Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 14, No. 2, 2025, pp.181-197
Online ISSN 2285 – 4177
ORIGINAL PAPERS

NAVIGATING CHALLENGES IN DRUG TESTING AND DISEASE MODELING: 3D SKIN ORGANOIDS VS. 2D CELL LINES

Elena RADU [1*], Laura-Elena ANDREI [1*], Ana-Maria ZAMFIRESCU [1**], Grațiela Grădișteanu-PÎRCĂLĂBIORU [1], Ming NI[3], Yi ZHANG[4], Ciprian ILIESCU [1,2,5**], Bianca-Maria TIHĂUAN [1,2]

[1]eBio-hub Center of Excellence in Bioengineering, National University of Science and Technology Politehnica Bucharest;
[2] Academy of Romanian Scientists, 3, Ilfov Street, 050045 Bucharest, Romania;
[3]GenScript, Zhenjiang, Jiangsu Province, P. R. China;
[4]School of Integrated Circuit Science and Engineering, University of Electronic Science and Technology of China, Sichuan, China.
[5] National Institute for Research and Development in Microtechnologies- IMT Bucharest, Romania

*Share first author – equal contribution
**Corresponding authors: anazamfirescu0803@upb.ro; ciprian.iliescu@upb.ro

Abstract. *Developing new and effective therapeutics relies on using robust preclinical models that can closely mimic human physiological and immunological responses. Two-dimensional (2D) cell culture systems fail to replicate the complex tissular architecture and specialized microenvironments, which limits their ability to translate early findings into clinical success. Three-dimensional (3D) organoid models have also several drawbacks, such as handling complexity, variability in growth and differentiation, scalability issues, and reliability concerns, particularly in the context of high-throughput drug screening. This review provides a critical examination of skin related 2D and 3D models in the context of drug testing and disease modeling, evaluating their respective strengths and limitations. We also explore emerging technologies (e.g. immune-competent skin organoids) designed to overcome current barriers in organoid research, particularly those aimed at enhancing throughput, standardization, and data reproducibility in drug screening and toxicity testing.*

Keywords: skin 3D organoids, 2D cell culture, drug discovery, diseases modelling, toxicity testing

DOI 10.56082/annalsarscibio.2025.2.181

Introduction

The well-documented limitations of conventional two-dimensional (2D) cell culture systems in accurately replicating human tissue physiology have underscored the urgent need for more advanced models. In this context, three-dimensional (3D) organoids—particularly skin organoids—have emerged as

promising tools, warranting a critical assessment of their role in drug screening and disease modeling.

Since its inception, organoid research has revolutionized the way biological assessments are conducted, offering enhanced *in vitro* relevance and improved translation to *in vivo* systems. One major limitation in evaluating the efficacy and potential toxicity of drugs, for example, was the reliance on 2D monolayer cultures or basic co-culture systems, which lacked the complexity of native tissue architecture. These simplified models not only limited clinical applicability but also generated misleading false positives that advanced prematurely to animal studies or microfluidic chip-based systems. Over time, pharmaceutical companies and research institutions have moved away from these approaches in favor of more physiologically relevant models, such as 3D organoids (Ghiță-Răileanu et al 2025).

As we now understand, developing new and effective therapeutics relies on using robust preclinical models that can closely mimic human physiological and immunological responses. Indeed, 2D cell culture systems have long served as the gold standard for *in vitro* drug screening due to their simplicity and scalability. However, their failure in replicating the complex tissular architecture and specialized microenvironments, has limited their ability to translate early findings into clinical success (Chouhury et al 2011). Not surprisingly, many drugs that perform well in 2D cultures ultimately fail in later stages of development.

In order to surpass these limitations, 3D organoid models are being considered as preclinical models due to their high physiological fidelity in replicating tissue organization and function (Yu et al 2016, Tong et al 2016): These models can be derived from stem cells (embryonic stem cells – ESCs, induced pluripotent stem cells – iPSCs, mesenchymal stem cells – MSCs, etc.) or primary tissues (skin biopsies, liver tissue, urothelial tissue, etc.) and recapitulate specific architectures and multicellular interactions, making them valuable tools for screening therapeutic effect of novel compounds, toxicity testing, and studying disease mechanisms.

As encountered with every biomimicry system, 3D organoid systems have some drawbacks, including handling complexity, variability in growth and differentiation, scalability, and reliability issues, particularly when it comes to high-throughput drug screening.

Here, we provide a critical examination of 2D and 3D skin-focused models in the context of drug testing and disease modeling, evaluating their respective strengths and limitations. We also explore emerging technologies such as microphysiological systems (so called skin-on-chip -SoC), 3D bioprinting, CRISPR-based, skin-on-chip (SoC) or artificial intelligence/machine learning

(AI/ML), aimed at overcoming current barriers in organoid research, particularly those designed to enhance throughput, standardization, and data reproducibility in drug efficacy screening and toxicity testing.

Brief history of two-dimensional and three-dimensional *in vitro* cell culture models

2D cell culture systems have been extensively used for studying cell motility, screening drugs in early discovery, and performing toxicity analyses, a technique that involves a significant level of simplicity and low maintenance costs. Although its use was of great significance for several decades, the restrictions it imposed in mimicking the *in vivo* environment forced researchers to push forward, because the controlled environment, uniformity of nutrients, and modified morphologies (Ballav et al., 2021) resulted in limited translation into pre-clinical advances.

3D cell culture systems have emerged as powerful alternatives to traditional 2D models, offering enhanced biological relevance by better replicating the structural, functional, and microenvironmental characteristics of native tissues. These systems can be broadly classified into spheroids – which consist of a single or multiple cell types and are useful for basic biological studies and high-throughput screening, and organoids – which are composed of various differentiated cell types that self-organize into tissue-like structures capable of mimicking the architecture and function of real organs. Depending on the complexity and intended application, 3D cultures can be developed using static approaches, (scaffold-based cultures), or dynamic ones such as constrained spheroids, or dynamic systems, often referred to as microphysiological systems which incorporate perfusion and mechanical stimuli via platforms like organ-on-a-chip (Ballav *et al*., 2021). These advanced culture systems are increasingly leveraged for two major biomedical directions: disease modeling by enabling the recreation of pathophysiological processes *in vitro*, and personalized medicine using patient-derived cells for customized drug testing and therapeutic development.

