3D living skin models: product development

Testing personal care products using clinical trials is the gold standard for proving efficacy, but is often seen as too expensive to be considered cost-effective. However, formal proof that a product works is highly motivating to consumers, as demonstrated by the runaway success of Boots Protect & Perfect anti-ageing serum in 2009. The combination of effective testing and marketing gave Protect & Perfect a distinct advantage over the competition, ushering in a new age in the marketing of personal care products, with cosmetic and personal care companies receiving increased pressure to provide solid evidence to support claims of product effectiveness. However, as proving efficacy remains discretionary for cosmetic and personal care products, for many manufacturers the primary motivating factor for testing non-regulated skin care products remains financial. Therefore, although companies can no longer rely entirely on marketing claims, there is still some reluctance to invest in R&D activities to help develop the stronger claims that attract the attention of consumers, even though there are now many laboratory and clinical methods available. Also, while reliable quantitative *in vitro* and *in vivo* methods have been developed to support many common claims (i.e. increased hydration, reduction in spots, reduced appearance of wrinkles, etc.), there is often a discrepancy between what is meant by the product claim for the consumer compared to the cosmetic scientist.

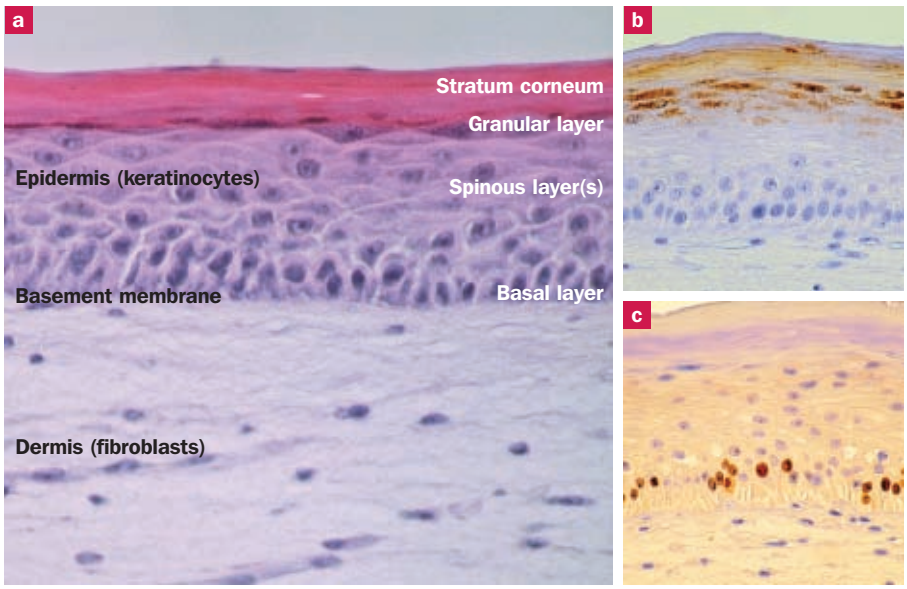

Figure 1: *Histological assessment of LabSkin showing* **a)** *a well differentiated epidermis with stratum corneum and active dermal fibroblasts (H&E stain),* **b)** *differentiation (filaggrin) and* **c)** *collagen IV production.*

The testing landscape

The most obvious point of reference for evaluating the effectiveness of cosmetic and personal care products are studies using human volunteer subjects. However, the capital cost of instrumentation, recruiting suitable panels of volunteers and executing the study is beyond the means of many companies. Therefore, the process is commonly outsourced to specialist Clinical Research Organisations (CROs), which often have specialist expertise in certain types of study. While a properly designed and conducted clinical study is undoubtedly the pinnacle of the testing pyramid, the dual issues of cost and ethics restricts its use to the end of the development process. Earlier phases of development such as discovery, mode of action and efficacy studies are better served by *in vitro* laboratory testing using model systems which are more cost effective for screening purposes and allow much more extensive investigations than would be ethically acceptable using human subjects. The usefulness and predictability of *in vitro* test systems for cosmetic and personal care applications has been enhanced significantly in recent years by the development of sophisticated three-dimensional living skin equivalent models as a stepping stone between simple *in vitro* laboratory assays and clinical studies. Therefore, the cosmetic and personal care industries have access to three distinct levels of efficacy testing for ingredient and product development (Table 1) within a testing continuum, with graded levels of cost, complexity and claims. With consumers and media no longer willing to accept advertised claims at face value without some confirmation and an increasing number of competing personal care brands producing similar products with similar benefits, verification of activity through testing is a cost-effective way to generate competitively advantageous consumer claims. Traditionally, the most successful brands at all price points are those that use R&D to generate advanced

