

Organoids/organs-on-chips towards biomimetic human artificial skin

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Abstract

As the largest organ in the human body, the skin protects the body from pathogens and harmful substances through physical, chemical, and immune barrier functions. However, accurately replicating the complex physiology of human skin in mouse models remains a significant challenge. Accurately replicating the complex physiology of human skin in mouse models remains a significant challenge, making the development of bionic artificial skin particularly important. In recent years, skin organoid and skin-on-a-chip technologies have greatly enhanced *in vitro* skin modeling, overcoming many limitations of traditional approaches. In this review, we comprehensively summarize important advances in research on skin organoids and skin-on-a-chip. First, we present the anatomical structures and functional roles of the different skin layers. We then highlight current construction techniques and research findings on skin organoids and skin-on-a-chip. We then discuss in detail the biomedical applications of these emerging technologies. However, current models of skin organoids and skin-on-a-chip still have limitations. Therefore, we summarize the key challenges and explore strategies to improve the complexity and maturation of skin models via the precise control over the microenvironment. In the future, with the advancement of bioengineering technology, skin organoids, and skin-on-a-chip will provide more powerful tools for skin disease research and treatment.

Keywords: Organoids-on-chips; Organs-on-chips; Skin organoids; Skin reconstruction; Cosmetics

Highlights

- Skin organoids and skin-on-a-chip technologies provide innovative platforms that closely replicate the structural and functional characteristics of native human skin.
- Integration of bioprinting, microfluidics, and stem cell engineering significantly improves the physiological relevance of artificial skin models.
- Organoids-on-chips, which combine organoids with microfluidic systems, enable precise control of the cellular microenvironment and enhance model complexity and translational potential.
- Despite ongoing challenges in standardization, vascularization, immune integration, and large-scale production, advances in biomaterials and bioengineering continue to drive progress toward clinical and industrial applications.

Background

As the largest organ of the human body, the skin functions as a crucial barrier that shields the body from external environment, safeguarding the body from pathogens and various harmful entities [1, 2]. In addition to its protective role, the skin is also involved in regulating body temperature, minimizing the loss of water and electrolytes, and limiting ultraviolet (UV) radiation absorption [3, 4]. Skin diseases rank the fourth leading cause of global nonfatal diseases, affecting nearly one-third of the world's population. Despite their prevalence and visibility, the impact of skin diseases is often underestimated [5]. Nowadays, increasing evidence have revealed that the skin conditions may serve as external indicators of a range of health issues [6]. Thus, it is highly anticipated to fully

understand the basic mechanisms of skin diseases. Our current understanding of skin diseases mainly depends on the conventional cell monolayers and animal models, which fail to replicate the complex physiological features of human skin, including cellular components, tissue structure, and function [7, 8]. Therefore, there is an urgent need to develop biological or synthetic substitute for human skin, namely biomimetic artificial skin, for wide applications in biological study, disease modeling, drug screening, and cosmetics.

Over the past two decades, organoids and organs-on-chips (OOC) have attracted worldwide attention due to their great potential to replicate the biological features of human organs under both physiological and pathological conditions [9]. By combining cell culture with microfluidics, these platforms

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offer improved recapitulation of the cellular composition, morphology, and function of human tissues, outperforming traditional 2D cultures and animal models [10, 11]. As miniaturized representations of human organ, OOC have been widely applied to model such as human brain, lung, heart, liver, intestine, and reproductive systems. Concurrently, organoids have emerged as 3D, self-assembling microtissues derived from either pluripotent or adult stem cells, representing another innovative approach in the field [12, 13]. Since H. Clevers first established intestinal organoids in 2009 [14], a wide range of organoids have been constructed, faithfully replicating the structural and functional complexities of the respective organ. Of particular, organoids offer powerful tools to investigate developmental processes and cellular dynamics [15], and they hold great promise in narrowing the gap between human physiology and animal models [16, 17]. Together, organoids and OOC are establishing a new paradigm in biomimetic modeling, with broad implications for understanding fundamental biology, elucidating disease mechanisms, and advancing precision medicine and high-throughput drug screening.

To date, there is increasing evidence that organoids and OOC propose versatile platforms to establish biomimetic artificial skin *in vitro* [18, 19]. Benefiting from the advantages of dermal organoids and skin-on-a-chips, current artificial skin models are now able to closely resemble native human skin in terms of cellular composition and structural organization. These developments signify a shift from basic *in vitro* skin models to more sophisticated systems incorporating the epidermis, dermis, and skin appendages, marking a significant milestone in the maturation of this field [20, 21].

This review aims to highlight the development of skin organoids and skin-on-a-chip as robust and versatile platforms for scientific research, with a particular focus on their ability to replicate key biophysical characteristics and diverse applications. In this review, we first provide a brief overview of the structure and functions of the skin, emphasizing its roles in both physiological and pathological conditions. Then, we summarize current strategies and advances in the construction of skin organoids and skin-on-a-chip. In addition, we provide insights into the applications of skin organoids and skin-on-a-chip in regenerative medicine, disease modelling, developmental biology and personalized medicine. Finally, we discuss future opportunities and challenges in this rapidly evolving field and offer perspectives on how these technologies may shape the future of skin research and therapeutic innovation.

Review

The snapshot of skin

Skin comprises three main layers: the epidermis, dermis, and subcutaneous layer (Figure 1). The epidermis is primarily composed of layered keratinocytes, which synthesize keratin, a protective protein [22, 23]. The dermis, located beneath the epidermis, is rich in connective tissues, with collagen being the predominant component, and it encapsulates fibroblasts [24]. Beneath this layer lies the subcutaneous tissue, which is primarily composed of adipocytes. The thickness of these layers varies across different regions of the human body, reflecting their diverse functional demands.

Epidermis

The epidermis is the outermost layer, composed of stratified squamous epithelial cells, primarily keratinocytes, which

undergo a progressive differentiation process as they migrate from the basal layer toward the surface [25]. It consists of four layers: basal (stratum germinativum), squamous (stratum spinosum), granular (stratum granulosum), and cornified (stratum corneum) [26]. The epidermis acts as a protective barrier against external stimuli, prevents water loss [27], and dynamically maintains homeostasis in response to environmental changes [28, 29].

Basement membrane

The basement membrane is a thin layer of extracellular matrix (ECM) that separates and anchors the epidermis to the underlying dermis [30]. Composed of key ECM components such as collagen IV, laminin, fibronectin, and proteoglycans, it provides structural support and regulates essential cellular processes including proliferation, differentiation, and migration [31]. It also plays a critical role in wound healing, tissue repair, and skin homeostasis. Its disruption is implicated in the pathogenesis of several skin diseases.

Dermis

The dermis, located directly the epidermis, is made up of collagen and elastic fibers and contains structures such as hair follicles, sweat, and sebaceous glands, as well as blood and lymph vessels. It is subdivided into two regions: the papillary dermis, which consists of loose connective tissue that forms dermal papillae, and the reticular dermis, which contains denser collagen and elastic fibers. This layer provides the skin with its elasticity, flexibility, and softness [32].

Hypodermis

The subcutaneous tissue, the skin's third layer, lies beneath the dermis and connects the skin to underlying tissues. It contains adipocytes, along with blood vessels and nerves [33]. This layer plays a crucial role in mechanical integrity, thermoregulation, and structural support [34]. In addition, it contributes to keratinocyte and fibroblast proliferation, facilitates wound healing, and supports the vascular supply to hair follicles [35].

Appendages

Skin appendages include hair follicles, sweat glands, and sebaceous glands. Hair serves multiple functions including physical protection, thermal insulation, sensory detection, and social signaling [36]. Sweat glands, which originate from the epidermis and form coiled tubular structures, secrete sweat containing urea, lactic acid, and other compounds that assist in thermoregulation [37]. Sebaceous glands, which are absent on palms, soles, and back of legs, secrete oils and contribute to local homeostasis through metabolic, endocrine, and immune functions [38]. Importantly, stem cells located at the base of hair follicles are essential for skin renewal, metabolism, and the wound healing process [39].

Organoids/organs-on-chips

Organs-on-chips are innovative *in vitro* microscale biomimetic platform capable of replicating the structural and functional characteristics of human organs [40]. Currently, a wide range of chips have been constructed, such as lung-on-chips, gut-on-chips, heart-on-chips and blood-brain barrier models [41]. Various fabrication techniques are employed to construct these microdevices, each with its own advantages and limitations. Soft lithography encompasses a set of microfabrication methods that utilize soft materials such as

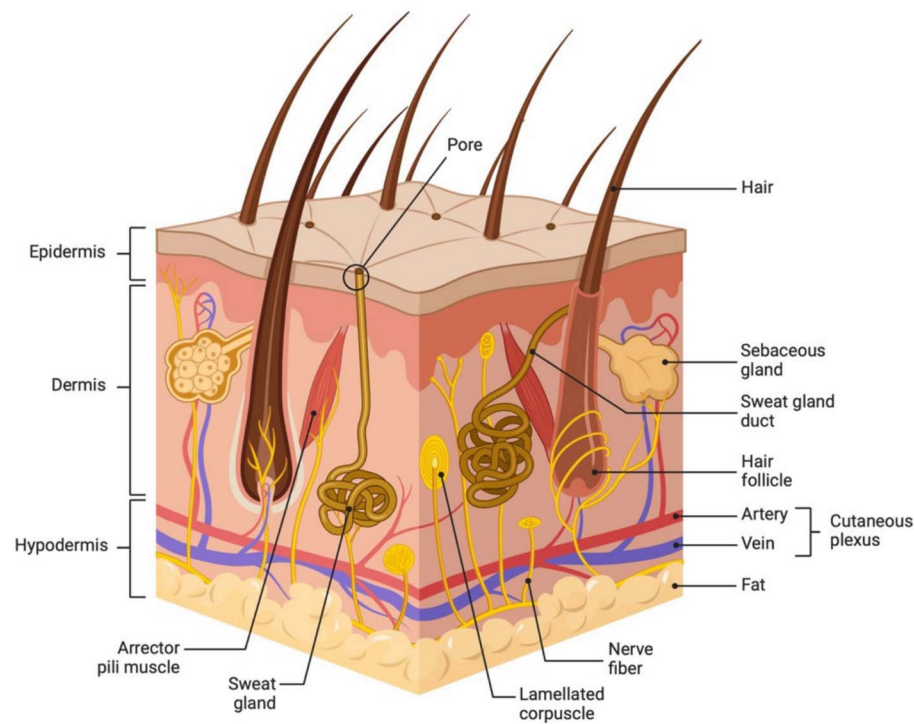


Figure 1. A diagrammatic illustration showing the fundamental structure of human skin, highlighting the various layers and their constituents. (created with [BioRender.com](#))

polydimethylsiloxane (PDMS) for molding and patterning. Due to its ease of processing, rapid prototyping capability, and optical transparency, PDMS has been widely adopted for laboratory-scale applications [42]. However, PDMS is permeable to gases, prone to dehydration, and can absorb small and hydrophobic molecules, limiting its reliability in drug testing where accurate dosing is critical [43]. 3D printing technology is an advanced approach for creating complex microchannel networks that mimic the 3D structure of human organs *in vivo* [44]. The technology enables spatial distribution control by assembling materials layer by layer, and supports both natural and synthetic biomaterials as printing inks. Several printing methods, such as micro-extrusion, inkjet, and laser-assisted techniques, are commonly used [45, 46]. Among these, laser-assisted technology has the highest resolution, while inkjet printing allows cells to be printed alongside their supporting matrix, making it suitable for commercial products and mass production of organ-on-chips [47]. Injection molding mainly use thermoplastics such as polymethyl methacrylate (PMMA), cyclic olefin copolymer (COC) or polycarbonate (PC) [48, 49]. These materials have excellent mechanical properties, optical transparency, and microfluidic compatibility, and are more robust than conventional materials. However, thermoplastics are hard and cannot absorb or permeate other molecules, which limits their application to some extent [50]. Collectively, the selection of method depends on the intended application, balancing complexity, physiological relevance, and feasibility.

