Since 1987
REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION | 38 (Sup1): 63-65 (2024) Rcpp

Received: 18 December 2023 | **Accepted**: 22 December 2023 | **Published**: 17 February 2024

Paper presented at the **1st Conference of the Hellenic Scientific Society of Aesthetics**
2-3 December 2023 | University of West Attica, Athens, Greece

Open Access | **Review Paper**

In vitro skin models. Challenges and Future Steps

Sophia Letsiou[1,*], Apostolos Beloukas[1], Efstathios Rallis[1], Vasiliki Kefala[1]

[1]Department of Biomedical Sciences, School of Health and Care Sciences, University of West Attica, Ag. Spyridonos Str., Egaleo 12243, Athens, Greece

***Corresponding author**
Sophia Letsiou, PhD, Department of Biomedical Sciences, School of Health and Care Sciences, University of West Attica, Ag. Spyridonos Str., Egaleo 12243, Athens, Greece.
Email: sletsiou@uniwa.gr

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

How to cite: Letsiou S., Beloukas A., Rallis E., Kefala V. In vitro skin models. Challenges and Future Steps. *Rev. Clin. Pharmacol. Pharmacokinet. Int. Ed.* 38(Sup1): 63-65 (2024).
https://doi.org/10.61873/FCXV3865

Publisher note: PHARMAKON-Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2024 by the authors.
Licensee PHARMAKON-Press, Athens, Greece. This is an open access article published under the terms and conditions of the Creative Commons Attribution (CC BY) license.

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.

REFERENCES

1. Sanabria-de la Torre R, Fernández-González A, Quiñones-Vico MI, Montero-Vilchez T, Arias-Santiago

S. Bioengineered Skin Intended as In Vitro Model for Pharmacosmetics, Skin Disease Study and Environmental Skin Impact Analysis. *Biomedicines* [Internet]. 2020 Oct 31;8(11):464.
http://dx.doi.org/10.3390/biomedicines8110464

2. Suhail S, Sardashti N, Jaiswal D, Rudraiah S, Misra M, Kumbar SG. Engineered Skin Tissue Equivalents for Product Evaluation and Therapeutic Applications. *Biotechnol J* [Internet]. 2019 Jul 17;14(7).
http://dx.doi.org/10.1002/biot.201900022

3. Deng M, Xu Y, Yu Z, Wang X, Cai Y, Zheng H, et al. Protective Effect of Fat Extract on UVB-Induced Photoaging In Vitro and In Vivo. *Oxid Med Cell Longev* [Internet]. 2019 Aug 18; 2019:1–11.
http://dx.doi.org/10.1155/2019/6146942

4. Stanton DN, Ganguli-Indra G, Indra AK, Karande P. Bioengineered Efficacy Models of Skin Disease: Advances in the Last 10 Years. *Pharmaceutics* [Internet]. 2022 Jan 28;14(2):319.
http://dx.doi.org/10.3390/pharmaceutics14020319

5. Hofmann E, Fink J, Pignet A-L, Schwarz A, Schellnegger M, Nischwitz SP, et al. Human In Vitro Skin Models for Wound Healing and Wound Healing Disorders. *Biomedicines* [Internet]. 2023 Mar 30;11(4):1056.
http://dx.doi.org/10.3390/biomedicines11041056

6. Sarkiri M, Fox S, Fratila-Apachitei L, Zadpoor A. Bioengineered Skin Intended for Skin Disease Modeling. *Int J Mol Sci* [Internet]. 2019 Mar 20;20(6):1407.**http://dx.doi.org/10.3390/ijms20061407**

7. Valdoz JC, Johnson BC, Jacobs DJ, Franks NA, Dodson EL, Sanders C, et al. The ECM: To Scaffold, or Not to Scaffold, That Is the Question. *Int J Mol Sci* [Internet]. 2021 Nov 24;22(23):12690.
http://dx.doi.org/10.3390/ijms222312690

8. Hofer M, Lutolf MP. Engineering organoids. *Nat Rev Mater* [Internet]. 2021 Feb 19;6(5):402–20.

9. Corrò C, Novellasdemunt L, Li VSW. A brief history of organoids. *Am J Physiol Physiol* [Internet]. 2020 Jul 1;319(1):C151–65.
http://dx.doi.org/10.1152/ajpcell.00120.2020

