Review

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Posted Date: 23 February 2024

doi: 10.20944/preprints202402.1340.v1

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Progress in the Application of 3D Skin Models for Cosmetic Assessment

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Abstract: With the development of organizational biology and forced from the EU Cosmetic Regulation (EU/1223/2009) with the complete ban of animal testing for cosmetic purposes, 3D skin models have been widely developed due to their similar physiological structure and metabolic function to native human tissue. 3D skin models for in vitro cosmetic evaluation are still promising alternative methods for evaluating phototoxicity, corrosivity and irritation. These models have been used for early screening and later validation of toxicity and efficacy evaluation by many well-known cosmetic companies. 3D skin models have become an important tool in cosmetic evaluation research. This article reviews the development of 3D skin models and their application in cosmetic evaluation. An outlook on the current application and future technological development directions in the cosmetic field is also presented.

Keywords: 3D skin model; cosmetics; in vitro assessment; efficacy evaluation

1. Introduction

The skin is the largest organ of the human body and plays a tremendously significant role in regulating body temperature, sensing external stimuli, and acting as a barrier to prevent environmental hazards such as mechanical stress, microorganisms, chemicals, and ultraviolet radiation [1]. Although animal models have greatly contributed to our basic understanding of skin mechanics, due to species differences, there is uncertainty when extrapolating animal test results to humans [2], as ca. 50% of drugs that passed positive animal trials proved to be toxic to humans and vice versa [3]. In terms of skin-related genes, humans and mice only have approximately 30% overlapping genes, and there are differences in the number of epithelial cell layers, hair follicles, and muscle layers compared to human skin [4,5]. In addition, there are concerns regarding eye irritation tests in rabbits, as they lack tear glands, their sensitivity is relatively low, and intrinsic mechanisms cannot be detected. Furthermore, the EU has completely banned animal testing in the cosmetics field since 2013, including the import and sale of cosmetic ingredients and finished products that have undergone animal testing.

In China, the phased approach to animal testing substitution was implemented in contrast to the EU's complete prohibition. Since 1997, alternative animal methods have been included as a key focus in fundamental research on laboratory animals during the "9th Five-Year Plan" and "10th Five-Year Plan" periods, and in 2014, China abolished the mandatory animal testing requirement for domestic nonspecial purpose cosmetics [6,7].

The development of tissue engineering technology, as well as the tremendous demand for animal replacement methods in the pharmaceutical and cosmetics industries, has led to the rapid development of in vitro reconstructed skin models in the past 20 years. Three-dimensional (3D) skin models have achieved a high degree of consistency with human skin in terms of gene expression, tissue structure, cell activity, and cell cytokine secretion [8]. They provide stable quantitative data with short experimental cycles and high throughput and comply with the principles of the 3Rs (replacement, reduction, refinement) [9], making them important tools for medicine [10] and cosmetic evaluation [2].

 doi:10.20944/preprints202402.1340.v1

2. 3D skin models and types

Skin modeling originated from the clinical treatment for burns [11,12], and since 1975, when Rheinwald and Green [13] successfully cultured human epidermal keratinocytes using 3T3 cells as a trophectoderm for the first time to overcome the challenge of culturing epidermal cells on a large scale in vitro, the study of skin substitutes has been widely developed [14,15]. The three main elements for constructing skin models are 1) seed cells (e.g., keratinocytes, fibroblasts, and epithelial cells), 2) scaffolding materials (e.g., treated allogeneic cell-free dermis, collagen glycosaminoglycans, collagen gels, and polyhydroxyacetic acid/polylactic acid mesh), and 3) media enriched with suitable growth factors [16–18].

