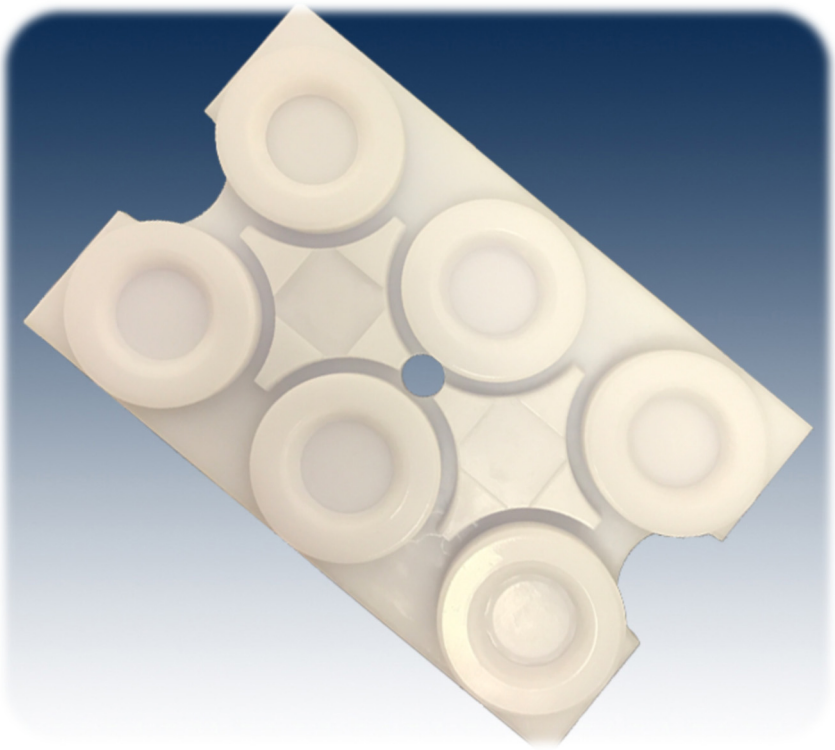
USER MANUAL

BioFlex® Cell Seeder



Optimize cell placement in each BioFlex® well!

10-31-17
Rev 1.0

Dynamic Culture for Tissue Engineering™

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INTRODUCTION

BioFlex® Cell Seeders (Fig. 1) confine cells during plating to the central area of a BioFlex® membrane. The central region of the membrane is positioned directly over the 25 mm diameter cylindrical loading posts in the Loading Station used during application of equibiaxial substrate strain. Cells plated in this manner are subjected to defined strains when using the equibiaxial posts in the Loading Stations (Fig. 2). The BioFlex® Cell Seeder™ is inserted into the BioFlex® Baseplate (Fig. 3), and the 6-well BioFlex® plate plus the gasket are placed on top of the Cell Seeder. Vacuum draws the membrane into the defined central region of the membrane during cell plating and adhesion. Once cells have adhered to the membrane, vacuum is released, and cells can be cultured in the usual manner.

Figure 1. 6-well BioFlex® Cell Seeder Station in a BioFlex® baseplate well.

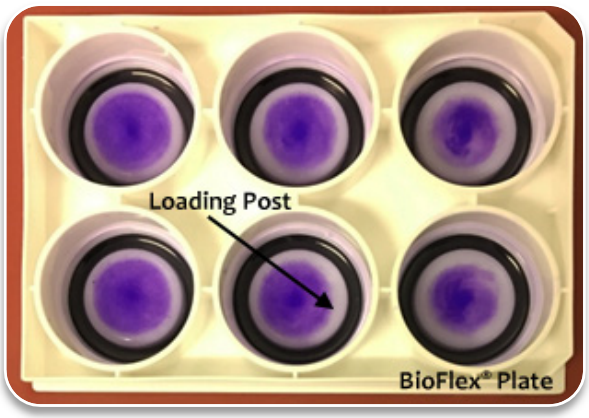

Figure 2. The results of using a BioFlex® Cell Seeder when plating cells to confine them to the area directly above the 25 mm cylindrical loading posts (purple = cells stained with Crystal Violet stain).