Accurate 3D organoids – a miniature, simplified versions of organs grown *in vitro* from stem cells, first appeared in scientific literature in the early 2000s, with a significant breakthrough in 2009. Up until then, researchers had been experimenting with 3D cultures of stem cells and tissue fragments for decades. The history of organoids dates back to 1907, with Wilson *et al* successful *in vitro* cultivation of dissociated sponge cells (Corrò, Novellasdemunt, and Li 2020). From then on, the approaches to such new endeavors concentrated on the isolation and reorganization of cells (Yang et al., 2023). In 2006–2007, advances in stem cell biology, including the development of induced iPSCs), laid the groundwork. Clevers' lab in the Netherlands reports that iPSCs grow intestinal organoids from a

single Lgr5+ adult stem cell (Sato *et al*, 2009). This was the first well-defined 3D organoid derived from adult stem cells that mimicked the structure and function of a real organ. This pivotal study paved the way for a wave of organoid research throughout the 2010s, resulting in the development of organoids for various organs, including the brain, liver, kidney, lung, pancreas, and others (Yang *et al*, 2023). These models have since become essential tools for studying human development, disease mechanisms, drug responses, and potential regenerative therapies.

Besides organoids, 3D cell culture systems encompass a range of techniques and methods used in research to replicate and mimic the *in vivo* microenvironment as physiologically relevant as possible. This includes hydrogel-based scaffolds (Rivero et al., 2020; Mavil-Guerrero et al., 2025), with a focus on cell proliferation, maturation, and the long-term physicochemical and mechanical stability of the systems. The common denominator is the development and evaluation of biomaterials suitable for supporting 3D culture environments in tissue-engineering applications. Besides this, a highly controlled systems that support the co-cultivation of cells are bioreactors. A bioreactor that mechanically guided 3D mesenchymal stem cell chondrogenesis using a novel thermo-reversible methylcellulose-based hydrogel (Cochis et al. 2017) was reported for potential use in cartilage repair. The novel systems focused on developing a 3D-printed perfusion bioreactor (3D-PBR) to facilitate the *in situ* growth and differentiation of human bone marrow (BM)-derived mesenchymal stem cells (MSCs), enabling co-culture with vascular cells (Jun et al. 2025). Organ-on-chip (OoC) systems are also intensely studied (Leung et al., 2022), particularly those that enable high-throughput screening and multifunctional organ representation (Lee et al., 2022).

To conclude, compared to 2D cell culture systems, 3D systems provide essential insights into cell-cell interaction, cell polarity, matrix interactions, and nutrient access, surpassing the uniformity, simplicity and cost encountered by 2D cell models (Fig. 1).

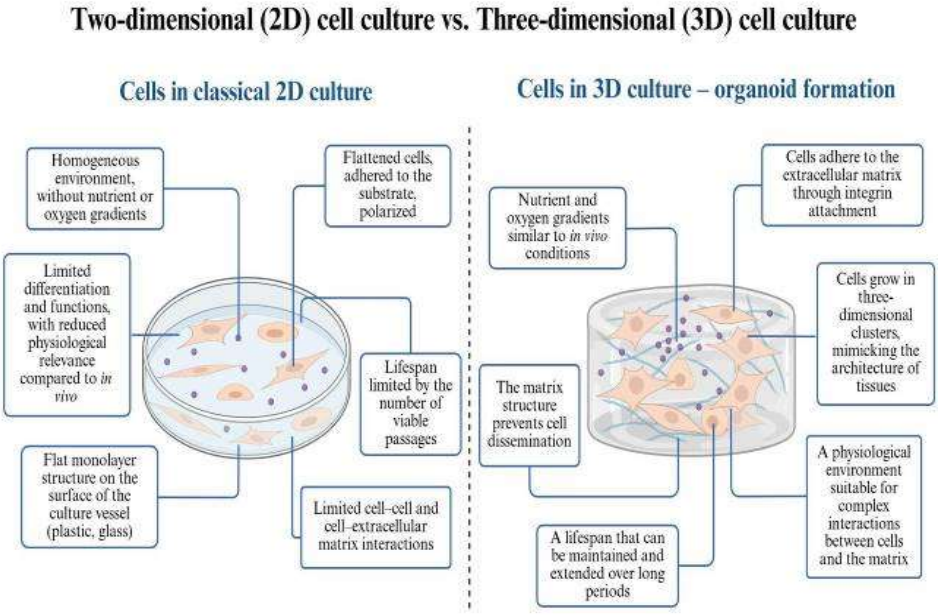

Fig. 1. Key structural, morphological, and physiological differences between 2D and 3D systems

3D Organoids in biomedical applications and drug discovery

Advantages in comparison with 2D models

3D cell cultures, including organoids, offer numerous advantages over conventional 2D models. Organoids have been shown to more accurately replicate the spatial architecture, cellular composition, and physiological functions of human organs while preserving the genetic and phenotypic heterogeneity of the tissue of origin (Cristobal et al., 2017). These 3D structures facilitate more realistic cell-cell interactions, thereby reflecting *in vivo* conditions with greater accuracy compared to 2D cultures (Lee et al., 2018).

Furthermore, 3D cultures exhibit augmented proliferative capacity, enhanced visualization, and facilitate high-throughput drug screening (Cristobal et al., 2017). These cultures are expedient, economical, and entail reduced ethical implications compared to animal models (Cruz et al., 2017). Due to these characteristics, organoids offer a superior platform for testing the efficacy and toxicity of therapeutics, as well as for developing personalized treatments in precision medicine (Pauli et al., 2017).

Specialized organoid approaches for skin

Skin organoids, like any other type of organoid, are created by using pluripotent stem cells or primary cells, and they have emerged as transformative tools in dermatological and developmental research. These three-dimensional structures replicate certain features of the skin, including the stratified epidermis, dermis, and appendages such as hair follicles and sebaceous glands. The development of skin organoids presents promising avenues for studying skin development, modeling diseases, testing drugs, and regenerative therapies.