For clinical treatment [14,19,20], skin models have been widely used for deep burns, diabetic leg ulcers, etc., including 1) epidermal substitutes, consisting of keratinocytes and biodegradable skeletal materials, represented by Epicel® (Genzyme Biosurgery) [21], Epidex® (Healiva Company) [22], and MySkin® (MySkin Inc.) [23]; 2) dermal substitutes, composed of cell-free dermal analogs represented by Integra® (Integra Life Sciences) [24], Biobrane® (Dow Hickman/Bertek Pharmaceuticals) [25], and Matriderm® (Dr. Otto Suwelack Skin & Health Care AG) [26] for cell-free dermis, and Dermagraft® (Advanced Tissue Sciences Inc.) [27] for fibroblast-containing dermis, and Alloderm® (Life Sciences Inc.) [28] for allogeneic acellular dermis; 3) combined dermis (full dermis, containing both epidermis and dermis) substitutes such as Apligraft®, also known as Graftskin, the first commercially available full dermis (Organogenesis Inc.) [29] and StrataGraft®, orphan drug for deep burns (Stratatech, Madison, WI)

3. 3D skin models for cosmetics

As dermal substitutes are mainly cell-free structural components, the 3D skin models used for the in vitro evaluation of cosmetics are epidermal and combined dermal models, as well as melanocytes constructed into models to form 3D melanin skin models [17]. To date, dozens of 3D skin model manufacturers have been submitted to the European Center for the Validation of Alternative Methods (ECVAM), and some of these commercial skin models have been validated and approved by the Organization for Economic Co-operation and Development (OECD) for use in cosmetic risk assessments [2,18] (Table 1). These models include the following: 1) epidermal models (RhE): Episkin (L'Oréal, France) [33–38], EpiEthic (L'Oréal, France) [39] [36,40–43]，Skin^{+} (Sterlab, France) [44], EpiDerm (MatTek, US) [36,37,43,45,46], epiCS (Henkel, Germany) [47], LabCyteTM EPI-MODEL (J-TEC, Japan) [43,48,49], KeraSkinTM (Biosolution, South Korea) [44,50]; 2) full model (FT): Phenion® FT (Henkel, Germany) [51]; 3) SkinEthic (L'Oréal, France) [52]， EpiOcular (MatTek, US) [53]， LabCyteTM CORNEA-MODEL24 (J-TEC, Japan) [54]，MCTT HCE (Biosolution, South Korea) [55].

 doi:10.20944/preprints202402.1340.v1

Table 1. Commercial 3D skin model included in the OECD.

Skin model	Commercial Name	Manufacture	Country	Method for manufacture	OECD
Epidermal skin	EpiSkin™	L'Oréal	France	Epidermal reconstruction model cultured in collagen medium using epidermal keratinocytes from skin tissue removed by mammoplasty as seed cells;	TG 431; TG 439
	EpiEthic™ RHE	L'Oréal	France	Epidermal reconstruction model seeded with epidermal keratinocytes from foreskin tissue and cultured in inert polycarbonate lipase medium	TG 431; TG 439
	epiCS®	Henkel	Germany	Epidermal reconstruction model seeded with epidermal keratinocytes from foreskin tissue and cultured in serum-free medium	TG 431; TG 439
	EpiDerm™	MatTek	America	Epidermal reconstruction model using epidermal keratinocytes from foreskin tissue as seed cells in serum-free medium	TG 431; TG 439; TG 498; Micronucleus assay under peer-review
	LabCyte™ EPI-MODEL	J-TEC	Japan	Keratinocytes from neonatal foreskin and 3T3-J2 grown in an inert filter matrix and cultured using an air-liquid surface approach	TG 431; TG 439
	Skin+	Sterlab	France	Human primary representative dermal keratinocytes were used as seed cells and cultured in an air-liquid surface approach	TG439
	KeraSkin™	Biosolution	Korea	cultured with human primary keratinocytes as seed cells and 3T3 cells as trophectoderm	TG439
Full skin	Phenion® FT	Henkel	Germany	Neonatal foreskin primary keratinocytes cultured in collagen scaffolding fibroblasts to form connective tissue as the basis of a multilayered epithelial cell layer cultured in air-liquid surface	Comet assay under peer-review

