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EDITORIAL

Fostering a healthy culture: Biological relevance of *in vitro* and *ex vivo* skin models

1 | BRIDGING THE GAP BETWEEN "SIMPLICITY" OF IN VITRO AND COMPLEXITY OF IN VIVO

The field of experimental dermatology research has dramatically benefited from the insights yielded by *in vivo* studies on animal models. Indeed, much of our understanding of the mechanisms that regulate embryonic skin development, adult skin homeostasis and physiological skin responses to stress, such as wound healing, has been educated by studies conducted in animal models, including mutant mice. These works become commonly published on the pages of *Experimental Dermatology* and, in fact, represent one of the core interests of the journal. Highlighting *in vivo* mouse model studies are recent works on hair follicle development and growth[1-5] and wound healing.[6-8] Further, studies in animal models often help to elucidate aspects of disease pathogenesis, and mouse models are used to investigate mechanisms of human skin conditions such as atopic dermatitis,[9-13] contact dermatitis,[14-16] psoriasis,[17,18] rosacea[19] and squamous cell carcinoma[20] to name a few.

On the other hand, not all human skin diseases or aspects of human skin physiology can be reliably modelled in rodents. This is not surprising, considering that humans and rodents are separated by an estimated 96 million years of evolution.[21] Addressing this fact, many studies are being conducted on patient-derived primary skin cells, including human keratinocytes,[22-25] melanocytes,[26-30] fibroblasts[31-34] and cell co-cultures.[35-37] However, typical *in vitro* culture conditions fail to replicate and, in fact, do not come close to imitating the biomechanical and biochemical complexity of the microenvironment in which cells exist and to which they respond to in native tissues. Further, ingredients in commonly used cell culture media and the two-dimensional constraints of growth on plastic result in cells being exposed to a lot of artificial cues, to which they adapt but also prominently alter their gene expression profile and functional activities in the process. Therefore, behaviours displayed by skin cells in two-dimensional cultures need to be comprehensively validated under more native-like conditions in order to be deemed biologically and physiologically relevant. Helping to bridge the gap, three-dimensional (3D) organotypic cultures and organ culture techniques have long been a highly instructive tool for investigating complex, tissue-level behaviours by skin cells.

2 | WHAT ORGANOTYPIC CULTURES CAN AND CAN'T DO

Typically composed of primary cells isolated from patient skin biopsies or surgical discard samples, the idea of studying 3D skin equivalents *in vitro* is an attractive premise for investigative dermatologists. From gaining molecular insight into essential aspects of skin development and homeostasis to preclinical testing of new drug candidates for skin diseases to their use as "tissue farms" to grow new skin substitutes for burn and trauma patients, 3D cultures are becoming a mainstay approach both in basic and translational dermatological research (Figure 1). Current 3D culture technologies include the following: (i) free-floating cultures of spherical organoids initiated from pluripotent stem cells; (ii) layered constructs consecutively assembled by seeding primary skin cells into extracellular matrix scaffolds[38] to contain stratified epidermis, sebocyte spheroids[39] or hair pegs[40]; (iii) freshly micro-dissected skin and hair follicle explant cultures[41-44]; and (iv) organ-on-a-chip cultures that incorporate capillary structures and allow active perfusion using microfluidics.[45,46]

However, so-called 3D skin "equivalent" cultures are not without limitations. For instance, despite their morphological similarity to native skin *in vivo*, their cellular composition is extremely simplified, and culture protocols can vary greatly, which affects reproducibility and interpretation. Depending on the protocol used, 3D cultures may not have a normal stratum corneum, an intact epidermal barrier, or proper lipid composition.[47] Critically, 3D cultures, no matter how sophisticated, lack system-level aspects of normal skin, such as fully functioning vasculature, immune system and innervation. Even though this may be partially compensated for with *ex vivo*-reinnervation protocols,[48] this puts limits on the maximum size that 3D cultures can achieve before experiencing hypoxia and simplifies their responses to external and neurotrophic stimuli.

