1 Artificial Skin Models for Animal-Free Testing: 3D Skin Reconstruct Approach, a Journey in the Past Two Decades

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Abstract

Growing ethical concerns regarding the use of animals in research have managed to the creation of several alternative procedures based on the refinement, reduction, and replacement, which Russell and Burch initially introduced in 1959. After that, since 2013, as animal experimentation ethics have been supported in the European Union, artificial skin models have fascinated interest as an alternative to use of animal model for testing for establishing the efficacy and toxicity of products. In addition to concerns for animal welfare, using animals in experiments should be reduced and avoided. Research on cosmetics and the skin is particularly important to objections to animal testing. Due to numerous constraints, including the fact that human and animal skins have distinct immune systems and anatomical makeup, investigations on animals may not correctly anticipate results in people. This chapter's major goal is to provide an overview of the strategies for creating 3D skin models, along with their advantages and disadvantages, as well as how new methods can be used to create constructions that are truly physiologically accurate and useful for preclinical innovation. In vivo animal testing for evaluating efficacy and safety in the beauty in the field of pharmaceutical sectors can be replaced with artificial skin models that closely resemble human skin. The primary investigations on cell-to-cell interactions, cell-matrix interactions, tissue creation, and development can also benefit from using 3D skin construct models. An integrated application of these approaches would give insight into the minimum use of animals in scientific experiments.

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A. B. Pant et al. (eds.), *Skin 3-D Models and Cosmetics Toxicity*,
https://doi.org/10.1007/978-981-99-2804-0_1

Keywords

3Rs · Artificial skin · 3D skin construct · European Union · Cell–matrix interaction

1.1 Introduction

The preamble of sophisticated 3D skin model development that is now being used by Global cosmetic research began more than 80 years ago. In the USA, in 1933, "an eyebrow and lash dye, Lash Lure" was introduced to the market. At first, no one thought an innocuous cosmetic product could have acute and even lethal repercussions. A chemical paraphenylenediamine, which was present in the product, was a substance that has not been thoroughly studied and can have significant effects on the face, eyelids, and eyes (McCally et al. 1933), and the impact of that compound was very intense. After using the dye, more than a dozen women lost their eyesight, and one of them contracted a fatal bacterial infection. After that, the U.S. Food and Drug Administration (FDA) authority has taken over cosmetics testing. Also, in 1936 publication introduced by "American Chamber of Horrors: The Truth about Food and Drug," Lamb (1936) emphasized the many examples where consumer goods show any change in terms of injury or even death. Even in 1938, the Federal Food, Drug and Cosmetic Act was passed by the U.S. Congress, which requires stricter regulations for cosmetic products (U.S. Congress 1934). Since then, cosmetic testing has been a crucial component of product development due to the possibility of adverse health consequences that could be severe due to high and frequent exposure. Following these instances that prompted consumer protection, animal testing quickly became required under American law (Zurlo et al. 1994). Due to this, the Scientific Committee on Consumer Safety (SCCS) decided to publish ad continuously update the SCCS Notes of Guidelines for Testing of Cosmetic Ingredients and their Safety Assessment (Bernauer et al. 2019). Since then, pharmacological and cosmetic product screening for skin research has been frequently done on animals (such as mice and pigs). Hence, the initial animal rights movement was established when animal testing became necessary.

1.2 Approach Toward an Animal Alternative

To overcome the problems with animal research and steer clear of unethical practices, alternative models to animal testing have been offered. In 1959, Russell and Burch published the first description of the fundamentals of human experimental research (Tannenbaum and Bennett 2015), which are elegantly referred by Russell and Burch as follows:

