

3D HaCAT SKIN MODEL DEVELOPMENT

Objective

Demonstrate the ability to grow HaCaT cells, an immortalized human keratinocyte cell line, on MatTek PermaCell 96-well insert plates to create a 3D epidermal tissue in a high-throughput platform.

Methods

Immortalized Human Keratinocytes (HaCaT) were seeded directly into two PermaCell Insert Plates (MatTek part #CCI96-PET-0.4) at 0.5×10^6 cells/cm² in 80µl of seeding medium per well. 300µl of seeding medium was added to the basolateral compartment. Both collagen coated and non-coated inserts were assessed for cell growth. Cultures were raised to the air-liquid interface (ALI) 24hrs after seeding by aspirating the media from the apical surface and were fed with specialized ALI media in the basolateral compartment (300µL/well) every day. Tissues were cultured at the air-liquid interface for an additional 10 days. Media and culture conditions paralleled those of Boelsma et al¹. Transepithelial electrical resistance (TEER) was measured on days 0, 1, 2, 3, 6, 7, 8, 9, and 10 of ALI culture. Individual tissues were fixed at these timepoints for histology assessment.

Results

Both collagen coated and uncoated inserts resulted in confluent monolayers that increased in both TEER values and number of layers over 10 days of culture at the ALI (Figures 1 and 2). Collagen coating did not promote improved cell growth or tight junction formation.

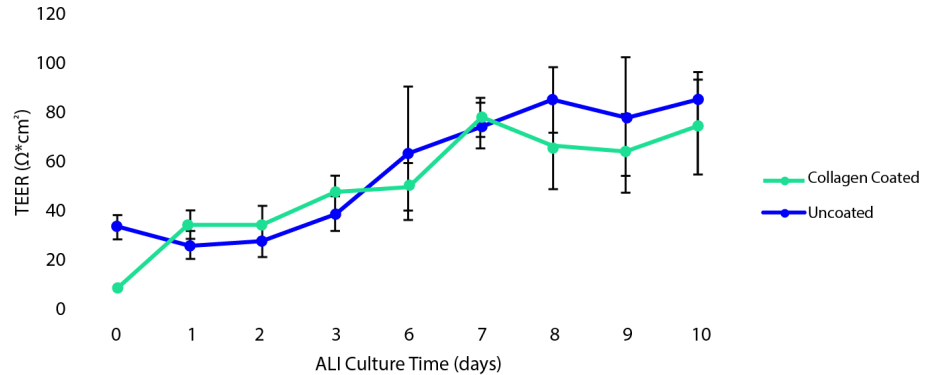


Figure 1. TEER (Ohms*cm²) of HaCaT tissue over a 10-day culture period comparing tissues cultured on uncoated and collagen-coated PermaCell 96-well insert plates.

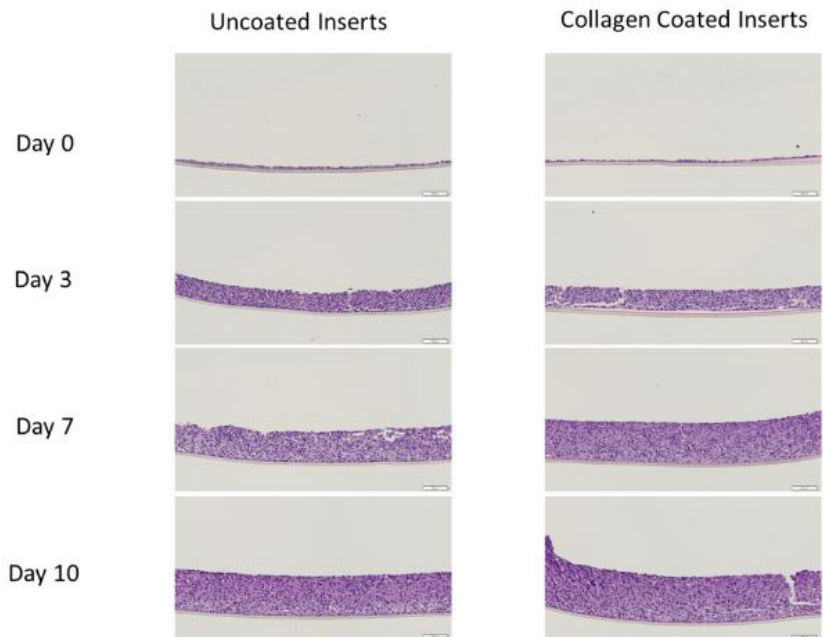


Figure 2. H&E images of 3D HaCaT tissues produced on collagen coated and uncoated PermaCell Insert Plates for up to 10 days at the ALI. Tissue thickness increases over time.

Conclusion

MatTek PermaCell 96-well Insert Plates provide researchers with a suitable system for developing 3D tissue culture models. The inserts can be coated with extracellular matrix proteins or utilized uncoated depending on the cells being cultured. While HaCaT cells grew similarly in both the uncoated (tissue culture treated) and collagen coated insert plates, other cell types may require specific coatings to improve cell growth and performance.

¹ Boelsma, E., Verhoeven, M. C. & Ponc, 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).