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In vitro skin models. Challenges and Future Steps

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Abstract
The in vitro models have great potential in skin-related research as well as in testing for active ingredients in cosmetics, dermocosmetics and pharmaceuticals. Human skin behavior can be simulated in vitro using a variety of methods ranging from cell monolayer models to complicated organotypic and bioengineered three-dimensional models. Moreover, skin in vitro models offer an excellent alternative to animal testing in cosmetics and some of them are validated to be used as preclinical as-says. However, the in vitro simulation of the whole skin together with its appendages is still in its early stages. In this article we discuss a short evolution of skin models with its challenges and its future.

KEYWORDS
in vitro models, skin models, three-dimensional models, cosmetics

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1. INTRODUCTION

The development of in vitro skin models that fully mimic the epidermis, dermis, and subcutis as well as its appendages, such as sweat glands, hair follicles, and the arrector pili muscle, is a major challenge in the field of in vitro skin models. We are still far from simulating the entire complexity of human skin, both structurally and cellularly [1,2]. However, the need for novel and efficient in vitro three-dimensional (3D) or even multi-dimensional human skin tissue equivalents has grown, not only for clinical applications like skin grafts, but also for research purposes, such as exploring the fundamental causes of skin diseases or evaluating the safety and efficacy of active agents in cosmetics, dermocosmetics and pharmaceuticals.

2. DISCUSSION

There are different in vitro skin models ranging

from simple cell-monolayer models to more complex such as tissue engineering approaches. Two-dimensional (2D) cultures of primary human keratinocytes and fibroblasts are used quite extensively as a skin model approach [3]. These cultures are useful for drug or cosmetics screening, cytotoxicity assays, and studying molecular mechanisms in homeostasis, skin aging, or diseases like cancer [2,4,5]. However, they do not accurately replicate cell-cell or cell-matrix communication and structural organization of the skin. It has been reported that extracellular matrix (ECM) components could be added to 2D cell models enabling a more representational approach close to in vivo [2,6,7].

Organoids, a simplified system in which cells grow in a 3D well chemically defined microenvironment made up of extracellular matrix (ECM) and media, are considered to be the evolution of more complex in vitro skin systems. The clusters of cells in these systems differentiate into distinct cell types that mimic the structure and function of the organ [8,9]. The traditional and most basic in vitro skin models are the 3D human skin equivalents (HSE) which are well established and are accessible on the market for testing of products [2]. Currently, a wide range of commercial in vitro HSE are available providing alternatives for skin sensitivity testing, toxicity testing, and drug screening such as ZK1350 [10], EpiDerm™ [11], T-skin™[12], MelanoDermTM, [13], EpiSkin™ [14], SkinEthic™ RHPE 45, and The Phenion™ FT Skin model [15]. The development of commercial in vitro assays for regulatory toxicology has been prompted the legislative shift toward non-animal testing (EU Regulation 1223/2009 and U.S. Federal Food, Drug, and Cosmetic Act, 2022). The OECD has adopted several validated epithelial only in vitro methods for skin corrosion and irritation (Test Guidelines 431 and 439, respectively) [16]. These in vitro 2D and 3D skin models, while helpful for certain cosmetic tests, are not representative of skin physiopathology and do not have a circulatory flow that replicates blood vessels, which is necessary for the distribution of nutrients and other molecules. In addition, as the human body is subjected to various stressors and environmental factors, normal skin growth takes place concomitantly. Thus, a variety of dynamic and microfluidic bioengineered devices, such as skin bioreactors and skin on chips, are being employed recently to promote and facilitate important physiological events for the formation of in vitro skin tissues. Skin bioreactors are complex bioengineered devices intended to simulate in-vivo like biophysiological stimuli at the bench scale to stimulate, mature, monitor and prolong healthy skin culture duration [17,18]. Moreover, skin on a chip are tiny devices that allow the application of various stimuli such as microflows, mechanical forces or chemical gradients to present more realistic models with a more accurate response to treatments and drugs [19]. In addition to bioreactors and state-of-the-art technology, 3D skin bioprinters allow for the reconstruction of human skin, including details such as sweat glands and hair follicles [20,21]. Research on skin grafting and regenerative medicine has previously employed these products in high-throughput research [2]. Presently available in vitro skin models attempt to mimic essential skin properties as flexibility, immunological response, and barrier function. However, biological and technical issues prevent the development of a more realistic model [22]. It worth to be noticed that the thickness of HSE models is attributed to the primary cells which exhibit donor-to-donor variance indicated by variations in individual responses. However, these multicellular models in order to be realistic, co-cultures systems should be developed raising issues on histocompatibility because of different cell types and donors. Additionally, skin functions such as body hydration, thermoregulation, and feeling (pain, itching), depend on the skin's appendages, including hair follicles, sebaceous, and sweat glands, as well as the brain sector. According to the current literature on this matter, the in vitro simulation of the melanogenesis process in skin or hair as well as the hair follicle development are two processes quite challenging with a variety of applications in medicine, dermocosmetics and pharmaceutics. Despite all the current advancements discussed above, which allow for the anatomical and physiological replication of both healthy or unhealthy in vitro HSE, there is no HSE available with widespread application.

3. CONCLUSION

In academia, many efforts are made in developing in vitro HSE to increase experimental throughput and tissue complexity, whilst improving biological and methodological aspects to successfully mimic human skin as well as skin diseases.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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