

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

H. Seeding glioblastoma neurospheres or dissociated glioblastoma stem cells

1. These cells grow in suspension as neurospheres, so they can be treated as aggregates.
2. Prepare nanofiber plates for cell 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. Glioblastoma neurospheres will usually require a pre- coating of fibronectin (5 µg/ml) or laminin (5 µg/ml) to adhere to the fibers.
 - 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
3. Stain the neurospheres with Green CellTracker in standard, non-nanofiber, 35mm dish. **Note:** Attempting to Stain cells on nanofibers may result in dyes binding to the fibers
 - a. To stain the aggregates, add Green CellTracker (Invitrogen C2925, 10 mM stock) at a final dilution 1/2000 for 60 minutes at 37 °C. This is the CellTracker dye that has the least or no effect on cell motility (CellTracker orange and the Vybrant dyes DiI and DiO reduce cell motility!).
 - b. To remove the excess dye, collect aggregates with a pipette and transfer to a clean 35 mm dish with culture medium. Collect them again (gently) and transfer them to a second clean 35mm dish with culture medium.
4. To plate whole neurospheres, proceed as indicated with cell aggregates in protocol *D. seeding Cell aggregates on nanofiber plates.*
5. To plate dissociated glioblastoma stem cells, dissociate the cells using accutase (Innovative Cell Technologies) and/or manual dissociation, count the cells and plate them as indicated in protocol *G. staining and seeding dissociated adherent cells on nanofiber plates.*

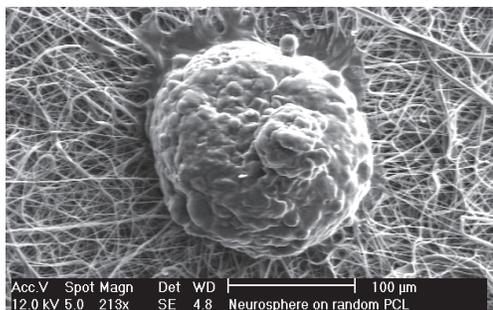


Figure H1. SEM image of Neurosphere on NanoECM™ (random PCL)