Seeding HeLa cells on grids

SaS

Grids: general workflow

- Charge glow grids
  (200, R1/2; but I used also R 2/2; 1.3/1.2 – I did not see differences)
- Optional: surface coating
- Optional: UV sterilization, aprox 1h
- Put grids on to dish
- Count cells
- Seed cells

I do not sterilize grids, but I usually have two dishes of cells (one to keep and second for experiment) and I always do it in the evening, clean everything properly and switch on UV

Charlie, Carlota do UV-sterilization: they often leave grids in hood under PBS in small dish for a few day so they are sterilized properly. Piotr do like 1 h.

Grids coating

I do it on a parafilm outside of cell culture hood
or directly on dish (PDMS coated dishes)

I do (for fibroblast):
- Fibronectin (stock 1mg/mL): 1:100 (10 µg/mL), 2h, r.t.
- Wash PBS

Piotr for Hela/grids (for FIB)
- Fibronectin: 25 µg/mL; 10 µL/grid; 37°C; 1h (until it is dry)

Trypsinisation:

6-cm dish
- Trypsin (T/E) 0.5 mL
- Medium 4.5 mL

10-cm dish
- Trypsin (T/E) 1 mL
- Medium 9 mL

Workflow
- Remove medium
- Wash (pre-warm) PBS 2x
- Add T/E
- Incubator (37°C, ≤5min)
- Check progress in microscope
- Add medium
- Count cells
- Seed cells

Seeding of HeLa cells

Both O/N

From my old exp.
- 3000 cells/cm^2: …. 96-well
- 8000 cells/cm^2: …. 96-well for MTT assay
- 1.6. x 10^6 …. 12 well for IF or FACS

Piotr seeds HeLa on grids
- 1.5. x 10^4 cells/cm

I think, it is good to do seeding exp. with a few grids) ;
from my experience, I would not be afraid to try lower concentrations; they tend to be together, but are robust (will be fine in lower concentrations)

Cells are often attached around 3h (based on fibroblast, but I would expect that also for Hela), another option would be to freeze grids after cells are attached, but do not let them divide. (WenLu and Carlota do that)

Grids on dish

I have ≤ 8 grids/ 3.5cm-dish or 12-well

I had problem to take grids out for plunge-freezing at beginning; good option is PDMS coating
e.g.
https://www.youtube.com/watch?v=nf7_BG2WBlc&ab_channel=SoftFluidicsLab%2FHashimotoGroup
I coated grids as well as dish with fibronectin

I have grids distributed across whole dish – usually grids along walls of dish has better seeding than that in the centre, but it is not rule ….

You can also consider Primo2

If you have expensive treatment, you can consideer that:
https://www.sciencedirect.com/science/article/pii/S1047847720302069#f0005
Charlie uses that.