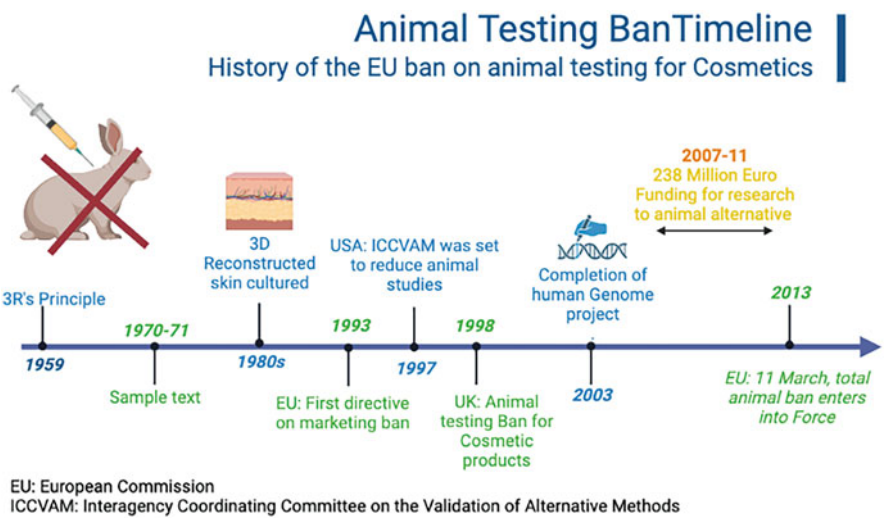

Fig. 1.1 EU timeline history for animal testing ban on cosmetics

First method is refinement, which involves the adoption of sophisticated techniques to prevent animal suffering or distress.

The second method is reduction, which involves employing methods to get similar amounts of data from fewer animals.

Third is substitute trials that do not include animals when possible. Three key factors for the development of non-animal testing emerged when the 3Rs were viewed from the current angle: ethical reasons, the absence of practical extrapolation, and economic considerations. When animal experimentation was eliminated, the search for human-relevant data at an affordable cost has been driven by the ethical consideration that they wanted to avoid (Silva and Tamburic 2022). The weight of ending animal testing has been placed on the cosmetics industry by animal welfare activism and public opinion, which has ultimately led to a fourth and crucial driver—animal testing restrictions. The result of these partnerships between researchers, NGOs, decision-makers, and the cosmetics business is the field of "New Approach Methodologies," which is constantly developing (NAMs).

Nonetheless, during the twentieth century, using animal testing models was common for determining the safety and effectiveness of novel medications or cosmetic elements (Semlin et al. 2011), yet because of growing worries about what happens to lab animals and because of ethical and scientific considerations (Ferdowsian and Gluck 2015), European legislators were compelled to strictly control and ultimately outlaw the use of animals in cosmetics testing. The laws governing animal testing have tightened up since the 1990s. A partial ban was enacted in the European Union (EU) in 1993 after the European Commission (EU) published the first legislation to restrict the use of animals in the cosmetic sector, whereas, in 1998 (Fig. 1.1), the UK became the first country in the world to completely outlaw cosmetics tested on animals, and 20 years later, in 2013, the EU

followed suit (CREDIT: European Commission). No animal testing on formulations or their ingredients is permitted as a result of the EU Cosmetics Regulation's stringent enforcement of the prohibition on animal testing and the concurrent marketing ban as of 2013 (Suhail et al. 2019; EC 2009). But, the biggest negatives were the high cost, time commitment, and, most critically, the painful process that animals must go through (Ahn et al. 2010; Cheluvappa et al. 2017). These issues were the primary driving force behind the ban on animal experimentation and the hunt for alternatives that may be used to evaluate the experimental endpoints. There are two different kinds of restrictions: I testing restrictions, which forbid the use of animals in the testing of cosmetics and cosmetic ingredients, and (ii) marketing restrictions, which forbid the sale of cosmetics and ingredients used in them in the EU. Numerous other nations have also issued bans. Nonetheless, it is still used in some nations' cosmetic markets, including those in the United States, where using animals in research is not outlawed.

Nevertheless, also its use is declining as consumers are becoming increasingly critical. These regulatory changes led to various alternatives for the replacement of in vivo animal tests, and together pharmaceutical and cosmetics companies started to find suitable skin models that could be used to access new formulations and other topical products (Jung et al. 2014; Catarino et al. 2018). The fundamental justification for seeking an animal-free option for skin research, aside from ethical considerations, is that animals do not exhibit the same physiological, structural, and biochemical behavior as human skin, which leads to high drug attrition rates in later phases of study. Organization for Economic Co-operation and Development (OECD) approved the toxicological testing strategies for skin sensitization, hazard assessment without animal testing. The first OECD Guide Line (GL) to include defined approaches (DAs) for skin sensitization study is (GL) No. 497, a new type of (non-test) guideline that employs combined data from non-animal methods to provide toxicological information for hazard and potency evaluations (EC 2002; OECD 2021a).

