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Isolation and Culture of Keratinocytes and Fibroblasts to Develop Artificial Skin Equivalent in Cats

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Abstract : The aim of this study was the isolation and culture of keratinocytes and fibroblasts from feline skin to ultimately create an artificial engineered skin (including dermis and epidermis) useful for the effective treatment of large cutaneous deficits in cats. Epidermal keratinocytes and dermal fibroblasts were freshly isolated from skin biopsies using an 8 mm biopsy punch obtained from 8 healthy cats that had undergone ovariohysterectomy. The owner's consent was obtained. All cats had a complete blood count and a serum biochemical analysis and were screened for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) preoperatively. The samples were cut into small pieces and incubated with collagenase (2 mg/ml) for 5-6 hours. Following digestion, cutaneous cells were filtered through a 100 μm cell strainer, washed with DMEM, and grown in DMEM supplemented with 10% FBS. The undigested epidermis was washed with DMEM and incubated with 0.05% Trypsin/0.02% EDTA (TE) solution. Keratinocytes recovered in the TE solution were filtered through a 100 μm and a 40 μm cell strainer and, following washing, were grown on a collagen type I matrix in DMEM: F12 (3:1) medium supplemented with 10% FBS, 1 μm hydrocortisone, 1 μm isoproterenol and 0.1 μm insulin. Both fibroblasts and keratinocytes were grown in a humidified atmosphere with 5% CO2 at 37oC. The medium was changed twice a week and cells were cultured up to passage 4. Cells were grown to 70-85% confluency, at which point they were trypsinized and subcultured in a 1:4 dilution. The majority of the cells in each passage were transferred to a freezing medium and stored at -80oC. Fibroblasts were frozen in DMEM supplemented with 30% FBS and 10% DMSO, whereas keratinocytes were frozen in a complete keratinocyte growth medium supplemented with 10% DMSO. Both cell types were thawed and successfully grown as described above. Therefore, we can create a bank of fibroblasts and keratinocytes, from which we can recover cells for further culture and use for the generation of skin equivalent in vitro. In conclusion, cutaneous cell isolation and cell culture and expansion were successfully developed. To the authors' best knowledge, this is the first study reporting isolation and culture of keratinocytes and fibroblasts from feline skin. However, these are preliminary results and thus, the development of autologous-engineered feline skin is still in process.

Keywords : cat, fibroblasts, keratinocytes, skin equivalent, wound
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