Depending on the research purpose, various strategies are employed to cultivate skin organoids in a dish. Bioengineering and bioprinting are primarily intended to ensure the correct placement of keratinocytes, fibroblasts, melanocytes, and stem cells in architectures that mimic the native stratified epidermis, dermis, and hypodermis. This technique enhances the structural fidelity of organoids, enabling the spatial patterning necessary for appendage development, including hair follicles and sebaceous glands. This approach yields reproducible skin constructs with enhanced structural fidelity, facilitating both translational grafting applications and drug screening (Lee et al., 2020; Hong et al., 2023).

However, the aforementioned skin organoids lack a specific feature of the primary tissue, that being vascularization. The skin relies on a constant supply of nutrients and oxygen, which are delivered through the blood vessels. A significant limitation of conventional skin organoids is the lack of vascularization, which restricts long-term culture and maturation. The recent integration of microfluidic systems, such as skin-on-a-chip platforms, enables the creation of vascularized organoids with perfusable channels that support angiogenesis and immune cell interactions. These systems enable more accurate modeling of inflammatory conditions, transdermal delivery, and wound healing, and they offer an ethically superior alternative to animal testing (Abaci et al., 2016; Derman et al., 2024).

Recently, the development of single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics has revolutionized our understanding of cellular heterogeneity and developmental trajectories within skin organoids. These tools enable researchers to map epidermal differentiation, melanocyte maturation, and appendage-specific signaling gradients, such as Wnt, BMP, and SHH (Ramovs et al., 2022; Stabel et al., 2023). This has been particularly valuable in assessing how closely organoids replicate fetal or adult human skin at the transcriptomic level.

Skin organoids have the great advantage of being able to form from patient-derived cells, and can be reprogrammed and induced into pluripotent stem cells (iPSCs). In this way, disease models can be created *in vitro* for molecular mechanistic research and testing of treatments. For example, CRISPR-Cas9 technology enables the precise genetic manipulation of skin organoids derived

from patient-specific induced pluripotent stem cells (iPSCs). This allows for the correction of monogenic disorders such as epidermolysis bullosa (COL7A1) or Netherton syndrome (SPINK5), the creation of disease models, and the insertion of fluorescent reporters for real-time tracking of differentiation and cell fate (Jacków et al., 2019; Nasrallah et al, 2024). Such models are now being explored in preclinical gene therapy pipelines and personalized dermatology.

Lately, skin organoid research has advanced by introducing the co-culturing of other cell types to add complexity to the model, because the skin is deeply intertwined with both the immune and nervous systems. Recent studies have integrated Langerhans cells, T cells, and nociceptors into skin organoids to investigate neuroimmune interactions, which are relevant in conditions such as atopic dermatitis and psoriasis. This co-culture approach provides mechanistic insights into pain, itch, and inflammation, moving beyond structural mimicry to functional disease modeling (Tang et al., 2024; Quilez et al, 2024).

Another breakthrough in organoid applications is the use of machine learning (ML) and AI tools for handling the variability and complexity in skin organoid data. Deep learning systems can quantify epidermal thickness, stratification, pigmentation, and even predict differentiation success based on imaging or transcriptomic profiles (Wang et al, 2024). These tools are also increasingly used in high-throughput screening setups to classify organoid morphology and response to drugs or cosmetic compounds.

Besides using skin organoids for drug testing or genetic manipulation, one other important application is studying the development and self-organization of the skin. Breakthroughs in differentiation protocols have enabled the self-organization of appendages such as hair follicles, sebaceous glands, and even pigment-producing units within skin organoids. In a pioneering study, Lee et al. (2020) demonstrated that pluripotent stem cells could give rise to hair-bearing skin with innervation and vascular integration.

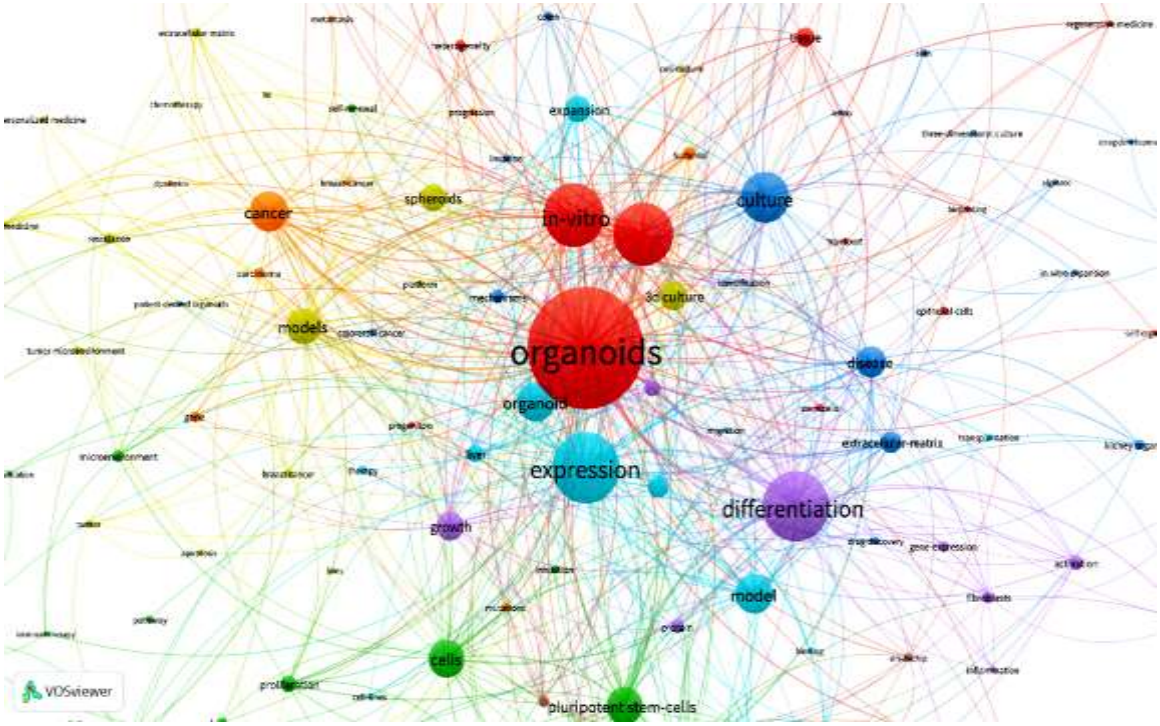

Fig. 2. VOSViewer Map representation of the keyword co-occurrence in the top-cited articles from Web of Science. Made by analyzing the bibliographic data of the 369 resulting articles, after applying advanced data searching of 2D cell culture, 3D cell culture, and organoids. The most occurring keyword in the most relevant papers in the field is organoids, which is a telling fact on the importance of this subject for further research.

