

## REVIEW ARTICLE

# Progress and perspectives on hydrogel-assisted skin organoids

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## Abstract

Skin organoids offer a powerful *in vitro* platform for modeling human skin physiology, disease mechanisms, and regenerative processes. However, faithfully recapitulating the multilayered architecture of skin, diverse appendages, and integrated vascular-neural networks remains a major challenge. As key extracellular matrix mimetics, hydrogels have emerged as central enablers in advancing skin organoid engineering by complementing passive self-organization with programmable biofabrication strategies. This review summarizes recent progress in hydrogel-assisted skin organoid engineering, highlighting how these systems enable the reconstruction of layered skin architectures, support the morphogenesis of skin appendages, and facilitate the integration of vascular and neural components, thereby progressively improving the structural and functional fidelity of skin organoids. These developments position hydrogel-based platforms as essential tools for advancing next-generation skin organoid models. By enabling more precise control over the microenvironment and tissue organization, hydrogel-assisted strategies are expected to accelerate the development of physiologically relevant skin organoids and expand their applications in regenerative medicine, drug discovery, and the study of complex skin disorders.

**Keywords:** Skin organoids; Hydrogels; Skin tissue engineering; 3D bioprinting; Extracellular matrix

## 1. Introduction

The skin is the largest organ of the human body and serves as the primary protective barrier.<sup>1</sup> It consists of three main layers: the epidermis, dermis, and hypodermis. The epidermis is continuously renewed by the proliferation and differentiation of stem cells in the basal layer. The dermis contains abundant fibroblasts that synthesize collagen and elastic fibers, thereby providing mechanical strength and elasticity. The hypodermis is mainly composed of adipose lobules and serves as a cushion, thermal insulator,

and energy reservoir. Skin appendages, including hair follicles, sebaceous glands, and sweat glands, are spatially organized within this layered architecture and supported by dense vascular and neural networks.<sup>2,3</sup> The coordinated organization and interaction of diverse cell types allow the skin to fulfill essential functions such as sensation, barrier maintenance, thermoregulation, immune surveillance, and metabolic secretion.<sup>4</sup> However, this close coupling between structure and function presents major challenges for existing biomedical models. In regenerative medicine, complex wounds such as deep burns and diabetic foot

ulcers are difficult to repair because full-thickness architecture and appendage functions are rarely restored. Consequently, regenerated tissue often lacks sensory and regulatory capacity and is associated with extensive scar formation.<sup>5,6</sup> In drug development and cosmetic testing, species-specific differences in stratum corneum thickness, hair follicle density, and metabolic enzyme profiles frequently reduce the predictive value of preclinical data.<sup>7-9</sup> Therefore, there is a strong demand for *in vitro* models that accurately recapitulate human skin barrier function, immune responses, and drug permeation behavior.

Organoid technology provides a promising route to overcome these challenges. Organoids are three-dimensional (3D), miniature tissues generated *in vitro* from pluripotent or adult stem cells through proliferation, differentiation, and self-organization in defined culture systems.<sup>10</sup> They recapitulate key aspects of native organ microarchitecture and physiological function. Since the first intestinal organoids were established in 2009, this technology has been extended to multiple systems, including the brain, liver, kidney, and skin.<sup>11-15</sup> Early attempts to generate skin organoids primarily exploited the intrinsic developmental potential of stem cells. Under defined biochemical cues, embryoid bodies were formed in suspension culture, partially overcoming the spatial limitations of two-dimensional (2D) systems and enabling a rudimentary simulation of *in vivo* development.<sup>16,17</sup> However, without external physical constraints and microenvironmental guidance, these aggregates—formed mainly through spontaneous cell–cell adhesion—often display unstable layering and disordered spatial orientation. This instability frequently results in uncontrolled contraction and morphological distortion. In addition, as organoids increase in size, limited diffusion of nutrients and oxygen leads to central necrosis, severely restricting their maturation, scalability, and long-term functional stability.<sup>18-20</sup>

