

## The Effects of Burn Blister Fluid on Cultured Keratinocytes

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Proceedings of the  
American Burn Association, 27<sup>th</sup> Annual Meeting  
April 19-22, 1995

We have previously shown that wound fluid collected from beneath occlusive dressing on partial thickness burn wounds and donor sites stimulated keratinocyte proliferation in vitro and contained growth factors that may be responsible for the enhanced wound healing. In this study, the effects of blister fluid on cultured keratinocytes were examined and quantitated to determine if similar growth enhancement characteristics occurred.

**Methods:** Nineteen burn blister fluids (BBF) at three different concentrations (2%, 10%, 20% in 20% fetal bovine serum / complete culture medium) were tested in triplicate using twelve populations of cultured keratinocytes. All blister fluids were collected from partial thickness burns within 72 hours of injury. Blister fluid was added on day 4 of keratinocyte culture. Cells were harvested three days later. Complete Culture Medium (20% fetal bovine serum) was used as a positive control fluid. Effect on proliferation and viability was assessed using trypan blue exclusion. Multiparameter flow cytometric analysis was used to quantitate effect on population kinetics and cell size distribution. Effect on keratinocyte differentiation was determined using immunohistochemical staining of differentiation markers and quantitation of cornified envelope formation.

**Results:** Relative to control fluid, BBF caused a variable effect on proliferation ranging from 67% inhibition to 103% stimulation with an overall 4% inhibition. Range of effect on keratinocyte viability was more narrow with a similar overall 4% reduction. Using flow cytometry to analyze RNA/DNA content and cell size, BBF caused a subtle shift in dividing cells. BBF did not significantly effect the expression of the differentiation markers filaggrin and involucrin. Finally, BBF did not alter terminal differentiation as seen by no effect on formation of cornified envelopes (BBF-9.1 +/-4.8%, control = 9.9 +/-6.6%).

**Conclusion:** Previous biochemical analysis has shown that BBF consists primarily of human serum filtrate with locally produced acute reactants. Our study suggests that BBF is biologically similar to serum and does not significantly alter keratinocyte proliferation or differentiation in vitro. Combined with the clinical observation that wounds underneath burn blister fluid heal well, our data supports that burn blisters should be left intact when possible.