

Establishment of Bovine Fibroblasts from Fetal Skin

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Materials

Fetal calf skin (≤ 2 months old)

D-PBS (-) (Dulbecco's Phosphate Buffered Saline without Calcium and Magnesium) + 1% (v/v) Antibiotic-Antimycotic (pen-strep-amphotericin)

BEF (Bovine Embryonic Fibroblast) medium-(89% (v/v) Dulbecco Modified Eagle medium + 10% (v/v) fetal bovine serum (FBS) + 1% antibiotic- antimycotic)

Freezing Medium-(90% (v/v) BEF medium, 10% (v/v) DMSO)

0.05% trypsin-EDTA (.53 mM) reagent

FBS

MMC (Mitomycin C) –stop cell cycles at G2 phase

60 mm Petri dish

100 mm Petri dish

15 ml sterile centrifuge tube

175 mm³ cell culture flasks

scalpel blades

Methods

- 1) Wipe the surface of fetal skin with 70% ethanol.
- 2) Cut a piece of skin (1 cm²) from the fetus using sterilized forceps and scissors. Avoid collection of subcutaneous fat and other tissues.
- 3) Transfer the piece of skin to a 15 mL tube containing DPBS+Anti-Anti. Rotate the tube thoroughly with DPBS + Anti-Anti.
- 4) Transfer the skin to a 60 mm Petri dish, and cut each piece into 5 mm x 5 mm (or smaller) square pieces using a scalpel blade. Cut the surface sharp and smooth (see Figure 1) or cell growth could be very slow.
- 5) Make perpendicular lines on the inside surface of a 100 mm petri dish using a scalpel blade.
- 6) Wash skin pieces with DPBS + Anti-Anti. Place skin pieces (about 10 pieces) onto the perpendicular lines with the skin surface facing up. After a few minutes, carefully add 10 mL of BEF medium (avoid disturbing the pieces, Figure 2).
- 7) Culture at 38.5°C and 5% CO₂ in air.

- 8) Check the cell growth every day and change medium every 2 days.
- 9) When outgrowing cells reach confluence, remove the skin pieces and passage the outgrowth into 175 mm³ flask.
 - a) Aspirate medium from the dish and remove skin pieces with sterile tweezers.
 - b) Wash cells with DPBS+Anti-Anti twice and then add 5 mL of 0.05% trypsin-EDTA reagent to dish followed by incubation at 38.5°C for 3 min.
 - c) Check that cells are floating free from the dish surface and then add 1.5 mL of FBS and transfer contents of the dish to a 15 ml centrifuge tube.
 - d) Centrifuge at 250 g for 5 min. Remove supernatant and resuspend cell pellet in 25 mL of BEF medium and seed cells into a 175 mm² flask.
 - e) Culture at 38.5°C and 5% CO₂ in air.
- 10) Passage cells again when confluence is reached (1:4 ratio).
- 11) To treat cells with mitomycin C, let cells reach 80-90% confluence, and then add fresh BEF medium with 10 µg/ml MMC and culture for 3 h at 38.5°C and 5% CO₂ in air. Wash cells with BEF medium three times to remove MMC and culture overnight with fresh BEF medium.
- 12) Wash MMC-treated BEF with DPBS, then incubate with Trypsin-EDTA (5 mL for 175cm² flask) for 3 minutes at 38.5°C and 5% CO₂ in air. Following this, add 15 mL BEF medium to stop the reaction.
- 13) Collect BEF in a 50 mL tube and centrifuge (250 x g for 5 minutes).
- 14) Resuspend BEF in 10 ml BEF medium and count the cell number.

(Ensure that following step #13 that all materials are kept at a low temperature to allow for the best chance of BEF survival)
- 15) Centrifuge BEF (250 x g for 5 minutes at 4°C) and resuspend in freezing medium with 1.5 x 10⁶ cells/mL.
- 16) Make 1 mL aliquots using cryovials and set the tubes in a freezing container filled with isopropanol for storage overnight in the -80°. Following the overnight storage, move to the cell culture liquid nitrogen tank for long-term storage.

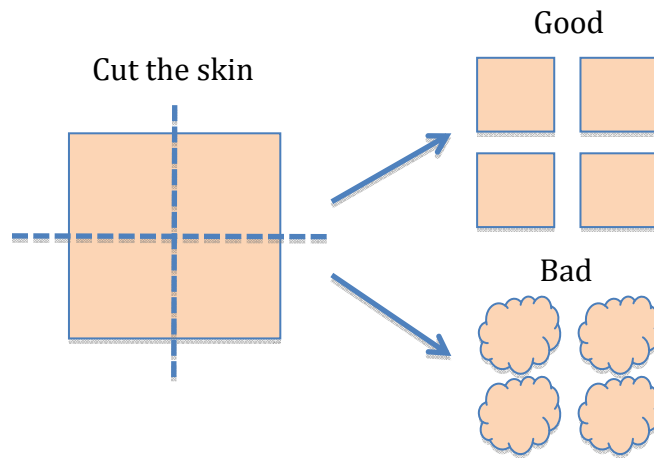


Figure 1. Example of technique for sectioning skin.

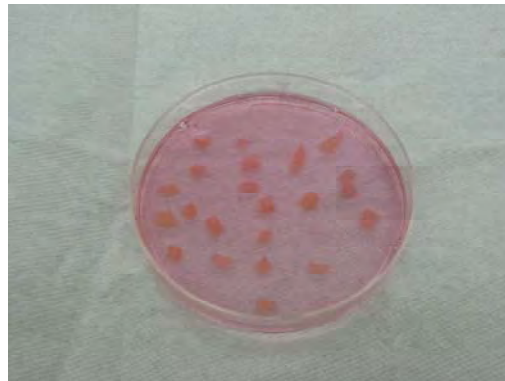


Figure 2. Example of culture for sectioning skin

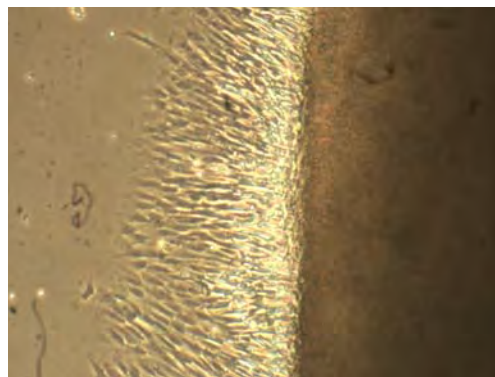


Figure 3. Example of outgrowth of fetal fibroblasts from skin (right). Photograph was taken at Day 1 of culture. (100X)

Created 5-31-2011; modified 8-15-2011