Irradiation and monitor cell viability

Technic: Irradiation of gold encapsulated cells

Goal: Monitor cell viability over 4 days after irradiation

Procedure:

Irradiation and preparation

1. Irradiate both the encapsulated and control cells at 2, 4, 6 and 8 Gy keeping one of each non irradiated.
2. Let them rest in the oven for a few hours.
3. Have the cells detached and resuspended in 15 mL tubes.
4. For each tube count the amount of cells: collect 30 µL and mix with 30 µL of Trypan Blue, then insert in a cell counting slide and count the amount of cells in 2x 9 circles.
5. Divide this number by 7.5 then take the inverse and multiply by 10. The result is the amount in mL of cells in the tube to pipet. If it exceeds 2, only take 2. Complete with culture medium to reach 2 mL.
6. Pour each solution in 6 well plates (BP35) so that 4 plates contain cells irradiated at 0, 2, 4, 6 and 8 Gy with gold, and 4 plates without gold.

MTT, to do each day on a duo of plates (with and without gold) for 4 days

7. Pour 1 mL of MTT solution in each well at 0.5 mg/mL
8. Incubate for one hour.
9. Empty each well then rinse with 1 mL of PBS.
10. Add 2 mL of DMSO in each well then let the plate agitate for 5 minutes.
11. Read at 544 nm the absorbance of each well by spectrophotometer.

Reagents and Solutions:

- PBS (calcium and magnesium free)
- Culture medium (89% RPMI + 10% SVF + 1% penicillin)
- Trypan Blue
- DMSO
- MTT (Thiazolyl Blue Tetrazolium Bromide)

  Prepare a 5 mg/mL solution of MTT in PBS. Aliquot in 2mL microtubes then put in a freezer in a light protected environment. Just before use defrost and dilute 1/10 in culture medium

- 10 BP100 dishes of MIAPaCa2 cells at less than 50% confluence, 5 encapsulated with gold nanoparticles and 5 controls

Equipment:

- Sterile microtubes
- Pipettes and sterile tips
- Petri dish rack
- Cell counting slides
- Incubator/shaker set to 37 °C
- Inverted microscope
- Irradiation chamber (done by a professional)

Estimate time:
Preparation and irradiation: ~4 hours
MTT each day: ~2 hours