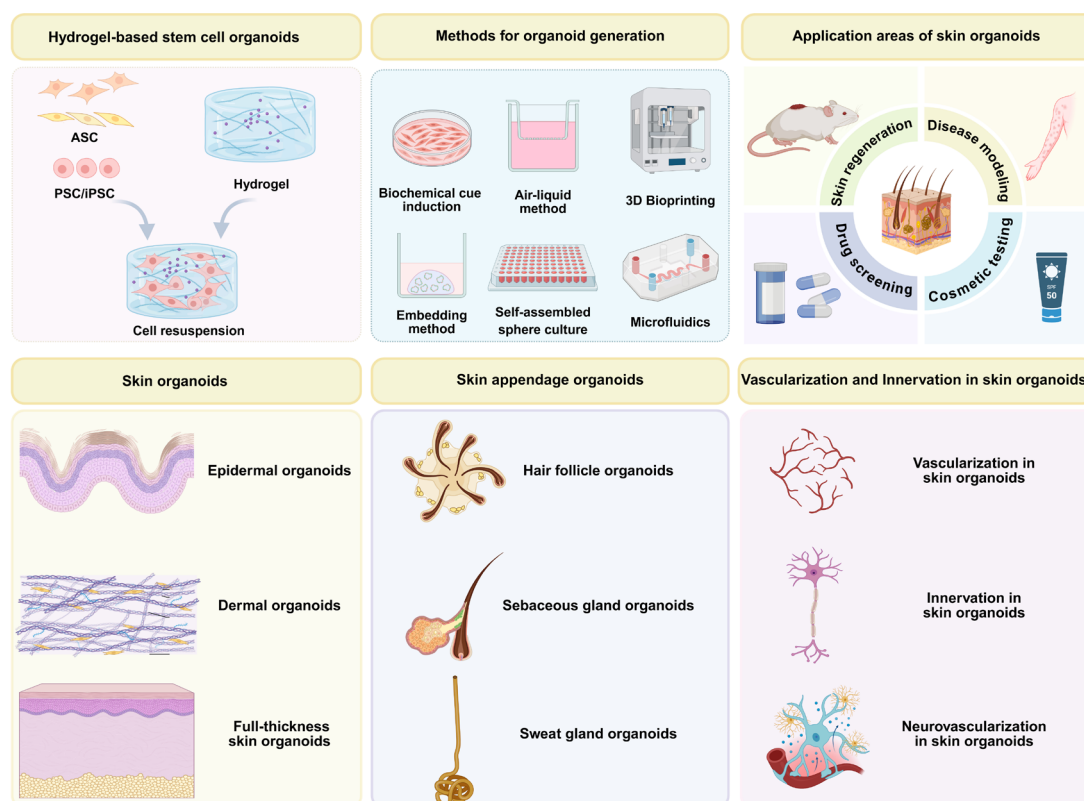
To address these limitations, it is essential to recreate an extracellular matrix (ECM)-like microenvironment *in vitro*. Beyond providing structural support, the ECM regulates cell fate through mechanical cues and biochemical signals.<sup>21</sup> Hydrogels have become a widely used ECM-mimicking platform due to their high water content, porous architecture, favorable biocompatibility, and tunable physicochemical properties.<sup>22-24</sup> Unlike the scaffold-free architectures characteristic of many organotypic models, hydrogel-assisted skin organoids utilize hydrated matrix networks that can actively guide the assembly of skin tissue. Hydrogels can function as adaptable scaffolds with predefined geometries. When integrated with bioprinting or microfluidic technologies, they enable precise spatial control and physical guidance of cells, supporting epidermal–dermal stratification and skin appendage formation.<sup>25-27</sup> In

addition, their porous structure facilitates mass transport and allows space for vascular network incorporation, which may improve the viability of larger organoids.<sup>28</sup> Moreover, ligand presentation and signaling molecule delivery within hydrogels can be rationally engineered to reproduce the biochemical microenvironments of distinct skin regions, thereby directing stem cell differentiation.<sup>29</sup> Together, these features position hydrogels as a key driver in the transition of skin organoid engineering from passive self-organization toward controllable and programmable biofabrication.