Organoids are 3D cell cultures that highly mimic the complex structure and functions of the organs. They are usually derived from adult stem cells (ASCs), and pluripotent stem cells (PSCs) [51, 52]. In 2009, a breakthrough work from Sato et al. showed that single Lgr5-positive adult intestinal stem cells could generate self-organizing 3D intestinal epithelial

organoids in Matrigel. Since then, protocols have been developed to establish human organoids, including mesendoderm (such as liver, pancreas, kidneys, and lungs) and neuroectoderm (including retina and brain), using either ASCs or PSCs. Compared to traditional 2D cell culture, organoids more accurately recapitulate *in vivo* organs in terms of cellular composition, spatial arrangement, and physiological properties and therefore show great potential in biomedical research. Currently, organoids have been applied in developmental biology, disease modeling, drug testing, personalized medicine, and transplantation therapy [52, 53]. For example, colorectal cancer organoids can be used to explore mutational signatures via whole genome sequencing [54], while brain organoids provide insights into neurodevelopment and neurodegenerative diseases [55].

Combining organoids and organ-on-chips enables the creation of more powerful and bionic engineered models, named as organoids-on-chips. Organoids provide highly realistic 3D tissue structures with cellular complexity and functional relevance, while organ-on-chips offer precise control over microenvironmental conditions, such as fluid dynamics, mechanical forces, and nutrient gradients [56]. The integration of these two technologies holds promise for bridging the gap between traditional *in vitro* studies and *in vivo* experiments. This combined approach not only enhances the accuracy of drug screening and toxicity testing but also enables the study of complex disease mechanisms in a controlled environment. For instance, patient-derived organoids-on-chips can be used to mimic individual-specific disease phenotypes and test therapeutic strategies [57]. Despite ongoing challenges such as scalability and long-term maintenance, organoids-on-chips holds immense potential for revolutionizing biomedical research and improving translational outcomes.

Table 1. Overview of present skin-on-a-chip research. Critical aspects of skin-on-a-chip including cell types, chip types, methodology and key findings are listed

Skin-on-a-chip types	Cell types involved	Device material	Manufacturing method	Dermal matrix	Key findings	References
Full-thickness skin-on-a-chip	Living human skin	PDMS	Cellular layering; ALI culture; Dynamic culture system	—	Designed a skin-on-a-chip pumpless microfluidic device	[62]
Epidermis-on-a-chip	Keratinocytes	PMMA	Microfluidic technology; ALI culture	PET	An iEoC with TEER to detect barrier function <i>in situ</i>	[67]
Epidermis-on-a-chip	Keratinocytes, melanocytes	PMMA	Microfluidic technology; ALI culture; Dynamic culture system	PET	A biomimetic EoC system for epidermis recreation and can be used to study skin irritation, permeability, drug safety tests and cosmetic evaluation	[68]
Keratinocyte-sensory neuron skin chip	Sensory neurons and keratinocytes	PDMS	Microfluidic technology; Dynamic culture system	Type I collagen	Development of a 3D microfluidic coculture system with a neuralized epidermal-like layer and the ability to simulate hyperglycemia	[69]
Full-thickness skin-on-a-chip	Keratinocytes, fibroblasts	PDMS	Microfluidic technology; ALI culture; Dynamic culture system	Endogenous dermal FDM	Constructed a 3D full-thickness SoC model which can be used to toxicity and permeation test	[70]
Vascularized HSE	Keratinocytes, fibroblasts, HUVECs	—	Microfluidic technology; ALI culture; Dynamic culture system	Type I collagen	Constructed the human skin equivalent with perfusable vascular channels	[71]
Full-thickness skin-on-a-chip	HaCaT, fibroblasts, HUVECs	PDMS	Layer-by-layer planting and dynamic cultivation	Fibronectin	Constructed skin-on-a-chip device to mimic skin inflammation and edema	[72]
Vascularized skin-on-a-chip	Keratinocytes, fibroblasts, endothelial cells and HUVECs	PDMS	Microfluidic technology; Dynamic culture system	Type I collagen	Development of a microfluidic skin chip with vascular structures and vascular endothelial cells	[73]

PMMA polymethyl methacrylate, PDMS polydimethylsiloxane, ALI air-liquid interface, PET polyethylene terephthalate, EoC epidermis-on-chips, HSE human skin equivalent, HaCaT human immortalized epidermal cells, FDM fibroblast-derived matrix.

Skin-on-a-chip models

Skin-on-a-chip (SoC) are bionic skin models based on microfluidics [58, 59]. Microfluidic platforms facilitate the cultivation of skin by regulating various physical and biochemical factors, including dynamic fluid movement, mechanical stress, and chemical concentration gradients [60–62]. During the development of skin models, the spatial arrangement of cells plays a key role in replicating the structure and function of human skin. Precisely arranging specific cell types is essential for mimicking key processes such as cell migration and cell–cell interactions. For example, interactions between neurons and keratinocytes, or the formation of vascular structures, is highly dependent on this spatial organization [63, 64]. While simple cell mixtures may be sufficient for basic toxicity assessment or drug screening, more complex physiological or pathological processes, such as chronic wound healing or inflammatory responses require structurally rigorous cellular layouts [65, 66]. Advances in 3D bioprinting and microfluidics have improved the

ability to achieve such precision, but these technologies also introduce greater complexity and higher costs. Research designs must therefore carefully balance the need for precise cell distribution, depending on the specific goals of the study. Here, we will describe different types of skin-on-a-chip according to skin structures (Table 1), like epidermis-on-chips, full-thickness SoC, vascularized SoC, and hair follicle chips [67–73].

Epidermis-on-chips

Disruption of epidermal homeostasis is intimately linked to the deterioration of skin health and is linked to the onset of chronic skin conditions and age-related changes, such as psoriasis [74], atopic dermatitis [75], ichthyosis [76], among others. Therefore, detecting and evaluating skin irritants, allergens, drug penetration, and other skin diseases has always been an important topic for epidermal homeostasis. Currently, there have been some reports to establish simplistic human skin models to evaluate barrier characteristics and drug

penetration using skin substitutes (i.e. commercial membranes) [77, 78]. However, these widely used porous membranes (e.g. nitrocellulose filtration membranes) are not ideal biological skin equivalents due to low barrier properties as well as the lack of hair follicles and sebaceous glands [79]. This highlights the growing need for more physiologically relevant, biomimetic skin models *in vitro*.

One widely used approach is the reconstructed human epidermis (RHE), a lab-grown skin tissue model in which human keratinocytes are cultured on an inert polycarbonate membrane [80]. The RHE structure exhibits various layers of both immature and mature keratinocytes, exhibiting noticeable levels of stratification and intercellular cohesion. This model allows for a more comprehensive evaluation of drug permeability and potential irritation within a controlled laboratory environment [81]. In the early 1990s, the first reconstructed human epidermis (RHE) models, EpiSkin® (developed by L'Oreal in Lyon, France) and EpiDerm® (developed by MatTek Corp. in Ashland, MA, USA), emerged and were validated as skin corrosion testing models by the European Centre for the Validation of Alternative Methods [82]. Following the publication of test guidelines by the Organization for Economic Co-operation and Development (OECD) in 2004, these models have been utilized for assessing skin irritancy [83]. In recent years, various RHE models have been developed, such as EST1000, Skinethic, and OS-Rep [84]. RHE models have also proven valuable for studying inflammatory skin diseases like psoriasis and atopic dermatitis (AD), enabling exploration of underlying molecular mechanisms and the development of therapeutic strategies [85]. It aids in the exploration of disease progression and assists in the identification of potential therapeutic agents. Jing Zhang et al. constructed an epidermis-on-chips (EoC) integrating a bubble removal device and a trans-epithelial electrical resistance (TEER) electrode for *in situ* detection of barrier function (Figure 2a) [67]. The model exhibited histological features similar to human epidermal structures and showed a robust barrier function with a TEER value of 3 k Ω cm². It successfully distinguished 10 known irritants and non-irritants, achieving a > 99% blocking rate for small molecules, and was capable of detecting cytokine-induced inflammatory responses. Qiwei Li et al. added a peristaltic pump to the EoC to enable dynamic addition and removal of test samples, expanding the EoC's scope for detecting substances related to pastes and semi-solids (Figure 2b). In addition, this EoC has a melanin simulation function that can be used to detect the whitening effect of cosmetics [68].

The skin is highly sensitive and densely innervated, containing a wide array of nerve endings capable of detecting sensations such as pain, temperature, and touch [86]. As a result, increasing attention is being directed toward integrating neural elements into microfluidic systems to develop more physiologically relevant engineered organ-on-a-chip (EOC) skin models [87]. Given the crucial role of skin-nerve interactions in both the physiology and pathology of the skin, the development of EOC systems that recapitulate the dynamic crosstalk between epidermal keratinocytes and sensory neurons is of great interest. For example, Jinchul Ahn and colleagues combined innervated epidermal keratinocyte layers with sensory nerves, serving as a co-culture model of sensory nerve epidermal keratinocytes [69]. By simulating conditions of elevated blood glucose, it was used to explore the pathophysiological roles of epidermal keratinocytes and

intraepidermal nerve fibers in the onset and progression of hyperglycaemia-induced cutaneous neuropathies. The device effectively replicates the dynamics involved in the growth and maturation of a neuro-innervated epidermal like layer, alongside facilitating the conveyance of local signals from epidermal keratinocytes to sensory neurons (Figure 2c). In conclusion, the recommended model offers a new *in vitro* solution for assessing skin irritation, evaluating drug permeation, and analyzing the effects of cosmetics.

Full-thickness SoC

Generally, the vast majority of current skin models are constructed by cultivating keratinocytes on porous membrane [88]. Although these models offer a consistent standard environment for pharmaceutical trials, they often fail to effectively simulate the interaction between the epidermis and dermis, limiting their broader applicability. Full-thickness skin models are expected to contain the epidermis (e.g. keratinocytes) and dermis layers (e.g. fibroblasts), resembling the epidermal-dermal crosstalk *in vitro* [70, 89]. Coculture systems have demonstrated that fibroblasts enhance the release of growth factors, thereby boosting the growth and specialization of keratinocytes [90]. Additionally, keratinocytes are known to secrete interleukin 1 (IL-1), which stimulates fibroblasts to produce metalloproteinases (MMPs) and keratinocyte growth factor (KGF) [91]. These factors play a crucial role in regulating the survival, multiplication, and specialization of keratinocytes, emphasizing the significance of interaction between the epidermal and dermal layers in determining cellular behavior and functionality [92]. Presently, a variety of commercially produced full-thickness skin models are available, such as EpiDerm-FT by MatTek Corp. in the USA, Phenion-FT by Henkel AG & Co. in Germany, StrataTest by Stratatech in the USA, and AST-2000 by cell systems in Germany [93]. More advanced full-thickness skin models (FTSms) have been created by placing keratinocytes over a dermal layer that includes fibroblasts within a 3D scaffold [94, 95]. It is worth mentioning that two full-thickness models named Phenion FT and Epiderm FT, have been used for different studies in environmental and age-dependent effect, skin penetration, UV irradiation effect, skin metabolism, genotoxicity, wound healing, disease mechanisms, and skin sensitization [96, 97]. Michel Bataillon and his team have created an innovative skin simulation system called T-Skin™, which combines an active epidermal layer formed by primary keratinocytes implanted in type I collagen gel and an active dermal layer implanted with primary fibroblasts. This model stands out due to its capability to replicate the architecture and cellular differentiation of normal human skin, notably displaying the characteristic layered organization, including the basal layer, stratum spinosum, stratum granulosum, and stratum corneum [98]. T-Skin™ offers an interesting and reliable method to evaluate the anti-aging effects of chemicals on the epidermis, the dermal-epidermal junction (DEJ), and the dermis. It has also become a crucial tool for the formulation of new cosmetic products and skin active ingredients.

