3D Spheroid Generation Protocol and Tips

Benefits of spheroids:

A 3D spheroid is a three-dimensional cell culture system that uses a collection of cells to mimic the natural internal environment of tissues and tumors.

Spheroids are generated by cell aggregation and subsequent compression together by their own extracellular matrix. The difference in oxygen between the spheroid's core and surface closely mimics the oxygen gradients found *in vivo*. Therefore, using cell spheroids rather than a dispersed monolayer of cells in tissue culture may enhance cellular functionality and provide more clinically relevant results. Construction of complex designs resembling those seen *in vivo* becomes possible by encapsulating cell spheroids within bioprinting-compatible hydrogels like Okagel [1].

Protocol:

Starting with a 2D cell culture in a flask:

0. Wash cell layer with 5mL of PBS making sure to cover surface area, then discard PBS;
1. Pre-warm 5mL of 0.1% trypsin-EDTA;
2. Add 5mL of 0.1% trypsin-EDTA to the cell monolayer ensure all cells are covered;
3. Return cells to a 37℃, 5% CO2 humidified incubator for 8-10 minutes;
4. Add the volume corresponding to the cell culture flask size of fresh culture media to the cell suspension to neutralize the effects of trypsin and prevent over trypsinization which may lead to cell lysis;
5. Transfer cell suspension to a 50mL centrifuge tube;
6. Centrifuge cell suspension for 5 minutes at 1120 rpm;
7. Discard supernatant and resuspend pellet in fresh cell medium;
8. Prepare trypan blue solution for live-dead cell assay by mixing trypan blue stain with cell suspension in a 1:1 ratio;
9. Count cells using a haemocytometer;
10. Dilute cell suspension to desired cell concentration NOTE: $2.5x10^6$ cells/mL is recommended;
11. Prepare Petri dish of choice by adding sterile PBS to the bottom lid, this will minimize evaporation of medium surrounding spheroid;
12. Add cell suspension to the lid of a slightly angled Petri dish;

13. Pipette 20µL of cell suspension onto the top lid of your Petri dish using a multichannel pipette
    NOTE: ensure spheroids are spaced appropriately from each other and side of dish;
14. Place top lid onto bottom of Petri dish and culture for desired incubation period;

Tip: It's preferred not to vortex or shake Okagel in order to avoid bubble formation, especially with the viscous higher concentrations. If bubbles occur, centrifugation at around 1500 rpm for 3 mins can help get rid of the bubbles.

References

[1] Nupura S Bhise *et al* (2016). A liver-on-a-chip platform with bioprinted hepatic spheroids. *Biofabrication, 8, 014101.* https://iopscience.iop.org/article/10.1088/1758-5090/8/1/014101