on the right. B) Schematic of a BioPress™ culture plate with a foam ring and sample holder in each well.

The sample diameter should be equal to or less than the inner diameter of the foam sample holder in the chamber, which is 5 mm (0.200"). If the user wishes to use a larger sample, they can remove the sample holder and place the sample within the larger ring that normally contains the sample holder (Fig. 4). This will allow a sample diameter of up to 13 mm (0.525").

### THREE-DIMENSIONAL CELL SAMPLES

Cells should be cultured according to your laboratory's established protocol for primary cultures or continuous cell lines in the medium of choice. In general:

1. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase, or other means.
2. Add serum containing media to the cells to neutralize the trypsin or collagenase.
3. Count cells and determine the number of cells needed for the three-dimensional culture. Three sample methods are described below.

**NOTE:** *Cell seeding density will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.*

### Sheet Punch of a Cell-Seeded Hydrogel

1. Prepare hydrogel (Collagel®, agarose, gelatin, alginate, etc.) according to the manufacturer's or laboratory's established protocol.
2. Suspend cells in the hydrogel at  $1 \times 10^4$  –  $1 \times 10^5$  cells/ml.
3. Allow hydrogel to polymerize in a sheet or plug that is 1-3 mm thick.
4. Using a 5 mm trephine punch (or similar tool), cut out a 5 mm diameter disc of the cell-seeded hydrogel.
5. Place sample in the inner ring of the sample holder as described in the next section, *Sample Placement into a BioPress™ Culture Plate*.



### ***Direct-Placement of a Cell-Seeded Hydrogel***

1. Prepare hydrogel (Collagel<sup>®</sup>, agarose, gelatin, alginate, etc.) according to the manufacturer's or laboratory's established protocol.
2. Suspend cells in the hydrogel at  $1 \times 10^4$  –  $1 \times 10^5$  cells/ml.
3. Pipette cell-hydrogel suspension into the foam sample holder of the BioPress<sup>™</sup> culture plate.
4. Allow the hydrogel to polymerize.

### ***Pellet Culture***

1. Using primary cultures or cell line, prepare a suspension of  $5 \times 10^5$  –  $5 \times 10^6$  cells.
2. Spin at 10,000 x g for 5 minutes.
3. Carefully transfer cell pellet to the center of the sample holder as described in the next section, *Sample Placement into a BioPress<sup>™</sup> Culture Plate*.

### **TISSUE EXPLANT SAMPLES**

1. Prepare tissue according to the laboratory's established protocol.
2. If applicable, cut a 1-3 mm thick sample of the tissue explant.
3. Using a 5 mm trephine punch (or similar tool), cut out a 5 mm diameter disc of tissue.
4. Place sample in the inner ring of the sample holder as described in the next section, *Sample Placement into a BioPress<sup>™</sup> Culture Plate*.

### **SAMPLE PLACEMENT INTO A BIOPRESS<sup>™</sup> CULTURE PLATE**

To prepare a sample for loading, use a pair of sterile forceps to place the tissue or gel sample into the center hole of the foam sample holder in the BioPress<sup>™</sup> compression plate. Do this for the remainder of the wells. Add 3-4 mL of culture medium to each well.

Prepare all stationary platens by adjusting the center screw (Fig. 2) such that the screw bottom (part that touches the sample) is exactly flush with the bottom of the platen body. Use a pair of sterile forceps and the two holes at the adjustable center of the platen to turn the center screw.

Place a stationary platen into each well of the compression plate (Fig. 2). Calculate the number of revolutions required to bring the bottom of the center screw to the point where it just touches the top of the sample by using the following equations:

*For sample height in millimeters,*

$$x = (3.11-h) / 0.62$$

where  $x$  is the number of 180 degree clockwise turns required for the center screw to touch the sample and  $h$  is the sample height in **millimeters**.