Yet, 3D cultures of the same primary skin cells[49] are still far more complex than their monolayer counterparts. For instance, a recent genome-wide methylation study showed no correlation between methylation status and transcriptome changes during keratinocyte differentiation in a monolayer culture,[22] despite other data showing significant methylation changes during *in vivo* skin morphogenesis.[50,51] Likewise, normal expression patterns of terminal marker genes are significantly impaired in Ca^{2+} induced differentiation of

primary keratinocytes.[23] Yet, the biological relevance of monolayer cultures can be substantially increased by co-culturing two or more cell types. For instance, a recently reported co-culture study of senescent dermal fibroblasts and macrophages identified macrophage-derived TNFα as an important trigger of senescent cell apoptosis and clearance.[35] Also, the co-culture of dermal fibroblasts and keratinocytes can model particulate matter exposure, where factors released by keratinocytes after heavy metal and hydrocarbon exposure can mediate dermal collagen degradation.[36] In other examples, co-cultures of vitiligo patient-derived $CD4^+$ and $CD8^+$ T cells,[52] or keratinocytes and T cells from psoriasis patients,[37] can be used to advance our understanding of skin disease immunopathogenesis.

3 | WHERE ORGANOTYPIC SKIN CULTURES "SHINE"

3D equivalent cultures are commonly used to study both basic and translational aspects of skin biology (Figure 1B). For example, relatively simple skin equivalents, featuring only keratinocytes and fibroblasts, have been recently used to study the effects of Dead Sea water minerals on terminal epidermal differentiation.[53] More complex 3D equivalents that also contain melanocytes can be used in studies on pigment-modulating components of cosmetic products[54] or to test the effect of organic environmental toxins on hyperpigmentation.[55] Recently, 3D equivalent cultures have also been used to evaluate the impact of microbiota on skin cell function.[56,57] Considering the relative simplicity of 3D equivalents, full-thickness skin biopsy explants are commonly used in follow-up validation experiments (Figure 1C). Recent examples of using skin explants include studies on penetration and metabolism of vitamin A derivatives[41] and the effect of cigarette smoke exposure on skin barrier permeability.[42]

Where 3D skin equivalents and skin explants really shine is in their ability to model aspects of human skin disorders, including dry skin,[58] radiodermatitis[59] or hidradenitis suppurativa[60] to name but a few. In this issue of *Experimental Dermatology*, Yoshida *et al*. report on modelling hand-foot skin reaction (HFSR),[61] a common side effect of anti-angiogenic therapies, where soles and palms experience hyperkeratosis, leading to debilitating pain, difficulties walking and object grasping.[62] Small-molecule tyrosine kinase inhibitors, such as sunitinib, are used as angiogenesis inhibitors against various types of cancer, and the occurrence of HFSR is often correlated with successful treatment.[63] Sunitinib-induced HFSR causes epidermal thickening, with spinous and granular layers thinning and, at times, separating from the reactive basal layer.[64]

Yoshida *et al*. analysed both monolayer cultures of keratinocytes and 3D skin equivalents treated with sunitinib and revealed decreased expression of basal gene *KRT6A* and terminal differentiation genes *SERPINB1* and *SPINK6*, suggesting the skin barrier is perturbed in HFSR.[61] p38 MAPK and ERK1/2 phosphorylation was also inhibited upon sunitinib treatment, suggesting that the MAPK pathway may control skin barrier gene expression. As subsequent MAPK signalling inhibition in monolayer keratinocyte cultures

