Perspective and Consensus Opinion: Good Practices for Using Organotypic Skin and Epidermal Equivalents in Experimental Dermatology Research

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TO THE EDITOR

Introduction

Organotypic cultures for human skin and its compartments (also called three-dimensional skin models, human skin equivalents, or human epidermal equivalents), generated from keratinocytes alone or keratinocytes plus fibroblasts derived from primary cell sources or immortalized cell lines, have become an important experimental approach to assess how changes in specific processes affect epidermal growth, differentiation, permeation, and barrier function. Innovations in this field have led to a wide variety in models based on different cell sources, dermal substrates, and cell culture media. For example, organotypic cultures generated with juvenile (foreskin) keratinocytes showcase different characteristics than those from adult (trunk) keratinocytes (Tjabringa et al., 2008). Furthermore, skin barrier properties in organotypic cultures differ depending on the culture conditions, including the composition of the culture medium (Ponec et al., 1997; Thakoersing et al., 2012), and may also be associated with the fibroblast cell source and interdonor variability of keratinocytes and fibroblast donor tissues. This variety can aid in providing evidence from different angles and thereby strengthen biological findings, for example, on gene and protein function and lipid composition involved in epidermal barrier function. However, these advantages only hold true if models faithfully mimic the epidermal compartment.

A robust discussion that followed the keynote session New Developments in Skin and Epidermal Equivalent Models at the 2019 Barrier Function of Mammalian Skin Gordon Research Conference pointed out that there is a clear need for a consensus on the quality standard and validation of

Table 1. Toolbox with Validation Parameters for Quality Assessment of General Tissue Structure

Parameter	Essential Assays	Expected Result	Optional Assays
Morphology	Histology (H&E staining or equivalent)	For HEE and HSE: ✓ Number of cell layers that approximate in vivo epidermis ✓ Flattening of keratinocytes and presence of keratohyalin granules in stratum granulosum ✓ Multiple layers of basket weave orthokeratotic SC Additional for HSE: ✓ Nuclei in the dermis indicating fibroblasts	- Safranin-O: SC layer quantification - Calcium gradient - pH gradient - Electron microscopy
Stratification	Immunohistochemistry Immunofluorescence	For HEE and HSE: Two markers per strata indicative for proper stratification: ✓ Basal layer: - Proliferative cells: e.g., Ki67, Click-iT EdU, or PCNA staining - Positive for KRT5 or KRT14 - Negative for KRT1 or KRT10 ✓ Suprabasal layers: - Positive for KRT1 or KRT10 - Terminal differentiation markers: e.g., IVL, LOR, FLG, LCE, or TGM Additional for HSE: ✓ Basement membrane proteins	Enzymatic activity

Abbreviations: HEE, human epidermal equivalent; HSE, human skin equivalent; PCNA, proliferating cell nuclear antigen; SC, stratum corneum.

Abbreviation: SC, stratum corneum

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Table 2. Toolbox with Validation Parameters Specific to Type of Epidermal Barrier Studied

Barrier Type	Essential Assays	Expected Result	Optimal and Specialized Assays	Expected Result
Inside-out: water barrier	TEWL	TEWL[1]: < 15 mg H_20/cm^2	Lanthanum penetration	Passage is blocked at the SC/SG interface (for lipid barrier) or SG1 or SG2 level (for TJ barrier)
	TEER	TEER[1]: 1,200–2,500 Ohm/cm^2		
Inside-out: barrier to larger molecules	Dye studies: larger dye molecules that model substances of interest	Dye penetration reveals an incompetent barrier to large molecules that may model passage of large molecules such as proteins	Dye studies: e.g., biotin tracers for TJ function	Passage is blocked at the SG1 or SG2 level
Outside-in: barrier permeation	Tracking labeled molecules: e.g., Lucifer yellow (see text)	Tracers will penetrate the viable epidermis if no SC is present or when the SC is severely compromised; however, it is not possible to rule out defects in barrier function with histologic exam of tracer alone	Diffusion studies	Competent or incompetent barrier will yield concentrations of permeants in the acceptor fluid comparable to that seen in diffusion cell studies of human skin. Can combine with ELISA assays
			Lipid composition: e.g., HPTLC, LC-MS	Lipid composition matches that seen in normal or diseased skin, depending on the model
Outside-in: barrier to microbes	Detection of microbes: DAPI, FISH, or fluorescence-labeled	Localization of microbes on top of SC or in viable epidermis	Epidermal expression of host defense molecules: AMPs, cytokines, chemokines	
	Inoculum and viability of microbes before and after coculture	CFU counts		
	Infection control	Culture medium in basolateral chamber should remain sterile (no CFU on agar plates)		

Abbreviations: AMP, antimicrobial peptide; CFU, colony forming units; HPTLC, high-performance thin layer chromatography–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; SC, stratum corneum; SG, stratum granulosum; TEER, transepithelial electrical resistance; TEWL, transepidermal water loss; TJ, tight junction.

