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3D organotypic culture: a powerful tool for research and application

Skin is one of the few human tissues that allow reconstruction in vitro to a high degree. 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. From this restricted survival - they commonly do not survive <4 weeks which equals only 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). Never the less these cultures provided an excellent potential to study short term effects requiring epidermal dermal interaction and/or are still used as test models for short term effects induced by factors, chemicals, or radiation. Attempts to optimize the life span of OTCs focused on modifying the composition of the dermal equivalents using e.g. hydrogels as a dermal scaffold. Due to a prolonged pre-cultivation time of these scaffolds, the overall cultivation period increased up to 45 days. However, true long-term epidermal regeneration was not reported. Only recently, we introduced scaffold-reinforced OTCs. Based on a non-woven meshwork of 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. However, this dermal construct still suffered from hydrolytic degradation. Therefore, the next generation of scaffold-reinforced OTCs was fabricated with inert cellulose-scaffolds, thereby providing a stable matrix. With this setting dermal fibro-blasts 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, ii) allows for genetically modified epidermal cells to develop their typical aberrant phenotypes, and iii) even provides a suitable environment for tumor cell invasion. In addition, these cultures can be modified to provide long-term wound healing models or extended by integrating other cell types, e.g. endothelial cells which under these condition undergo largely complete maturation and thus allow for the analysis of the complex inter-action program of keratinocytes, fibroblasts and endothelial cells.

These models are not restricted to epidermis but also support proliferation and differentiation of medullary thymic epithelial cells and as shown for the first time, allow for their promiscuous gene expression in vitro. Additionally, cell-derived matrix (CDM) models are used. For these scaffold-free dermal equivalents high numbers of fibroblasts are precultivated for 3 to 4 weeks in order to establish a matrix-rich dermal equivalent. Also these CDMs are suitable for epidermal long-term regeneration and tumor cell invasion, thus providing a suitable alternative to scaffold-based OTCs. Further-more, the translucent nature of these dermal equivalents now allows for life cell imaging also of complex in vitro models.