Development of a full thickness in vitro human skin equivalent (EpiDerm-FT) for sulfur mustard research. Hayden, Patrick J.[1]; Petrali, John P.[2]; Hamilton, Tracey A.[2]; Kubilus, Joseph[1]; Smith, William J.[2]; Klausner, Mitchell[1]. 1. MatTek Corp., Ashland, MA, USA. 2. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA.

Skin exposure to the chemical warfare agent bis-(β-chloroethyl)sulfide (mustard gas, HD) causes vesication at the basement membrane (BM) of the dermal/epidermal junction (DEJ) that is difficult to heal. Efforts to develop prophylactic and/or therapeutic treatments for HD wounds have been hampered by lack of adequate human skin models. Therefore, an in vitro skin model was developed for HD research. Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce highly differentiated full-thickness skin equivalents (EpiDerm-FT). Histologic examination of EpiDerm-FT shows a collagen dermis populated by viable FB and an epidermis of stratified KC including basal, spinous, granular and stratum corneum components. Examination of the DEJ by transmission electron microscopy (TEM) revealed a well-developed BM. Hemidesmosomes exist at the basal membranes of KC, with associated tonofilaments extending into the cytoplasm. Well-defined lamina densa, lamina lucida and fine anchoring filaments are present beneath the basal KC. Anchoring fibrils connect the lamina densa to the underlying collagen matrix. Immunohistochemical analysis of BM proteins was also performed. Protein markers of hemidesmosomes (α6 integrin), lamina lucida (laminin 5), lamina densa (collagen IV) and anchoring fibrils (collagen VII) are localized to the DEJ. EpiDerm-FT was exposed to HD for 8 minutes and evaluated at 6 and 12 hours post-exposure. Histological evaluation revealed typical HD targeting of basal KC (cytopathology, condensed chromatin, pyknotic nuclei, increased eosinophilia) and epidermal cleavage at the DEJ. TEM show the lamina densa of the BM was largely intact. EpiDerm-FT overcomes shortcomings of previous models in terms of providing a wall to wall tissue as well as appropriate in vivo-like basement membrane development. These attributes will enable more realistic in vitro toxicological studies of dermal/epidermal phenomena including HD research.

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