Given the structural complexity of the skin, hydrogel-based strategies have been developed to address its distinct anatomical components. For reconstruction of the layered architecture, hydrogel design primarily focuses on biomimicry and mechanical support by reproducing the mechanical gradients and basement membrane–related cues at the epidermal–dermal interface to preserve tissue integrity.<sup>30,31</sup> For skin appendages such as hair follicles and sweat glands, material design shifts toward induction and spatial confinement. By providing defined biochemical signals and localized microenvironments, hydrogels can initiate stem cell–driven morphogenetic processes.<sup>32-34</sup> In the case of microstructures including vascular and neural networks, hydrogels are required to guide and integrate tissue organization. Predefined physical channels combined with biochemical cues can promote vascular perfusion and directed neural ingrowth.<sup>35,36</sup> To clarify these targeted engineering approaches, this article adopts a hierarchical framework that progresses from layered tissue architecture to appendage morphogenesis and ultimately to neurovascular integration supporting organoid function. We review advances in hydrogel-assisted reconstruction of layered and multilayered skin, followed by strategies for inducing complex skin appendages, and conclude with approaches for building vascularized and innervated micronetworks. An overview of hydrogel-assisted strategies for skin organoid construction and their major biomedical applications is summarized in [Figure 1](#).

## 2. Hydrogel-assisted reconstruction of layered skin architecture for organoid engineering

The reconstruction of layered skin architecture represents the structural foundation of skin organoid engineering. Native skin exhibits a highly organized epidermal–dermal hierarchy, in which spatial organization, mechanical support, and cell–matrix interactions collectively govern tissue homeostasis and barrier function.<sup>37</sup> Hydrogel-based systems have emerged as versatile platforms for guiding stratification by providing tunable biochemical and biophysical cues, while advanced biofabrication techniques further enable spatial control beyond spontaneous self-organization. Together, these approaches allow the



**Figure 1.** The schematic illustrates the use of stem cells and hydrogels to construct stratified skin, appendages, and neurovascular networks via various biofabrication technologies, as well as their applications in regenerative medicine, disease modeling, and cosmeceutical evaluation. Image created by the authors.

Abbreviations: ASC: Adipose-derived stem cells; iPSC: Induced pluripotent stem cells; PSC: Pluripotent stem cells.

generation of skin constructs with improved architectural fidelity and reproducibility, forming the basis for more complex functional integration (Figure 2).

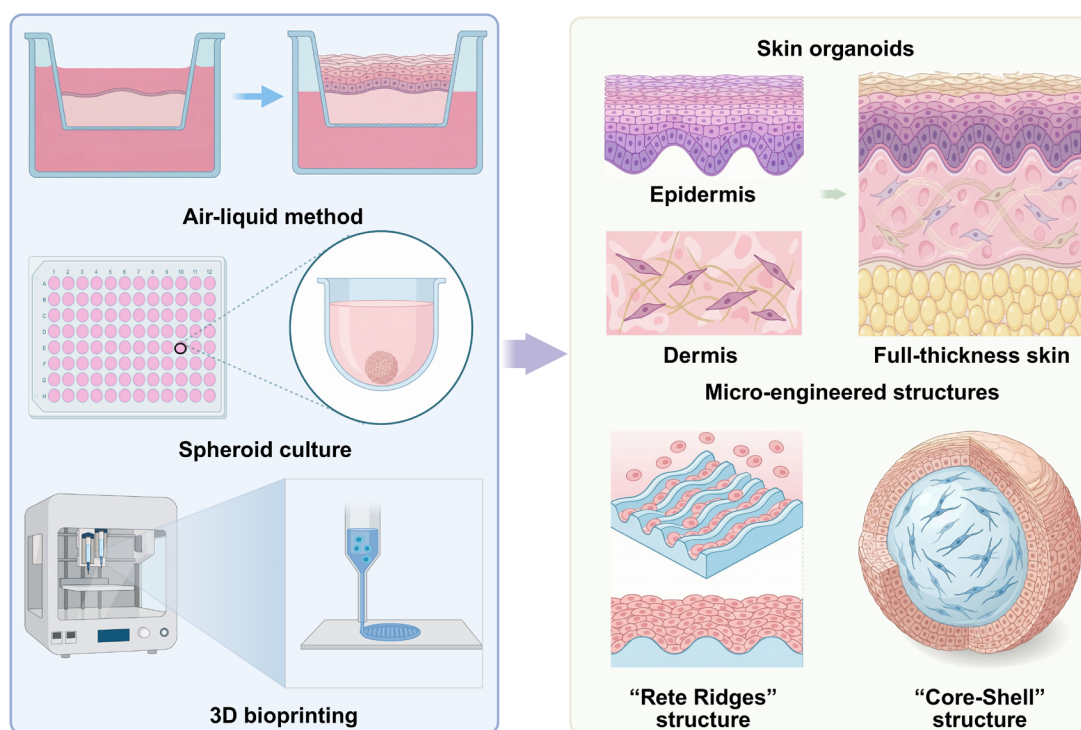
## 2.1. Epidermal reconstruction

The mammalian epidermis is a stratified squamous epithelium composed of basal, spinous, granular, and cornified layers. As the primary barrier against physical, chemical, and microbial insults, its structural integrity and continuous renewal are essential for skin homeostasis.<sup>38</sup> Reconstructing this highly organized architecture *in vitro* is therefore a central goal of skin organoid research.