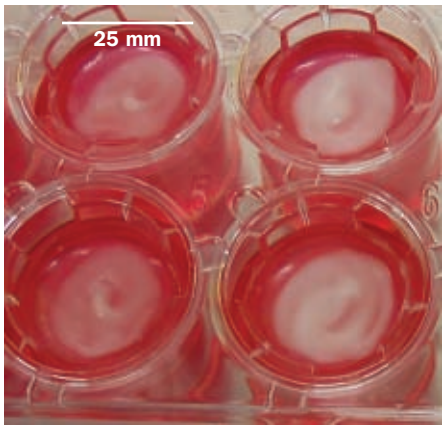

Figure 2: *Direct application of skincare products onto LabSkin. The size (4.5 cm²) and strength of the material allows 'real-world' application protocols to be used i.e twice daily, for 5 days using a fingertip.*

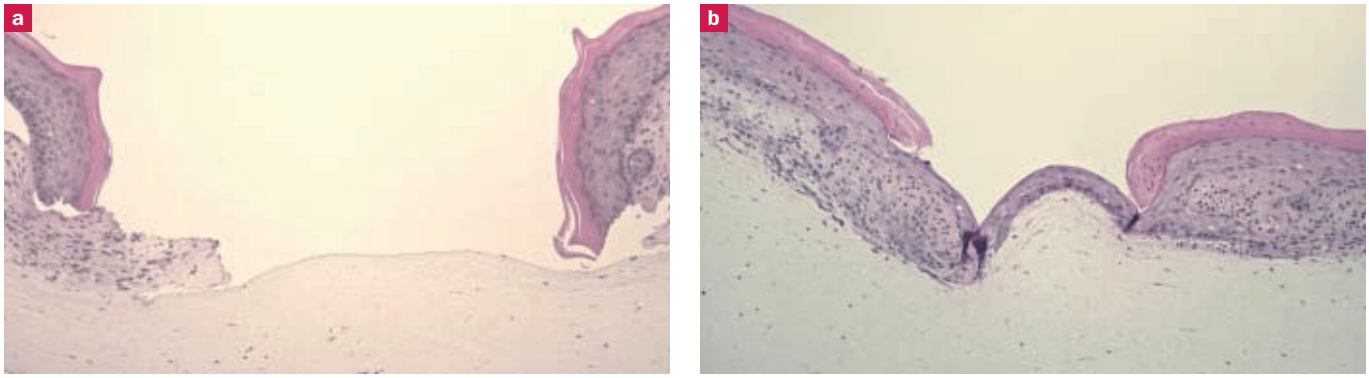

Figure 3: *Assessment of Wound Healing using LabSkin* **a)** *immediately following wound,* **b)** *after 24 hours.*

formulations and back up their products claims with scientific proof.

In vitro laboratory testing

Laboratory testing of biological, physical and chemical properties of cosmetics and personal care ingredients and products is an established, routine procedure in the process of product development. The tests can range from highly specific assays with unique targets to outcomes assessed by changes in a trend in an unspecified endpoint. Depending upon the outcome being measured, the data collected can be highly predictive of activity on skin (rare) or an indicator of possible activity (common). Therefore, in many cases it is the lack of relevance to the 'real-world' situation that is the major flaw in this approach. However, when the shortcomings are appreciated and the data is used with care, these relatively simple tests are useful, cost-effective tools which are used extensively in early-phase screening and can also be used for claims support when appropriate.

Clinical testing

It is quite obvious that clinical testing is the *de facto* standard for skin care products and can potentially give the strongest claims. However, as with most things in life, it is not always that simple. While it is true that some clinical volunteer studies for cosmetic and personal care products approach the standards of GCP Clinical Trials, unfortunately many are woefully inadequate in design and execution. Obviously the complexity of doing a clinical study properly, coupled with the high cost, means that for many the hurdles are too great to make this a cost effective option. And even when resources allow, the study design may need to be compromised in order to fit in with what is ethically acceptable to ask volunteers to do when testing a cosmetic or personal care product. For instance, while photographing changes in facial wrinkles is acceptable when testing a product making claims for anti-ageing, it would not be acceptable to take biopsy material to determine whether