*For sample height in inches,*

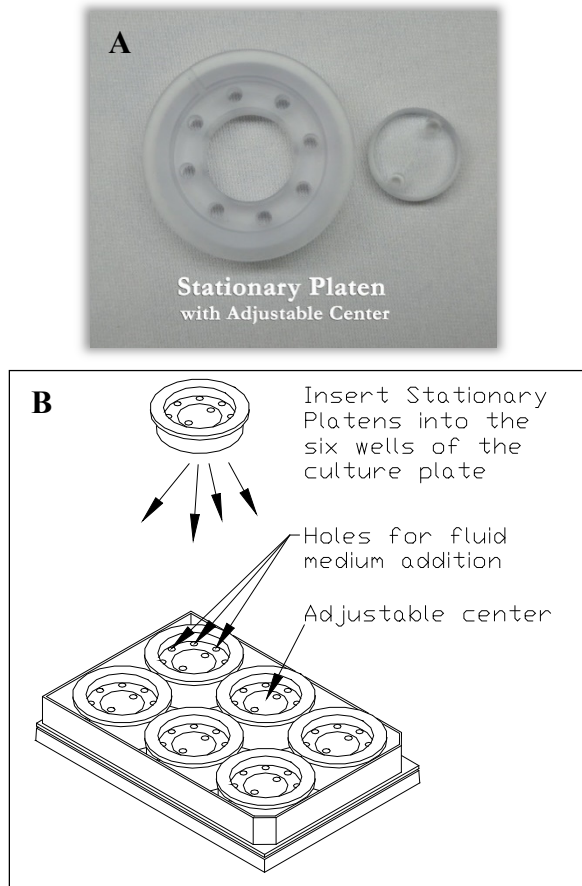
$$x = (0.122-h) / 0.025$$

where  $x$  is the number of 180 degree clockwise turns required for the center screw to touch the sample and  $h$  is sample height in **inches**.

***NOTE:*** *If the sample height is larger than 3.11 mm (0.122"), 'x' in the above equations will be a negative number. In this case, the bottom of the center screw will compress the sample when it is flush with the bottom of the platen body as the platen is first inserted into the well. To compensate for this, the center screw can be turned counterclockwise 'x' number of turns; however, it should be noted that the sample will no longer be fully compressible, as the base of the sample holder will no longer be able to contact the base of the center screw.*



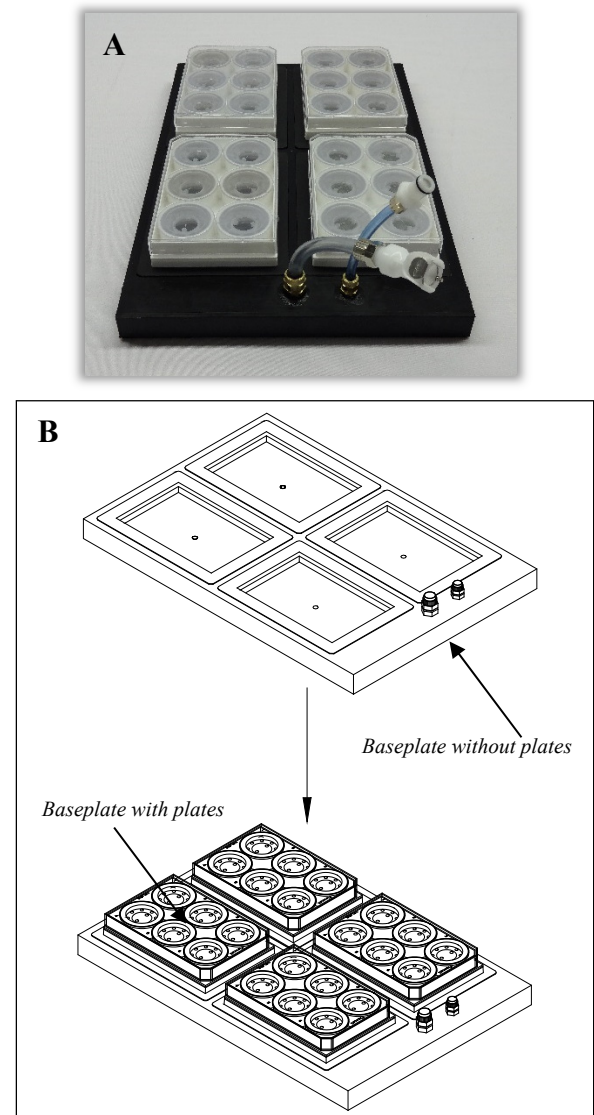
Using sterile forceps, turn the center screw clockwise the number of turns required by the above appropriate equation. Be careful not to screw the center down too far as this will preload the sample and give inaccurate force readings. Add or remove fluid medium through any of the eight holes around the inner periphery of the platen (Fig. 2).



**Figure 2.** A) Stationary platen with an adjustable center. B) Schematic of the stationary platen in a BioPress™ culture plate.

Center the BioPress™ compression plates on top of the four black gaskets in the compression baseplate (Fig. 3). Adjust the eight clamping pads in the clamping system to their maximum height and turn them so that the bottom of each pad is parallel with the surface of the baseplate. Place the Plexiglas® window into the clamping system

(Fig. 4), evenly along the bottom four clamping bars.