1.3 Merits/Demerits of Elective Methods

Replacement could be the best way to dodge animal torment and endure. Although it is hard to completely replace animal research, combining two or three in vivo procedures should be required as an elective method. Investigational projects are consistently applied with limited funds and time. Selecting elective non-animal experiments can uniquely spare both fetched and time. Besides, in a few cases, elective methods are way well suited than the test in animal since they permit higher quality tests to be required. Animal and human skins are endlessly distinctive regarding architecture and immune response; conjointly, animal skin often has a significantly lower life expectancy than human skin (Van Gele et al. 2011). Therefore, it is outlandish to know precisely what happens within the experimental animal body, and elective in vitro methodology can more obviously uncover the overall mechanisms involved. The comprehensive immune response of animal experiments

plays the most crucial role. Ironically, elective methods held back on this front, which is why animals are quiet often used for different dermatological assessment and research. Here is the development of other optional plans by focusing on the use of elective an animal alternative model for cosmetic research.

Biochemical methods, 2D and 3D cell cultures, genomics research, and the creation of in bioinformatics-based in silico simulations of skin models are some specific/other potential alternatives to animal models (Nakamura et al. 2018; Yun et al. 2018). Different methods, such as cell culture or artificial skin models, have been developed to produce and construct biological skin models that replicate the human skin's very complex and stratified structure. These models use synthetic or natural biomaterial-based scaffolds (Przekora 2020); even though they have numerous drawbacks, their careful and skillful handling makes these methods indispensable for futuristic dermatology research. EpiDerm® (MatTek Corporation, USA), EpiSkin® (L'Oreal, France), epiCS® (CellSystems, Germany), Holoderm®, and SkinEthic® (SkinEthics, France) were the most popular epidermis models based on the utilization of human skin cells (Mao et al. 2003; Whang et al. 2005; You et al. 2012). More recently, a few advanced skin models were commercialized, including NeoDerm® (Tego Science, Korea), Phenion® (Henkel, Germany), Genoskin Ex Vivo (USA) (summarized in Table 1.1). A collagen matrix comprising human fibroblasts and an epidermal overlay made of human keratinocytes serve as the foundation for sophisticated skin models (Ackermann et al. 2010; Kano et al. 2010). The representation of the full thickness of human skin's structural makeup is crucial in engineered skin models and the skin's cellular components. Complex human skin models with the proper cell compositions and matrix structure can be made using a number of techniques, such as electrospinning, three-dimensional (3D) bioprinting, and microfluidic systems (Kempf et al. 2011; Koch et al. 2012; Atac et al. 2013). A significant tool for understanding cell–cell, cell–matrix, and dermal–epithelial interactions in dermatology, as well as for assessing the safety of novel drug formulations or cosmetic elements, is the 3D engineered skin model (Suhail et al. 2019; Lee et al. 2014a, b).

1.4 Skin Equivalent: 3D Skin Model, a Valuable Alternative to Animal Tests

There has been an enormous increase in dermatology research using skin equivalents for fundamental and industrial research in the past several years and for clinical applications (Choudhury and Das 2021). Tissue-engineered human skin equivalents have been produced to regenerate the skin's main structural and functional behavior in vitro. These synthetically built skin substitutes, made up of epidermal and dermal layers, are known as skin equivalents (Dellambra et al. 2019). They are made from primary cells (keratinocytes, fibroblasts, and/or stem cells) and extracellular matrix (ECM) components in a manner that closely resembles natural skin, allowing for the study of their effect. They have been broadly utilized for skin homeostasis studies and its alterations and also for generating therapeutic tools, which can be used for

