A DIY MODEL FOR GENERATION OF ROBUST 3D EPIDERMAL SKIN EQUIVALENTS COMPOSED OF NORMAL HUMAN PRIMARY EPIDERMAL KERATINOCYTES

life technologies
A Thermo Fisher Scientific Brand

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ABSTRACT

Three dimensional models composed of primary human cells enable *in vitro* modeling of complex human tissue and organ systems. In humans skin is the largest organ and is critical in maintaining an appropriate environmental barrier, wound healing and thermal regulation. Because of these properties physiologically relevant models of human skin are important in both basic research and clinical applications. In addition, 3D organotypic models are increasingly being used to supplant animal testing in product development of cosmetic and consumer products, providing more accurate, reproducible, and cost effective solutions. Here, we present an "off the shelf" solution for the generation of 3D epidermal skin models composed of normal human primary epidermal keratinocytes derived from either adult or neonatal skin; pairing an optimized protocol with set of commercially available reagents. Experiments were conducted to modify the protocol published by Poumay et al. in 2004 to minimize donor to donor variation and provide workflow flexibility enabling direct seeding into 3D cell culture inserts with cryopreserved human epidermal keratinocytes (HEKs). Numerous parameters were evaluated, including different extracellular matrices, growth media and supplements, cell culture insert type and brand, cell seeding density, passage number as well as stratification conditions. From these experiments, we show that seeding of expanded neonatal HEK (HEKn) or adult HEK (HEKa) into Nunc™ Cell Culture Inserts with Polycarbonate Membrane and cultured in EpiLife® medium supplemented with Human Keratinocyte Growth Supplement in the presence of FGF7 Recombinant Human Protein, Ascorbic Acid, and $CaCl_2$ resulted in consistent generation of 3D epidermal skin equivalents. Models produced using the optimized protocol display physiologically relevant morphology, displaying comparable number of cell layers and stratification to human epidermis. Basal cells are shown to express keratin 14, while suprabasal cells are shown to express keratin 10 and filaggrin. Furthermore, models were evaluated for their ability to correctly identify corrosivity and irritancy potential of a small panel of chemicals. Together, this protocol allows for generation of robust 3D skin model equivalents, which may be used for modeling of complex physiological processes in basic research.

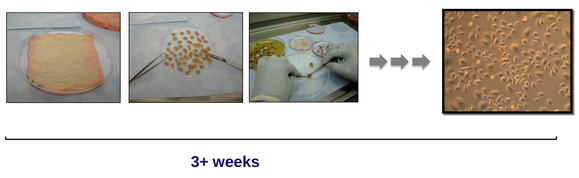
INTRODUCTION

3D epithelial skin models provide a physiologically relevant system to study various aspects of dermal biology, including wound healing, drug delivery and metabolism, aging and consumer products testing. Several challenges have limited their wider application- cost, complex and lengthy production protocols and the relatively short working lifespan of these models are major factors.

Here we present data demonstrating a standardized workflow using "off the shelf" cells, media and reagents can be used create reproducible and robust 3D epithelial skin models.

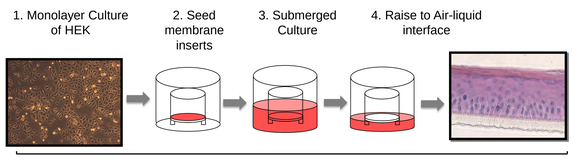
Figure 1. Primary HEK isolation/expansion and 3D epithelial skin model generation workflow

A. Procurement, dissociation, expansion and characterization of primary keratinocytes is lengthy and complex process



3+ weeks

B. Outline of multistep protocol for generation of 3D Epithelial skin models

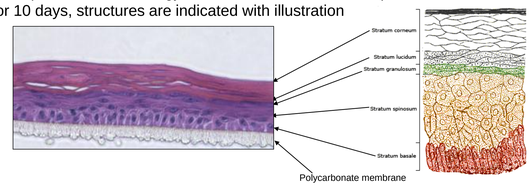


~ 3 weeks

RESULTS

Figure 2. Physiological relevance of 3D epithelial skin models derived from normal human epidermal keratinocytes

A. Representative histology for 3D culture raised to air liquid interface for 10 days, structures are indicated with illustration



B. Immunofluorescent staining of sectioned 3D models (upper panels) or adult human skin (lower panels). Basal marker Cytokeratin (CK)14, Differentiation Marker (CK)10