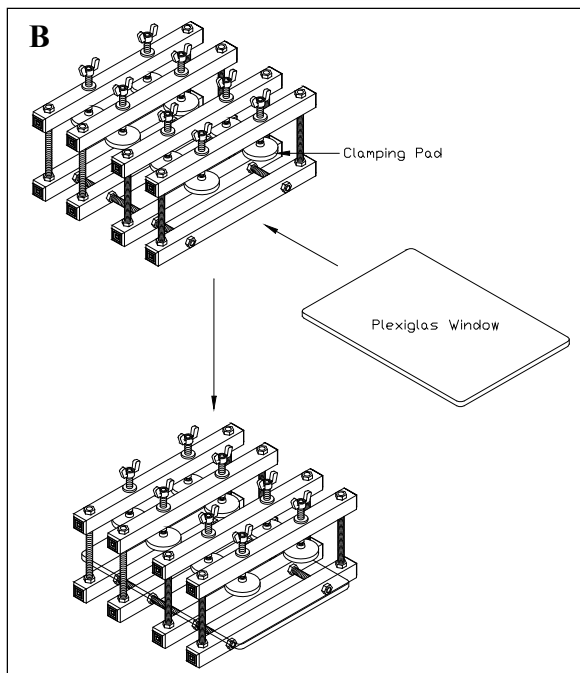
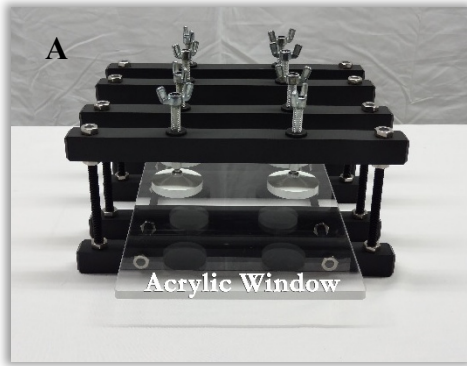
**Figure 3.** A) Compression baseplate with compression plates. B) Schematic of compression baseplate with and without BioPress™ plates

**NOTE:** The Plexiglas window is used as a backing support for the baseplate to enable it to withstand high pressures. Never operate the compression baseplate without this window in place.

Carefully slide the baseplate/plate assembly into the clamping system so that the four BioPress™ plates are centered underneath the



eight clamping pads (Fig. 5). Check the Plexiglas® window and move as necessary to center it under the baseplate. Turn the wing nuts on the clamping pads until they are finger tight. This will compress the plates downward onto the gaskets, creating a seal.

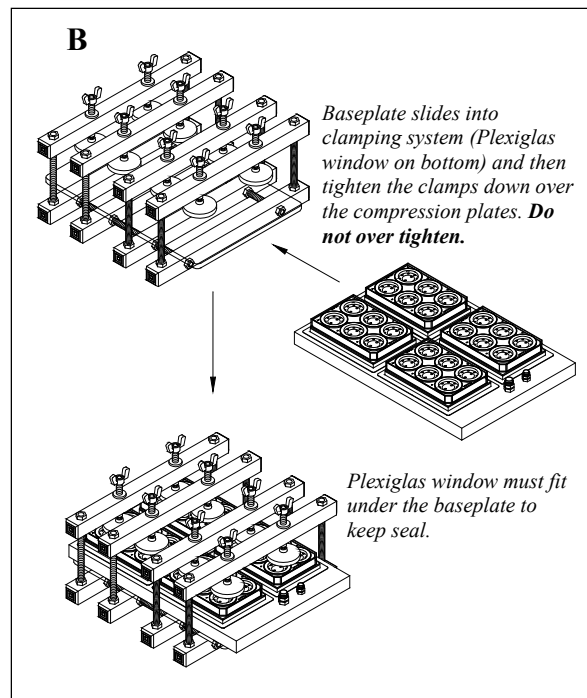
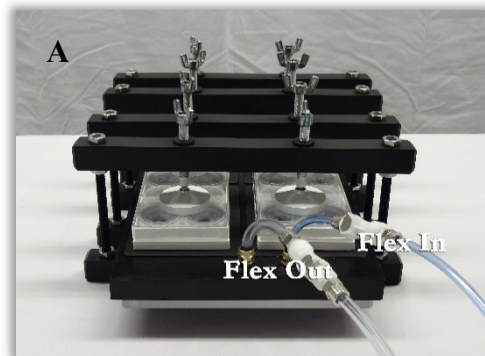


**Figure 4.** Clamping system with Plexiglas® window

**NOTE:** If using less than 4 plates in an experiment, use an old plate or support in place of the missing plate(s) and tighten the clamping pads onto this surface. This will ensure that the baseplate is seeing an even and sufficient pressure across its surface and will prevent baseplate blowout.

Place the entire assembly into the incubator. Attach the *FLEX IN* and *FLEX OUT* quick disconnect fittings to the fittings on the compression baseplate.

For more information, see the instructional video, *Compression Baseplate Assembly*, accessible on our website (<http://www.flexcellint.com/videos-instruct.htm>). Samples are now ready for compression loading. Please see the Compression User Manual for further instructions on running a compression regimen.



**Figure 5.** Clamping system with baseplate and culture plates