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	SkinEthic™ HCE	L'Oréal	France	Corneal epithelial model without keratinization using cells from immortalized human corneal epithelial tissue as seed cells, cultured in air-liquid surface culture	TG492; TG492B
Corneal Epithelium	EpiOcular™	MatTek	France	Corneal epithelial model without corneal stroma, seeded with cells from neonatal foreskin epithelial tissue and cultured in air-liquid surface culture with serum-free DMEM (EGF, insulin, HYD, etc.).	TG 492
	LabCyte™ CORNEA-MODEL24	J-TEC	Japan	Normal human corneal epithelial cells from Rocky Mountain Lions Eye Bank, USA	TG 492
	MCTT HCE™	Biosolution	Korea	Prepared from corneal epithelial cells from corneal limbal tissue remaining after corneal transplantation	TG 492

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The most widely used in cosmetics are EpiSkin™ and SkinEthic™ from L'Oréal [36] (Table 2). EpiSkin was constructed in 1991 by Estelle Tinois-Tessonneaud et al. from Imedex and was developed with financial support from ECVAM to replace in vivo rabbit skin stimulation experiments; it was acquired by L'Oréal in April 1997 and is described as a "type I bovine collagen matrix, representing the dermis, surfaced with afilm of type IV human collagen, upon which is laid, after 13 days in culture, stratified differentiated epidermis derived from second passage human keratinocytes" for irritation. To make the model more suitable for drug penetration, the keratinocytes were cultured for 20 days before being transferred to a collagen substrate. SkinEthic was constructed by Rodsy M, Clauss LC et al. of SkinEthic in 1990 and acquired by Episkin, a division of L'Oréal. In 2006, normal human keratinocyte-forming cells were cultured on inert polycarbonate filter membranes on an air-liquid surface for 17 days to reconstruct the epidermis. In addition, EpiDerm™, marketed by MatTek in 1993, is also a classic model [36,43] and was described as "epidermis reconstituted by air lifted culture of normal human keratinocytes for 17 days in chemically defined medium on inert polycarbonate filters". In addition, J-TEC's LabCyte™ from Japan, which was subsequently founded, has cells derived from the Rocky Mountain Lions Eye Bank in the U.S., and KeraSkin™ from South Korea, which uses immortalized lineage 3T3 cells as a trophectoderm, is distinct from the conventional combination of primary keratinocytes and primary fibroblasts.

In addition, StrataTest of Stratatech and Graftskin™ of Organogenesis® LSE™ are also used for safety testing and have achieved satisfactory results, but they are still mainly based on therapeutic 3D skin models; additionally, their artificial skin is dedicated to the treatment of skin burns or diabetic foot, and artificial skin is currently available through FDA filing.

To develop and screen for cosmetics that are more suitable for Orientals and to solve the problems of storage, transportation and timeliness of 3D skin models, two series of skin models from L'Oréal localized in Shanghai were used. Henkel from Germany is also looking for further cooperation with local companies such as BioCell for transformation. In China, EpiKuits from BioCell, which was confirmed to be comparable to the 3D skin model-related methods listed in Table 1, has passed many rounds of "Me too" method verification and is especially suitable for Chinese cosmetic research because of the characteristics of oriental skin. In addition, the Chinese cosmetic companies JALA [56] and Beijing Forelandpharma have also reported 3D skin models [6].

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Table 2. Information on manufacturers of 3D skin models for in vitro testing of commercial cosmetics.