Table 1.1 Commercially available reconstructed skin models

Name of reconstructed skin model	Cells used for culture	Structures	OECD approved	Provider	Place
EpiSkin™	Keratinocytes	Cultured on a matrix	TG439, TG431	EpiSkin SNC	Lyon, France
EpiDerm™	Keratinocytes	Based on tissue culture inserts	TG431, TG439	MatTek	Ashland, MA, USA
SkinEthic™ RHE	Keratinocytes	Cultured on polycarbonate filter	TG431, TG439		EPISKIN Labs, France
epiCS™	Keratinocytes	Cultured on tissue culture inserts	TG431	Cell Systems	Troisdorf, Germany
SkinEthic™ RHPE	Keratinocytes Melanocytes	Pigmented epidermis			
T-Skin™	Keratinocytes Fibroblasts	Full-thickness model			
EpiDerm™ FT	Keratinocytes Fibroblasts	Full-thickness model			
MelanoDerm™	Keratinocytes Melanocytes	Pigmented epidermis			
epiCS™-M	Keratinocytes Melanocytes	Pigmented epidermis			
Ex Vivo Skin	From surgical discard	Full-thickness model			
Naïve Ex Vivo Skin	Donated surgical skin	Full-thickness model		Genoskin, USA	

chronic skin lesions (Martínez-Santamaría et al. 2012). Right now, the finest substitute tool for animal research is three-dimensional (3D) reconstructed living human skin analogs. They have been extensively used to study a variety of dermatological research because these can reproduce close resemblance of structural and functional properties with natural human skin. With the aid of a computer-controlled 3D printer, tissues and organs can be created by precisely positioning living cells, biological components, and biochemicals (Fig. 1.2). Therefore, 3D printing method has developed as a propitious tool to assemble structural matrix and attracted the attention of skincare companies. There are several methods to mimic skin models, including electrospinning, 3D printing, and even microfluidic devices, which show the human skin's structure in much more detail. Table 1.2 enlightens the various procedures used for the fabrication of artificial skin model using different biomaterials and their applications in cosmetic research (Yun et al. 2018). When used to create tissue-engineered constructs, 3D bioprinting imparts high accuracy, reproducibility, and good control over a scaffold's internal structure and external shape (Koch et al. 2010, 2012).

The skin is known to be a multilayered structure containing various cell types, and thus, 3D bioprinting could provide the opportunity to deposit cells in this

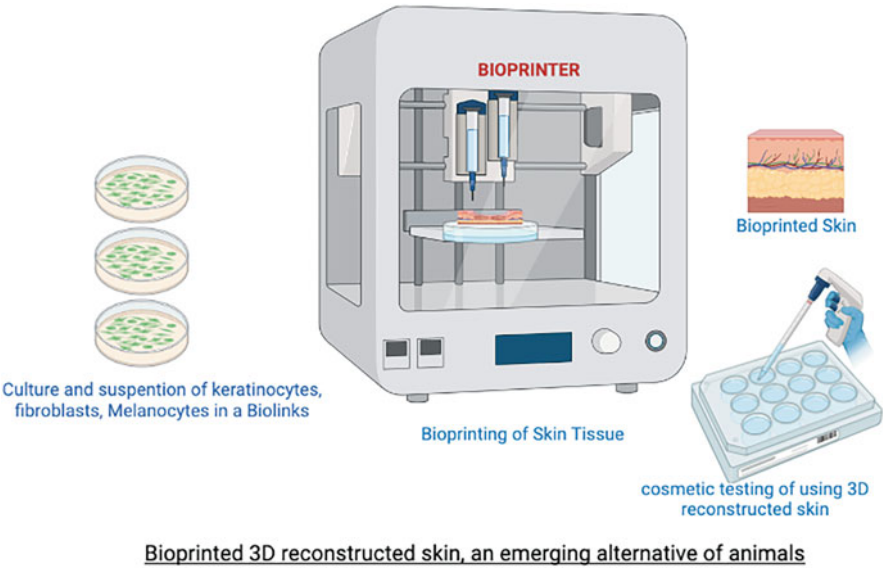

Fig. 1.2 Bioprinting used for bioprinted 3D reconstructed skin

arrangement. Multilayer artificial skins were created by depositing a collagen type I from rat tail hydrogel precursor, fibroblasts, and keratinocytes using a layer-by-layer printing process. Lee et al. (2014b) described the creation of 3D manufactured skin models using collagen and human skin cells assembled, layer by layer. Primarily, collagen layer was printed, followed by both the cells' (keratinocytes and fibroblasts) deposition on the top of every particular collagen matrix. In the reconstructed skin model construct, cell viability was great (>94%), and at 14 days following air–liquid interface culture, completely mature skin tissue displayed 3–7 different cell layers in the epidermis. In a different study, Ng et al. (2016) created skin constructs using a gelatin–chitosan bioink with good printability and antibacterial properties. The dermal region and portions of the outer epidermal layer were printed in three dimensions at around 400 μm. The fibroblasts from human foreskin showed a spindle-like morphology on the 5% gelatin–chitosan, and more viable cells were seen on hydrogels.