10. Liebsch M, Döring B, Donelly TA, Logemann P, Rheins LA, Spielmann H. application of the human dermal model skin2 ZK 1350 to phototoxicity and skin corrosivity testing. *Toxicol Vitr*. 1995;
http://dx.doi.org/10.1016/0887-2333(95)00042-7

11. Líšková A. Evaluation of phototoxic and cytotoxic potential of TiO2 nanosheets in a 3D reconstructed human skin model. *ALTEX*. 2020;
http://dx.doi.org/10.14573/altex.1910012

12. Bataillon M, Lelièvre D, Chapuis A, Thillou F, Autourde JB, Durand S, et al. Characterization of a new reconstructed full thickness skin model, t-skinTM, and its application for investigations of anti-aging compounds. *Int J Mol Sci*. 2019;
http://dx.doi.org/10.3390/ijms20092240

13. Park DJ, Jeon G, Bang SH, Kim SY, Wee JH, Kim YH, et al. Cellular Lysosomes' Activity for Melanin Reduction on Artificial Skin Tissue. *Mol Biotechnol.* 2020; **http://dx.doi.org/10.1007/s12033-019-00235-w**

14. Chen L, Li K, Liu Q, Quiles JL, Filosa R, Kamal MA, et al. Protective effects of raspberry on the oxidative damage in HepG2 cells through Keap1/Nrf2-dependent signaling pathway. *Food Chem Toxicol* [Internet]. 2019;133(July):110781.
http://dx.doi.org/10.1016/j.fct.2019.110781

15. Pfuhler S, Pirow R, Downs TR, Haase A, Hewitt N, Luch A, et al. Validation of the 3D reconstructed human skin Comet assay, an animal-free alternative for following-up positive results from standard in vitro genotoxicity assays. *Mutagenesis*. 2020;
http://dx.doi.org/10.1093/mutage/geaa009

16. Kandárová H, Liebsch M, Schmidt E, Genschow E, Traue D, Spielmann H, et al. Assessment of the Skin Irritation Potential of Chemicals by Using the SkinEthic Reconstructed Human Epidermal Model and the Common Skin Irritation Protocol Evaluated in the ECVAM Skin Irritation Validation Study. *Altern to Lab Anim* [Internet]. 2006 Aug 1;34(4):393–406.
http://dx.doi.org/10.1177/026119290603400407

17. Wahlsten A, Rütsche D, Nanni M, Giampietro C, Biedermann T, Reichmann E, et al. Mechanical stimulation induces rapid fibroblast proliferation and accelerates the early maturation of human skin substitutes. *Biomaterials* [Internet]. 2021 Jun; 273:120779.
http://dx.doi.org/10.1016/j.biomaterials.2021.120779

18. Tokuyama E, Nagai Y, Takahashi K, Kimata Y, Naruse K. Mechanical Stretch on Human Skin Equivalents Increases the Epidermal Thickness and Develops the Basement Membrane. Egles C, editor. *PLoS One* [Internet]. 2015 Nov 3;10(11):e0141989.
http://dx.doi.org/10.1371/journal.pone.0141989

19. Mori N, Morimoto Y, Takeuchi S. Skin integrated with perfusable vascular channels on a chip. *Biomaterials* [Internet]. 2017 Feb;116:48–56.
http://dx.doi.org/10.1016/j.biomaterials.2016.11.031

20. Nanmo A, Yan L, Asaba T, Wan L, Kageyama T, Fukuda J. Bioprinting of hair follicle germs for hair regenerative medicine. Acta Biomater [Internet]. 2023 Jul;165:50–9.
http://dx.doi.org/10.1016/j.actbio.2022.06.021

21. Song HJ, Lim HY, Chun W, Choi KC, Lee T, Sung JH, et al. Development of 3D skin-equivalent in a pumpless microfluidic chip. *J Ind Eng Chem* [Internet]. 2018 Apr;60:355–9.
http://dx.doi.org/10.1016/j.jiec.2017.11.022

22. Klicks J, von Molitor E, Ertongur-Fauth T, Rudolf R, Hafner M. In vitro skin three-dimensional models and their applications. *J Cell Biotechnol* [Internet]. 2017 Nov 13;3(1):21–39. **http://dx.doi.org/10.3233/jcb-179004**