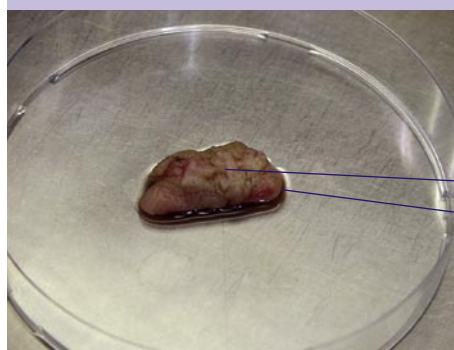


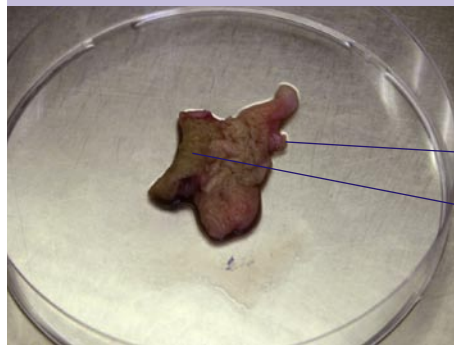
# Isolation of human dermal microvascular endothelial cells (HDMECs) from foreskin tissue

## 2.1.4.2 Supplement to CD31 MicroBead Kit booklet



1. Orientate the biopsy so that the epidermal side is facing upwards. (Step 3.)

Epidermis  
Dermis



2. Identify the inner and outer sides of the foreskin. (Step 4.)

The inside  
(lighter color)  
The outside  
(darker color)



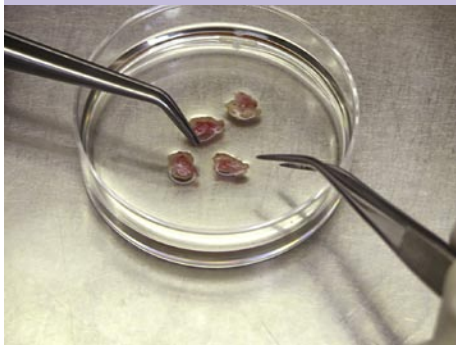
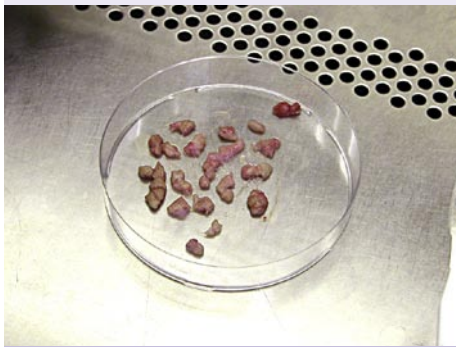
3. Separate the two sides by carefully cutting between them with a scalpel. (Step 4.)



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4. Subdivide both pieces into smaller pieces (approx. 4-5 mm<sup>2</sup>). (Step 5.)



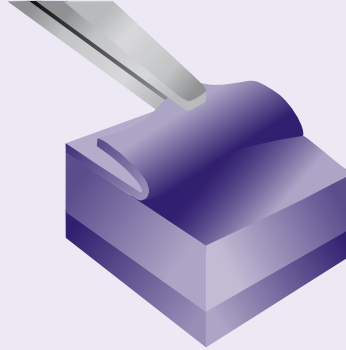
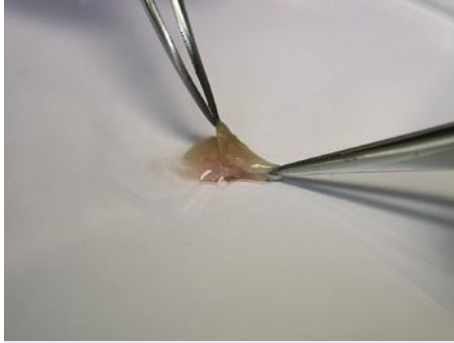
5. Place all pieces into a petri dish containing Dispase II with the epidermal side facing downwards. Incubate overnight (12-24 h) at 4 °C. (Step 6 and 7.)



