Cell Culture Protocol for NHDFs on Topographic Surfaces

Version: 1.0

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Linked to Manuscript: Micellangelo: In Silico Cell Morphology Generation with Deep Generative Models

Repository : https://github.com/cbite/Micellangelo/

# 1. Reagents and Materials

• NHDF cells (Lonza, CC-2511)  
• DMEM (Gibco, Fisher Scientific, 42430)  
• FBS (Sigma-Aldrich)  
• Penicillin/Streptomycin (Gibco)  
• Trypsin-EDTA (0.05%, Gibco)  
• PBS (Phosphate Buffered Saline)

# 2. Equipment

• T75 flasks (vented cap, sterile)  
• 24-well plates (for topography)  
• Hemocytometer or NucleoCounter  
• Pipettes and sterile tips  
• Biosafety cabinet (Class II)  
• Centrifuge (swing-bucket rotor)  
• 37°C, 5% CO₂ incubator

# 3. Cell Thawing and Expansion

1. Thaw one vial of NHDFs (passage 1) rapidly in a 37°C water bath until a small ice pellet remains.  
2. Disinfect vial with 70% ethanol and transfer to a sterile tube.  
3. Add 4 mL of cold DMEM + 10% FBS dropwise.  
4. Centrifuge at 300 × g for 3 minutes.  
5. Aspirate DMSO-containing supernatant and resuspend pellet in 12 mL fresh medium.  
6. Transfer 5\*105 cells to T75 flask and incubate at 37°C, 5% CO₂.

# 4. Routine Culture and Maintenance

• Seeding density – 6.666 cells per cm2  
• Change medium every 2–3 days.  
• Passage cells at ~80% confluency.  
• For the screen use cells of passage 4.  
• Doubling time: 36–48 hours.

# 5. Cell Harvest and Counting

1. Rinse with PBS, add Trypsin-EDTA, incubate 5 minutes at 37°C.  
2. Neutralize with medium, centrifuge at 300 × g for 5 minutes.  
3. Resuspend in fresh medium and count cells.  
4. Proceed with seeding.

# 6. Experimental Seeding on Topographic Surfaces

1. Prepare 1,053 cells/cm² (~2,000 cells/well, distribute from the master mix, total number of wells for the experiment +2 mL).  
2. Add 1 mL cell suspension to each well.  
3. Rest in biosafety cabinet for 10 minutes.  
4. Incubate (37°C, 5% CO₂.) for 48 hours without media change.

# 7. Fixation, Immunostaining and Mounting

1. Wash wells 3× with PBS.  
2. Fix cells in 4% paraformaldehyde (Thermo Fisher, methanol-free) for 20 minutes at room temperature.  
3. Wash cells 3× with PBS.  
4. Permeabilize with 0.5% Triton X-100 in PBS for 15 minutes.  
5. Block with 3% BSA + 0.3 M glycine in PBS for 45 minutes at room temperature.  
6. Incubate overnight at 4°C with mouse anti-YAP primary antibody (Santa Cruz, sc-101199, lot #G3119), diluted 1:500 in blocking buffer.  
7. Wash cells 3× with PBS.  
8. Incubate for 1 hour at room temperature with Alexa Fluor 488-conjugated goat anti-mouse IgG2a (Invitrogen, A21131), 1:400 dilution.  
9. Stain F-actin using phalloidin (Abcam, ab176756 or ab176759), 1:1000 in PBS, for 15 minutes at room temperature.  
10. Counterstain nuclei with DAPI (Invitrogen, D1306), 1:1000 for 8 minutes.  
11. Wash 3× with PBS between all steps.  
12. After staining, place each topographic insert flat onto a clean microscope slide (cell side facing UP).  
13. Add 15 μL of Mowiol 4-88 mounting medium directly onto the top of each insert.  
14. Carefully place a coverslip over the insert, lowering it from one edge to avoid air bubbles.  
15. Seal the corners of the coverslip using clear nail polish.  
16. Store mounted slides at 4°C, protected from light, until imaging.

⚠️ Safety Note: PFA, Triton X-100, phalloidin, and DAPI are hazardous chemicals. Use gloves, lab coat, and eye protection. Perform all fixation and staining steps in a certified fume hood. Dispose of chemical waste in accordance with institutional guidelines.