Country	Manufacturer	Commercial skin models	Manufacturer Information
China	BioCell	EpiKutis™	Founded in 2014, started the development of 3D cells in the early 90 s with the support of tissue engineering research and development of the Fourth Military Medical University and the Xi'an Institute of Tissue Engineering and Regenerative Medicine
French	L'Oréal	EpiSkin™	EpiSkin was constructed in 1991 by Estelle Tinois-Tessonneaud et al. of Imedex and acquired by L'Oreal in April 1997.
		SkinEthic™	SkinEthic was built in 1990 by Rodsy M, Clauss LC et al. It was originally part of SkinEthic, which was acquired by L'Oreal's Episkin in 2006.
Germany	Sterlab	Skin+®	The Sterlab Group was founded in 1977 and focuses on the development and application of implantable medical devices.
	Henkel	epiCS®	epiCS® was developed by SkinInVitro GmbH in Germany, acquired by Henkel in November 2020, and is now part of Phenion.
		Phenion®	Phenion® was developed by Henkel in cooperation with the Johann WoKgang Goethe University in Frankfurt and a group of professors through the public–private partnership Phenion GmbH. in 2007, K.R. Mewes and M. Raus, among others, developed the Phenion® model. in January 2009, Phenion GmbH became part of the Henkel Group.
America	MatTek	EpiDerm™	MatTek was founded in 1985 and Epiderm was launched in 1993.
Korea	Biosolution	MCTT HCE™ KeraSkin™	Biosolution founded in 2000, KeraSkin™ launched in 2002, MCTT HCETM launched in 2008.

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Japan	J-TEC	LabCyte™	J-TEC founded in 1999, LabCyteTM EPI-MODEL launched in 2005

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4. Current application of 3D skin models for cosmetic evaluation

In terms of safety evaluation, the application of 3D skin models in the field of cosmetics in China is mainly based on experimental research and group standards, and there is no authoritative standard collection information available. China has also established corresponding group standards for safety evaluation, and 7 of the 10 group standards related to 3D skin have been released related to safety evaluation (Table 3). The main models applied are the epidermal model EpiKuis® and the corneal model BioOcular® from BiCell, as well as the full dermal model Phenion® FT of Henkel, and the methods are mainly based on the existing OECD guidelines: skin irritation (TG439), skin corrosion (TG431), eye irritation (TG492), genotoxicity (TG492B), phototoxicity (TG498), Micronucleus Experiment and Comet Experiment (peer-reviewed).

In terms of efficacy evaluation, the certification of cosmetic efficacy in China has been in a state of disorder and blind obedience before, and the only authoritative standard evaluation is for moisturizing efficacy (QB/T4256-2011 "Guidelines for the Evaluation of Cosmetic Moisturizing Efficacy"). However, with the strengthening of regulatory efforts, many standards are being upgraded. Among the group standards, the Shanghai Daily Chemicals Industry Association released three group standards in 2019 for whitening efficacy (MelaKutis®), moisturizing efficacy (EpiKutis), and barrier efficacy (EpiKutis®). Therefore, in addition to a series of safety assessments, research on the application of 3D skin models for evaluating the efficacy of cosmetics has experienced explosive growth in recent years [31,57–59].

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Table 3. Group standard using 3D skin models as an evaluation tool.

Group Name	Standard No.	Standard Name	Release Date	Endpoint of the Evaluation	Skin Model	Manufacture
Shanghai Daily Chemistry Trade Association	T/SHRH 008—2018	Cosmetics-*In Vitro* Skin Irritation Test Method (SIT)	2018/12/10	Safety-Skin irritation (SIT)	EpiKuis®	BioCell Biotech
Shanghai Daily Chemistry Trade Association	T/SHRH 007—2018	Cosmetics-*In Vitro* Skin Irritation Test Method (ET-50)	2018/12/10	Safety-Skin irritation (ET-50)	EpiKuis®	BioCell Biotech
Shanghai Daily Chemistry Trade Association	T/SHRH 012—2018	Cosmetics Eye Irritation test - *In vitro* Reconstructed human Cornea-like Epithelium (RhCE) test method	2018/12/10	Safety-Eye irritation	Reconstructed human Cornea-like Epithelium	Not specified
Shanghai Daily Chemistry Trade Association	T/SHRH 009—2018	Cosmetics -3D Skin Comet Assay	2018/12/10	Safety-Genotoxicity	Phenion®FT	Henkel
Shanghai Daily Chemistry Trade Association	T/SHRH 024—2019	Eye irritation test of cosmetics-*In vitro* reconstructed cornea model-Time to toxicity test method (ET50)	2019/12/31	Safety-Eye irritation (ET50)	BioOcullar®	BioCell Biotech
Shanghai Daily Chemistry Trade Association	T/SHRH 022—2019	Moisturizing efficacy test of cosmetics-*In vitro* reconstructed human skin test method	2019/12/31	Moisturizing efficacy	EpiKutis®	BioCell Biotech