Here, reconstruction focuses on rebuilding epidermal microenvironments that support skin organoid engineering. Many current approaches rely on basement membrane-derived hydrogels, such as Matrigel, as 3D scaffolds to support epidermal self-organization. Primary keratinocytes or induced pluripotent stem cell (iPSC)-derived epidermal progenitors are encapsulated within these matrices, often in combination with air-liquid interface (ALI) culture, to promote stratified differentiation.<sup>39,40</sup> Using these strategies, epidermal organoids have been generated and shown to exhibit functional barrier properties. For example, Wang

*et al.*<sup>40</sup> established human primary epidermal organoids by embedding foreskin-derived keratinocytes or integrin  $\alpha 6$ -high basal stem cells in Matrigel, enabling long-term expansion and effective modeling of trichophyton rubrum infection. Similarly, Kwak *et al.*<sup>41</sup> generated iPSC-derived epidermal organoids via directed differentiation and 3D culture, and demonstrated that organoid-derived extracellular vesicles promote cell migration, angiogenesis, and wound healing.

Despite these advances, clinical translation remains constrained by the xenogeneic origin and batch variability of animal-derived matrices such as Matrigel, underscoring the need for engineered hydrogel alternatives.<sup>42</sup> Moreover, the lack of immune components, including Langerhans cells, and functional vascular networks limits the ability of current models to recapitulate complex immune responses and to overcome barriers related to graft survival.<sup>43,44</sup> The development of standardized, xeno-free, and vascularized scaffolds combined with multi-lineage co-culture strategies will therefore be critical for bridging epidermal organoid platforms with clinically translatable, full-thickness skin equivalents.



**Figure 2.** Biofabrication strategies, including air-liquid interface, spheroid culture, and 3D bioprinting, enable the reconstruction of stratified skin layers and advanced biomimetic architectures, such as Rete Ridges and core-shell structures. Image created by the authors.

## 2.2. Dermal reconstruction

The dermis serves as the supporting structure of the skin, composed primarily of fibroblasts and their secreted ECM, undertaking critical functions such as mechanical support and dynamic ECM remodeling, as well as providing a developmental microenvironment for epidermal appendages.<sup>45</sup> To accurately reproduce this structure *in vitro*, researchers are dedicated to developing bioactive scaffolds that promote dermal ECM deposition and organization by fibroblasts. Zhou *et al.*<sup>46</sup> adopted a hydrogel system based on the co-assembly of aromatic peptide amphiphiles to construct a dermal model. Although chemically simple, this material provided critical integrin-binding sites; during 14 days of 3D culture, it successfully induced encapsulated human dermal fibroblasts to spontaneously deposit and organize dense networks of fibronectin and type I collagen. More importantly, this system did not induce differentiation into myofibroblasts during gel contraction, demonstrating that precise molecular design can drive dermal fibroblasts to complete near-physiological matrix reconstruction *in vitro* without the need for complex growth factors.<sup>46</sup> Additionally, studies have reported that recombinant human collagen can promote fibroblast migration and upregulate the expression of various repair-related growth factors; dermal equivalents constructed via transglutaminase crosslinking further exhibited excellent

repair efficacy in murine full-thickness defect models.<sup>47</sup>

Beyond the biological activity of chemical components, the physical microstructure and mechanical properties of the scaffold play a decisive role in determining dermal fibroblast phenotype and fibrotic outcomes. Addressing the issue where increased stiffness in traditional bulk hydrogels often leads to fibrosis risks, investigators utilized 3D printing technology to fabricate gelatin methacryloyl (GelMA) scaffolds with controllable pore size and porosity. Studies have found that while increasing the bulk stiffness of the matrix material typically activates the fibroblast-to-myofibroblast transition and leads to the upregulation of fibrosis-related proteins, the porous microarchitecture introduced by 3D printing can effectively antagonize this mechanically induced effect. This porous architecture not only supported healthy fibroblast physiological homeostasis and spatial distribution but also significantly suppressed the activation of fibrotic phenotypes, providing a key structural strategy for designing dermal equivalents that combine mechanical manipulability with scarless healing potential.<sup>48</sup>

