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

F. Preparation and culture of cell spheroids on nanofibers

Cell spheroids are prepared by culturing dissociated cells on non-adhesive surfaces. Examples include culturing dissociated cells on hydrophobic cultureware (e.g., Corning Flat Bottom Ultra-Low Attachment Microplates, cat #3474) or culturing on 1% hard agar-coated plates.

1. Prepare agar plates to make cell spheroids
    a. Prepare a solution of 1% w/v molecular biology-grade agarose in DMEM. This will require dissolving the agarose carefully by heating in a microwave or heated bath
    b. Let the agarose solution cool down to ~50 ºC and filter through a large 0.22 um filter to sterilize the solution. You may need to fraction the agarose in small volumes and pass each volume through a separate filter to prevent clogging
    c. Rapidly pipette the sterile agarose solution in 35 mm culture plates (1.5 ml / plate). Let them cool and gellify in a biosafety hood
    d. Wrap in Saran Wrap (groups of 4-5 dishes together) and store at 4 ºC for up to two weeks. Discard plates if the agarose shows cracks after storage
    e. To prepare spheroids, warm the agarose plates to room temperature in a biosafety hood and add 1 ml of prewarmed culture medium per plate
    f. Let the medium soak into the agarose and discard the medium after 1h. Refill the plates with1ml fresh medium
    g. Seed dissociated cells at 50,000 - 75,000 cells/plate and return to culture incubator. Monitor development of cell aggregates over time (24 - 72h). Carefully dislodge cells with a Pasteur pipette if they form large chains, to enhance the formation of individual aggregates.
2. Culture cells on hydrophobic surfaces until spheroids of 200-300 µm diameter can be easily observed under the microscope
3. Transfer the spheroids to a different vessel or conical tube and stain them with a tracking dye for 60 min at 37 ºC following the manufacturer's recommendations (recommended dyes: Cell- Tracker dyes, Invitrogen, used at 5 µM final concentration in culture medium)
4. Let the spheroids sediment to the bottom of the tube and remove as much of the staining solution as possible. Rinse the spheroids once with pre-warmed culture medium
5. Transfer the spheroids to a 35 mm or 60 mm culture dish to manipulate them
6. Using a dissection microscope aspirate each spheroid individually (using a 200 µl tip) and seed them in individual wells of nanofiber-coated plates. Let the spheroids attach to the wells for 1h at room temperature in the biosafety hood (make sure the wells have sufficient liquid to avoid drying the spheroids)
7. Complete the culture medium in each well to the desired volume and return the plates to the culture incubator