Creating complex epidermis and dermis structures via 3D printing offers hope for skin tissue engineering, but many technical obstacles still exist. The difficulties in fabrication of 3D skin constructions with high resolution and printability include biomaterials restrictions due to biocompatibility, biodegradability, and physico-chemical qualities (Zhu et al. 2016). Additionally, the conditions of 3D printing should be tuned to reduce stress-related cellular and biological component damage during the deposition phase (Patra and Young 2016).

Table 1.2 Various skin reconstruct fabrication methods, model, and their application

Methods	Cell type used for culture	Structure	Use in cosmetic industry/ dermatology research	Skin equivalent model	References
Freeze dying	Primary human dermal fibroblasts	Dermis	Skin tissue engineering	Chitosan sheet	Mohd Hilmi et al. (2013)
	Human foreskin fibroblasts Human keratinocytes	Dermis epidermis	Skin tissue engineering	Collagen scaffold	Ahn et al. (2010)
3D printing	Human neonatal fibroblasts	Dermis epidermis	Skin tissue engineering Bioink	Gelatin–chitosan scaffold	Ng et al. (2016)
	Human fibroblasts human keratinocytes	Dermis epidermis	Bioink	Human plasma-derived fibrin matrix	Cubo et al. (2016)
	Human fribroblasts HaCaT cells	Dermis epidermis	Bioink drug transdermal test skin regeneration	Collagen scaffold	Lee et al. (2014b)
Electrospinning	Normal human fibroblasts	Dermis	Skin tissue engineering	Silk fibroin nanofibrous matrix	Lee et al. (2014a)
	Normal human fibroblasts Normal human keratinocytes	Dermis epidermis	Skin tissue engineering	Chitin nanofibrous matrix	Noh et al. (2006)
Electrospinning/ freeze-drying	–	Dermis epidermis	Antibacterial wound dressing	Bilayer TiO_2-chitosan/human adipose-derived ECM sheet	Woo et al. (2015)
Microfluidic device	Human fibroblasts and dermal keratinocytes Blood vessel endothelial cellsHuman umbilical vein endothelial cells (HUVECs), HaCaT cells	Dermis epidermis vascular layer	Drug toxicity test	Skin-on-a-chip model using polydimethylsiloxane (PDMS) and polyester membranes	Wufuer et al. (2016)
	Human keratinocytes human dermal fibroblasts			Skin chip model using PDMS and collagen hydrogel	Lee et al. (2017)

1.5 Skin Equivalents As Far

1.5.1 In Vitro Reconstituted Epidermis

In the 1980s, collagen gel and multilayered human epidermal keratinocytes (isolated and serially reproduced in vitro from a tiny skin biopsy) were used to culture human fibroblasts for 3D culture for the first time (Bell et al. 1981). After that, as an alternative, essential reconstructed skin culture comprising cultured keratinocytes on a mesh is started to be utilized for assessing the safety of home and personal care products (Triglia et al. 1991). Though the more enhanced performance of in vitro reconstitutes, the epidermis can be obtained using biological matrices for keratinocyte seeding, such as a fibrin substrate that permits keratinocyte stem cell conservation and growth factor delivery (Hynds et al. 2018; Wang et al. 2017). However, as demonstrated by several clinical trials, a genuine dermis is necessary for improvement in the histological quality of the newly regenerated skin and cell engraftment (Tavakoli and Klar 2021).