## 2.3. Full-thickness skin reconstruction

Following the successful *in vitro* construction of isolated epidermal and dermal tissues, the subsequent goal is to integrate these diverse tissues into anatomically complete skin models. Native skin is not merely a combination

of epidermis and dermis but a complex entity integrated with the hypodermis, a layer critical for maintaining morphology and wound healing that was frequently overlooked in early ALI models.<sup>49-51</sup> Moreover, constructing multilayered models is not simply a matter of cell stacking; it faces challenges such as dermal matrix contraction, insufficient interlayer adhesion, and a lack of biomimetic microstructures. Consequently, current research focuses on two primary dimensions: utilizing biofabrication to incorporate the hypodermis to perfect the anatomical structure, and optimizing hydrogel materials to enhance the mechanical stability and physiological fidelity of the dermis-epidermis unit.

Addressing anatomical integrity, 3D bioprinting offers the potential to include the hypodermis, which is often omitted due to technical difficulties. Researchers have begun constructing complex “hypodermis-dermis-epidermis” tri-layer structures. For instance, Jorgensen *et al.*<sup>52</sup> utilized different cell populations suspended in fibrinogen-composite bioinks to successfully print a construct comprising a basal hypodermal layer of pre-adipocytes, an intermediate dermal layer of fibroblasts, and a superficial epidermal layer of keratinocytes and melanocytes. This full-thickness design not only anatomically approximated native tissue but also demonstrated accelerated re-epithelialization and significantly reduced wound contraction upon transplantation, supporting the synergistic role of the hypodermis-dermis-epidermis hierarchy in simulating native repair mechanisms.<sup>52</sup>

Mere layering is insufficient to build high-quality skin models; instead, it is the stability of the basic dermis-epidermis unit that determines the success of the multilayer model.<sup>53</sup> Traditional type I collagen gels are prone to excessive contraction during fibroblast culture, leading to deformation. To address this, Li *et al.* have mechanically optimized hydrogel matrices. For example, a composite hydrogel system composed of GelMA, hyaluronic acid methacrylate (HAMA), and type I collagen was developed. By adjusting the GelMA concentration to optimize the elastic modulus to approximately 2.27 kPa, which served as an effective *in vitro* engineering optimum for this specific material system. At this optimized stiffness, the matrix could accommodate fibroblast growth and spreading while effectively resisting cell-generated contraction forces. Seeding keratinocytes onto this stable dermal base, followed by ALI culture, successfully established a full-thickness skin model with clear basal, spinous, granular, and cornified layers that maintained long-term structural integrity.<sup>54</sup> Additionally, Bacakova *et al.*<sup>55</sup> adopted a “soft-hard” strategy, using nanofibrous poly-L-lactic acid membranes for mechanical strength and collagen for bioactivity. This not only promoted dermal fibroblast adhesion and migration but also induced the formation

of a highly mitotically active basal layer in the epidermis, providing a more robust graft for full-thickness injury repair.<sup>55</sup>

Furthermore, to further enhance the biomimetic depth of multilayer models, research attention has deepened into the micro-topographical reconstruction of the dermal-epidermal junction. Native skin utilizes undulated Rete Ridges to enhance interlayer adhesion and maintain the stem cell microenvironment, a feature that simple planar stacking cannot replicate.<sup>56,57</sup> Specifically, researchers employed micromolding to construct GelMA-PEGDA hydrogels with Rete Ridge microstructures. For example, studies utilizing UV-curing 3D printers to fabricate resin molds replicated undulated micropatterns onto scaffolds, identifying an optimal ratio of 10% GelMA and 2% PEGDA. Experiments showed that this formulation offered excellent structural support and appropriate degradation rates, supporting the co-culture of human keratinocytes and fibroblasts on the micropatterned surface to construct an epidermis with biomimetic undulated morphology, which also significantly promoted wound healing *in vivo*.<sup>58</sup> Building on this, to further optimize cell adhesion and development, another study grafted acrylated Arg-Gly-Asp peptides onto the hydrogel surface. RNA sequencing and immunofluorescence analysis confirmed that this interface design, combining physical microstructure with biochemical modification, significantly upregulated genes associated with basement membrane formation and epidermal stem cell maintenance, effectively simulating the native stem cell niche.<sup>59</sup> This means that future full-thickness skin construction requires not only macroscopic layering but also the synchronous introduction of such dual physical and biochemical biomimetic designs at the microscopic interface.