These models hold promise not only for cosmetic testing but also for regenerative transplantation in patients with burns or wounds. Skin organoids have also been successfully adapted to model infectious diseases, such as EV-A71, Mpox, and Staphylococcus aureus, allowing for the real-time study of viral entry, immune response, and antimicrobial efficacy (Li et al., 2023; Li et al., 2025; Xie et al., 2023). Additionally, their use in skin aging studies has illuminated pathways involved in collagen degradation, senescence, and barrier dysfunction, providing platforms for anti-aging drug discovery (Wang et al., 2024).

Emerging technologies in skin organoids research

Recent advances in tissue engineering, imaging, and genomics are propelling the development of increasingly sophisticated skin organoid models. These emerging technologies aim to enhance physiological relevance, reproducibility, and clinical applicability by overcoming limitations such as poor vascularization, lack of immune components, or insufficient maturation. Below, we explore some of the most promising directions.

Vascularized Skin Organoids Using Organoid-on-a-Chip Platforms

The integration of skin organoids with microfluidic organ-on-a-chip platforms is enabling the development of perfusable, vascularized skin models. By incorporating endothelial cells and simulating flow dynamics, these platforms enable more accurate modeling of dermal capillary beds and nutrient diffusion, which is essential for long-term culture and tissue viability (Derman et al., 2025). Techniques include co-culturing with iPSC-derived endothelial cells and using microfabricated channels that mimic vascular geometry (Hong et al., 2023). These approaches have shown promise in creating stratified, vascularized skin layers that closely mimic in vivo histology (Hong et al, 2023).

3D and 4D Bioprinting for Architectural Precision and Dynamic Remodeling

3D bioprinting has enabled the layer-by-layer deposition of multiple skin cell types (e.g., keratinocytes, fibroblasts, melanocytes) in biomimetic patterns using ECM-mimicking hydrogels. Advanced techniques, such as 4D bioprinting, incorporate time as a dimension, enabling constructs to undergo programmed shape changes or self-assembly in response to environmental cues, including pH or temperature. This is particularly useful for simulating wound healing dynamics or scar formation, as well as for producing large-scale skin grafts with embedded vasculature and appendages, such as sweat glands (Zhang et al., 2024).

Multi-Omics and Spatial Transcriptomics Integration

Single-cell and spatial transcriptomics technologies are now routinely applied to skin organoids to profile differentiation dynamics, epidermal stratification, and dermal-epidermal crosstalk. For example, transcriptomic analysis has helped identify basal progenitor cell zones and melanocyte stem cell niches within skin organoids (Lee et al., 2020; Hong et al, 2023). When combined with proteomics and metabolomics, these tools enable high-resolution mapping of pathways involved in barrier function, immune signaling, and aging.

CRISPR-Based Functional Genomics and Disease Modeling

The use of CRISPR/Cas9 in skin organoids enables the precise modeling of genetic skin diseases, such as epidermolysis bullosa or psoriasis, by introducing disease-relevant mutations in keratin genes or cytokine pathways (Jacków et al., 2019). Gene editing is also employed for lineage tracing, fluorescent reporter tagging, and rescuing disease phenotypes using isogenic control lines, making skin organoids powerful tools for therapeutic screening and personalized medicin.

Immune-Competent Skin Organoids

One major limitation of conventional organoid models is the absence of immune cells. Recent strategies now integrate Langerhans cells, T cells, and macrophages into skin organoid cultures, either through co-differentiation or transplantation of immune precursors. This is particularly relevant for studying inflammatory skin diseases (e.g., atopic dermatitis), tumor-immune interactions in melanoma, and responses to immunotherapies (Jung et al., 2022; Lee et al., 2021). Incorporating immune cells has also improved the modeling of pathogen-host interactions, such as viral entry through skin epithelia.

AI and Machine Learning for Skin Organoid Analysis and Design

Artificial intelligence (AI) is being employed to analyze high-content imaging data from skin organoid cultures. Convolutional neural networks (CNNs) can classify morphological phenotypes (e.g., layer thickness, pigmentation) and predict outcomes of differentiation protocols (Bai et al., 2024). Generative AI tools are also being developed to design optimal bioprinting patterns or ECM compositions for improved organoid self-organization, accelerating hypothesis generation and protocol optimization.

Ex vivo models used in toxicity testing and drug screening

Ex vivo cellular models, either derived from explants, engineered from spheroids, and monocultures, or OoCs, are currently reported as preferred options for toxicity testing and drug screening applications. In the case of epidermal and transdermal evaluations, whether for drug discovery or testing, we benefit from several *in-house* and commercially available skin-mimicking models. Among the first reported was the reconstructed human epidermis (RHE) (Rosdy and Clauss 1990), adopted by OECD guidelines as a reliable test method for *in vitro* skin irritation (TG 439) in 2010, through the European Centre for the Validation of Alternative Methods (ECVAM) validation study (Alépée et al. 2010).

Recent models report a medium-throughput drug-screening platform (METPlatform) based on organotypic cultures. This platform was used specifically to evaluate inhibitors against metastases growing *in situ* (Zhu et al., 2022), for example. With this approach, authors focus on brain metastasis, displaying how ex vivo organotypic cultures can bridge the gap between *in vitro* and *in vivo* models, providing a more physiologically relevant environment for drug screening and identifying novel therapeutic targets.

