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D) Seeding cell aggregates on nanofiber plates:

- Aggregates must be manually picked one by one using a dissecting microscope (preferably with epifluorescence illumination, but a simple epi-illumination works well). Because this scope is rarely inside a culture hood, it must be located in a relatively isolated and very clean area of the laboratory. Use lab coat, hairnet, gloves and mouth cover to avoid contamination of the aggregates. Surround the microscope with clean bench paper and sterilize the whole area with 70% ethanol. You can also keep a lighted Bunsen burner close to the microscope to generate a continuous sterile area.
- Using the dissecting scope, carefully pick up one stained aggregate from the 35mm dish in 1 µl of medium, using a 10 µl pipette with a 10 µl tip.
- Transfer the aggregate to a well in the nanofiber plate under the dissecting scope, carefully "seeding" the aggregate onto the fibers in the center of the well. It is best to bring the pipette tip as close to the fibers as possible -without touching them- before ejecting the aggregate. This prevents the aggregate from floating away towards the edge of the well.
- After adding the aggregates, cover the plate and allow aggregates to attach without movement for 20-25min.
- Complete the culture medium in the wells to 500 µl by adding 250 µl medium with or without test compounds. This allows the addition of test compounds without disrupting the aggregates.
- Image the aggregates in a fluorescence microscope (usually at 4X and/or 10X). This will be the t=0h images. Further imaging can be done at different times (usually t=8h or t=24h), or by time-lapse microscopy.

E) Staining and seeding dissociated adherent cells on nanofiber plates

- Prepare nanofiber plates as indicated in section C.
- Stain adherent cells in their culture dishes using Green CellTracker (Invitrogen, 10mM 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.
- Rinse cells again and trypsinize. Count and apply directly to the wells of the nanofiber plate. Usual amounts are:
    - For imaging: 1,000-2,000 cells in 96well plate (in a final volume = 100 µl)
      5,000-10,000 cells in 24well plate (in a final volume = 500 µl)
    - For cell viability experiments: start with ~4,000 cells / 100 µl in a 96well plate
- 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 20min at 37 °C and rinse twice before imaging. Calcein bleaches very quickly under fluorescence so it cannot be used for long-time exposures or time-lapse experiments!

F) Seeding glioblastoma neurospheres or dissociated glioblastoma stem cells:

- These cells grow in suspension as neurospheres, so they can be treated as aggregates.
- Prepare nanofiber plates as indicated in section C. Glioblastoma neurospheres will usually require a pre-coating of fibronectin (5µg/ml) or laminin (5 µg/ml) to adhere to the fibers.
- Stain the neurospheres with Green CellTracker as indicated in section B.
- To plate whole neurospheres, proceed as indicated with cell aggregates in section D.
- 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 section E.