In comparison to RHE models, full-thickness skin models exhibit superior barrier functionality [99, 100]. Gopu Sriram et al. used dynamic perfusion and microenvironmental control to generate a thicker epidermal layer than the static culture equivalent skin, improving epidermogenesis and morphological differentiation and enhancing barrier

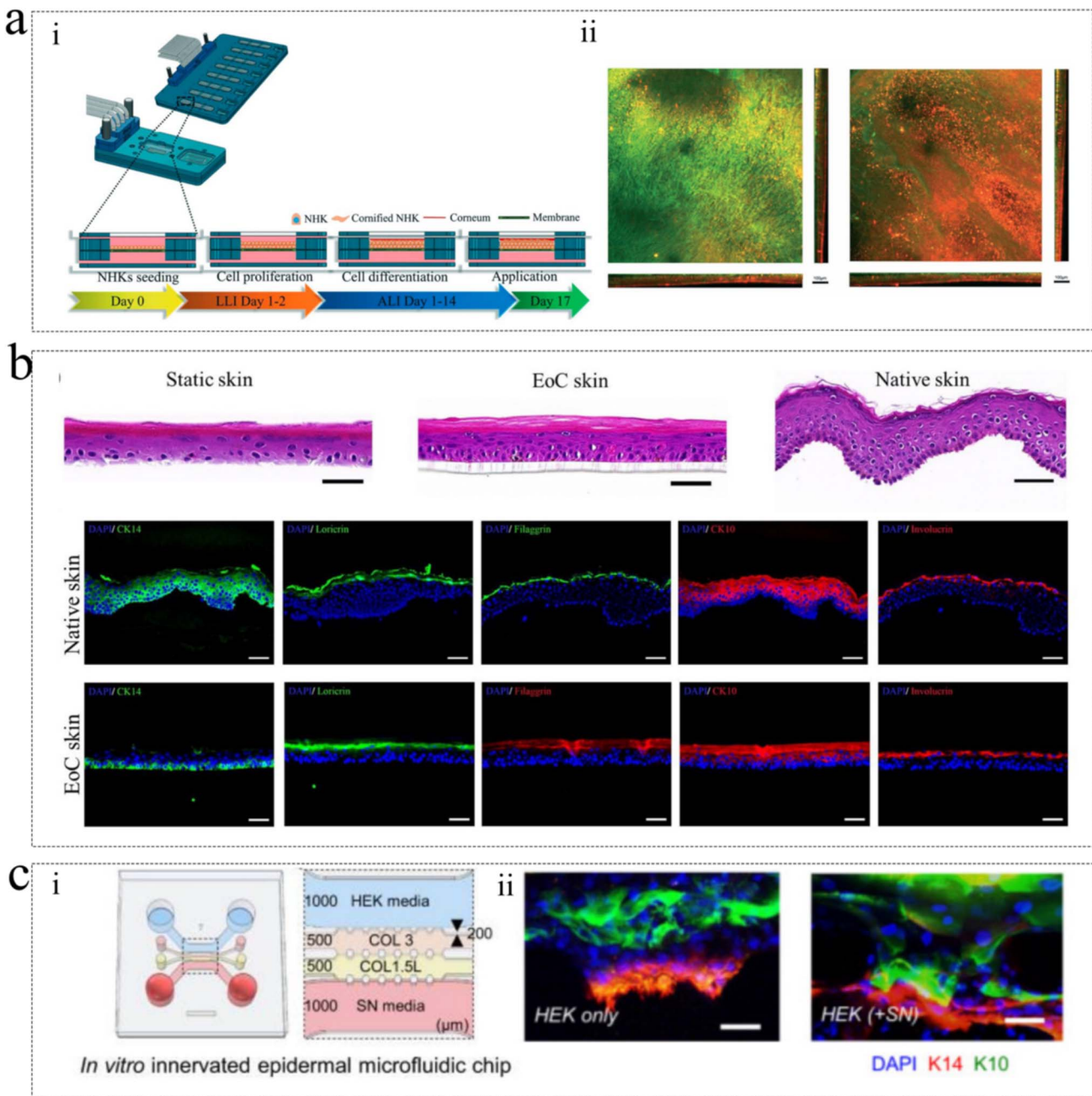


Figure 2. Design and representative results of the multi-chamber chip, neuralized epidermal chip. **(a)** (i) Shows the multi-chamber design of the chip and its overall appearance as an iEOC device and the progression from primary cells to a complete epidermal layer in a multi-chamber culture system. (ii) confocal spectroscopy of epidermal equivalents. Adapted with permission. [67] Copyright 2021, Royal Society of Chemistry. **(b)** Representative images and difference of static skin, EoC skin and human skin after 14 days of *in vitro* maturation. Adapted with permission. [68] Copyright 2023, sage journals. **(c)** (i) schematic diagram of the compartment design of the neuralized epidermal chip. (ii) quantitative comparison of differentiation of epidermal layers containing only HEK cells versus SN neuronal cells. Adapted with permission. [69] Copyright 2023, Springer Nature. *NHK* normal human keratinocytes, *SN* sensory neurons, *HEK* human epidermal keratinocytes, *COL 3* collagen I at 3 mg/ml concentration, *COL 1.5 L* collagen I at 1.5 mg/mL with 10% laminin

capacity [101]. This model utilized a fibrin hydrogel as the dermal matrix, effectively addressing the issue of collagen shrinkage commonly observed in traditional collagen-based static cultures. This design also enabled direct 3D co-culture and facilitated integrated assessments of tissue integrity and permeability (Figure 3a). Freia F. Schmidt et al. constructed an advanced skin model including subcutaneous layer, dermis and epidermis [102]. The subcutaneous layer consisted of a mixture of collagen gel and adipocytes; the dermis was a

mixture of fibroblasts and collagen gel; and the epidermis consisted of keratinocytes (Figure 3b). To overcome the issue of epidermal atrophy frequently seen in standard models, keratinocyte-forming cells were seeded onto a PET membrane positioned between the dermis and epidermis. This modification resulted in higher cell viability, improved prediction of irritant responses, and enhanced resistance to chemical penetration. These findings suggest that the construction of comprehensive, advanced skin-on-a-chip can

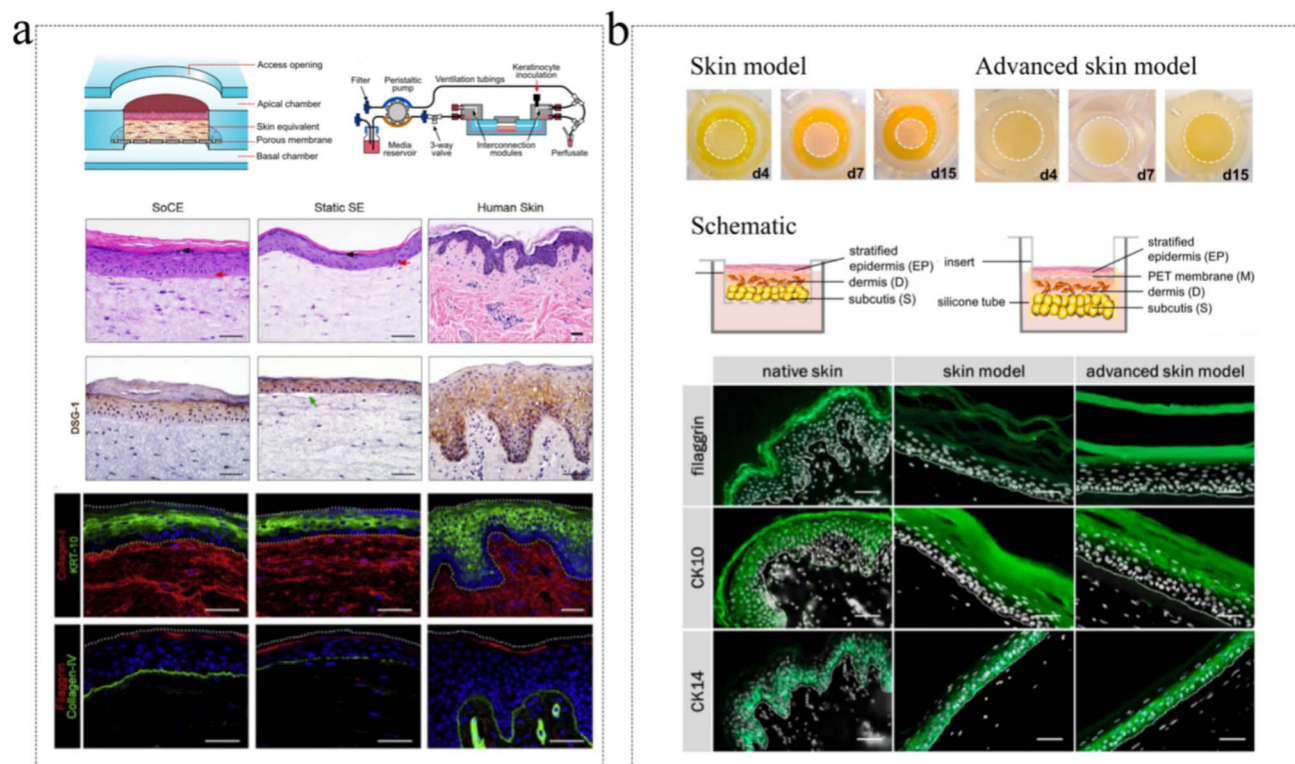


Figure 3. Development and evaluation of a full-thickness skin chip model. (a) The design of a full thickness skin model co-cultured with human fibroblasts and N/TERT keratinocytes and skin culture process and the experimental setup used to perform osmotic testing. Adapted with permission. [101] Copyright 2017, Elsevier. (b) The steps of constructing the skin model as well as further development of an advanced skin model and also shows tissue model after 4, 7, and 15 days. Adapted with permission. [102] Copyright 2020, Frontiers Media S.A. SoCE skin-on-chip equivalent, SE skin equivalents, DSG-1 Desmoglein-1, CK10 Cytokeratin 10, CK14 Cytokeratin 14

help to mimic the human skin barrier and function, thereby offering improved platforms for drug testing and serving as a promising alternative to animal models.

3D bioprinting technology has emerged as a powerful technique for generating skin models with distinct, biomimetic layered architecture. Ramasamy et al. utilized extrusion-based bioprinting to fabricate a dermal layer by depositing a collagen I bio-ink containing human dermal fibroblasts atop a polycaprolactone (PCL)-collagen composite, followed by sequential printing of human keratinocytes to form the epidermal layer [103]. This bioprinted construct demonstrated robust barrier integrity, minimal matrix contraction, and improved reproducibility, cellular viability, and differentiation compared to conventional manual cell seeding methods. In addition, Admane et al. used silk-gelatin bioink to construct full-thickness model that is highly similar to human skin, especially the distinctive wavy pattern at the DEJ. Transcriptomic and proteomic analyses showed that the constructed model has a great similarity to human skin [104]. The model provides a highly promising platform for exploring the complex physiology of the skin, offering new solutions for regenerative medicine and drug testing.

Vascularized SoC

The construction of *in vitro* skin models with vascular channels has attracted great attention, given the essential role that vascular networks play in maintaining skin function [105, 106]. In human skin, blood vessels extend horizontally across the dermis and at the interface between the dermis and subcutaneous tissue [107]. The vascular network of blood

vessels represents a crucial part of the dermis, tasked with delivering oxygen and nutrients to the skin cells [108]. The deepest layer of the skin, known as the hypodermis, consists of vascularized fatty tissue, playing a key role in maintaining body heat and storing fat [109, 110].

Despite the crucial role of the vascular networks in human skin, the majority of *in vitro* skin models lack functional blood vessel networks, failing to replicate the internal blood flow that plays a significant role in the emergence and progression of skin conditions [111]. Meanwhile, conventional skin models are difficult to elucidate some basic mechanisms related to human vascular diseases due to the differences in size and the lack of physiologically relevant fluid dynamics [112, 113]. Over the past decade, advances in bioengineering have enabled the development of vascularized *in vitro* systems. Notably, the emergence of microfluidic organ-on-a-chip (OOC) platforms has significantly accelerated the creation of extracorporeal vascular systems. These platforms offer remarkable flexibility in creating a permissive microenvironment for vascular network formation and maturation. This is achieved through the precise regulation of dynamic flow conditions, 3D hydrogel matrices, cell co-culture systems, and chemical gradient distributions [114, 115]. As a result, microfluidic devices are not only powerful tools for elucidating the fundamental mechanisms of vascular development, but also serve as experimental platforms for modeling vascular diseases and conducting drug screening under physiologically relevant conditions [116, 117].