Key breakthroughs reported include the already well-researched bio-printing techniques (Armbrecht and Dittrich 2017; Xiang et al 2022), OoC, gene editing with the CRISPR-Cas9 system (Sun et al. 2023), advanced imaging techniques,

synthetic biomaterials, and their role, application, and perspectives in the furtherance of biomedical research and medication development (Dave et al. 2025).

However, scientific progress must be supported by adequate legislation. Therefore, the implementation of *ex vivo* tools into drug testing legislation is evolving, driven by the desire to improve the predictivity of preclinical studies, reduce reliance on animal testing (the 3Rs: Replace, Reduce, Refine), and accelerate the drug development process. The first progress towards this goal was made by the FDA Modernization Act 2.0 (Han 2023), which permits the utilization of alternative testing platforms, including cell-based assays (like human induced pluripotent stem cells - iPSCs), organoids, OoCs, and advanced artificial intelligence (AI) methods, to assess drug safety and effectiveness during the preclinical phase. This represents a significant shift away from the previous requirement for mandatory animal testing of all drugs before human trials. European Medicines Agency (EMA) has been actively encouraging the incorporation of alternative methods, including ex vivo models, by supporting institutes and researchers with targeted funds for developing such tools. In one of their latest reports, EMA focuses on the regulatory acceptance and implementation of **New Approach Methodologies (NAMs)** in the development of human medicines (European Medicines Agency (EMA) 2025). It provides an overview of current trends, ongoing initiatives, and challenges/opportunities from a regulatory perspective, offering recommendations to facilitate the integration of these innovative methods (which include ex vivo models) and reduce reliance on animal testing (the 3Rs principles).

Challenges and Perspective

Despite the significant progress made in the development and application of 3D skin organoid models, several challenges continue to limit their full integration into mainstream drug discovery and disease modeling pipelines. These obstacles span technical, biological, and regulatory domains, often hindering reproducibility, scalability, and clinical translation (Dave et al. 2025). At the same time, emerging technologies and innovative approaches are actively addressing these limitations, paving the way for more robust, physiologically relevant, and ethically sound models. The following table provides an overview of the main challenges associated with 3D organoids, alongside current perspectives and potential solutions aimed at overcoming these hurdles (Table 1).

Table 1. Overview of the challenges and perspectives reported in the development and application of 3D skin organoid models

Challenges	Perspectives / Solutions
Complex handling and growth variability of 3D organoids	Development of standardized protocols and automation tools to improve reproducibility and scalability (Lee et al. 2024; "CellXpress.Ai Automated Cell Culture System | Molecular Devices" n.d.)
Limited vascularization impairs nutrient delivery and maturation	Integration of vascularized organoid-on-a-chip platforms to simulate perfusion and support tissue viability (Quintard et al. 2024)
Absence of immune components in traditional skin organoids	Co-culturing with immune cells (e.g., Langerhans, T cells, macrophages) for better modeling of inflammation and immune response (Van Os, Engelhardt, and Guenat 2023; Moon, Kim, and Shin 2021)
Structural and functional limitations	3D and 4D bioprinting technologies enabling precise architecture and dynamic remodeling of skin organoids (Zhang et al. 2024; Kennedy et al. 2025; Phang et al. 2022)
Limited insight into cellular heterogeneity and signaling	Use of multi-omics approaches and spatial transcriptomics for mapping differentiation and functional pathways (Li et al. 2025)
Genetic disease modeling constraints	CRISPR-Cas9 for creating skin disease-specific mutations, functional genomics, and personalized therapeutic testing (Wang et al. 2023; Abdelnour et al. 2021; Rossi et al. 2024)
Variability in morphology and drug response	Application of AI/ML to classify skin morphology, predict outcomes, and design optimal culture conditions (Ahammed, Mamun, and Uddin 2022; Abbas et al. 2025)
Legislative and regulatory barriers	Adoption of the FDA Modernization Act 2.0 and EMA support for New Approach Methodologies (NAMs) (Van Norman 2019; European Medicines Agency (EMA) 2025)

Conclusions

2D cell culture systems, although still used and considered relevant for some assays, have been surpassed in complexity, biological relevance, mimicry, and *in vivo* comparability by 3D systems.

Skin organoids represent a transformative platform in dermatological and developmental research, leveraging pluripotent stem cells and highly interdisciplinary technologies, such as bioengineering, microfluidics, and CRISPR-Cas9, to recapitulate skin structure and function. Innovations such as vascularized OoC systems, 3D/4D bioprinting, single-cell RNA sequencing, and AI-driven analysis have overcome limitations like poor vascularization and lack of immune components, enhancing physiological relevance. These models enable precise disease modeling, drug screening, and regenerative therapies for conditions like epidermolysis bullosa, atopic dermatitis, and skin aging, while also advancing our understanding of skin development and pathogen interactions. As these technologies evolve, skin organoids hold immense potential for personalized medicine and ethical alternatives to animal testing, paving the way for clinical applications and novel therapeutic strategies.

As seen, 3D systems are reported in numerous forms, from scaffolds to explants, ex *vivo* reconstructs, spheroids, or OoC. Their use is benefiting from growing legislative support, with organizations such as the FDA and EMA implementing new methodologies to integrate alternative testing platforms.

Recent advances in high-throughput platforms have enabled the study of multi-organ systems, providing unprecedented insights into the molecular effects of new drugs and a rapid pathway for laboratory-to-clinical studies.

Authors' contributions: ER – writing the original draft, methodology, data analysis; L-EA – writing the original draft, software, data analysis; A-MZ – conceptualization, writing the original draft, formal analysis; GGP – writing - review & editing; YZ – writing - review & editing CI– writing - review & editing, supervision; MN - writing - review & editing, formal analysis; B-MT – conceptualization, writing the original draft, writing - review & editing, supervision.