1.5.2 Development of Full-Thickness Skin Equivalents (FTSE)

The reconstituted epidermis has its own limitations; therefore, the 3D organotypic models have been produced to overcome these. FTSE also promotes cellular communication between the dermal and epidermal layers, making it possible to use it as a more complicated model to study processes like skin formation, infection, or wound healing. Epidermal models still offer highly standardized conditions for risk assessment (Reuter et al. 2017). By seeding primary keratinocytes on a de-epidermized dermis, full-thickness skin equivalents (FTSEs) are created (Zhang and Michniak-Kohn 2012; Singh et al. 2020; Reijnders et al. 2015) or on biodegradable polymer substrates (natural origin hydrogels or synthetic hydrogels) incorporating human dermal fibroblasts. The basal layer keratinocytes also rigorously control the proliferation. Although hydrogel production can be optimized, no perfect bioink exists to produce a hydrogel that mimics the structural, mechanical, and biochemical characteristics of native skin in a medically meaningful manner. Thus, an additional focus on hydrogel composition is vital. Another approach that may be considered is utilizing the biochemical pathways for physiological polymer formation to create bioink materials with increased physiological pertinence (Randall et al. 2018). It accurately reflects the physiological aspect, the structural and mechanical elements of 3D skin creations, including appendages and macrostructures like glands and vasculature, which are crucial. Additionally, integrating technologies like MESW and 3D bioprinting open up new possibilities for merging synthetic and natural matrix that produce the tissue environment required for cell survival while offering structural support. To prevent dermal framework shrinkage in long-lasting cultures, fibrin or a composite silk–collagen matrix should be preferred (Janani et al. 2019). At the confluence point of air and liquid, the skin equivalents are present in an uncovered position to cultivate entire dermis differentiation and to facilitate full

epidermal differentiation and stratification (Roger et al. 2019). However, these skin equivalents are comparatively more porous than native human skin (Bouwstra and Ponec 2006). At the epidermal basal layer, co-seeded keratinocytes and melanocytes merge as a single entity to regenerate their physiological distribution (Cichorek et al. 2013). Full-thickness skin model (human skin equivalent HSE, FTSE) offers several benefits: (1) Since most models are composed of primary human cells, inter-species extrapolation is avoided; (2) repeated application of formulations can be performed in contrast to ex vivo human skin for at least several weeks; (3) as it is ready to use, it does not require advanced knowledge of cell culture technique; (4) It has become the great alternative to animal models for research and development applications in regulatory toxicology and in the cosmetic industry (Zhang and Michniak-Kohn 2012; Brohem et al. 2011; Groeber et al. 2011). Besides these applications, models of skin have been employed frequently to investigate the cellular and molecular mechanisms governing cutaneous disease.

1.5.3 Most Recent Skin Equivalents

Unfortunately, with regards to the production of 3D skin, analysts confront numerous issues that vexed researchers in the mid-twentieth century, i.e., complicated structures like glands and tactile corpuscles, physiological oxygen, and nutrient delivery via a perfused vasculature, as well as easily available and repeatable 3D model for use in research laboratories. A significant barrier to furthering our understanding of the skin is the absence of skin appendages in skin grafts. Therefore, skin appendages are being incorporated into full-thickness skin equivalents to produce the latest generation skin equivalents. Adipocytes at the time of maturity are well co-cultured with fibroblasts and keratinocytes. It improves the stability between epidermal growth and differentiation and develops a more competent epidermal barrier (Vig et al. 2017; Yang et al. 2019). As of late, an "endogenous" HSE was produced by employing a active whirligig culture of fibroblasts fixed in a temporary matrix made of gelatin microspheres, which is steadily damaged. In contrast, fibroblasts congregate with the extracellular matrix (ECM) and lead to improved epidermal barrier function (Tracy et al. 2016). The main drawback of skin equivalents is their lack of a functional vascular system. Vascularizing skin structures for therapeutic applications is vital since it is required for good and long-lasting structure and function. However, vascular skin equivalents are not helpful as models to study the main features of diseased skin, i.e., leukocyte trafficking across vascular endothelium or testing the skin's ability to absorb an intravenously supplied chemical. Black et al. created the first skin analog with a capillary-like architecture in 1998 (Black et al. 1999). The main use has been to improve the graft uptake in a clinical setting or to examine angiogenic and angiostatic drugs (Veith et al. 2019; Gradin et al. 2021; Shahin et al. 2020). The vascularized tissue generation was performed using decellularized porcine small bowel segments due to their tendency to have a collagen matrix scaffold showing the structures of native vascular network and is repopulated with endothelial progenitor