In order to construct vascular networks, several studies have utilized stem cells or endothelial cells to induce angiogenesis

through exposure to vascular endothelial growth factor (VEGF) within transwell systems [118]. In such models, intradermal capillary networks can be efficiently formed at the base of dermal constructs [119]. However, these static models do not fully reflect physiological conditions, largely due to the absence of dynamic flow, which is critical for functional vasculature. To address this limitation, various strategies have been developed to establish vascularized tissues with perfusion capabilities, mimicking those observed in the native dermis. Recently, Mori's team applied 3D printing technology to construct a skin equivalent model with a perfusable vascular network (Figure 4a). In this model, nylon filaments were used to form hollow channels within a collagen hydrogel matrix populated with dermal fibroblasts. Human umbilical vein endothelial cells (HUVECs) were then seeded onto the inner surfaces of the channels, allowing the formation of functional vascular structures [71]. The dermal and epidermal compartments were composed of fibroblasts and keratinocytes, respectively. This model successfully simulated blood flow by enabling nutrient and oxygen delivery through perfusable channels, thereby enhancing cell density and tissue development. The system proved useful for studying skin biology and drug absorption. Notably, the study demonstrated that vascular perfusion significantly influences the percutaneous absorption of compounds, as evidenced by the diffusion of drugs from the epidermis into the vascular channels. These findings underscore the promising potential of vascularized human skin equivalents (HSEs) on-a-chip in advancing skin tissue engineering and *in vitro* drug testing.

Cultivating skin equivalents on microvascularized substrates has been shown to significantly enhance epidermal stratification and cellular differentiation. Christian and his team investigated the impact of chip design on the formation of microvascular networks by configuring the peripheral channels of the chip in a circular or hexagonal pattern (Figure 4b). A fibrin gel embedded with endothelial cells was injected into these microchannels to promote vascular network formation. Skin equivalents cultured in chips with circular channel designs exhibited superior stratification and more uniform tissue architecture compared to those in hexagonal configurations. These findings highlight the critical role of well-organized vascular networks in supporting balanced tissue development through uniform nutrient and oxygen distribution [120]. To further improve the physiological relevance of the model, pericytes were incorporated into the vascular compartment. Beyond their initial impact on vascular morphology, pericytes also enhanced the integration of the vascularized system with adjacent tissue compartments, contributing to the construction of more complex, physiologically relevant skin models. In this system, the dermal layer was formed by embedding fibroblasts within a collagen matrix positioned above the vascularized gel, while keratinocytes were seeded on top to generate a stratified epidermis. The resulting model is designed to emulate vascularized human skin, making it suitable for studying the effects of systemic drug delivery, as well as investigating how microvascularization influences epidermal differentiation and function.

Hair follicle chips

Human hair follicle is a complex organ that remains difficult to regenerate, significantly limiting our understanding

of the mechanisms of hair follicle development and hair growth [121, 122]. Thus, it is highly desirable to establish biomimetic hair follicle models. OOC technology offers a promising platform for inducing hair growth by enabling precise, dynamic, and spatiotemporal control over the cellular microenvironment, including the distribution of growth factors and signaling molecules [123]. To date, many studies have proposed a series of hair follicle chips, broadening the potential for wide applications. Tatsuto Kageyama et al combined mouse epidermal cells with mouse/human mesenchymal cells in a suspension and allocated them into microwells on a specially designed array plate. They discovered that these self-organized hair follicle germs (ssHFGs) could effectively generate hair follicles and shafts when transplanted under the skin of nude mice. This discovery enabled the production of around 5000 ssHFGs on a large scale using a microwell-array chip fabricated from oxygen-permeable silicone [124]. Abaci et al utilized 3D bioprinting to fabricate a structured microenvironment for culturing fibroblasts, keratinocytes, and dermal papilla cells (DPCs) within a human skin construct [125]. This platform enabled the controlled self-aggregation of DPCs within an extracellular matrix that mimics physiological conditions. The resulting interactions between epidermal and mesenchymal layers facilitated the *in vitro* development of hair follicles from hair stem cells.

Skin organoids

Skin organoids represent advanced 3D stem cell-derived culture systems that replicate the structural and functional complexity of human skin, making them invaluable for studying skin development, regeneration, and disease mechanisms. Compared with skin-on-a-chip models, skin organoids more accurately mimic 3D physiological skin architecture, including the formation of intricate accessory structures such as hair follicles and sweat glands, which are critical for comprehensive skin modeling. However, skin organoids currently face limitations in fully recapitulating vascularization and innervation, which are essential for the enhanced functional maturation [126]. Despite these limitations, skin organoids and skin-on-a-chip platforms serve distinct yet complementary roles in advancing the fields of regenerative medicine, disease modeling, and drug development, collectively driving innovation in skin science. This section aims to underscore key contributions in the field of skin organoids, spanning from the 3D epidermis to accessory organs (Table 2) [127–133]. Selected advancements in this area will be discussed in detail to illustrate their significance and potential applications.

Epidermis and dermis-based organoids

Conventional methods for cultivating human epidermal cells frequently rely on mouse fibroblasts to provide a feeder layer, alongside growth media supplemented with fetal bovine serum or extracts from bovine pituitary glands [127, 134, 135]. These practices introduce undefined elements or foreign substances that could adversely impact clinical safety and add complexity to the analysis of skin disease mechanisms [136]. While 3D cultures, air-liquid interface (ALI) setups, or ex vivo grafting of mouse and human epidermis permit investigation into the impacts of external pathogens, these methods are constrained by a finite duration of viability [137, 138]. However, the reliable human epidermis models

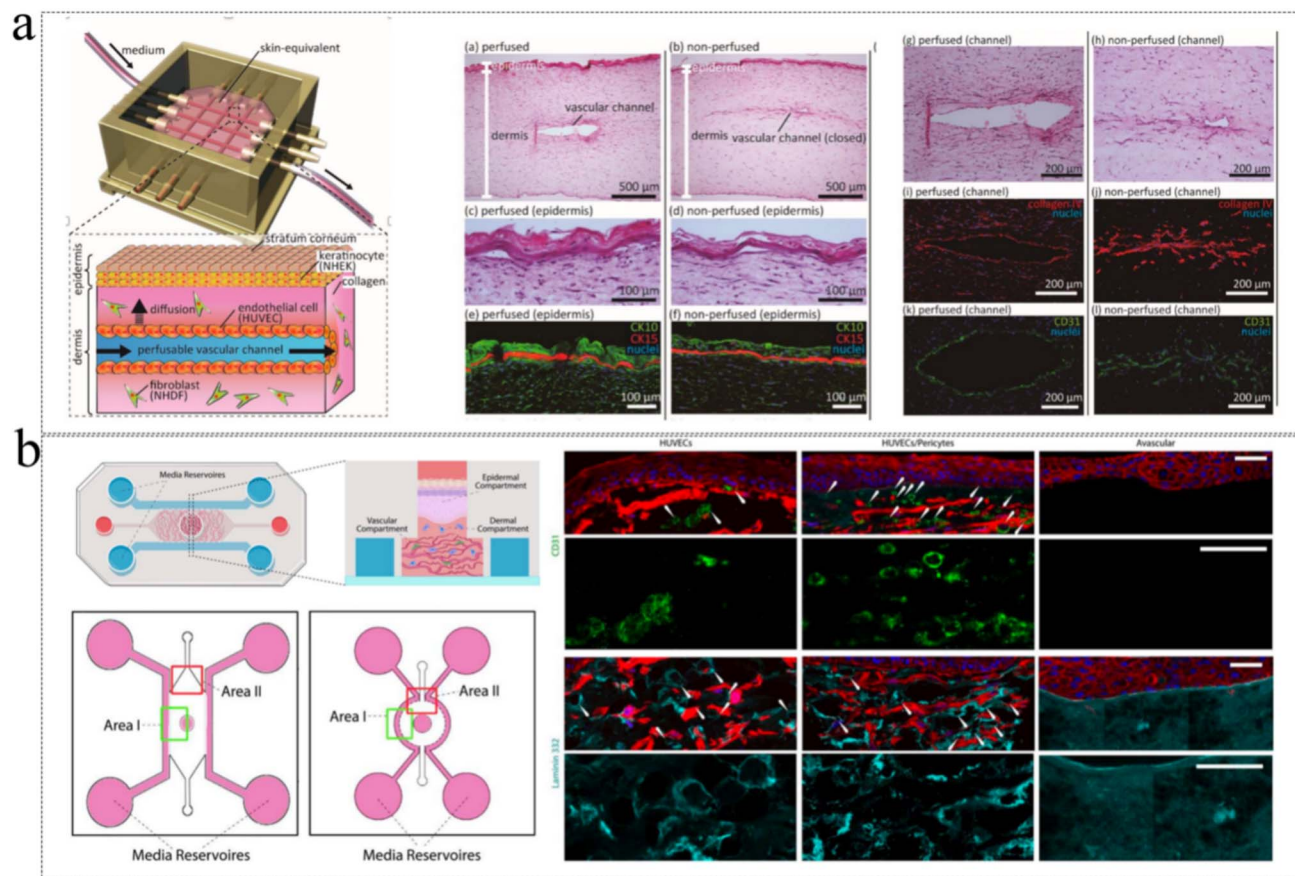


Figure 4. Microvascular skin-on-a-chip model for simulation of human skin structure and barrier function assessment. **(a)** The design and functionality of the skin vascular equivalents and showing the importance of perfusion in development of skin equivalents and their vascular channels. Adapted with permission. [71] Copyright 2016, Elsevier. **(b)** Design and structure of the two different skin vascular skin-on-a-chip model. Adapted with permission. [120] Copyright 2022, Frontiers Media S.A. HUVEC human umbilical vein endothelial cell

based on human epidermal organoids have not yet been reported. Here, we enumerate the current models on epidermal organoids and dermis-based organoids [139–144]. Xuan Wang and colleagues successfully developed human primary epidermal organoids (hPEOs) derived from human interfollicular epidermis in a reproducible and scalable manner [139]. These hPEOs consist of proliferative stem/progenitor cells capable of differentiating into various epidermal lineages, closely mimicking the stratified structure of human epidermis (Figure 5a). Remarkably, hPEOs support simultaneous epidermal proliferation and stratification, making them an ideal platform for studying epidermal homeostasis and cell fate decisions at single-cell resolution.

Traditional *in vitro* skin reconstruction strategies have generally involved the separate differentiation of keratinocytes and fibroblasts from pluripotent stem cells (PSCs), followed by their combination to form bilayered skin equivalents [142]. In a notable example, Itoh et al. (2011) generated induced pluripotent stem cells (iPSCs) from the skin fibroblasts of both healthy individuals and patients with recessive dystrophic epidermolysis bullosa (RDEB). These iPSCs were successfully differentiated into keratinocytes, which were then used to construct 3D skin equivalents. Keratinocytes derived from RDEB patients failed to express type VII collagen, resulting in epidermal blistering and parakeratosis, thereby confirming the disease phenotype *in vitro*. This

work not only validates the regenerative capacity of iPSC-derived keratinocytes but also highlights the potential of gene correction approaches in treating RDEB and related genetic skin disorders [143]. Patricia Ebner-Peking and colleagues engineered skin constructs by co-culturing iPSC-derived fibroblasts, keratinocytes, and endothelial progenitor cells with human platelet lysates (Figure 5b). When implanted into a full-thickness skin wound model in mice, this three-cell-type suspension gave rise to stratified human skin with neovascularization, successfully integrating with the host vasculature [140]. This self-assembly approach offers a novel strategy to generate functional skin organoids with great potential in tissue repair and regeneration.