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Shanghai Daily Chemistry Trade Association	T/SHRH 021—2019	Cosmetics whitening efficacy test-*In vitro* Reconstructed human skin containing melanocytes test method	2019/12/31	Whitening efficacy	MelaKutis®	BioCell Biotech
Shanghai Daily Chemistry Trade Association	T/SHRH 023—2019	Skin barrier efficacy test of cosmetics - In vitro reconstructed human skin test method	2019/12/31	Skin barrier efficacy	EpiKutis®	BioCell Biotech
ZheJiang Health Products & Cosmetics Industry Association	T/ZHCA 009—2019	Method of skin irritation test *in vitro* for face cream cosmetic products-*In vitro* skin irritation test with reconstructed skin model	2022/1/4	Safety-Skin irritation	EpiKuis® RHE	BioCell Biotech
ZheJiang Health Products & Cosmetics Industry Association	T/ZHCA 013—2021	Method of eye irritation test *in vitro* for facial cleanser cosmetic products—*In vitro* eye irritation test with reconstructed human cornea-like epithelium model	2022/1/4	Safety-Eye irritation	BioOcullar®	BioCell Biotech

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4.1. Whitening effect

Due to the characteristics of skin color and the pursuit of whitening in Eastern countries, the whitening effect is a popular research field. The method in the group standard (T/SHRH 021—2019) is to evaluate the cosmetic raw materials and products. The brightening effect of the analyte is determined by the apparent chromaticity and L* value, the melanin transport inhibition of the analyte is determined by the distribution of melanin particles, and the melanin synthesis inhibition of the analyte is determined by the melanin content. In addition, Ning et al. [60] used MelaKutis for apparent chromaticity testing, L* value testing, and melanin content testing and investigated the whitening efficacy of cosmetic compositions containing tranexamic acid, ascorbyl glucoside (AA2G), niacinamide and hydroxyethylpiperazine ethanesulfonic acid (HEPES), and it was shown that the efficacy of composition A with four components was significantly better than that of whitening cosmetic compositions containing only 3 or 2 of these components and that all four components had a synergistic effect. He et al. [61] used MelaKutis to evaluate the whitening efficacy of aloe vera flower extract through the L* value, melanin content and apparent detection results of the model, and the results showed that the apparent color of the sample group became whiter, the L value significantly increased ($P<0.01$), and the melanin content decreased ($P<0.01$), which confirmed the whitening effect of aloe vera flower extract. Jiang et al. [62] evaluated the whitening efficacy of α-arbutin by the natural blackening mode of MelaKuits without the use of extra-UV irradiation, and the indicators were similar to those of the group standard. The results showed that 10 mg/mL α-arbutin was cytotoxic and could accelerate blackening after acting on the skin model; however, it had a whitening effect after continuous action for 3 or 4 days at concentrations of 2 mg/mL and 1 mg/mL, and it was also found that the skin model would fall off at D5, indicating that the skin model was self-limited. In terms of the application and expansion of skin models. H. Ko et al. [63] confirmed the whitening efficacy of the melaninogenic inhibitor TCTE by L* detection using MelanoDerm™ (MatTek), a dark skin donor-derived melanogenic skin model, and identified the target at the molecular level through a 3D epidermal model, revealing that the molecular mechanism of TCTE against melanin production is partial inhibition of PGC-1a-dependent PPARr.