[1]Values are for information only. They might vary depending on the environment and how measurements are performed as well as device used.

which organotypic models are suitable for skin barrier research. This group has extensive experience and interest in using organotypic skin and epidermal equivalents in experimental dermatology research (Leman et al., 2019; Maione et al., 2015; Niehues et al., 2018; Petrova et al., 2014). Continued discussion during and after the Gordon Conference about validation parameters required to assess the quality of the models and their suitability in skin barrier research led to the following consensus.

Principles behind the consensus

- Given the genetic diversity in donors, we recommend that at least 3–4 biological donors of primary cells (keratinocytes and/or fibroblasts) should be used to validate results obtained by immortalized cell lines or technical replicates of primary cells or cell lines.
- Although we do not need to standardize culture protocols, we should standardize what data we expect to see in a model and the information needed to understand how the model was developed (e.g., cell sources and donor characteristics, culture medium, seeding density, and time frame of culturing). At a minimum, this should be presented as information in Materials and Methods, a figure in the body of the report, or Supplementary Data.
- A model should replicate what is seen in skin, should allow the experimenters to study mechanisms, and should be able to predict changes that can be tested in skin.
- Given the heterogeneity of culture characteristics, any model presented should define standard baseline characteristics of organotypic models, including morphological parameters, biophysical parameters, and functional parameters (Tables 1 and 2). Depending on the process studied, models also may include more specialized information (e.g., transcriptome, proteome, and transport data).
- Models should be used as a tool to predict results in skin. Using models to solve biological questions without validating corresponding results in skin is not recommended.

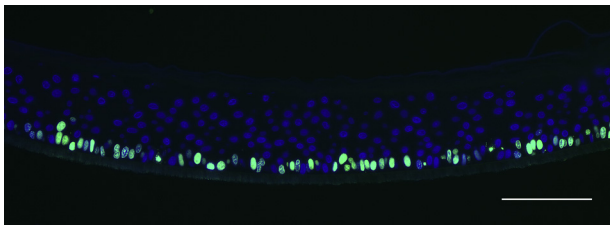

Figure 1. Proliferation in HEEs. HEEs are stained with the fluorescent assay, Click-iT EdU (Thermo Fisher Scientific, Waltham, MA), in which the modified thymidine analogue EdU is fluorescently labeled with Alexa Fluor dye. During the S-phase of the cell cycle, the modified thymidine will be incorporated, enabling the visualization of cells (fluorescently labeled) that underwent proliferation from the time in between addition of the EdU and harvesting of the HEEs. HEE, human epidermal equivalent.

Validation parameters required to assess the quality of the models and their suitability in skin barrier research

Histology.

- H&E staining of the skin equivalent and microscopic imaging at high quality and resolution to enable morphology assessment.
- Histology should explicitly address specific epidermal features of the model compared with native skin.

Immunohistochemistry or immunofluorescence for differentiation and proliferation markers.

- Proliferation is mostly confined to basal cells, using proliferative markers such as Ki67, proliferating cell nuclear antigen, or Click-iT EdU (Figure 1).
- Differentiation markers for each stratum (KRT5/KRT14, KRT1/KRT10, IVL, LOR, FLG, LCE, and TGM).
- Similar to National Institutes of Health Rigor and Reproducibility standards, antibody use should be justified for rigor and reproducibility in the Materials and Methods section.

Penetration ability.

- Fluorescent dyes are preferable to use as paracellular tracers. Of the common hydrophilic dyes (Calcium green, Oregon green, and Lucifer yellow), Lucifer yellow has the advantage of tolerating fixation. When the stratum corneum (SC) is present with a certain degree of barrier function, dyes placed on the SC surface will be retained in the SC and will not be seen in the viable epidermis.
- Conversely, a tracer will penetrate the viable epidermis if no SC is present or when the SC is severely compromised. However, it is not possible to rule out defects in barrier function with histologic examination of tracer molecules alone.
- When analyzing the skin barrier, a quantitative measure such as performing diffusion studies (e.g., Franz Cell chamber) is preferred.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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