NHDF Propagation

Materials

- DMEM high glucose
- Peniciline/streptomycine (100x)
- FBS
- Tryple
- 2% Gelatine
- PBS

Media preparation

- DMEM high glucose
- 1% peniciline/streptomycine
- 10% FBS

Dish coating

- Dilute 2% stock of Gelatine to 0.1% in PBS
- Sterile filter
- Incubate 30 min. at 37 ∘ C
- Aspirate gelatine from the plates

Cells Thawing * Transfer cells from liquid nitrogen storage on dry ice into cell culture room * Spray with ethanol, immerse in water bath * Let thawing for 1-2 min. (check visually, remove when there is only a little piece of ice left inside the vial) * Transfer content of the vial into a 15mL tube * Dropwise add cold culture medium, shake gently between the media addition (first 5 ml) * Add 5 more mL to reach 10-12 ml * Colllect cells by centrifugation at 300g for 5 minutes * Discard the supernatant into waste * Flick the tube to release the cell pellet

Cells counting

- Resuspend in 1mL of media
- take 10uL of susupension, mix with 10uL of trypan blue, inject into the counting slide
- count cells

Cells seeding

- recomended seeding density is 2x104 cells/ cm2
- dilute cells to proper concentration, gently pipette onto the plate

Cell Passaging

- Prepare fresh dishes with gelatine coating (-30 min.)
- Aspirate the media
- Wash with PBS
- Add Tryple (1mL per 20cm2)
- Incubate for 5 min. at 37 ∘ C
- Visually check the cells detachment
- Gently collect using P1000 pipette tip
- Collect to 10mL of medium

- Colllect cells by centrifugation at 300g for 5 minutes
- Discard the supernatant into waste
- Flick the tube to release the cell pellet
- Resuspend in 1 ml of medium
- Count cells
- Resuspend in fresh media to desired concentration • Seed onto the dishes

Recomended cell densities

Sparse cells

5 000 -20 000 cells/cm2

Dense cells

> 50 000 cells/cm2