4.2. Moisturizing efficacy

In the national standard (QB/T4256-2011), moisturizing testing is conducted directly in the arm and evaluated by quantitative detection by a capacitance moisture analyzer, which is time-consuming because of the participation of volunteers who need to be well organized and coordinated. The method in the group standard (T/SHRH 022—2019) is to analyze the contents of the skin's natural moisturizing factors pyrrolidone carboxylic acid (PCA) and urocanic acid (UCA) to evaluate the deep moisturizing function of skin biology. Yang et al. [64] used epidermal model Epikuits to test the moistening efficacy of polysaccharides extracted from the space-flight bred Spirulina Platensis mutant strain H11 and found that the production of trans-uric acid in the 3D epidermal model increased by 156.43% at a concentration of 1%, which was much greater than that of the wild-type strain (16.5%), showing excellent moisturizing ability. Wang et al. [65] also used Epikuits to evaluate the moistening effect of the compound moisturizer, and the moisturizing efficacy of the compound moisturizer in different cosmetic formulations was compared. The results showed that the water content of the skin models that had applied the moisturizer containing the moisturizer was significantly greater than that of the blank control ($P<0.01$).

4.3. Skin barrier efficacy

The group standard (T/SHRH 023—2019) method applies the irritant sodium dodecyl sulfate (SLS) to the model to cause damage to the skin barrier and the model tissue. The ability of the analyte to improve the skin barrier was evaluated by the ET50 value (the exposure time required for the analyte to cause a 50% reduction in cell viability at a fixed concentration) to characterize the tolerance potential of the skin. Fang et al. [66] used SLS to construct an epidermal skin EpiKuits injury model, and the results showed that phytosterol esters had no obvious effect on tissue viability or tissue

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morphology in SLS-induced skin injury models and had no significant inhibitory effect on the expression of PEG2 and IL-1a; moreover, when the concentration of phytosterol esters was greater than 1%, phytosterol esters significantly inhibited the expression of TNFa and promoted the expression of FLG. Fan et al. [67] used donkey milk powder to treat a skin model of SLS-EpiKuits damage and evaluated its skin irritation and barrier repair effects by detecting tissue viability (ET50) and IL-1a release. In terms of the application and expansion of skin models, Amano et al. [19] used a self-built TESTSKIN skin model and human normal keratinocytes to integrate gene, protein, metabolism, and osmotic barrier data and evaluated the barrier effect of the JAK inhibitor JTE-052, indicating that the downregulation of IL-4 and IL-13 was related to keratinocyte differentiation and that STAT3 and STAT6 were related to keratinocyte differentiation and the production of chemokines, respectively. In addition, studies have shown that [57] the detection of the FLG gene or the expression of proteins that are constituents of the cornified envelope (CE) structure (Envoplakin, Periplakin, TGM1, K1/10, Involucrin, Loricrin, SPRs, Filaggrin, Repetin, etc.) is also an important indicator of barrier efficacy.

4.4. Comprehensive evaluation

The 3D skin model has a wide range of evaluation, and its advantages in comprehensive evaluation and application are highlighted, which is reflected in the comprehensive evaluation of anti-aging efficacy. Michelet et al. [35] used Episkin to prove the anti-aging efficacy of LR2412 with a hyaluronidase system (HAS, HAS2, HAS3), stratum corneum thickness (marker Ki67) and dermal thickness. Li et al. [68] used the 3D epi-breaking model EpiKuits to evaluate the anti-eczema and anti-inflammatory effects of camellia oil-containing moisturizing milk on infant skin, and the results showed that as the tissue viability increased, the IL-1a and IL-8 content decreased, morphological damage to the SLS-induced diaper-like rash model improved, the barrier index ET50 increased, and the TSLP content decreased, which confirmed the anti-eczema and anti-inflammatory efficacy of camellia oil milk. Cai et al. [69] constructed a 3D full-thickness skin model using human epidermal keratinocytes and dermal fibroblasts. Photodamage was induced by UVB and UVA, and the cell viability and morphology of the sections were observed under a microscope. The expression of collagen, barrier-related proteins (LCE-A1, CLDN-1 and FLG) and stress-related enzymes (MMP-1 and NQO-1) in the sections was also observed. After the administration of 0.025% ceramide complex, the viability of the skin was well sustained; type I collagen, FLG, CLDN and LCE-A1 increased by 45%, 108%, 67% and 1835%, respectively; and MMP-1 and NQO-1 decreased by 45% and 29%, respectively.