Finally, beyond traditional planar layering, an innovative “core-shell” strategy has been proposed: utilizing rotating bioreactors or microfluidics to construct spheroid organoids with an inner “core” of dermal fibroblasts and collagen matrix, encapsulated by an outer “shell” of keratinocytes. This structure ingeniously mimics the spatial relationship of the dermis supporting the epidermis at the microscale and successfully establishes mature epidermal barrier function. This hydrogel-based spheroid multilayer model provides an efficient, scalable, and novel geometric paradigm for large-scale skin physiological research and drug testing, serving as an alternative to planar stacking.<sup>60</sup>

Notably, many full-thickness platforms discussed here are engineered constructs that inform the design of next-generation skin organoids. Despite substantial progress in reconstructing anatomical organization and physical stability through advanced biofabrication and material optimization, native skin function transcends simple layered architecture. As a highly synergistic ecosystem,

skin perception, thermoregulation, and homeostasis rely heavily on the physiological activities of appendages like hair follicles, sweat glands, and sebaceous glands, as well as dynamic neurovascular support.<sup>61,62</sup> Most current multilayer models resolve the “layering” issue but still lack the organic “inside-out” integration of these key components.<sup>63</sup> Therefore, establishing stable multilayer scaffolds that further introduce and integrate these functional units to construct active, sensing, and regulating skin systems represents the next critical threshold for translating skin organoid research from the laboratory to clinical application.

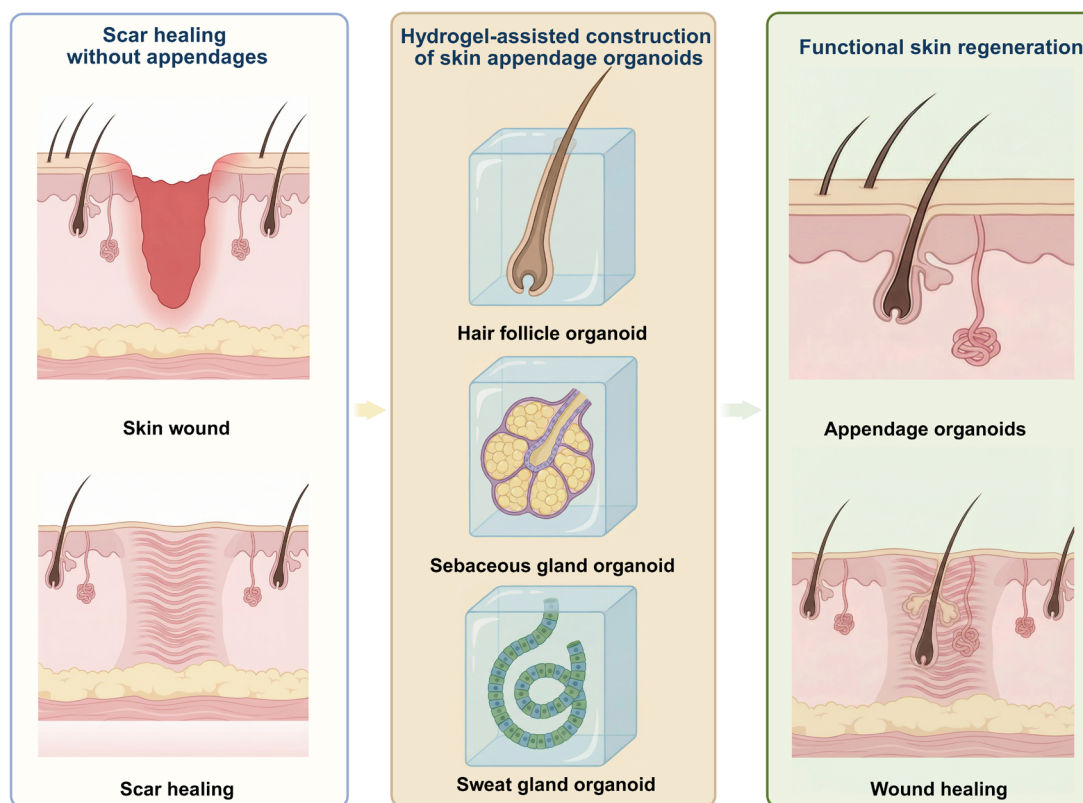
### 3. Hydrogel-assisted engineering of skin appendages

