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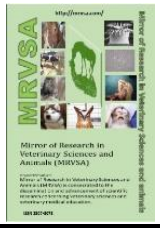

Culture technique of rabbit primary epidermal keratinocytes

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Abstract

The epidermis is the protective covering outer layer of the mammalian skin. The epidermal cells are stratified squamous epithelia which undergo continuous differentiation of loss and replacement of cells. Ninety per cent of epidermal cells consist of keratinocytes that are found in the basal layer of the stratified epithelium called epidermis. Keratinocytes are responsible for forming tight junctions with the nerves of the skin as well as in the process of wound healing. This article highlights the method of isolation and culture of rabbit primary epidermal keratinocytes *in vitro*. Approximately 2cm x 2cm oval shaped line was drawn on the dorsum of the rabbit to mark the surgical area. Then, the skin was carefully excised using a surgical blade and the target skin specimens harvested from the rabbits were placed in transport medium comprising of Dulbecco's Modified Eagle Medium (DMEM) and 1% of antibiotic-antimycotic solution. The specimens were transferred into a petri dish containing 70% ethanol and washed for 5 min followed by a wash in 1 x Dulbecco's Phosphate Buffered Saline (DBPS). Then, the skin specimens were placed in DMEM and minced into small pieces using a scalpel. The minced pieces were placed in a centrifuge tube containing 0.6% Dispase and 1% antibiotic-antimycotic solution overnight at 4°C in a horizontal orientation. The epidermis layer (whitish, semi-transparent) was separated from the dermis (pink, opaque, gooey) with the aid of curved forceps by fixing the dermis with one pair of forceps while detaching the epidermis with the second pair. The cells were cultured at a density of 4 x 10^4 cells/cm^2 in culture flask at 37°C and 5% CO_2. The cell morphology of the keratinocytes was analyzed using inverted microscope.

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