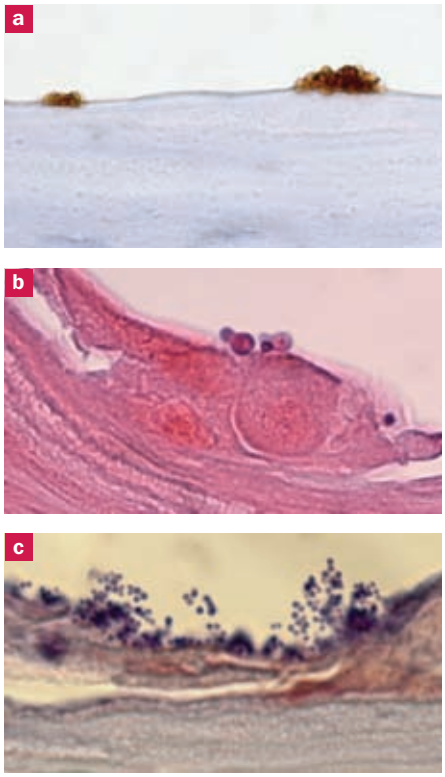

Figure 4: *Microbial colonisation of LabSkin* **a)** Propionibacterium acnes, **b)** Malassezia restricta *showing budding,* **c)** Staphylococcus epidermidis.

the product has had an effect on skin structure. Consequently, most clinical studies for cosmetics and personal care products are somewhat conservative in design, relying heavily on instrumental and expert clinical grading assessments.

In vitro living skin equivalent testing

Testing cosmetic and personal care ingredients and products to support claims is a difficult balance between inexpensive (and often non-predictive) laboratory tests and expensive (and often restrictive) clinical methods. The short-comings of laboratory tests are exacerbated by the transition from mainly aqueous and/or solution/suspension systems to the lipid-rich surface of the skin. This transition from laboratory to skin is notoriously risky, but fortunately living skin equivalent (LSE) test models are available to bridge this gap.

Over the years, a range of model systems have been developed both as in-house tools in research laboratories and as commercial consumable products. While the obvious objective of development has been to produce ever more complex model systems in an attempt to replicate the structure and function of human skin, simpler models with limited predictive capacity are also useful, being inherently more reproducible, reliable and cost-effective. However, in all cases the primary objective is to produce a layered cellular structure with some inherent structural and functional barrier qualities similar to human skin. The functions of the skin are pretty self-evident. In its simplest form, it is a physical barrier to the changeable external environment, retaining nutrients and preventing dehydration. However, it is also a complex, dynamic living interface with primary immunological and protection functions, with the capacity to self-repair.

Technological innovation in this area has been driven by a number of factors, including clinical need (skin replacement for burns patients), research requirements (understanding biological mechanisms of the skin) and product material testing (cosmetics, personal products, toxicology). For example, the banning of animal testing of cosmetics in 2009 was a major driver in the development of LSE technologies that were suitable alternatives. Consequently,

Table 1: Options for testing cosmetic and personal care products.

Execution	Test method	Predictivity	Cost factor
Simple	*In vitro* laboratory testing	Low	1
	In vitro living skin equivalent testing	Good	10
Complex	Clinical testing	High	100

Table 2: R&D options for cosmetic and personal care products using LabSkin *in vitro* living skin model.		
Assay	**Effect**	**End-point measurement**
In vitro testing for anti-inflammatory activity	Soothing	Increase in IL-10 or reduction in IL-1α
In vitro testing for wound healing effects	Promotes skin repair	Rate of epidermal regeneration
In vitro anti-bacterial activity against pathogens	Antimicrobial	Clinical pathogens - panel MICs
In vitro effect on commensal microflora	Microbe balancing	Commensal microbe mix
In vitro irritation potential	Safe for use on skin	IL-1α, IL-6, IL-8, TNFα
In vitro irritation potential/penetration	Suitable for sensitive skin	IL-1α, IL-6, IL-8, TNFα
In vitro effects on structural dermal proteins	Anti-ageing	Fibrillin, collagen

