

Preparation of various cells from vessels / tissue (explant technique)

Materials:

- autoclaved forcipies, scissors
- sterile scalpells, pincets, needles
- HBSS (Sigma H-6136) containing antibiotics and 0,01M Hepes
- M199 (Sigma M-5017) containing antibiotics, 20% FCS (=NECM)
- Gelatine solution (1% in PBS)
- all solutions prewarmed to 37°C
- tissue culture plastic (centrifuge tubes, dishes, flasks, cell-scrapers, ...)

Procedure:

1. Rinse vessel (tissue) with HBSS
2. Cut vessel (tissue) into small pieces (approx. 3x3mm)
3. Scratch the surface of a petri-dish coated with 1% gelatine with a needle
4. Place pieces into the petri-dish on the scratches
5. Cover the pieces with 1-2 drops of NECM
6. Incubate at 37°C, 5% CO₂
7. Add two drops of NECM every 24 hours
8. Add 8ml of NECM after 5-7 days
9. Remove pieces after cells attaching
10. Grow, split and freeze as usual