Acknowledgment

We acknowledge the European Union's Horizon Europe Research and Innovation framework programme 2021–2027, under the Coordination and Support Actions, HORIZON-WIDERA-2022-TALENTS-01 (grant agreement - 101087007 – eBio-hub). Funded by the European Union. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them**.** The authors also acknowledge the support from the grant of the Romanian Ministry of Research, Innovation and Digitalization, CCCDI–UEFISCDI, PN-IV-P8-8.1-PRE-HE-ORG-2023-0054- Contract no. 17PHE/2023- Contract no 17PHE/2023 and the support of the Academy of Romanian Scientists through the project "*Skin-on-chip platform for in vitro testing of nanoparticles*", Contract no. 54/ 17.05.2024.

REFERENCES

[1]. Abaci, H. E., Guo, Z., Coffman, A., Gillette, B., Lee, W. H., Sia, S. K. & Christiano, A. M., 2016. Human skin constructs with spatially controlled vasculature using primary and iPSC-derived endothelial cells. *Advanced Healthcare Materials*, 5(14), pp. 1800–1807. doi:10.1002/adhm.201500936.

[2]. Abbas, Sagheer, Fahad Ahmed, Wasim Ahmad Khan, Munir Ahmad, Muhammad Adnan Khan, and Taher M. Ghazal. 2025. "Intelligent Skin Disease Prediction System Using Transfer Learning and Explainable Artificial Intelligence." *Scientific Reports 2025 15:1* 15 (1): 1–13. https://doi.org/10.1038/s41598-024-83966-4.Abdelnour, Sameh A., Long Xie, Abdallah A. Hassanin, Erwei Zuo, and Yangqing Lu. 2021. "The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Inherited Diseases." *Frontiers in Cell and Developmental Biology* 9 (December): 699597. https://doi.org/10.3389/FCELL.2021.699597.

[3]. Ahammed, Mostafiz, Md Al Mamun, and Mohammad Shorif Uddin. 2022. "A Machine Learning Approach for Skin Disease Detection and Classification Using Image Segmentation." *Healthcare Analytics* 2 (November): 100122. https://doi.org/10.1016/J.HEALTH.2022.100122.

[4]. Alépée, N., Tornier, C., Robert, C., Amsellem, C., Roux, M. H., Doucet, O., Pachot, J., Méloni, M. & de Brugerolle de Fraissinette, A., 2010. A catch-up validation study on reconstructed human epidermis (SkinEthicTM RHE) for full replacement of the Draize skin irritation test. *Toxicology in Vitro*, 24(1), pp. 257–266. doi:10.1016/j.tiv.2009.08.024.

[5]. Armbrecht, L. & Dittrich, P. S., 2017. Recent advances in the analysis of single cells. *Analytical Chemistry*, 89(1), pp. 2–21. doi:10.1021/acs.analchem.6b04255.

[6]. Bai, L., Wu, Y., Li, G., Zhang, W., Zhang, H. & Su, J., 2024. AI-enabled organoids: Construction, analysis, and application. *Bioactive Materials*, 31, pp. 525–548. doi:10.1016/j.bioactmat.2023.09.005.

[7]. Ballav, S., Deshmukh, A. J., Siddiqui, S., Aich, J. & Basu, S., 2021. Two-dimensional and three-dimensional cell culture and their applications. In: *IntechOpen*. doi:10.5772/intechopen.100382.

[8]. "CellXpress.Ai Automated Cell Culture System | Molecular Devices." n.d. Accessed July 24, 2025. https://www.moleculardevices.com/products/3d-biology/cellxpress-ai-automated-cell-culture-system#overview.

[9]. Choudhury D, Mo X, Iliescu C, Tan LL, Tong WH, Yu H. Exploitation of physical and chemical constraints for three-dimensional microtissue construction in microfluidics. Biomicrofluidics. 2011 Jun;5(2):22203. doi: 10.1063/1.3593407. Epub 2011 Jun 29. PMID: 21799710; PMCID: PMC3145229.

[10]. Cochis, A., Grad, S., Stoddart, M. J., Farè, S., Altomare, L., Azzimonti, B., Alini, M. & Rimondini, L., 2017. Bioreactor mechanically guided 3D mesenchymal stem cell chondrogenesis using a biocompatible novel thermo-reversible methylcellulose-based hydrogel. *Scientific Reports*, 7, p. 45018. doi:10.1038/srep45018.

[11]. Corrò, C., Novellasdemunt, L. & Li, V. S. W., 2020. A brief history of organoids. *American Journal of Physiology - Cell Physiology*, 319(1), pp. C151–C165. doi:10.1152/ajpcell.00120.2020.

[12]. Dave, R., Pandey, K., Patel, R., Gour, N. & Bhatia, D., 2025. Leveraging 3D cell culture and AI technologies for next-generation drug discovery. *Cell Biomaterials*, 1(3), p. 100050. doi:10.1016/j.celbio.2025.100050.

[13]. Dave, Raj, Kshipra Pandey, Ritu Patel, Nidhi Gour, and Dhiraj Bhatia. 2025. "Leveraging 3D Cell Culture and AI Technologies for Next-Generation Drug Discovery." *Cell*

Biomaterials 1 (3): 100050. https://doi.org/10.1016/J.CELBIO.2025.100050.

[14]. Derman, I. D., Rivera, T., Garriga Cerda, L., Singh, Y. P., Saini, S., Abaci, H. E., & Ozbolat, I. T. (2024). Advancements in 3D skin bioprinting: Processes, bioinks, applications and sensor integration. *International Journal of Extreme Manufacturing*, 7(1), 012009. https://doi.org/10.1088/2631-7990/ad878c

[15]. European Medicines Agency (EMA), 2025. New approach methodologies (NAMs). [Online] Available at: https://www.ema.europa.eu/en/committees/working-parties-other-groups/eu-innovation-network-eu.

[16]. Ghiță-Răileanu, M., Tihăuan, B. M., Lixandru-Petre, I. O., Gheorghiu, G. L., Gradisteanu-Pircalabioru, G., Cioca, G., Iliescu, F. S., Iliescu, C., 2025. Skin-on-chip: Quo vadis? APL Bioengineering, 9(4), 041502. doi:10.1063/5.0268706.