Recent progress in the study of skin organoid self-organization has highlighted the crucial role of mechanochemical interactions [141, 144]. Earlier models failed to accurately represent the dynamic interplay between mechanical forces and biochemical signals that are vital for skin development and wound healing. Addressing this gap, Mingxing Lei et al. utilized PSC-derived skin organoids cultured in a bioreactor system to simulate physiological mechanical conditions such as stretching and compression [145]. By adding various inhibitors and enhancers, they found that the formation of skin organoids is delicately governed by a synergy of mechanical and chemical factors (Figure 5c). These insights not only deepen our understanding of skin physiology

Table 2. Overview of present skin organoids research. Critical aspects of skin organoids, detailed essential elements are concluded, including the types of stem cells, cell types involved, skin organoids types, methodology and key findings

The types of stem cells	Cell types involved	Skin organoids types	Manufacturing method	Dermal matrix	Key findings	References
ASC	Keratinocytes	3D Epidermal Organoids	Matrix-supported culture	BME	Adult murine epidermal stem cells can be expanded and differentiated effectively in 3D organoid cultures over a long period	[127]
ASC	New born mouse cells:epidermal and dermal cells	3D skin organoids	ALI culture	—	A method for investigating skin development by using specific inhibitors to modify the function of crucial molecules during different stages of skin formation.	[128]
PSC	mouse PSCs, ESCs	Skin organoids with hair follicles, adipocytes and sebaceous glands	Matrigel embedding; Growth factors: EGF, bFGF, EDA; Small Molecule Compound: TGFβ inhibitor(A8301), Forskolin,BMP4.	Matrigel	Skin organoids constructed using mPSC are able to spontaneously generate de novo hair follicles	[129]
ASC	Dermal Papilla Cells	3D hair follicle organoids	Droplet culture	—	3D culture reprograms dermal papilla cells, enabling them to induce de novo human hair follicle growth	[130]
ASC	Sweat gland cells	Sweat gland organoid	Matrigel embedding:ALI culture; Growth factors: EGF, bFGF, EDA.	Matrigel tailored	Sweat gland organoids effectively support rapid cutaneous wound healing, promote sweat gland regeneration, and restore function.	[131]
PSC	hESC	Hair-bearing human skin organoid	Matrigel embedding; Directed differentiation: BMP4, TGFβ inhibitor, FGF.	Matrigel	Construction of self-assembled human skin organoids with hair <i>in vitro</i> that can be used to reconstruct skin <i>in vivo</i>	[132]
PSC	hiPSCs	Atopic dermatitis organoid	ALI culture:Activation of Wnt signaling pathway by GSK3β inhibitor (CHIR99021)	Matrigel	Wnt-activated human skin organoids can be used to simulate <i>Staphylococcus aureus</i> skin colonization and infection	[133]

BME, basement membrane extract; ASC, adult stem cells; PSC, pluripotent stem cells; ESC, embryonic stem cell; 3D, three-dimensional.

but also lay the groundwork to create more accurate skin models for medical research, pharmaceutical testing, and tissue engineering. Looking ahead, a critical next step will be the scaling-up and clinical translation of these models, enabling their use in personalized medicine and advanced therapeutic strategies.

Hair follicles organoids

In human body, most organs including HF comprise multiple cell types. To generate HF organoids, HF epithelial cells and mesenchymal progenitor cells are required for the formation of complex structures [146, 147]. TATSUTO et al. developed a scalable method to prepare hair follicle germs using a flow-focusing microfluidic device (Figure 6a). By channeling a

collagen solution containing epithelial and mesenchymal cells through the device, they produced Janus-structured collagen microspheres. During incubation, cellular traction forces induced microsphere contraction, leading to the formation of dense, cell-rich HF organoids. These organoids exhibited robust hair regeneration when transplanted into nude mice. Compared to conventional methods, this approach allowed high-throughput generation of tissue grafts and improved regenerative efficiency by optimizing flow rates to regulate cell density [148]. In Sitian Xie’s study, hair follicle organoids were successfully generated by the self-assembly of neonatal mouse epidermal and dermal cells. These organoids exhibited intricate structural details, including vesicular upper-segment structures, well-defined lower segments, and distinct hair bulb

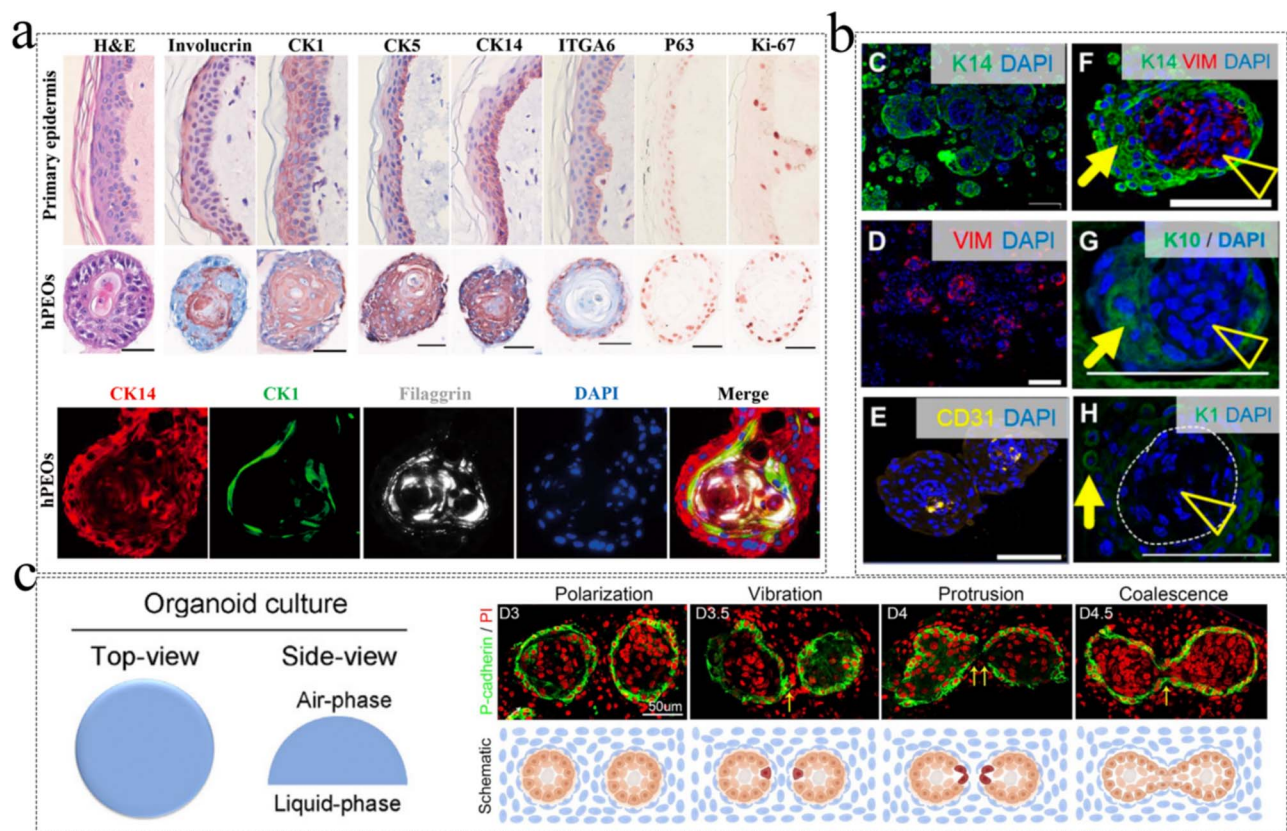


Figure 5. Dynamic processes and intercellular interactions in the construction of skin organoids. (a) Human primary epidermal tissues and epidermal-like organs (hPEOs) derived from human pluripotent stem cells. Adapted with permission. [139] Copyright 2021, Springer Nature. (b) Immunostaining of organoids harvested on Day 6 confirmed the dermal core structure and epidermal stratification properties. [140] Copyright 2021, Ivyspring international Publisher. (c) A top or side perspective view of a skin organoid culture setup and successive stages of organoid self-organization, including polarization, vibration, protrusion, and polymerization. Adapted with permission. [141] Copyright 2023, National Academy of Sciences

formations (Figure 6b). The hair follicle organoid exhibited several distinct compartments and, closely resembling the structure of native anagen hair follicles. Moreover, the expression of molecular proteins in model was consistent with native anagen HF [149]. To explore more details for HF organoid formation, Lei et al. revealed that dissociated neonatal mouse epidermal and dermal cells could self-organize *in vitro* to reconstruct skin following six distinct morphological stages including cell aggregation, polarized vesicle formation, vesicle fusion, planar skin formation, and follicular progenitor induction. When transplanted onto the backs of nude mice, these engineered tissues developed into structurally intact skin, and hair follicles, in which the hair follicles displayed structural and cyclic growth characteristics consistent with those of native hair follicles [128]. Furthermore, the study found that adult mouse skin cells lost their capacity for hair follicle regeneration due to premature differentiation, however, environmental reprogramming successfully restored their self-organizing ability, promoting hair follicle formation. These findings not only enhance the understanding of skin self-organization but also offer innovative strategies for regenerative medicine and hair follicle restoration. More recently, Jiyeon Lee et al. used mouse mPSCs to self-organize into structures that contain both epidermal and dermal layers, autonomously producing hair follicles, sebaceous glands, and adipocytes [129]. These organoids successfully recapitulated HF developmental stages, from primordia formation to bulb maturation, and were

also capable of differentiating into melanocytes and other skin-related cell types, highlighting their strong potential in regenerative medicine (Figure 6c). This technology may one day be applied to treat burn injuries, chronic ulcers, and even for cosmetic skin reconstruction.

Beyond animal models, several studies have reported the successful generation of human skin tissues with hair follicles from human stem cells [130]. Quynh Nhu et al. proposed a planktonic-adherent co-culture system that utilizes human iPSCs to form embryoid bodies in a planktonic environment, followed by the induction of hair follicle development under adherent conditions (Figure 6d). This strategy yielded mature organoids comprising epidermis, dermis, and outer root sheaths, along with functional sensory neural networks, including Schwann cells and sensory neurons, integrated with epidermal and HF progenitor cells [150]. Min-Ji Kim et al. further advanced this field by establishing UV-induced photodamage models in HF organoids. Their system successfully reproduced key features of skin barrier impairment, such as extracellular matrix degradation and inflammatory responses [151]. UV exposure caused structural and degenerative damage to the hair follicles. Notably, when treated with exosomes derived from human umbilical cord blood-derived mesenchymal stem cells (MSCs), these organoids showed reversal of damage and promoted hair follicle regeneration. This model serves not only as a valuable platform for studying disease pathology, but also for personalized therapeutic screening.

