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

G. Staining and seeding dissociated adherent cells on nanofiber plates

Note: Some researchers have experienced non-specific labeling of nanofibers when attempting to stain cells that have been seeded on nanofiber plates. For best results, stain cells in standard cell culture products prior to seeding on nanofibers or purchase fluoresce expressing cells.

1. Stain adherent cells in their culture dishes using Green CellTracker (Invitrogen, 10 mM stock) at a final dilution 1/2000 (in culture medium) for 60 minutes at 37 ˚C. Rinse cells once with fresh culture medium to remove excess dye.
2. Prepare nanofiber plates for culture
    a. Remove nanofiber plate from the sterile pouch and place inside a biosafety hood.
    b. Rinse the plate 2-3 times with sterile water or PBS and allow fibers to air-dry.
    c. 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
    d. 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)
        i. For other coatings, and as a general preferred method, use coating protocol provided by the coating manufacturer.
    e. After pre-incubation, rinse the plate 2x with sterile PBS (100 mM phosphate buffer saline solutions) and 1x with desired culture medium
    f. 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.
3. Rinse cells (still in standard culture dishes) again and trypsinize. Count and apply cells directly to the wells of the nanofiber plate. Usual amounts are:
    o For imaging: 1,000-2,000 cells in 96 well plate (in a final volume = 100 µl) 5,000-10,000 cells in 24 well plate (in a final volume = 500 µl)
    o For cell viability experiments: start with ~4,000 cells / 100 µl in a 96 well plate
4. If cells were not pre-stained before plating, wait until they are adhered and stain them with a solution of calcein-AM (Invitrogen, dilution 1/1000 from stock). Add calcein to the cells for 20 min at 37 ºC and rinse twice before imaging. Calcein bleaches very quickly under fluorescence so it cannot be used for long-time exposures or time-lapse experiments!