Beyond reconstructing layered skin structures, the incorporation of skin appendages is essential for achieving functional skin regeneration. Skin appendages play indispensable roles in thermoregulation, barrier maintenance, and tissue homeostasis, yet are largely absent in conventional wound healing outcomes. Hydrogel-based organoid systems provide a permissive microenvironment for recapitulating appendage morphogenesis by supporting

epithelial–mesenchymal interactions and lineage-specific differentiation. Recent advances highlight how engineered hydrogels can facilitate the formation and integration of skin appendage organoids, thereby enhancing the functional completeness of regenerated skin (Figure 3).

#### 3.1. Hair follicle organoids

Hair follicles are essential skin appendages whose organoid engineering critically depends on reciprocal interactions between dermal papilla cells (DPCs) and epithelial cells within a specialized microenvironment. Central to this process is the recapitulation of epithelial–mesenchymal interactions observed *in vivo*.<sup>64</sup> As 3D scaffolds that mimic the physical and biochemical properties of the ECM, hydrogels play an indispensable role in hair follicle engineering.<sup>65</sup> To date, a range of natural and synthetic hydrogel systems has been employed, with type I collagen and Matrigel remaining the most widely used foundational materials. Kageyama *et al.*<sup>66</sup> showed that both type I collagen and Matrigel provide permissive microenvironments for hair follicle morphogenesis, supporting the formation of follicle-like structures and enabling *in vitro* evaluation of hair growth–promoting agents such as minoxidil. Building on these systems, Abreu *et al.*<sup>67</sup> introduced microscopy-guided



**Figure 3.** Facilitating functional skin regeneration by synthesizing hair follicle, sebaceous gland, and sweat gland organoids within hydrogel scaffolds, thereby overcoming the structural deficiencies of conventional scar healing. Image created by the authors.

laser ablation to generate microscale subcompartments within collagen matrices, allowing precise spatial control over the aggregation and reorganization of DPCs and keratinocytes.

To better capture the complexity of native skin and enhance cellular function, composite hydrogels have increasingly been explored. Gupta *et al.*<sup>68</sup> developed a silk fibroin–gelatin composite hydrogel to encapsulate human DPCs, keratinocytes, and stem cells. This system exhibited follicle-like architecture and recreated hypoxic niche conditions, resulting in enhanced expression of DPC-specific genes and increased ECM production. In parallel, fibrin- and polysaccharide-based hydrogels have also been widely applied. Chen *et al.*<sup>69</sup> reported that skin-derived precursor cells (SKPs) embedded in fibrin hydrogels maintained stemness and proliferative capacity and induced hair regeneration after transplantation without teratoma formation. Similarly, Lin *et al.*<sup>70</sup> employed a layer-by-layer self-assembly strategy to construct nanoscale ECM-like coatings of alginate and gelatin on DPC surfaces, preserving bioactivity while enabling efficient cell encapsulation. *In vivo* implantation confirmed robust hair follicle regeneration, highlighting the potential of this approach for alopecia treatment.<sup>70</sup>

Advances in tissue engineering have further enabled hydrogels to support scalable fabrication and structural optimization of hair follicle organoids. Sugiyama *et al.*<sup>71</sup> developed a microfluidic platform to generate bilayer collagen microbeads containing mesenchymal and epithelial cells, which spontaneously contracted into hair germ-like structures and exhibited strong hair regenerative capacity after transplantation. Addressing the issue of directional hair growth, Nanmo *et al.*<sup>72</sup> employed 3D bioprinting to fabricate millimeter-scale, spatially ordered hair follicle germ-like grafts, and found that collagen microdroplets encapsulating mesenchymal stem cells (MSCs) and epidermal stem cells were deposited in defined spatial arrangements, enabling newly formed hair shafts to grow along predefined orientations following implantation in nude mice.