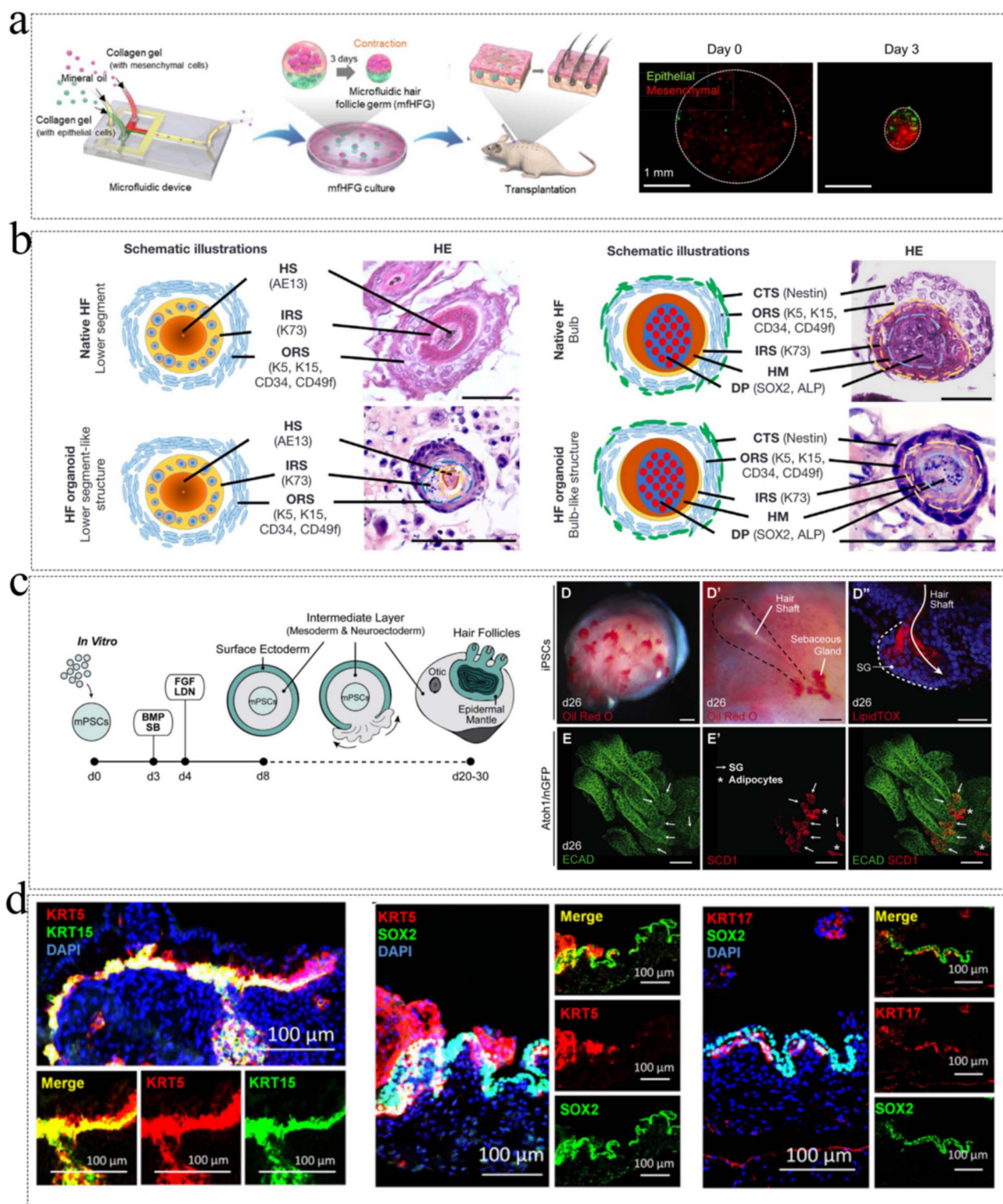


Figure 6. Detailed illustration and histological analysis of hair follicle organoids and native structures. **(a)** microfluidic setup for the generation of hair follicle germs. Adapted with permission. [148] Copyright 2024, American Chemical Society. **(b)** Comparison of hair follicle organoids and native hair follicles, highlighting structural equivalency. Adapted with permission. [149] Copyright 2022, AME Publishing Company. **(c)** The cultivation process of mouse skin organoids and visualization of sebaceous gland-like (SG-like) structures at Day 24 in the organoid model. Adapted with permission. [129] Copyright 2018, Elsevier. **(d)** Immunostaining of skin organoids on Day 100 highlighted high frequency epidermal and outer root sheaths, granular layer, dermal condensate cells, dermal papilla cells, epithelial cells and hair follicles. Adapted with permission. [150] Copyright 2022, The Organoid Society. *HS* hair shaft, *SG* sebaceous gland, *ORS* outer root sheath, *CTS* connective tissue sheath, *IRS* inner root sheath, *HM* hair matrix, *DP* dermal papillae, *SB* SB431542, *LDN* LDN-193189

The construction of hair follicle organoids relies on the self-organizing ability of cells to form multilayered structures, such as outer root sheaths, inner root sheaths, hair shafts, and dermal papillae, and these structures are closely resemble those of natural hair follicles and express relevant molecular markers [146]. Compared to hair follicle chips, hair follicle organoids exhibit higher biological fidelity, making them particularly suitable for studying hair follicle development, cycling, and regeneration mechanisms, as well as exploring the behavior and differentiation of hair follicle stem cells [152]. Importantly, HF organoids can be maintained in long-term cultures, enabling observation of dynamic events during development. However, the construction process of hair follicle organoids is complex, and it remains challenging to precisely control the external microenvironment, such as fluid shear stress and oxygen gradients. In addition, standardization and large-scale manufacturing are still limited, which currently restricts their broader translational applications.

Sweat glands organoids

Similar to other skin elements, sweat glands are derived from epidermal progenitor cells [153, 154]. They share a limited regenerative capability akin to hair follicles when responding to injuries [155, 156]. In a pioneering work, Diao and colleagues pioneered a technique for generating sweat gland organoids (SGOs) from epithelial cells harvested from the sweat glands in the dermal layer of adult mouse paw pads. When these cells were cultured in a Matrigel scaffold, they proliferated into SGOs with significant growth capabilities. Notably, the organoids retained sweat gland stem cells with bipotent differentiation capacity, allowing them to give rise to both sweat gland and epidermal cells [131]. This characteristic holds significant promise for promoting wound repair and enabling the on-site reconstruction of structures akin to sweat glands in the skin.

In a subsequent advancement in 2021, Sun et al. successfully reprogrammed human epidermal keratinocytes into sweat gland (SwG) cells, which closely resembled primary human SwG cells in morphology, gene expression, and function [157]. They further constructed sweat gland organoid that exhibited characteristics highly similar to native sweat glands. Upon transplantation into a mouse skin injury model, these organoids differentiated into functional sweat glands *in vivo*. This advance opens new avenues for repairing damaged skin tissues, offering promising prospects for personalized regenerative therapies in patients with extensive skin loss or sweat gland dysfunction.

Sebaceous glands organoids

Sebaceous glands (SGs) are skin appendages that secrete lipid-rich mixtures, and abnormalities in sebaceous gland function may lead to a range of skin problems such as acne, seborrheic dermatitis, and skin barrier dysfunction. Currently, SG organoids can be induced from PSCs and primary SG ASCs [158]. Feldman et al. isolated Blimp1+ cells from mouse tissue and demonstrated their capacity to form sebaceous gland organoids *in vitro*. Using this model, they identified c-Myc as a central regulator of sebocyte proliferation and differentiation, and successfully established a disease model for acne, enabling the evaluation of current therapeutic agents [159]. Meanwhile, Lee et al. utilized hPSCs to generate skin organoids comprising stratified epidermis, adipocyte-rich dermis, hair follicles, and sebaceous glands [132]. These SGs were

primarily localized in the upper follicular region and exhibited a multilobular morphology, closely resembling adult sebaceous glands. This organoid platform effectively recapitulates human skin development and appendage formation, providing a valuable tool for disease modeling, drug screening, and the development of targeted therapies for sebaceous gland-associated conditions.

Applications of the skin organoids/SoC

Biological basic research

Skin organoids serve as crucial tools for delving into the mysteries of skin formation, supplying a wealth of resources to examine the initial stages of human skin growth and address the shortcomings of traditional research methodologies [160, 161]. The exploration of developmental signaling mechanisms, well-documented in mouse models, remains underexplored in human contexts. Future studies promise to shed light on how microenvironmental factors and interactions between cells influence the maturation of stem and progenitor cells in skin development, particularly in the complex initiation of skin appendages like hair follicles [162, 163]. These processes are regulated by a variety of signaling networks, such as Wnt, FGF, and BMP, which in the initial differentiation of ectodermal and mesenchymal cell lineages [164, 165].

Furthermore, reproducing the regional heterogeneity of human skin within organoid cultures presents a compelling avenue for embryological studies [166]. Human skin varies significantly across different body parts, with variations like the thicker, sweat gland-rich skin of the foot soles compared to the thinner, appendage-free skin of eyelids and lips [167]. Investigating these regional differences through organoid models could significantly advance our comprehension of dermal and subdermal development. Additionally, juxtaposing human skin organoids with those derived from animals, including organoids based on animal models, can unveil critical understanding into the developmental parallels or divergences among species [168, 169]. The creation of hair follicle organoids from both mouse and human iPSCs exemplifies this, offering a rich field for studying the developmental and disease-related characteristics inherent to these organoid types. Such research could provide further data to support or question findings from rodent-based studies, enhancing our grasp of skin organogenesis and its implications for human health.

Despite promising advances, cultivating skin models remains a complex and time-consuming process, requiring meticulous control over mechanical, chemical, and environmental cues to accurately mimic native skin architecture and function. Moreover, manipulation and sampling of these models may lead to alterations in cellular morphology and metabolism, potentially impacting experimental outcomes [170]. Compared with conventional 2D and 3D culture platforms, SoC technologies offer the unique advantage of enabling real-time observation of skin development, stratification, and barrier integrity. One widely used technique is trans-epithelial electrical resistance (TEER), a non-invasive and rapid method to assess the tight junction integrity of epithelial monolayers. TEER measurements involve applying a weak current across a permeable membrane and calculating impedance ($\Omega \cdot \text{cm}^2$) using Ohm's law. The resulting values directly reflect the barrier strength of the cell layer, offering a dynamic assessment of epithelial maturation and tight junction function [171, 172]. Furthermore, N. Van Helleputte

et al. proposed a novel SOC with multifunctional *in situ* monitor system, enabling to monitor metabolic parameters in skin tissue over time. The integrated biosensors can quickly and directly detect dynamic changes in analyte levels, contributing to our deep understanding of epidermal barrier function [173]. Additionally, the design of microfluidics with Raman spectroscopy has been reported, and offers rich information about biological samples. Sriram implemented Raman spectroscopy analyses to assess the hydration and keratin levels in the skin model, indicative of the stratum corneum's status, necessitating the detachment of skin specimens from the chip for examination [101]. In another study, Wufuer analyzed the responses of SOC models during the inflammation and edema processes, based on cytokine levels in fluid collected from the outlet [72]. While current bioanalytical techniques are advancing, the direct incorporation of biosensors into SoC platforms remains an area in need of further development. Nevertheless, it is increasingly evident that next-generation skin organoid and SoC platforms will not only enable dynamic, real-time monitoring of tissue differentiation and function but also serve as powerful tools for studying disease mechanisms, screening pharmaceuticals, and advancing personalized medicine. Disease modeling.

The utilization of skin organoids and SoC technologies has opened new avenues for disease modeling, drug discovery, genotype–phenotype correlation, and the development of condition-specific biobanks for future personalized therapies [174]. Despite the nascent stage of disease modeling within this domain, there are emerging instances where organoid/SOC systems have been deployed for the exploration of both innate and externally induced skin ailments. Recently, skin organoid cultures were shown to mimic genetic skin disorders using patient-derived or gene-edited healthy donor hiPSCs, such as epidermolysis bullosa. These models facilitate to investigate molecular mechanisms underlying skin diseases, and thereby are highly advantageous to identify novel therapeutic targets for diseases or testing drug/gene therapies. Jung et al. initially investigated if the key features of atopic dermatitis could be replicated in ALI skin organoids following infection with *Staphylococcus aureus*. Utilizing a Transwell permeable support in their ALI culture technique, they ensured the cell's basal surface remained immersed in nutrient-rich culture medium, while the top surface was exposed to air, enhancing oxygen supply. Their findings indicated that *S. aureus* was capable of breaching the epidermal layer to reach and infect the underlying dermis, ultimately leading to a compromised epidermal barrier. This process was associated with a surge in inflammatory cytokines originating from both the epidermal and dermal layers [133]. Overall, these changes well recapitulated the phenotype observed in AD patients.

In parallel, SoC platforms have made significant contributions to the study of wound healing, transdermal drug delivery, and inflammatory responses. For instance, Sun and colleagues developed a microfluidic chip for electrically stimulated wound healing (ESWHC) to assess the impacts of both chemical and electrical stimulations on the migration of fibroblasts. In essence, this chip comprises a top chamber that generates an electric field and a bottom chamber designated for fibroblast seeding. The ESWHC offers an *in vitro* environment that closely replicates the *in vivo* conditions of a wound, employing NIH-3 T3 fibroblasts as the model

cells to investigate the healing process's reliance on serum and electrical stimulation [175]. To simulate skin inflammation, Wufuer and their team crafted a multi-layered SOC microdevice that facilitates the co-culture of keratinocytes (forming the epidermal layer), fibroblasts (constituting the dermis), and venous endothelial cells (making up the endothelial layer). By infusing tumor necrosis factor- α (TNF- α), this microsystem successfully mimics critical aspects of vascular lesions in the skin, such as changes in endothelial cytoskeletal actin and the creation of intercellular gaps [72]. Together, these studies have displayed the potential of organoids and chips in skin disease modeling, which can help us to better understand the disease pathology and evaluate potential therapeutic pathways more correlated than animal models.

Skin reconstruction and wound healing

Previous studies have demonstrated that disaggregated human fetal skin cells are capable of forming hair bearing skin when transplanted into immunodeficient mice [176]. Generating hair follicles using hPSCs often requires intricate bio fabrication methods or the formation of chimeric models [177]. These approaches typically involve the xenografting of human and mouse epidermal or dermal cells onto immunodeficient hosts to support skin tissue development. Achieving substantial vascularization and promoting morphological maturation are critical for driving skin organoids toward an adult-like state [178, 179]. Skin organoids generated from PSCs contain intact hair follicle structures and sebaceous glands, which can mimic the dynamics of the hair follicle growth cycle (anagen, regression and resting phases) and provide an ideal model for studying the etiology of alopecia (e.g. follicular degradation, immune attack, and genetic mutation). Of particular, hair follicles generated in organoids exhibit integration ability and functional recovery in the human skin, suggesting the potential in follicular regeneration therapies. Benefiting from the advantages of skin-on-a-chip, they can be applied to study the interactions between different cells during hair follicle regeneration and provide theoretical support for hair follicle transplantation and regeneration therapy [180].