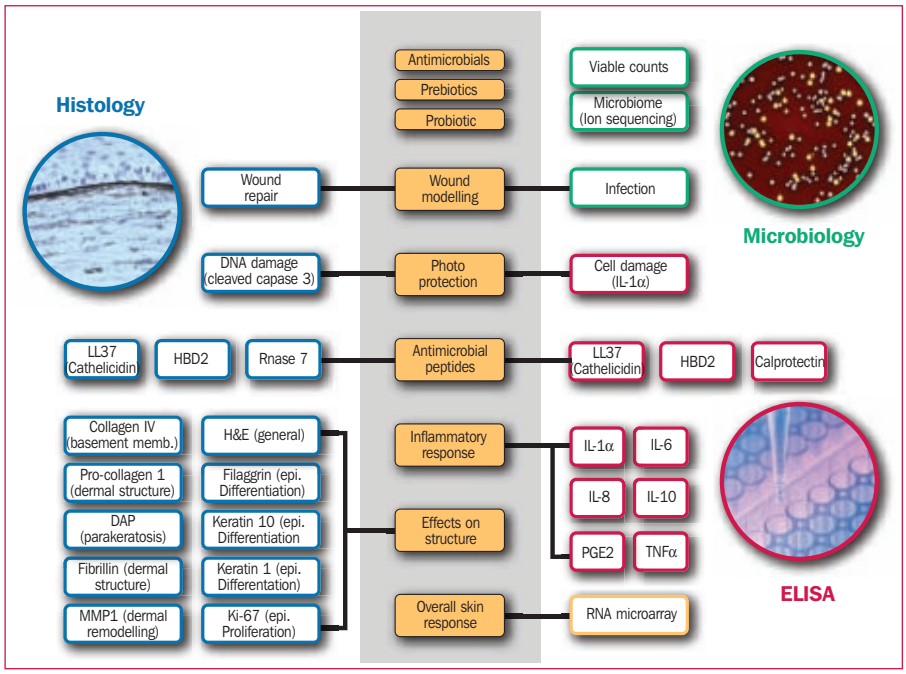

Figure 5: *Overview of the current testing capabilities of LabSkin.*

LSE models for skin irritancy and corrosivity testing were developed prior to the deadline (MatTek's EpiDerm and SkinEthic's RHE & EpiSkin) and acceptance of these models as a suitable replacement for irritation testing in animals is a pivotal step in moving away from reliance on animal and/or human testing. While these models provide a solution to the specific problem of skin irritancy, they are differentiated epidermal models which lack a dermal component. This makes them relatively simple compared to 'full thickness' models (epidermis and dermis), as the interaction between keratinocytes in the epidermis and fibroblasts in the dermis is vital to skin structure and function at many levels.

The combination of epidermal and dermal components in 3D models contributes to better differentiation of the epidermis and tempers the immunological reactivity of keratinocytes. The LabSkin full thickness living skin equivalent model, developed by Evocutis over the last 10 years, is an advanced complex model with highly developed epidermal differentiation (Fig. 1) and a robust skin-like structure which enables a more intuitive approach to testing (Fig. 2) and the use of physical procedures such as wounding (Fig. 3), which are not practical with other, less structurally robust models. A direct consequence of the highly structured architecture of LabSkin is that the surface is relatively dry compared to other 3D skin models (MatTek's EpiDermFT, Phenion Full Thickness Skin Model) which makes it ideal for growing microorganisms on the surface (Fig. 4). This makes it an invaluable tool for R&D in the areas of dandruff, acne, foot care, underarm odour, hand and body wash, hygiene and any personal care application where there is a microbial component. Additionally, it is of particular value in helping to understand how the natural microflora of the skin may impact on skin health in a similar manner as to how the gut microflora affects digestive health. With the technology of tissue-cultured, three-dimensional models of human skin such as LabSkin, it is possible to explore direct effects on skin biology (Fig. 5) and relate these to real-world effects (Fig. 6 & Table 2).

Conclusion

Living skin equivalents are a mature and exciting technology, which are cost-effective, reliable and reproducible, with the dual benefits of being more predictive than many standard laboratory tests and allowing a much greater range of endpoints to be assessed than can be achieved in cosmetic clinical studies. The potential for open-ended development of the models will encourage continued evolution of the technology to help develop better clinical solutions, improved research outcomes and more predictive test systems. PC

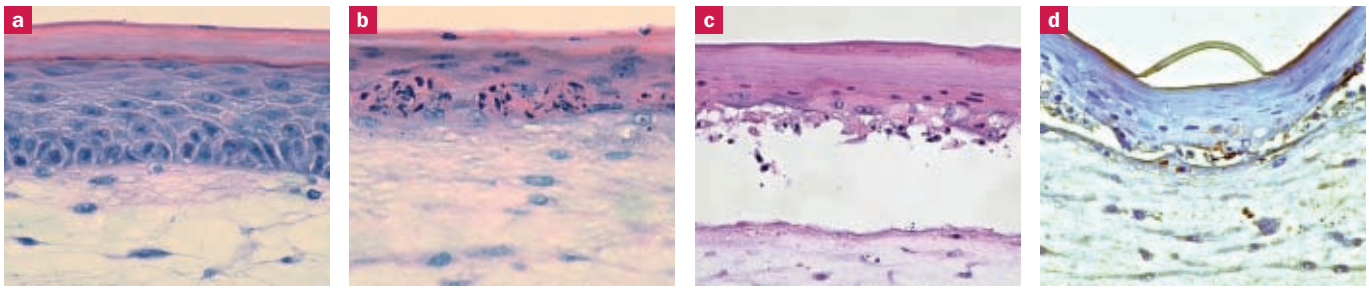

Figure 6: *Photo-Damage Assessment using LabSkin following exposure to UVB* **a)** *SPF protected (no damage),* **b)** *unprotected showing 'sun burn' cells,* **c)** *unprotected showing apoptotic cells,* **d)** *unprotected showing DNA damage (blue).*