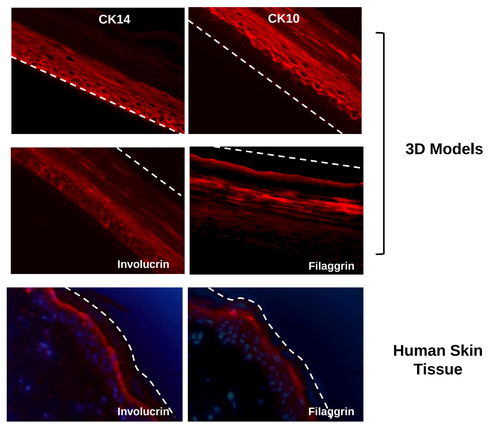
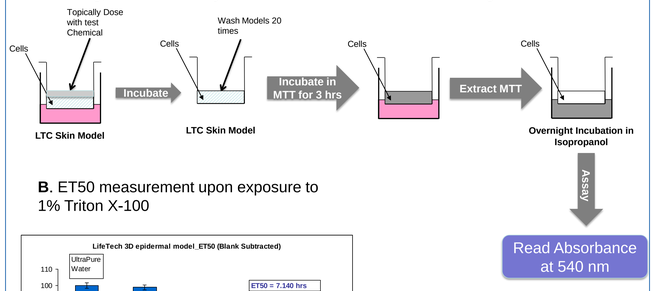
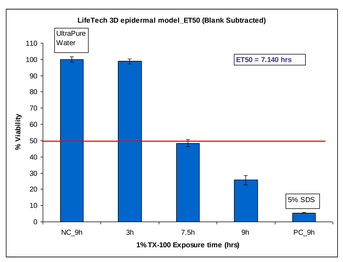


Figure 3. ET-50: Assessment of barrier properties of 3D epithelial skin models

A. Dosing, wash and assay workflow for ET50 determination using 1% Triton X-100

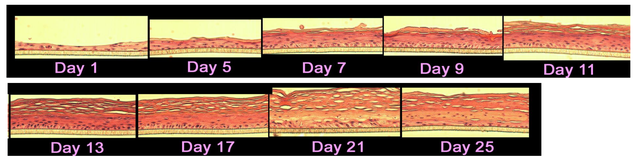


B. ET50 measurement upon exposure to 1% Triton X-100



C. Representative Histology (H&E) and TEER values

Figure 4. Assessing impact of time at the air liquid interface on 3D epithelial model stratification

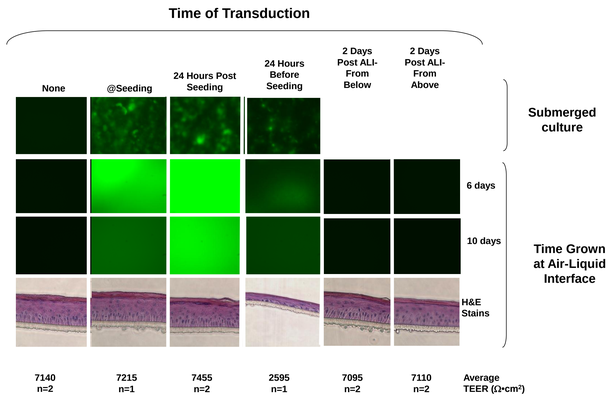


- Equivalent HEKa models were incubated for indicated days at the air liquid interface
- Inserts were removed, fixed & embedded then sectioned for H&E staining
- Pronounced thickening of the stratum corneum is observed around day 13 and beyond

Figure 5. Reconstructed human epidermis with or without human neonatal melanocytes (HEMn)

- Primary HEMn were combined with HEKa at a ratio of ~7:100 prior to seeding in trans-well inserts
- Following stratification (0 hr), cell inserts were photographed and then incubated with L-DOPA to stimulate melanin (pigmentation) production – *upper panels*
- Cell inserts were then photographed, relative to controls and then fixed and sectioned for histological analysis (H&E)- *middle and lower panels*

Figure 6. Combining HEKs and BacMam to create engineered epidermal skin models- *proof of concept*



- Primary HEKn were transduced with BacMam 2.0 transduction controls at indicated points in 3D model protocol
- BacMam encoded GFP expression was assessed prior to seeding into trans-well inserts *(top row)* and following 6 or 10 days at air liquid interface *(middle rows)*
- Equivalent stratification and TEER values were observed for all conditions *(bottom row)*

Table 1. Thermo Fisher Scientific reagents needed to create DIY 3D epithelial skin models

Material type	description	SKU
Cells	HEKa (adult)	C0055C
	HEKn (neonatal)	C0015C
Media & Supplements	Epilife®	MEPI500CA
	HKGS	S0015
Reagent	Coating Matrix Kit	R011K
	DPBS	14190
	100X Antibiotic-Antimycotic	15240
	TrypLE™	12604
	KGF	PHG0094
Inserts	Polycarbonate cell culture inserts (NUNC)	12-565-010

Note- additional reagents required: $CaCl_2$ and Ascorbic Acid

CONCLUSIONS

- Primary Human Keratinocytes can be used to reliably produce 3D epithelial skin models
- Adult and neonatal HEKs demonstrate comparable performance
- Thermo Fisher Scientific provides essential "off the shelf" materials and reagents necessary for model production

REFERENCES

1. Poumay et al 2004

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