[17]. Han, J. J., 2023. FDA Modernization Act 2.0 allows for alternatives to animal testing. *Artificial Organs*, 47(3), pp. 449–450. doi:10.1111/aor.14503.

[18]. Hong, ZX., *et al.* Bioengineered skin organoids: from development to applications. *Military Med Res* 10, 40 (2023). https://doi.org/10.1186/s40779-023-00475-7

[19]. Jacków, J., Titeux, M., Portier, S., Charbonnier, F., Kieffer, Y., Lazou, J., ... Hovnanian, A. (2019). Gene-corrected fibroblast therapy for recessive dystrophic epidermolysis bullosa using a self-inactivating lentiviral vector. *Journal of Investigative Dermatology*, 139(5), 977–987. https://doi.org/10.1016/j.jid.2016.02.811

[20]. Jun, B. H., Torrez, J. E., Ross, D. J., Patterson, B. M., Ishak, M. O., Rodriguez, A. M., Harris, J. F. & Davis-Anderson, K. L., 2025. Fabrication of a novel 3D-printed perfusion bioreactor for complex cell culture models. *Scientific Reports*, 15(1), pp. 1–14. doi:10.1038/s41598-025-94093-z.

[21]. Kennedy, Senthil Maharaj, M. Sekar, A. Vasanthanathan, and K. Amudhan. 2025. "4D Bioprinting for Personalized Medicine, Innovations in Implant Fabrication and Regenerative Therapies." *Polymer-Plastics Technology and Materials*, August. https://doi.org/10.1080/25740881.2025.2483763.

[22]. Lee, J., Rabbani, C. C., Gao, H., Steinhart, M. R., Woodruff, B. M., Pflum, Z. E., ... Koehler, K. R. (2020). Hair-bearing human skin generated entirely from pluripotent stem cells. *Nature*, 582(7812), 399–404. https://doi.org/10.1038/s41586-020-2352-3

[23]. Lee, Hana, Seunghye Yang, Kyung Jin Lee, Si Na Kim, Ji Seon Jeong, Ki Young Kim, Cho Rok Jung, et al. 2024. "Standardization and Quality Assessment for Human Intestinal Organoids." *Frontiers in Cell and Developmental Biology* 12 (September): 1383893. https://doi.org/10.3389/FCELL.2024.1383893/BIBTEX.

[24]. Lee, S. R., Kim, Y., Kim, S., Kim, J., Park, S., Rhee, S., Park, D. & others, 2022. U-IMPACT: A universal 3D microfluidic cell culture platform. *Microsystems and Nanoengineering*, 8(1), pp. 1–12. doi:10.1038/s41378-022-00431-w.

[25]. Li, J., Ma, J., Cao, R. *et al.* A skin organoid-based infection platform identifies an inhibitor specific for HFMD. *Nat Commun* 16, 2513 (2025). https://doi.org/10.1038/s41467-025-57610-2

[26]. Li, P., Pachis, S.T., Xu, G. *et al.* Mpox virus infection and drug treatment modelled in human skin organoids. *Nat Microbiol* 8, 2067–2079 (2023). https://doi.org/10.1038/s41564-023-01489-6

[27]. Li, Ying, Guangchang Cai, Fuqun Chen, Kepei Wen, and Le Ou-Yang. 2025. "Unveiling Spatial Domains from Spatial Multi-Omics Data Using Dual-Graph Regularized Ensemble Learning." *Communications Biology* 8 (1): 1–14. https://doi.org/10.1038/S42003-025-08372-6;SUBJMETA=114,1305,2401,631;KWRD=DATA+INTEGRATION,MACHINE+LEARNING.

[28]. Leung, C. M., de Haan, P., Ronaldson-Bouchard, K., Kim, G. A., Ko, J., Rho, H. S., Chen, Z. & others, 2022. A guide to the organ-on-a-chip. *Nature Reviews Methods Primers*, 2(1), pp. 1–29. doi:10.1038/s43586-022-00118-6.

[29]. Mavil-Guerrero, E., Romo-Herrera, J. M., Quiñonez-Angulo, P., Flores-Ruiz, F. J., Morales-Narváez, E., Soltero, J. F. A., Juarez-Moreno, K., 2025. Enhanced cell proliferation and maturation using carboxylated bacterial nanocellulose scaffolds for 3D cell culture. *ACS Applied Materials and Interfaces*, 17(11), pp. 16632–16643. doi:10.1021/acsami.4c22475.

[30]. Moon, Sujin, Dong Hyun Kim, and Jung U. Shin. 2021. "In Vitro Models Mimicking Immune Response in the Skin." *Yonsei Medical Journal* 62 (11): 969. https://doi.org/10.3349/YMJ.2021.62.11.969.

[31]. Nasrallah, A., Rezvani, HR., Kobaisi, F. et al. Generation and characterization of CRISPR-Cas9-mediated XPC gene knockout in human skin cells. *Sci Rep* 14, 30879 (2024). https://doi.org/10.1038/s41598-024-81675-6

[32]. Norman, Gail A. Van. 2019. "Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is It Time to Rethink Our Current Approach?" *JACC: Basic to Translational Science* 4 (7): 845. https://doi.org/10.1016/J.JACBTS.2019.10.008.

[33]. Os, Lisette Van, Britta Engelhardt, and Olivier T. Guenat. 2023. "Integration of Immune Cells in Organs-on-Chips: A Tutorial." *Frontiers in Bioengineering and Biotechnology* 11 (June): 1191104. https://doi.org/10.3389/FBIOE.2023.1191104/XML.

[34]. Phang, Shou Jin, Soumyadeep Basak, Huey Xhin Teh, Gopinath Packirisamy, Mh Busra Fauzi, Umah Rani Kuppusamy, Yun Ping Neo, and Mee Lee Looi. 2022. "Advancements in Extracellular Matrix-Based Biomaterials and Biofabrication of 3D Organotypic Skin Models." *ACS Biomaterials Science and Engineering* 8 (8): 3220–41. https://doi.org/10.1021/ACSBIOMATERIALS.2C00342/ASSET/IMAGES/MEDIUM/AB2C00342_0008.GIF.Quintard, Clément, Emily Tubbs, Gustav Jonsson, Jie Jiao, Jun Wang, Nicolas Werschler, Camille Laporte, et al. 2024. "A Microfluidic Platform Integrating Functional Vascularized Organoids-on-Chip." *Nature Communications 2024 15:1* 15 (1): 1–16. https://doi.org/10.1038/s41467-024-45710-4.