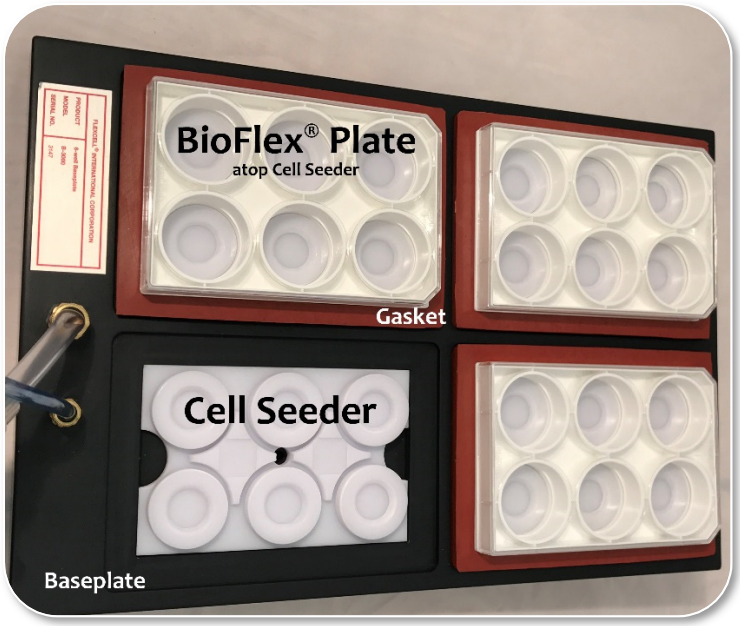

Figure 3. BioFlex® Cell Seeders in a BioFlex® Baseplate.

INSTRUCTIONS

1. Insert the BioFlex® Cell Seeder into a BioFlex® Baseplate, similar to how the 25 mm equibiaxial Loading Stations are placed in the baseplate. *Optional:* Add silicone lubricant to the upper rim of a Cell Seeder post to aid membrane gliding into the Cell Seeder body.
2. Place a BioFlex® plate into its respective gasket positioned over the Cell Seeder, ensuring that the wells in the plate align concentrically over the posts on the Cell Seeder.
3. Using the FX-5000™ Tension System, create and start a regimen with the following settings:
    - SHAPE: Static
    - MIN: 0.0
    - MAX: 15.0
    - FREQ: 1 Hz (*this value must still be entered even though the regimen is static*)
    - DURATION: Equal to the seeding time (see step 5 below) plus the time needed to plate the cells into the well.
    - PLATFORM: BFlx Loading Station (25mm)
4. The recommended suspension volume when using a 6-well, BioFlex® Cell Seeder for an individual well is 1 mL medium plus cells. This volume is large enough to allow uniform distribution of the media-cell suspension over the growth surface area when dispensing cells and medium with a 1000 µL micropipette. Also, this volume is small enough to retain the media-cell suspension within the boundary of the Cell Seeder form when handling the baseplate.
5. Due to the limited suspension volume, it is advised that the user observe the seeding time required for the cells to adhere to the membrane. A seeding time of at least two hours is recommended, but the required time is dependent on the type of cells being used.
6. After the cells have adhered to the substrate, remove the vacuum gradually. It is recommended to create a slow vacuum release regimen that reduces the vacuum a set percent every "n" seconds. An example regimen is outlined below in Table 1.
7. Slowly add 1 mL of fresh medium to each well to increase the volume for proper aspiration of non-adherent cells. Then, insert an aspirator tip near the side of each well (adjacent to the seeded cells) and aspirate the media containing non-adherent cells.
8. Add 3 mL of fresh media to each well and return the BioFlex® plate to the incubator until you are ready to apply strain.

Table 1. Sample regimen parameters for releasing the vacuum pressure slowly.

Step	Shape	Min	Max	Freq	DC%	dd:hh:mm:ss	Back To	Repeat
1	Static	0.0	15.0	1.0	50.0	00:02:30:00	0	0
2	Static	0.0	13.0	1.0	50.0	00:00:00:06	0	0
3	Static	0.0	11.0	1.0	50.0	00:00:00:06	0	0
4	Static	0.0	9.0	1.0	50.0	00:00:00:06	0	0
5	Static	0.0	7.0	1.0	50.0	00:00:00:06	0	0
6	Static	0.0	5.0	1.0	50.0	00:00:00:06	0	0
7	Static	0.0	3.0	1.0	50.0	00:00:00:06	0	0
8	Static	0.0	1.0	1.0	50.0	00:00:00:06	0	0

In this example, we recommend a two hour adherence time plus a 30 minute set-up time (Step 1). Following seeding, this example releases the strain by 1-2% every six seconds.