Dermatotoxicological and other Safety Testing Methods without Animals – State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

Long-term culture of scaffold-dependent skin models

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Skin is one of the few human tissues that allows a high degree of in vitro reconstruction. Accordingly, 3D organotypic cultures (OTCs) or also called raft cultures are extensively used since a long time. OTCs were primarily established by growing human keratinocytes air-exposed on de-epidermized dermis or dermal equivalents made up of type I collagen gels and containing mouse 3T3 cells or human dermal fibroblasts. Although these models provided important insights into the regulation of epidermal differentiation, epithelial-mesen-chymal interactions, and wound healing processes, their limited lifespan remained a major drawback. Because of the restricted survival – these cultures commonly do not survive >4 weeks which equals about one epidermal regeneration cycle - it was suggested that explant and „air-lift" cultures promote differentiation, but not retention of stem cells (de Luca et al. 2006). Inhibiting degradation of the matrix already disproved this hypothesis and demonstrated the importance of an optimal matrix for long-term epidermal regeneration.

Based on a non-woven meshwork of a modified hyaluronic acid meshwork fibers (Hyalograft®) the integrated fibroblasts established an authentic dermal matrix, thus promoting the transition from a wound healing- to a homeostatic-type epidermis with the potential for epidermal long-term (>12 weeks) regeneration. Still suffering from hydrolytic degradation, next generation scaffold-reinforced OTCs were fabricated with inert cellulose-scaffolds, thereby providing a stable matrix. With this setting dermal fibroblasts build a functional stromal tissue thereby preparing the ground for a proper stem cell niche which i) supports long-term epidermal stem cell maintenance and regular epithelial regeneration for >16 weeks, ii) allows for serial epidermal transplantation, iii. study wound healing in a physiological environment of tissue homeostasis, and iv. allowing for reconstruction of skin aging models.