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

E. Preparation of agar plates to make cell spheroids

1. 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
2. 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
3. Rapidly pipette the sterile agarose solution in 35 mm culture plates (1.5 ml / plate). Let them cool and gellify in a biosafety hood
4. 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
5. To prepare spheroids, warm the agarose plates to room temperature in a biosafety hood and add 1 ml of prewarmed culture medium per plate
6. Let the medium soak into the agarose and discard the medium after 1h. Refill the plates with1ml fresh medium
7. 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.