cells. With its characteristic endothelial differentiation structures, it is able to form a vascular network (Schanz et al. 2010). The attainment of typical skin architecture can accelerate because of such system, which supports the 3D skin under immersed conditions and at the air-lift liquid interface (Groeber et al. 2011). As of late, dermal fibroblasts have been utilized for neural stem cell generation through direct reprogramming and to obtain a neuroimmune-cutaneous system. These have been added to skin substitutes made of silk collagen that contain immune and adipose cells. A major setback is cultivated primary skin cells' low proliferation potential and scarcity of these skin analogs. On the other hand, using induced pluripotent stem cells (iPSCs) is very crucial as these can differentiate into different skin cells, with dermal papilla and sensory neuron cells (Abaci et al. 2018). These have also been used to generate FTSE. Therefore, it could be an incredible source for massive-scale generation of distinctive skin cell sorts, with improved reproducibility.

1.6 Future Perspective: Next-Generation Skin Equivalents, a More Advanced Way Ahead!

With so many advancements in the skin research field, it is tough to optimize a method where an organotypic model can be developed, summarizing human skin's whole complexity and similarity. Once immune-competent cells are effectively integrated into hydrogels and inside a circulating vasculature tissue culture, modeling of skin disease will become a reality (Randall et al. 2018). Integrating various biofabrication techniques, such as electrospinning and bioprinting, will target both appendages to increase the possibility of producing a functional skin and the consolidation of immune cells within the skin model for specific formulations and other preclinical applications (Fig. 1.2). The next step toward developing the skin equivalent within microfluidic may provide much better models, which can mimic skin function even more efficiently (van den Broek et al. 2017). In previous days, silicone microfabrication and micromachining techniques were used to produce microfluidic devices (Preetam et al. 2022). On the other hand, a comparatively cheaper and easier way to develop microfluid devices is to use biocompatible silicone rubber poly(dimethylsiloxane) (PDMS) (Torino et al. 2018). Due to perfused vascular structure of microfluidic platforms, it became easier to mimic in vivo physical force applied by blood flow (shear stress), which is necessary to regulate endothelial cell gene expression, morphology, proliferation, and apoptosis (D'Arcangelo et al. 2016; Osaki et al. 2018). Skin analogs, skin biopsies, or explants of individual hair follicles have all been cultured in a dynamically perfused bioreactor based on chip chamber by subjecting them to varying mechanical shear stress. Long-term composite skin equivalents can be maintained, and multiple tests can be performed without device disassembly/tissue disruption.

Furthermore, histological procedures and other analyses can be performed after removing the tissue from the device. Epidermal stratification, differentiation, and barrier functions can be improved by allowing dynamic perfusion and a finely controlled region that is exposed to air movement and gas composition in

microfluidic systems (Sriram et al. 2018). Microfluidic devices play an important role to create skin immuno-competent models. To represent human dendritic cells, a keratinocyte cell line HaCaT has been cultured as an epidermis barrier model on one side using a bi-channel device and on the other side using a human leukemic monocyte lymphoma cell line (U937) (Ramadan and Ting 2016). The effects of UV irradiation are evaluated by measuring an integrated magnetic-bead immunological test and trans-epithelial electrical resistance.

Similar to this, a model based on three microfluidic channels was developed to simulate skin inflammation and edema for drug testing (Wufuer et al. 2016), and to further improve skin equivalent complexity, 3D bioprinting technology has been applied. Indeed, to build a similar structure as native human skin, deposition of various cell types and biomaterials has been permitted by this fully automated system (Ng et al. 2016; Lee et al. 2017). Using 3D bioprinting, Abaci and colleagues created a 3D skin with perusable, made of both primary and iPSC-derived endothelial cells (Abaci et al. 2016). They also worked on a pumpless "skin-on-a-chip" model (Abaci et al. 2015). A HUVEC-coated nylon wires were used to produce perfusable skin equivalent when inserting within the dermal compartment and using 3D bioprinting. The benefit of this model is that, after medication delivery, it demonstrates effective percutaneous penetration in the endothelialized tubes (Mori et al. 2017). The organization of skin appendage age niches in mini-organoids may be recreated using 3D bioprinting technology. The difference in density, anatomy, and function of different fibroblast subpopulations can easily be observed in the native dermis. However, the difference in the composition of extracellular matrices is also predominant (Sriram et al. 2015). By fusing various fibroblast subpopulations with various extracellular matrix elements, 3D bioprinting technology can be an effective tool for recreating the dermal natural composition. Altogether, 3D bioprinting technology can be very supportive to summarize the local dermal composition by combining diverse fibroblasts with various extracellular matrix components and can achieve a way to make a connection between in vitro models of different tissues and the skin "human-on-a-chip" system for drug screening. Recently, skin has been connected with organs such as kidney, liver, and intestine (Risueño et al. 2021).

