Isolation of Primary Human Keratinocytes from Foreskin

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[Abstract] Keratinocytes are the primary constituents of human skin, the functional barrier between our bodies and the external environment. The balance between keratinocyte differentiation and self-renewal is crucial to skin homeostasis. Primary keratinocyte culture serves as a tractable model for understanding human epithelial cell differentiation as well as self-renewal.

Materials and Reagents

1. Foreskin
2. 0.05% Trypsin (Life Technologies, Invitrogen™, catalog number: 25300-054)
3. 2x dispase (Life Technologies, Invitrogen™, catalog number: 17105-041)
4. DMEM (Life Technologies, Invitrogen™, catalog number: 11965-092)
5. Fetal bovine serum (FBS) (Life Technologies, Invitrogen™, catalog number: 16000-044)
6. Defined keratinocyte serum-free medium (Life Technologies, Invitrogen™, catalog number: 10744-019)
7. 154 medium (Life Technologies, Invitrogen™, catalog number: M-154-500)
8. Penicillin and streptomycin (Life Technologies, Invitrogen™, catalog number: 10378-016)
9. Phosphate buffered saline (PBS)
10. DMEM cultural medium (see Recipes)
11. 50: 50 medium (see Recipes)

Equipment

1. Centrifuges
2. Incubator (37 °C and 5% CO2)
3. Sterile petridish
4. Cell culture hood
5. Cell culture dishes
6. Scissors
7. Forceps
8. 50 ml falcon tube
9. Disposable sterile cell culture strainer
Procedure

1. In a sterile petridish, remove vascular and adipose tissue from foreskin using scissors and forceps.
   
   Note: All manipulations should be done with sterile instruments and in the hood.
2. Incubate prepared foreskin with 10 ml of 1x dispase for 12-18 h at +4 °C. 2x dispase stock should be diluted in PBS, containing penicillin (500 units/ml) and streptomycin (50 μg/ml).
3. Peel off the epidermis and place into 2-3 ml of 0.05% trypsin. Incubate at 37 °C for 15 min in a 50 ml falcon tube.
4. Quench trypsin by adding 2 volumes of DMEM, containing 10% FBS. Invert falcon tube several times to detach keratinocytes from the dermis.
5. Pass solution through a sterile sieve and centrifuge filtered solution at 1,000 rpm and 37 °C to pellet cells.
6. Aspirate medium, gently resuspend cells in 30 ml of 50:50 medium supplemented with penicillin and streptomycin and plate on 3, 10 cm dishes.
7. Incubate at 37 °C and 5% CO₂ for several days until colonies grow out. Change the medium every 2 days.

Recipes

1. DMEM cultural medium
   DMEM supplemented with 10% FBS.
2. 50:50 medium
   1:1 mixture of defined KSF medium and 154 medium

References