5. Future Perspectives

Skin constructs manufactured using the biomimetic approach, especially the statistical skin model, have reached a high level of complexity in biological terms, and it has become a trend of the industry to carry out cosmetic testing on the model. The 3D skin model includes basic epidermal and dermal structures and can include melanocytes and Langerhans cells.

In recent years, significant progress has been made in the field of 3D skin model construction, in addition to conventional 3D skin models. 3D bioprinting materials were first fabricated in 2009 [70], and the appreciable advantage of preoperative planning of complex operations involving the use of computed tomography or magnetic resonance imaging data has shown the potential to revolutionize cosmetic testing. Due to computer-driven bioprinting, cells and biomaterials can be deposited precisely and consistently [3,71,72]. The technology is still in the developing stage, and a skin equivalent that contains all skin elements has not yet been printed. However, a growing number of printable biomaterials have emerged that expedite the manufacturing process with the development of bioprinting, which mainly involves chemically functionalized polymers and reinforcement strategies through molecular blending and post-printing interventions, i.e., ionic, covalent, or light entanglement, to enhance the mechanical properties of the construct and facilitate layer-by-layer deposition, which could also ensure an appropriate biodegradation rate with no toxicity or immunogenicity [73,74]. Based on bioprinting, a skin-on-a-chip (SOC) device was developed that has

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the advantages of dynamic perfusion and microfluidic devices and is herein termed the dynamic skin model. Current research on skin models cultured on SOC still focuses mainly on improving skin substitutes and culture conditions. The skin models created by 3D bioprinting technology and other technologies are not yet mature in terms of the printing procedure and the bioinks used for self-safety concerns.

Despite significant progress in the development of 3D skin models for use in cosmetic safety assessments, there is no standard for 3D skin model creation, including cell source, cell type and culture time. Due to the high complexity of the whole system, 3D skin models lack skin appendages such as blood vessels, hair follicles, sweat glands, sebaceous glands, muscles, and nerves [75]; therefore, representativeness is not yet known, and there remains an absence of OECD-approved animal-free alternatives for assessing dermal absorption, a measure of how a chemical substance can become systemically available by penetrating or permeating the skin [76,77]. In summary, there is still a long way to go to mimic the complex structure of living skin. For cosmetic applications, the 3D skin model needs to be continuously optimized for the following reasons: 1) Consistency: The primary cells used to construct the 3D skin model are not derived from the same donor, and there are differences in the measurement indicators of the models [78,79]. 2) Baseline: the critical baseline for cosmetic applications through different commercial skin models, and the commonly accepted scaling law still needs to be developed [80]. 3) Model growth time: The growth time of 3D models is usually approximately 30 days, which is shorter than that of real human skin and is self-limited [62]. The long-term effect of the test substance is difficult to assess. 4) Scale: Due to the limitations of cell donors and consistency considerations, large-scale production is urgently needed [81,82]. 5) Price: The growth of skin models requires a variety of growth factors, and the price is high; however, for cosmetic applications, it is mainly for testing but not for curing, and a high price decreases the willingness for cosmetic testing.

Funding: This research was funded by 2024 Jiangsu Provincial Market Supervision Administration Science and Technology Plan Project, grant number KJ2024018.

Conflicts of Interest: The authors declare no conflicts of interest.

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