Nanofiber SOLUTIONS

Culture and analysis of glioma cells on nanofibers:

Protocol graciously provided by: Jessica de Jesus, Paula Agudelo-Garcia, Dr. Mariano Viapiano

A) Preparation of agar plates to make aggregates of adherent cells

- In a 50ml conical tube dissolve 0.4g agarose GPG/LE (American Bioanalytical AB00972) in 40 ml high-glucose DMEM (Invitrogen). Use DMEM without serum or antibiotics.
- The agarose won't dissolve completely. Put the mixture in a boiling water bath and watch until agarose is completely dissolved (keep tube loosely covered).
- Let the dissolved agar cool down to ~50 °C (otherwise it will break the filter) and filter-sterilize in a culture hood using a 0.22 µm filter (Millipore Steriflip).
- Quickly pipette dissolved agar into 35mm culture dishes (1.5 ml/dish), you need to do this quickly before the agar solidifies.
- Half-cover the dishes and leave them in the hood until the agar solidifies.
- Wrap the plates in saran-wrap (4-5 plates together) and store at 4 °C until use (they last ~3 weeks before the agar starts cracking).

B) Preparation of aggregates of adherent cells

- Incubate agar plates at 37 °C for 1h with 2ml of culture medium for the desired cell type. This will hydrate the agar, otherwise the cells will attach to the plate.
- Remove the medium after 1h (it will remove debris in the agar) and add 1-2 ml fresh culture medium.
- Trypsinize and count cells. Add 50,000-70,000 cells/agar plate.
- Rock agar plates gently to evenly disperse the cells. If possible do this every few hours to prevent formation of loose sheets instead of tight aggregates. Fast and continuous rocking (with a cell culture rocker in the incubator) may aggregate all the cells in the center of the agar plate, so it's not recommended (but can be tested for each cell type).
- Incubate at 37 °C in a CO_2 incubator for at least 24h.
- 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!).
- To remove the excess dye, collect aggregates with a pipette and transfer to a clean 35mm dish with culture medium. Collect them again (gently!) and transfer them to a second clean 35mm dish with culture medium.

C) Preparation of nanofiber plates for culture

- Wash the wells 3 times with autoclaved water or sterile PBS before use, to remove debris left by the nanofiber manufacturing process. Allow plates to air dry.
- If needed, apply a substrate pre-coating before seeding cell aggregates. Substances previously tested for pre-coating include bovine plasma fibronectin (5 µg/ml, Calbiochem), mouse laminin (5 µg/ml, Invitrogen), human collagen-IV (5 µg/ml, Sigma-Aldrich) and hyaluronan (100 µg/ml, Calbiochem).
- For precoating, add 200 µl of substrate solution at the desired concentration to each well and incubate for 2h at room temperature. Rinse twice with sterile PBS.
- Add 250 µl culture medium to the plate and keep it in the hood ready to receive aggregates.