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Organotypic Skin Cultures: A Human Model for Basic Studies

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Aim. To produce organotypic skin cultures using human skin samples as a source of keratinocytes and fibroblasts.
Methods. Keratinocytes and fibroblasts from human skin samples were separated by warm trypsine and collagenase, respectively. Keratinocytes were plated in tissue culture dishes in keratinocyte serum free medium supplemented with epidermal growth factor and bovine pituitary extract, and were grown until confluence. Fibroblasts were cultured in Dulbecco's medium (DMEM) supplemented with fetal bovine serum and hydrocortisone. A mixture of fibroblasts, rat dermal collagen type I, E tissue culture medium, and reconstitution buffer were used as a dermal equivalent. Keratinocytes were plated on the top of the dermal equivalent and cultured for 10 days in organotypic culture dishes on stainless steel grids in the supplemented DMEM medium. The cultures were fixed in formaline, embedded in paraffin, stained with hematoxylin and eosin, and immunohistochemically stained with anti-cytokeratin and anti-HLA-DR antibody.
Results. Cultured keratinocytes in organotypic skin cultures expressed the majority of the cytokeratins seen in the normal stratified epithelium. Consistent with previous studies, organotypic skin cultures did not show antigen-presenting Langerhans cells.
Conclusion. Human skin from patients who underwent thoracic surgery can be used to produce organotypic skin cultures. This artificial skin can serve as a basis for future basic science studies and as a skin transplantation model.

Key words: artificial skin; fibroblasts; kerationcytes; organ culture; skin substitutes