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PermaCell Inserts - 8mm | Application Note

3D HaCaT SKIN MODEL DEVELOPMENT

Objective

Demonstrate the ability to grow HaCaT cells, an immortalized human keratinocyte cell line, on MatTek PermaCell 8mm Inserts to create an in vitro 3D skin model.

Methods

Immortalized Human Keratinocytes (HaCaT) were expanded for 5 days in monoculture. After 5 days, cells were trypsinized and seeded onto PermaCell 8mm Inserts (MatTek part #CCI24-PET-0.4) at a density of $0.5x10^6$ cells/cm^2. Both collagen coated and uncoated inserts were assessed for cell growth. Following cell seeding, cultures remained submerged for 5 days with media changes every 2-3 days. On day 6, the culture inserts with cells were raised to the air-liquid interface (ALI) and fed with specialized media in the basolateral compartment every 2-3 days. The tissues were grown at the ALI for an additional 14 days. Media and culture conditions paralleled those of Boelsma et al[1]. Barrier function was evaluated by measurement of Transepithelial Electrical Resistance (TEER) and tissues were collected for histological analysis on days 0, 4, 7, 11 and 14 of ALI culture. Inserts collected on ALI day 7 were assessed for E-cadherin (a cellular adhesion marker), Keratin 14 (a marker of proliferative basal cells), and DAPI (nuclear/DNA stain) using immunofluorescent (IF) staining.

Results

The culture of HaCaT cells on both collagen-coated or uncoated PermaCell 8mm Inserts resulted in the formation of 3D skin tissues. Over a 14-day culture period at the ALI, both TEER (data not shown) and epidermal thickness increased (Figure 1). Immunofluorescence data demonstrated the presence of both E-Cadherin and Keratin 14 throughout the multi-layered 3D tissue (Figure 2).

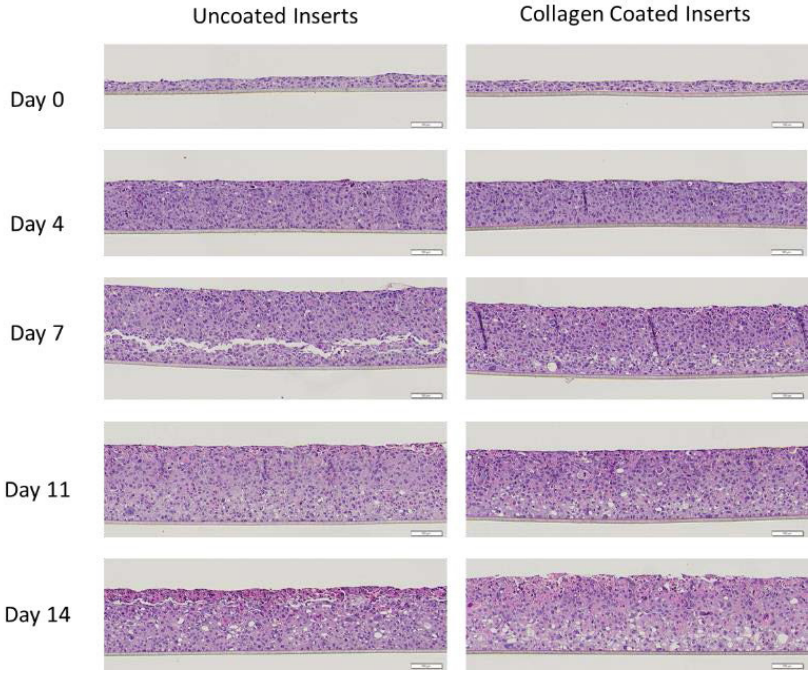

Figure 1. H&E stained histological cross-sections of 3D HaCaT tissues cultured on collagen coated and uncoated PermaCell Inserts cultured for up to 14 days at the ALI. Tissue thickness increases over time.

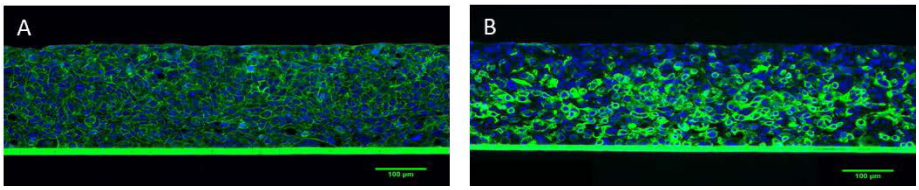

Figure 2. IF images of 3D HaCaT tissues cultured on collagen coated PermaCell Inserts for 7 days at the ALI. **A**. DAPI (blue) and E-Cadherin (green) and **B**. DAPI (blue) and Keratin 14 (green).

Conclusion

MatTek PermaCell 8mm Inserts provide researchers with a suitable system for developing 3D cell culture models. The inserts can be coated with extracellular matrix proteins or utilized uncoated depending on the cells being cultured. PermaCell Inserts are offered with three different membrane options to suit specific cell type requirements, including polyethylene terephthalate (PET), polycarbonate (PC), and polytetrafluoroethylene (PTFE). The PermaCell Inserts utilize high pore density membrane (1×10^8 pores/cm^2) which support the growth of highly stratified, 3D tissues.

1 Boelsma, E., Verhoeven, M. C. & Ponec, M. Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT). J Invest Dermatol 112, 489-498, doi:10.1046/j.1523-1747.1999.00545.x (1999).