Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)

Pre-Cell seeding:

A. Preparing nanofibers for cell culture

1. Remove nanofiber plate from the sterile pouch and place inside a biosafety hood.
2. Rinse the plate 2-3 times with sterile water or PBS and allow fibers to air-dry.
3. After washing fibers may be pre-incubated in media and biological components of interest for at least 30 minutes and up to 24 hours at 37 ˚C, aspirating off the media and finally adding your cells and media
4. For cells that have low attachment to plastic ware (e.x. neural and tumor stem cells), coat the nanofibers with suitable ECM protein, e.x. fibronectin (5-10 µg/ml) or laminin-1 (5- 20 µg/ml), for 2h at room temperature (alternatively coating can be done overnight at 2-8 ºC)
    a. For other coatings, and as a general preferred method, use coating protocol provided by the coating manufacturer.
5. After pre-incubation, rinse the plate 2x with sterile PBS (100 mM phosphate buffer saline solutions) and 1x with desired culture medium
6. The scaffolds are now ready for cell culture; cells can be dissociated by conventional methods and pipetted into the wells or spheroids can be applied manually.
7. Initial cell densities of 10^4-10^6 cells/cm^2 are suggested (the cell density can be more or less based on your cell type and experimental needs).
8. During longer-term experiments, exchange the media at normal rates (rates suggested by media supplier).

Recommendations:

Culture Plate	Bottom Area (cm^2)	Lower Number of Cells	Upper Number of Cells
384-well	0.1	1000 cells/well	100,000 cells/well
96-well	0.35	3500 cells/well	350,000 cells/well
24-well	1.9	19,000 cells/well	1,900,000 cells/well
6-well	9.7	97,000 cells/well	9,700,000 cells/well