Severe skin injuries, such as burns and extensive wounds, can profoundly impair a patient's quality of life and, in extreme cases, be life-threatening [181, 182]. A variety of engineered strategies have been developed for wound healing [179, 180]. Integrating skin organoids and SoC systems with biomaterials has emerged as a promising approach for enhancing skin repair and tissue regeneration. Mingyang Chang developed a novel automated micro-atomizing device (AMAD) for the efficient delivery of human epidermal organoids. The sprayed epidermal organoids accelerated wound healing, reduced wound area, and promoted epidermal regeneration in a mouse model of full-thickness skin injury. This study demonstrated that the AMAD can be used to apply epidermal organoids to large or irregular wounds in a portable and efficient manner. The therapeutic efficacy can be further enhanced in the future by optimizing the organoid composition, such as incorporating mesenchymal and endothelial cell precursors, providing an innovative strategy for regenerative medicine in severe skin injuries [183]. Pappalardo et al. combined biomaterials with tissue engineering techniques to expand skin tissues uniformly without physical boundaries [184]. This skin model not only approximates natural skin in both morphology and function,

but also enhances the interaction between keratinocytes and fibroblasts, improving the mechanical strength and tissue integration. Additionally, the study explored the effects of different biomaterials on skin growth and regeneration, offering new strategies for personalized skin grafting and chronic wound repair in the future.

Drug screening and testing

For decades, the skin has been used as a gateway for transdermal and local drug delivery, and is also the endpoint of many cosmetics [185]. The availability of reliable skin models is critical for assessing the efficacy and safety of pharmaceutical and cosmetic compounds. Since 2009, the European Union has banned the use of animal testing for cosmetic ingredients [186], significantly increasing the demand for alternative, ethical, and accurate methods for chemical safety evaluations [187]. The fields of pharmacology, toxicology, and cosmetics urgently require reliable, repeatable, and high-throughput alternative methods to replace animal experiments. Skin organoids and SoC platforms, as highly biomimetic human skin models, offer a cost-effective and efficient manner for chemical screening and testing, and are expected to replace ethically complex animal experiments (Figure 7). Abaci et al. created a microfluidic system to facilitate long-term ALI culture of a full thickness human skin model, which composed of epidermis and dermal layers. Based on this system, the toxic effects of doxorubicin on skin have been investigated in terms of cell conditions and tissue structures [188]. In a recent study, Alberti et al. introduced a microfluidic permeation array (μ FPA) to improve upon the Franz diffusion cell, a traditional system for skin permeability testing [189]. The μ FPA enabled simultaneous evaluation of six compounds, with adjustable flow rates simulating cutaneous blood circulation. Its performance was validated by examining the permeation of caffeine, salicylic acid, and testosterone through internally developed full-thickness skin equivalents.

In addition to producing the improved biomimetic skin models, SoC platform combined with embedded microsensors also provides the opportunity to automatically record key diagnostic parameters in real-time, thereby simplifying the drug testing process [190, 191]. Sriram et al. developed a full thickness human chip skin model that integrates a fibrin-derived dermal matrix, enhancing the recapitulation of human skin's architecture and functionality. In contrast to traditional human skin equivalent (HSE) cultures, this apparatus offers enhanced epidermal formation, superior epidermal differentiation, accelerated maturation timelines, and more robust skin junctions in HSEs. Moreover, evaluations of TEER and caffeine permeability tests reveal that the HSEs in the chip showcase superior barrier efficacy. This highlights their capability for economical, large-scale assessments of drug permeability and toxicity [101]. Additionally, Zhang Jing et al. designed an integrated epidermal-on-chip (iEOC) system capable of accurately distinguishing 10 known toxins and non-toxins using MTT-based viability assays, consistent with OECD standards [67]. The preliminary detection of inflammatory cytokines and other biological analysis further demonstrated the varied responses induced by different stimuli. This high-fidelity chip based epidermal inflammation may be a potential substitute for *in vitro* skin irritation assessment.

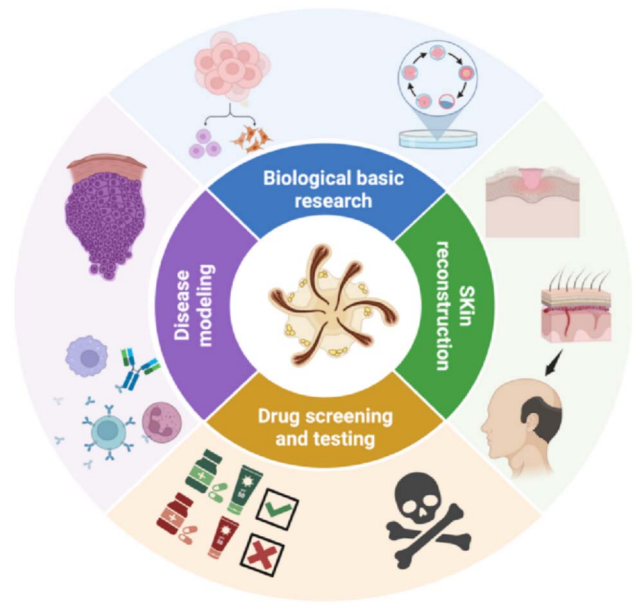


Figure 7. Application of skin organoids/skin-on-a-chip. Skin organoids/skin-on-a-chip are mainly used in (a) biological basic research, (b) disease modeling, (c) skin reconstruction and wound healing, as well as (d) drug screening and testing. (Created with Biorender.com)

In addition to SOC, skin organoids can be applied for drug testing. Xuebo Tong et al. constructed mouse primary epidermal organoids to study their susceptibility to methicillin-resistant *S. aureus* USA3030 infection, a common agent in skin and soft tissue infections [192]. Using this platform, vancomycin was shown to significantly improve cell survival and reduce bacterial penetration in a dose-dependent manner, demonstrating the organoid model's feasibility for antibiotic screening.

Challenges and perspectives

The development and functional maturity of the skin involves the regulation of complex microenvironments, including the formation of extracellular matrix, key signaling pathway, mechanical stresses and reciprocal communication between cells [193, 194]. While current systems hold significant promise for applications in organogenesis, pharmaceutical evaluation, and regenerative medicine, a considerable gap remains between *in vitro* skin organoid development and native human skin physiology. Potential source of skin cells are scarce. The absence of essential cell types makes it challenging to accurately mimic the intricate architecture of natural skin. Moreover, the extended time needed for their differentiation and maturation limits their immediate use in clinical scenarios requiring prompt intervention [195]. Additionally, the constrained size and short lifespan of these organoids curtail their widespread use and availability [196]. Therefore, building a highly biomimetic microenvironment is crucial, including its performance like defined hydrogel, vascular network, immune system, and microbial community dynamics.

Defined hydrogel

The extracellular matrix (ECM) plays a pivotal role in the intricate and dynamic architecture necessary for wound healing and the regeneration of dermal tissues. ECM is a crucial

component of the skin, providing structural support for cells while regulating processes such as proliferation, differentiation, and migration. In the epidermis, ECM is primarily located in the basement membrane, where it plays a key role in the attachment and differentiation of keratinocytes [197]. In the dermis, ECM is mainly composed of type I and III collagen, elastin, and glycosaminoglycans (GAGs), which contribute to the skin's strength, elasticity, and moisture retention [198, 199]. During injury repair, ECM facilitates cell migration and tissue regeneration through dynamic remodeling. However, excessive ECM deposition can result in scarring, such as keloids and hypertrophic scars. Additionally, abnormalities in ECM composition have been linked to various skin diseases, including psoriasis and atopic dermatitis. In recent years, ECM-like biomaterials, such as collagen scaffolds and bio-printed ECM hydrogels, have gained significant attention in skin regenerative medicine, offering new strategies for skin repair and tissue engineering. ECM acts as a physical scaffold, important for maintaining tissue shape and structural integrity [200, 201]. Matrigel, derived from Engelbreth-Holm-Swarm mouse sarcoma cell secretions, serves as a widely utilized culture substrate [202, 203], facilitating the development of skin appendages like hair follicles and sweat glands. However, Matrigel presents notable drawbacks, including batch-to-batch variability, animal-origin components, limited tunability, and challenges in scalability [204, 205], often resulting in organoids with poor reproducibility, variable cellular composition, and uncontrolled morphologies [206]. To address these obstacles, researchers are investigating well-defined substitutes for this complex matrix, focusing on natural, synthetic, or hybrid polymer-based hydrogels (Figure 8a), which have proven highly effective in mimicking skin structures [207]. Hydrogels are particularly advantageous for emulating the natural ECM environment, offering precise control over both biochemical and physical properties through their inherent biocompatibility and adjustable features such as permeability, elasticity, stiffness, and chemical functionality.

Engineered extracellular matrix like biomaterials have been developed to better mimic the microenvironment of natural skin, providing the structural and biochemical signals required for cell growth and tissue maturation. Decellularized ECM (dECM) stand out as 3D, natural biomaterials derived by extracting cellular elements, preserving the inherent structure and components of the ECM [208, 209]. These scaffolds are rich in biologically active molecules, such as proteoglycans, growth factors, cytokines, and adhesive proteins, showcasing excellent biocompatibility, low risk of immune reaction, and straightforward procurement [210, 211]. For example, Kim et al. employed adipose-derived dECM to support adipocyte differentiation in the subcutaneous layer, while tuning the collagen-to-fibrinogen ratio to optimize mechanical properties. Supplementation with VEGF, TGF- β , and other growth factors enhanced angiogenesis and cellular integration, improving the performance of skin models [212]. In the research conducted by Yaling Yu and colleagues, a novel approach was taken to create a covalently crosslinked dermal ECM hydrogel, named dECMMA, utilizing the amide bond formation between methacryloyl groups and amino groups under UV light activation. This innovative dECMMA hydrogel was shown to activate SOX9-positive hair follicle stem cells, encourage hair follicle growth [213], and support scarless healing, while also significantly promoting angiogenesis.

Engineered hydrogels offer greater tunability in terms of mechanical and chemical properties. For example, synthetic or natural hydrogels such as polyethylene glycol (PEG), alginate, hyaluronic acid, etc., can be adjusted in terms of their cross-linking density and the addition of bioactive molecules such as the integrin-binding peptide, RGD, to mimic the stiffness and chemical environment of different tissues. This customization allows researchers to optimize according to specific tissue microenvironments, which has been done with some success in liver, kidney, and brain organoid models [214, 215]. In a study by Justin J Y Tan et al, two engineered hydrogels, PEGDA and GelMA, were used in a co-culture skin model to compare their effects on heterogeneous spheroids of dermal papilla cells and keratinocytes [216]. It was found that PEGDA was difficult to maintain the integrity of the co-culture model due to its low adherence, as evidenced by easy shedding of keratinocytes. In contrast, GelMA hydrogel was able to maintain cell attachment and interactions, especially during prolonged incubation, as evidenced by the stability of the sphere structure and the promoted expression of genes related to hair follicle morphogenesis. Thus, GelMA has significant advantages in mimicking the hair follicle microenvironment and maintaining cellular functionality, and future studies may further optimize GelMA to enhance its potential for in skin engineering.

Despite significant progress, the widespread adoption of biomimetic hydrogels still faces technical challenges. Designing standardized, tunable hydrogels capable of reproducing the complex fibrous ECM remains difficult, particularly at industrial scales [217, 218]. An alternative approach involves simplifying synthetic scaffolds to provide minimal cues, relying on cell-autonomous mechanisms to further elaborate the matrix [219, 220]. These materials must be versatile enough to cater to multiple purposes, accommodating various cell types and applications [221]. Consequently, a more profound investigation and refinement of such biomimetic hydrogels are essential for realizing their potential in efficient cell culture practices and industrial-scale production.