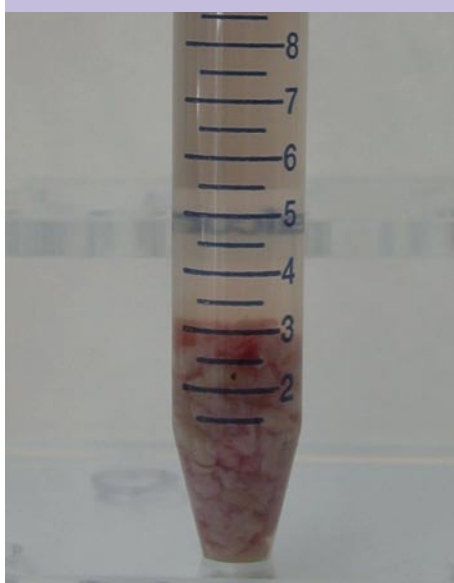
6. After digestion the pieces have a swollen morphology.



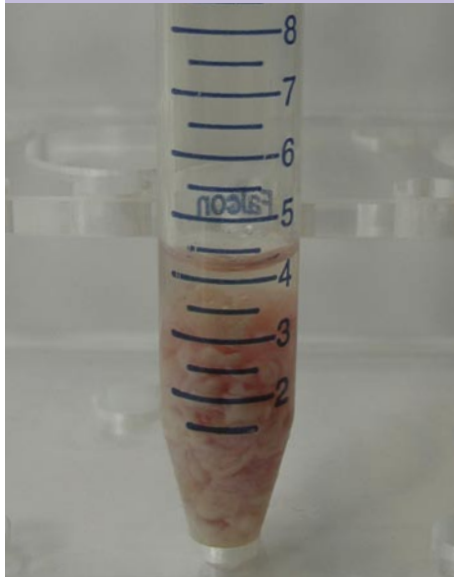
7. Separate the dermis from the epidermis by peeling back the epidermal layer of all pieces using sterile tweezers. (Step 9.)



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8. Collect the dermal layers in 10 mL HepesBSS. Let the pieces settle to the bottom. (Step 10.)



9. Aspirate the supernatant.

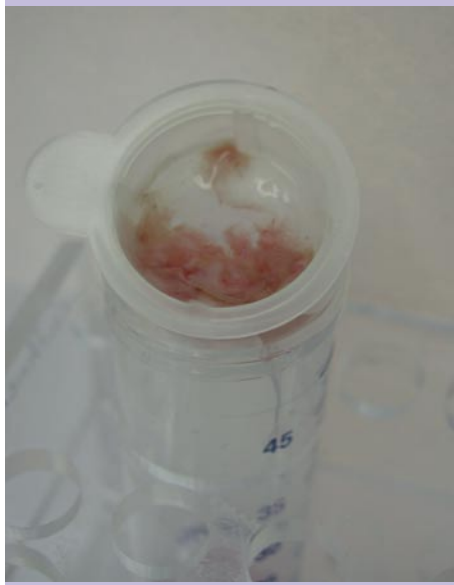


10. Apply 5 mL pre-warmed Collagenase Type 1a solution and close the tube. Incubate for 75 min (maximum) in a 37 °C water bath.

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11. After digestion, the cell suspension should be slightly viscous, though it is normal to still observe undigested parts of skin tissue.



12. Dilute the cell suspension with 10 mL EndoGMMV and pass through a 70 µm nylon filter. Centrifuge filtrate at 300 xg for 3–5 min.

13. Resuspend pellet carefully in EndoGMMV and culture the cells at 37 °C, 5% CO<sub>2</sub> and > 95% humidity for a minimum of 24 h before purification with the CD31 MicroBead Kit.



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