Some pigmentation experiments utilize human melanocytes in the base layer of multifaceted epidermal keratinocytes. Skin aging-related studies about wrinkles and elasticity of skin employ some full-thickness skin models including keratinocytes and fibroblasts. OECD records a few skin constructs as options to animal experiments for chemical testing in their technical guidelines (TG): TG431, a skin corrosion test, and TG439 (OECD 2019), a skin irritation test (OECD 2021b).

However contrary, 3D skin models cannot be used for drug penetrability tests because of lipid proportions in these 3D remodeled skin models that are not accurate compared to in ex vivo human skin ex plant and thus exhibit an increase in drug penetrance up to 5–50 fold in these models. The pivotal restriction of 3D skin cultures is their confinement of having a beneficial barrier and competent immune response. In some studies of 3D reconstructed skin models, the incorporation of immune cells has been thorough. Duval et al. (2003) have studied skin aging to

access UV-induced skin damage and skin modifications and, for this, have used reconstructed skin containing Langerhans cells. In another study by Pageon et al. (2017), reconstructed skin containing monocytes was utilized to evaluate the glycation reaction, which is a partial reason for skin aging.

There are several advantages of 3D skin equivalents for both cosmetic and pharmaceutical industries. Each new substance/drug had to undergo various in vitro safety checks before each clinical study, and therefore, the cosmetic industry researchers may evaluate the medicines/chemicals by using 3D bioprinter-fabricated skin models, whereas, prior to any kind of marketing of cosmetic formulations, it is of utmost importance to evaluate the potential toxic and allergic effect of the same (Sarkiri et al. 2019). All of these needs and their ethical approach make 3D bioprinted skin a great tool to execute evaluation and screening of pharmaceutical and cosmetic products. Additionally, 3D skin bioprinting may be used to examine how well drugs and other active substances penetrate and absorb through skin. Global giants in the cosmetics industry, like L'Oreal and Proctor and Gamble, were interested in this technology and invested in the study and creation of 3D bioprinted skin models.

1.7 Conclusion

A PubMed search revealed references to "alternative to animal model" in every subject, indicating the significant and growing interest in studies utilizing alternatives to animals. The number of non-animal research has increased from 628 in 2007 to 212 in 1997 and 1219 in 2017 respectively. The skin equivalent strategy appears to be the most effective method now available, having advanced from systems that just consisted of keratinocytes seeded on a medium to more intricate cell and matrix combinations. An improvement in recreating the skin's structural, functional, and molecular network features is made possible by 3D bioprinting and microfluidic tools. The corresponding models replicate skin architecture and blood flow effects more precisely. The difficult task of collecting a more precise understanding of biological systems and appropriately resolving issues of cost, time, and ethics calls for the further development of in vitro skin systems. Forthcoming substitute technologies should ideally be able to simulate skin inside the framework of an artificial body, simulating certain connections with other organs. There is so much work to be done, but that will be very worthwhile. The straightforward hand-poured hydrogel matrix will also become obsolete in the twenty-first century with the adoption of 3D printing for usage in biological procedures providing a new benchmark for creating 3D tissue constructs. The necessary physiologically relevant skin components, such as the ECM and microbiome, can currently be produced using bioengineering techniques. Still, future advancements in these techniques and the creation of completely new ones will allow the cost-effective and repeatable in vitro production of physiological skin. A distinct physiological matrix and microenvironment, the addition of extra specific type of cells, and the simplicity of manufacture using novel fabrication processes are

crucial components to creating a more accurate 3D skin model. Future researchers would not have to worry about choosing sources and techniques, and all skin aging research can be carried out utilizing comprehensive skin simulation models. It is critically necessary to collect reliable analyses of human data and conduct extensive and thorough sampling to realize this ideal model.

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