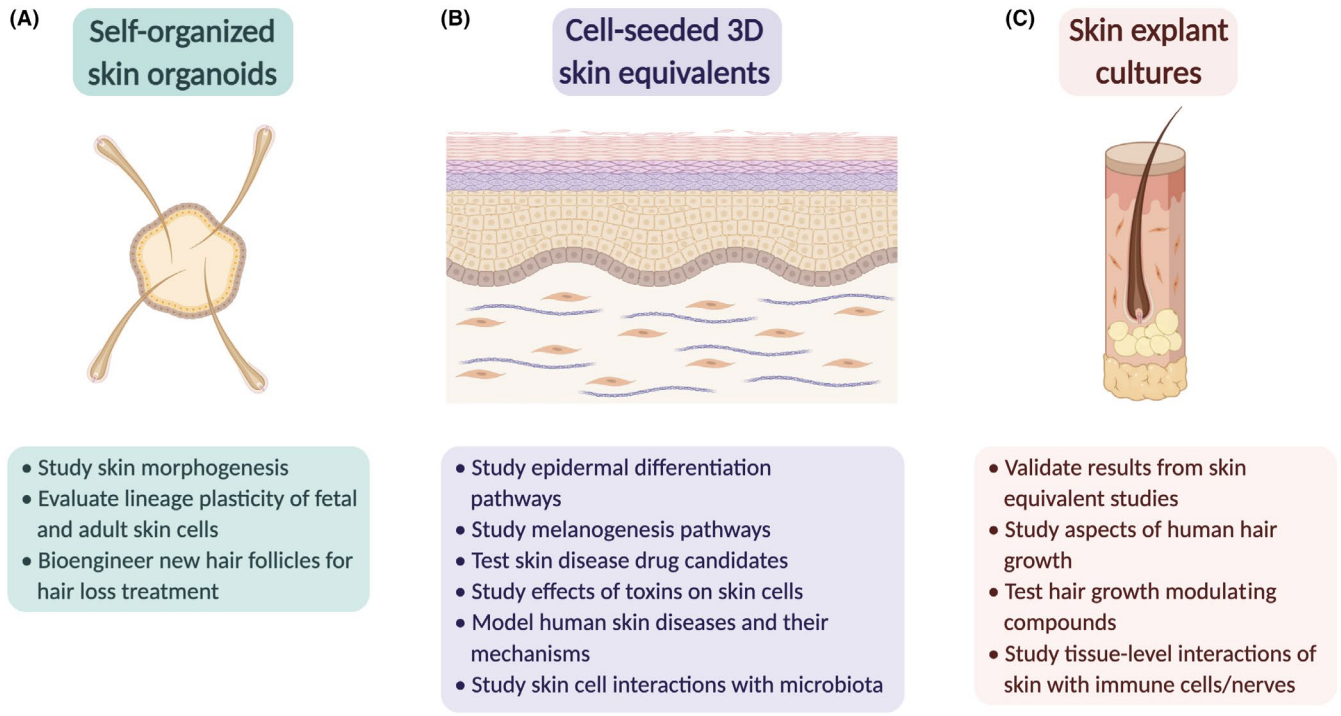

FIGURE 1 *In vitro* and *ex vivo* skin culture models in dermatological research. Common applications are listed for (A) self-organized skin organoids, (B) skin equivalents produced by seeding primary cells into extracellular matrix scaffolds and (C) explant cultures of freshly microdissected skin and/or hair follicles

phenocopied sunitinib treatment, the authors reasoned that activation of MAPK signalling could rescue the sunitinib-induced gene expression changes. Nuclear CTNNB1 can promote both p38 MAPK and ERK1/2 activity, whereas GSKB suppresses nuclear CTNNB1. Using an inhibitor to GSK3B with and without sunitinib, the authors showed that MAPK signalling and skin barrier gene expression changes can both be rescued, suggesting that GSK3B inhibitors may be a clinically relevant treatment to HFSR.

Another common skin disorder ripe for *in vitro* modelling is *acne vulgaris*, where genetic predispositions, hormonal changes, stress and environmental factors lead to the formation of microcomedones,[65-67] many of which can become inflamed.[68] Inflammatory microcomedones are caused by hyperseborrhoea, where fatty acid composition of sebum becomes altered, leading to accumulation of peroxidized squalene and favouring expansion of *C. acnes*.[66,69,70] The condition is aggravated by hair duct clogging with overproduced and abnormally differentiated epidermis. In this issue of *Experimental Dermatology*, Laclaverie *et al*. developed and characterized a 3D skin equivalent model for *acne vulgaris*.[71] The authors used primary keratinocyte- and fibroblast-seeded 3D skin equivalents that were subsequently treated with peroxidized squalene and/or *C. acnes*. They showed that combined treatment results in inflammatory, skin defense and remodelling gene expression changes analogous to those seen in acneic skin. The authors then tested different phylotypes of *C. acnes* strains isolated from healthy and acneic patients and found that strain IA1, commonly found in acne-prone skin, causes the major hallmarks of acne including hyper-keratinization, inflammation and altered barrier function.