[35]. Quintard, Clément, Emily Tubbs, Gustav Jonsson, Jie Jiao, Jun Wang, Nicolas Werschler, Camille Laporte, et al. 2024. "A Microfluidic Platform Integrating Functional Vascularized Organoids-on-Chip." *Nature Communications 2024 15:1* 15 (1): 1–16. https://doi.org/10.1038/s41467-024-45710-4.

[36]. Ramovs, V., Janssen, H., Fuentes, I., Pitaval, A., Rachidi, W., de Sousa Lopes, S. M. C., ... Raymond, K. (2022). Characterization of the epidermal-dermal junction in hiPSC-derived skin organoids. *Stem Cell Reports*, 17(6), 1279–1288. https://doi.org/10.1016/j.stemcr.2022.04.008

[37]. Rivero, R. E., Capella, V., Liaudat, A. C., Bosch, P., Barbero, C. A., Rodríguez, N. & Rivarola, C. R., 2020. Mechanical and physicochemical behavior of a 3D hydrogel scaffold during cell growth and proliferation. *RSC Advances*, 10(10), pp. 5827–5837. doi:10.1039/c9ra08162c.

[38]. Rosdy, M. & Clauss, L. C., 1990. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface. *Journal of Investigative Dermatology*, 95(4), pp. 409–414. doi:10.1111/1523-1747.ep12555510.

[39]. Rossi, Stefano, Ellie Louise Richards, Gisela Orozco, and Stephen Eyre. 2024. "Functional Genomics in Psoriasis." *International Journal of Molecular Sciences* 25 (13): 7349. https://doi.org/10.3390/IJMS25137349.

[40]. Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., van Es, J. H. & others, 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*, 459(7244), pp. 262–265. doi:10.1038/nature07935.

[41]. Sun, L., Bian, F., Xu, D., Luo, Y., Wang, Y. & Zhao, Y., 2023. Tailoring biomaterials for biomimetic organs-on-chips. *Materials Horizons*, 10(11), pp. 4724–4745. doi:10.1039/d3mh00755c.

[42]. Tang, C. T., Lim, C. Y., & Lay, K. (2024). Modelling human hair follicles—Lessons from animal models and beyond. *Biology*, 13(5), 312. https://doi.org/10.3390/biology13050312

[43]. Tong WH, Fang Y, Yan J, Hong X, Hari Singh N, Wang SR, Nugraha B, Xia L, Fong ELS, Iliescu C, Yu H. Constrained spheroids for prolonged hepatocyte culture. Biomaterials. 2016 Feb;80:106-120. doi: 10.1016/j.biomaterials.2015.11.036. Epub 2015 Dec 3. PMID: 26708088.

[44]. Wang, X.-Y., Jia, Q.-N., Li, J., & Zheng, H.-Y. (2024). Organoids as Tools for Investigating Skin Aging: Mechanisms, Applications, and Insights. *Biomolecules*, 14(11), 1436. https://doi.org/10.3390/biom14111436

[45]. Wang, Xianqing, Xi Wang, Yinghao Li, Sigen A, Bei Qiu, Albina Bushmalyova, Zhonglei He, Wenxin Wang, and Irene Lara-Sáez. 2023. "CRISPR-Cas9-Based Non-Viral Gene Editing Therapy for Topical Treatment of Recessive Dystrophic Epidermolysis Bullosa." *Molecular Therapy Methods and Clinical Development* 31 (December). https://doi.org/10.1016/J.OMTM.2023.101134/ASSET/9ABC117D-0A84-46AF-AA54-D4F99C6F59FD/MAIN.ASSETS/GR5.JPG.

[46]. Xiang, Y., Miller, K., Guan, J., Kiratitanaporn, W., Tang, M. & Chen, S., 2022. 3D bioprinting of complex tissues in vitro: State-of-the-art and future perspectives. *Archives of Toxicology*, 96(3), pp. 691–710. doi:10.1007/s00204-021-03212-y.

[47]. Xie, X., Tong, X., Li, Z. et al. Use of mouse primary epidermal organoids for USA300 infection modeling and drug screening. *Cell Death Dis* 14, 15 (2023). https://doi.org/10.1038/s41419-022-05525-x

[48]. Yang, S., Hu, H., Kung, H., Zou, R., Dai, Y., Hu, Y., Wang, T., Lv, T., Yu, J. & Li, F., 2023. Organoids: The current status and biomedical applications. *MedComm*, 4(3), p. e274. doi:10.1002/mco2.274.

[49]. Yu, F., Deng, R., Hao Tong, W. *et al.* A perfusion incubator liver chip for 3D cell culture with application on chronic hepatotoxicity testing. *Sci Rep* **7**, 14528 (2017). https://doi.org/10.1038/s41598-017-13848-5

[50]. Yu F, Zhuo S, Qu Y, Choudhury D, Wang Z, Iliescu C, Yu H. On chip two-photon metabolic imaging for drug toxicity testing. Biomicrofluidics. 2017 May 11;11(3):034108. doi: 10.1063/1.4983615. PMID: 28529673; PMCID: PMC5426952.

[51]. Zhang, Tao, Shihao Sheng, Weihuang Cai, Huijian Yang, Jiameng Li, Luyu Niu, Wanzhuo Chen, et al. 2024. "3-D Bioprinted Human-Derived Skin Organoids Accelerate Full-Thickness Skin Defects Repair." *Bioactive Materials* 42 (December): 257–69. https://doi.org/10.1016/J.BIOACTMAT.2024.08.036.

[52]. Zhu, L., Retana, D., García-Gómez, P., Álvaro-Espinosa, L., Priego, N., Masmudi-Martín, M., Yebra, N. & others, 2022. A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Molecular Medicine*, 14(3), p. e14552. doi:10.15252/emmm.202114552.