Vascular network

Considering the unavoidable occurrence of central necrosis in organoids as they expand to substantial sizes due to hypoxia, the creation of perfusable vascular networks to mitigate hypoxia and enhance viability in skin models is imperative [222]. Consequently, a variety of approaches have been devised to enhance or expedite vascular development within engineered tissues. Compared to normal skin models, these vascularized skin organoids and chips facilitates the cross-talk among vascular, epidermal, and dermal part [223].

Various *in vitro* methodologies have been developed to address the challenge of integrating perfusable vascular networks into engineered tissues (Figure 8b), including the sparse seeding of cells into scaffolds or hydrogels, the cell sheet technique, the repurposing of biological vascular structures, bioprinting, and the patterning of vascular networks for construct perfusion [73, 224, 225]. In a recent study, Patricia et al. introduced an innovative approach to skin organoid development by harnessing the self-assembly capabilities of proge-nitor-enriched skin fibroblasts, keratinocytes, and endothelial progenitor cells. The self-organization of these FSOs was supported by a complex mixture of human platelet-derived factors, including extracellular vesicles, which facilitated intercellular

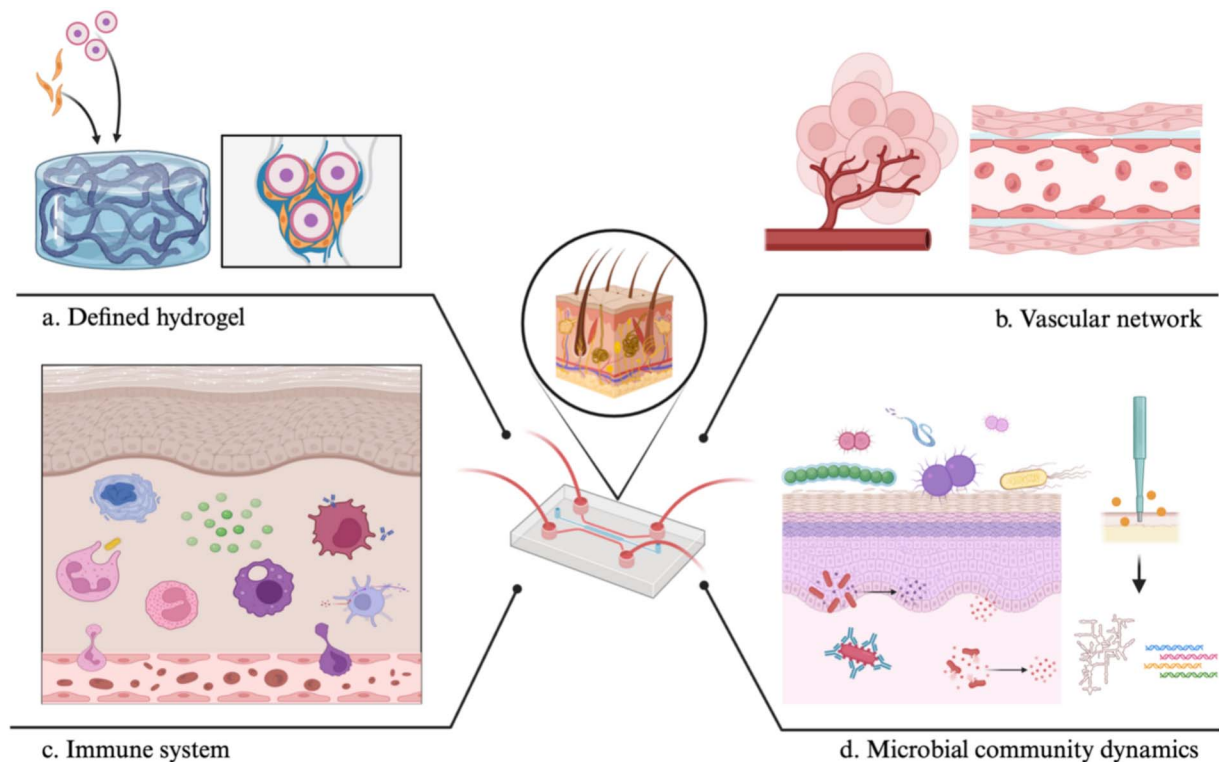


Figure 8. Challenges and prospects for skin organoids and skin-on-a-chip. **(a)** The composition of the hydrogel determines its biocompatibility and tunable properties, providing support for the extracellular matrix. **(b)** Constructing a vascular network prolongs the life span of the culture system and promotes the physiological function of the skin organoids and skin-on-a-chip. **(c)** Incorporation of an immune component facilitates the study of autoimmune dermatological disorders. **(d)** Incorporation of the microbial community mimics the onset of dermatological disorders and changes in permeability. (Created with Biorender.com)

communication and tissue structuring. Importantly, this approach enabled the efficient and scalable generation of FSOs from both adult progenitor cells and hiPSC-derived lineages, representing a significant advancement in the field. These FSOs hold great promise for personalized medicine, particularly in cell type-specific pharmaceutical testing, and offer a reproducible platform for modeling vascularized human skin equivalents.

Additionally, the micro-flow control device offers an effective platform for creating vascular chips *in vitro*, particularly in the context of skin-on-a-chip technologies. Through the manipulation of microflow control mechanisms, hydrogels, and fluid shear forces, a more detailed comprehension of how blood interacts with vessels and skin layers can be achieved. Currently, researchers have made significant progress in vascular regeneration; however, further improvements are needed to enable long-term functionality and to accurately replicate the dynamic variations achievable in high-capacity microfluidic control systems. Although efforts to develop vascularized skin models are underway, this area of research remains in its early stages. The advent of modern tissue engineering technologies has facilitated the creation of vascularized human skin equivalents (vHSEs), marking a promising step toward more physiologically relevant skin models [226]. However, consistently producing vHSEs that boast excellent barrier functions, enduring vasculature, and robust durability is still a formidable challenge. Future studies will focus on enhancing vascularization by integrating microfluidic devices, advanced biomaterials, and other multidisciplinary technologies. Approaches such as cell

self-assembly and bioprinting can be employed to design more efficient vascular networks, ensuring that tissues receive adequate nutrients and oxygen. Microfluidic devices, meanwhile, facilitate angiogenesis and enable personalized drug testing by precisely modulating culture conditions. The application of novel biomaterials is anticipated to further enhance vascular functionality and durability. Moreover, the integration of biomaterials science, microfluidic engineering, and stem cell technology is expected to facilitate the development of fully functional, vascularized organoids and chips. These advanced models will offer more accurate representations of the complex physiology of human skin and hold great promise in areas such as cutaneous angiogenesis, wound healing, and immune system research. Additionally, their compatibility with high-throughput screening and personalized medicine positions them as indispensable tools in disease modeling and regenerative medicine [227, 228]. Achieving this milestone will significantly enhance the utility of organoid technology, making it an indispensable resource for expanding its applications.

Immune system

Skin, being the largest organ, harbors a diverse array of immunocompetent cells that intricately regulate signaling pathways to uphold a delicate balance between immunogenicity and tolerance [229, 230]. This dynamic immune milieu plays a pivotal role in shaping allergic responses and sensitivities. However, the mechanisms governing these pathways have often been overlooked and underutilized in the context of therapeutic development. For instance, autoimmune skin conditions like psoriasis entail complex interplays

between immune and non-immune cells, with underlying mechanisms remaining unclear [231, 232]. Thus, integrating immune elements into skin organoid and chip models holds promise for accelerating discoveries in understanding these mechanisms and devising innovative treatments (Figure 8c). In the realm of skin tumor organoids, co-culturing patient-derived immune cells with the tumor organoid has been utilized. This strategy establishes a more faithful model that accurately reproduces the interplay between tumor cells and the immune system [233]. A notable example is the work by Ramadan and Ting, who developed an immune-competent keratinocyte-on-chip model demonstrating the dynamic crosstalk between the HaCaT keratinocyte cell line and the U937 monocytic cell line within a dual-channel microfluidic system [234]. This model stands out as the sole skin-on-a-chip system documented to incorporate an immune element. The authors highlight its utility for testing substances through the application of LPS and UV stimuli. The impact of these stimuli is evaluated using TEER measurements and a magnetic bead immune assay, both seamlessly integrated within the device.

Up until now, there have been relatively few accounts of *in vitro*, tissue-engineered 3D skin models that incorporate primary cells within microfluidic apparatuses. In the creation of these 3D skins, the matching of cell types to their original donors has often been overlooked in many studies. However, as the complexity of 3D skin models increases, the significance of aligning donors may become more apparent [235]. This is particularly relevant for models incorporating immune cells, such as T cells, where using donor-matched cells may be critical to avoid unexpected inflammatory responses [236, 237]. In the future, replacing various cell types with iPSC-derived counterparts and integrating immune components into skin organoids and chips will be instrumental in advancing our understanding of inflammatory skin diseases and evaluating therapeutic efficacy.

Microbial community dynamics

In recent years, people have increasingly recognized the important role of microbial communities in human health. The human skin hosts a vast community of bacteria, fungi, and viruses, collectively known as the skin microbiota [238]. This microbiome plays a crucial role in reinforcing the skin's barrier function and maintaining its equilibrium [239]. Indeed, research has shown variations in microbiome compositions across different skin areas (Figure 8d), with specific skin disorders being linked to changes in the microbiome [240]. Recent investigations into the microbiome have uncovered that *S. aureus* colonizes 95% of individuals with Atopic Dermatitis (AD). To simulate AD, scientists have applied *S. aureus* directly onto the surface of ALI skin organoids [241, 242]. Structural damage was observed in the epidermal and dermal layers of *S. aureus*-infected models, a hallmark of AD pathology. Researchers have also explored the effects of co-cultured skin microbiota. In particular, by treating a 3D epidermal model with *Cutibacterium acnes* (*C. acnes*) and peroxidized squalene, the system effectively mimics the pathophysiological conditions of acne-prone skin. This model exhibits an increase in inflammatory markers, a reduction in claudin-1 expression, and disruption of epidermal barrier integrity. Within the context of skin-on-a-chip platforms, Gijs Rikken introduced an innovative approach for the safe and controlled application

of topical bacterial cultures to 3D human epidermal equivalents, advancing the study of host-microbe interactions under physiologically relevant conditions [243]. This approach minimizes the risk of infections beneath the surface layer, enabling the *in vitro* examination of infectious and virulent bacterial strains.

While these models serve as a solid starting point for exploring the skin microbiome and related pathologies, incorporating additional microorganisms from the skin and sebaceous glands, along with introducing flow or other mechanical aspects into the model, would provide a more accurate representation of human skin. These advanced skin replicas provide a platform for conducting studies on permeability or for mimicking various diseases triggered by changes in the microbiome [244, 245]. Moreover, the stability of commensal microbial communities is notably influenced by *in vitro* cell culture environments, often resulting in a shift towards aerobic bacterial predominance following the culture duration. Persistent endeavors to refine culture methodologies and conditions to simulate the *in vitro* skin barrier's resemblance more closely to that of authentic skin will likewise impact the dynamics between microbes and epidermal keratinocytes in organotypic model systems. Consequently, this continues to present a significant challenge.

Conclusions

In conclusion, although skin organoid and skin-on-a-chip technologies are still in their early stages, their potential is undeniable. Current models have yet to fully satisfy the diverse requirements of clinical and research applications, but bioengineering techniques offer the potential to optimize the structure and functional fidelity of synthetic skin. Future advancements must address a range of technical and ethical challenges, including the vascularization, innervation, and immune integration, as well as the standardization necessary for personalized medicine and industrial production. Additionally, ethical concerns must extend to reducing the use of animal-derived materials and safeguarding the privacy and ethical rights of patient-derived cells. Future research should prioritize the integration of multidisciplinary technologies to enhance complexity and physiological relevance of these models, thereby facilitating the development of large-scale, bioengineered skin tissues. Through cross-disciplinary collaboration, these technologies are well positioned to evolve into applicable and reliable medical tool, driving innovation in regenerative medicine, drug development, and disease modeling.

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Author contributions

X. Chen and Y. Zhu conceived the study. Y. Huang and X. Wu co-wrote the manuscript. Y. Xu and N. Yang contribute to the graphic visualization. P. Xi and Y. wang contribute to literature search. All authors read and approved the final manuscript.

Conflict of interest

The author(s) declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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