4 | GROWING HAIRS IN A PETRI DISH

The dynamic biology of hair follicles poses a particular challenge for their organotypic culture, yet *in vitro* approaches have been actively pursued in the field driven by many differences between human scalp and mouse pelage hairs and by the unmet demand to bioengineer new hair follicles to treat hair loss. With regard to hair bioengineering, competent hair-fated epithelial and mesenchymal skin cells, such as those derived from neonatal mice, can efficiently self-organize into many new hair follicles when injected subcutaneously into host mice. However, such injections produce "hairy cysts" that lack proper follicle orientation and are, thus, not therapeutically viable.[72,73]

To overcome this fundamental limitation, Paik *et al*. recently reported an approach in which neonatal mouse dermal and epidermal cells are cultured within the collagen scaffold prior to being grafted.[74] This approach produces high densities of hair follicles that maintain near-normal orientation perpendicular to the skin surface. In an effort to translate similar tissue engineering approaches to the human system, Weber *et al*. developed a 3D model for co-culturing human neonatal foreskin keratinocytes with human foetal scalp dermal cells that permits their self-organization into hair peg-like structures.[40] When grafted after *in vitro* self-assembly to nude mice, at least some peg-like structures matured towards functional human hair follicles. To this end, recent advances in pluripotent stem cell differentiation protocols have enabled production of human hair follicle-bearing organoids fully *in vitro* (Figure 1A).[75] Progress on this and similar *in vitro* hair-bearing organoid systems is discussed in a recent *Experimental Dermatology* Viewpoint article.

Cultures of freshly micro-dissected human scalp hair follicles, which are a special variant of skin explant culture, have been successfully used to study aspects of human hair biology not displayed in the animal models and have been instructive for our understanding of human hair follicle physiology and pathology (Figure 1C).[76,77] Studies on human hair follicle organ cultures are commonly featured in this journal, including recent works that examined hair growth-promoting effect of dermal papilla-derived exosomes,[78] regulation of hair follicle immune privilege[79] and modulation of hair growth by the blue light-sensitive circadian clock factor CRY1.[80]

While explant cultures of freshly dissected skin and hair follicles are arguably the most physiologically complete type of *ex vivo* culture models, they still suffer from being cut-off from numerous microenvironmental and systemic inputs that regulate their physiology *in vivo*. To this end, a recent study showed that growth and pigmentation parameters of scalp hair follicles *in vitro* are improved if they are cultured with the adjacent piece of dermal adipose tissue that secretes hepatocyte growth factor.[81] Sensory nerves are another important source of regulatory signalling molecules, from which skin explants become severed in culture. To this end, a co-culture with rat dorsal root ganglions (rDRGs) permits partial sensory reinnervation of human skin explants *in vitro*,[48] where "trophic" effects of rDRGs have been shown to positively affect epidermal proliferation and induce activation of resident mast cells.[82]

Taken together, the above examples clearly show that organotypic cultures have already evolved to a level of technical sophistication that permits studying aspects of normal skin physiology and conducting meaningful disease modelling. As the citations listed here demonstrate, *Experimental Dermatology* prides itself for being at the forefront of this area in skin biology research. At present, experimental dermatologists have the "luxury" to choose from several models, ranging from self-organizing skin organoids to cell-seeded extracellular matrix scaffolds to freshly micro-dissected skin or appendage explants. Looking into the future, technical efforts aimed at better imitating system-level inputs will eventually advance organotypic cultures towards true organ-on-a-chip level, when complex skin functions, such as hair growth cycling, and progression of major skin diseases will be modelled fully *ex vivo*.

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