Application Forum

The establishment of *in vivo*-like tissue culture conditions in ThinCert™ tissue culture products

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INTRODUCTION

For many years animal experimentation provided the singular means to access intact and living tissue for biological and pharmaceutical research. This situation changed with the introduction of cell culture methods that allowed tissue reconstruction from individual cells *in vitro*. Such approaches typically involve the use of porous membrane supports and/or three-dimensional collagen gels to re-create *in vivo*-like growth in cell culture environments^{1, 2}. Today, reconstructed intestinal and oral epithelia, airway epithelium, skin and cornea models are frequently used to assess corrosive and irritant effects of substances and to study drug delivery and pathology.

TISSUE RECONSTRUCTION USING THINCERT™ CELL CULTURE PRODUCTS

One of the main challenges in tissue reconstruction is to restore specific tissue functions *in vitro*. Depending on the particular tissue type and scientific question such critical

features may comprise the barrier function, the capability of substance transport and the potential to express certain marker genes and signaling molecules. Optimal cell culture conditions must be well-established to re-create native function and maintain such features *in vitro*.

For instance, it has been determined that cultivated epidermal keratinocytes differentiate and form a coherent *stratum corneum* only if they are exposed to air^{3, 4}. Specific cell culture labware, such as ThinCert™ cell culture inserts with porous membrane supports, allows cultivation of epidermal cells at the air-liquid-interface (air-lift-culture) to ensure the formation of a *stratum corneum*.

Fig. 1 illustrates the two major steps of the formation of a skin equivalent *in vitro*, including the submersed cultivation of the dermis equivalent and the air-lift-culture of the epidermal layer. For improved nutrient supply the air-lift-culture can be performed in the novel ThinCert™Plate. This plate permits air-lift-cultivations with larger volumes of culture medium. The outcome is an improved nutrient supply to the cultivated tissue with reduced frequency of medium exchange, resulting in an improved quality of the generated tissue (Fig. 2).

Fig. 1: Reconstruction of a full thickness skin model.

A collagen gel containing primary dermal fibroblasts is cultivated under submersed conditions on the porous membrane of a ThinCert™ cell culture insert (A). Subsequently, primary epidermal keratinocytes are seeded on top of the dermis equivalent. At this stage cell culture medium is filled only to the level of the insert membrane to allow cultivation at the air-liquid-interface and formation of a *stratum corneum* (B). HE-staining of a reconstructed full thickness skin (C).

Fig. 2: Improved nutrient supply with the ThinCert™Plate.

A gingival epithelium was reconstructed from oral squamous cells using ThinCert™ cell culture inserts in combination with either conventional well plates or the novel ThinCert™Plate. The enlarged culture medium reservoir of the ThinCert™Plate enabled medium exchanges to be reduced from every day, as required by the conventional plate, to every 4th day. In addition to reducing the frequency of medium exchange, a significantly higher glucose concentration was maintained in the ThinCert™Plate as compared to the standard condition (A, glucose measurements were performed prior to medium changes). Moreover, tissue generated in the ThinCert™Plate appeared thicker and had more cell layers (B, HE-staining of the gingival epithelium after 29 days in culture).

CONCLUSIONS

ThinCert™ cell culture inserts enable the reconstruction of functional, multi-layered tissues from individual cells *in vitro*. Tissues may be cultivated at the air-liquid-interface with improved cellular oxygen supply, providing differentiation stimuli for development of air-exposed terminal structures such as the epidermal *stratum corneum*.

REFERENCES

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LINKS

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