Despite substantial progress in hair follicle organoid engineering, efficient and minimally invasive delivery of fragile organoids remains a major barrier to clinical translation. Zheng *et al.*<sup>73</sup> addressed this barrier by developing GelMA-based cryomicroneedles capable of delivering hair follicle organoids directly into the skin. These microneedles penetrate the epidermis and subsequently dissolve *in situ*, releasing viable organoids without residual polymer debris. Organoids delivered via this platform retained high viability, self-organization, and differentiation capacity, and successfully induced the formation of biomimetic hair clusters *in vivo*. This strategy highlights the potential of combining hydrogel-based

organoids with minimally invasive delivery technologies for clinical hair regeneration.<sup>73</sup>

### 3.2. Sebaceous gland organoids

Sebaceous glands are critical skin appendages that play an indispensable role in barrier maintenance, lubrication, and immune defense.<sup>74</sup> Recently, researchers have leveraged hydrogel-based 3D microenvironments, particularly Matrigel, to overcome the limitations of traditional cultures. By mimicking the *in vivo* basement membrane niche, these systems enable organoid formation with gland-like architecture and functional recapitulation of sebaceous gland organoids, providing a robust platform for investigating acne pathology and screening therapeutics.

To reconstruct a “semi-*vivo*” microenvironment, Yoshida *et al.*<sup>75</sup> utilized Matrigel as both a scaffold and overlay to establish a 3D organoid model using the SZ95 human sebaceous gland cell line. In contrast to 2D monolayer cultures, Matrigel-embedded cells self-assembled into organoids characterized by distinct basement membranes and complex “gland-in-gland” architectures. Notably, this model elucidated the regulatory mechanisms of the inflammatory microenvironment; specifically, the pro-inflammatory mediator prostaglandin E2 was found to significantly activate the canonical Wnt/ $\beta$ -catenin signaling pathway within the 3D context, thereby driving sebocyte proliferation and lipid synthesis, confirming the unique capacity of hydrogel systems to recapitulate *in vivo* signal transduction.<sup>75</sup>

To enhance clinical relevance and address ethnic disparities, Liu *et al.*<sup>76</sup> developed and characterized a novel Asian-derived immortalized sebaceous gland cell line (XL-i-20) and explored its potential for organoid generation in Matrigel. The study demonstrated that XL-i-20 organoids maintained in Matrigel retained the androgen receptor expression and lipid synthesis functions of primary cells while exhibiting high sensitivity to photodynamic therapy (ALA-PDT). Experimental data confirmed that this 3D model effectively simulated the clinical inhibition of sebum secretion and mTOR signaling by ALA-PDT, underscoring the value of combining specific cell sources with hydrogel scaffolds for personalized drug screening and mechanistic studies of acne treatment.<sup>76</sup>

Furthermore, addressing the stem cell regulation central to sebaceous gland regeneration, Feldman *et al.*<sup>77</sup> established a murine organoid system derived from Blimp1+ progenitor cells using Matrigel supplemented with a defined growth factor cocktail. High-precision lipidomics revealed that only organoids differentiated under 3D hydrogel conditions synthesized a specific lipid profile highly consistent with native sebaceous glands. Interestingly, this model identified c-Myc as a pivotal

transcription factor regulating organoid proliferation and differentiation. Collectively, these findings indicate that hydrogel-based 3D sebaceous gland organoids not only bridge the physiological gap left by planar cultures but also offer a standardized experimental paradigm for elucidating lipid metabolic networks and advancing regenerative therapies.

### 3.3. Sweat gland organoids

Sweat glands are essential skin appendages responsible for thermoregulation and metabolic waste excretion.<sup>78</sup> A central limitation in sweat gland organoid engineering lies in maintaining lineage-specific cellular phenotypes *in vitro*. Previous studies have indicated that isolated sweat gland stem/progenitor cells tend to rapidly transition toward a keratinocyte-like phenotype under conventional 2D monolayer culture conditions, accompanied by the loss of sweat gland-specific phenotypic characteristics.<sup>79</sup> To re-establish the physical and biochemical niche of the ECM, Diao *et al.*<sup>80</sup> employed hydrogel-based matrices such as Matrigel to enable 3D expansion and phenotypic maintenance of sweat gland epithelial cells. They showed that isolated murine sweat gland cells embedded in Matrigel, in the presence of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and bone morphogenetic protein 4 (BMP4), could self-organize into glandular organoids. This 3D hydrogel microenvironment effectively prevented dedifferentiation while preserving bidirectional differentiation potential toward both sweat gland and epidermal lineages.<sup>80</sup> Similarly, for human sweat gland cells, hydrogel-based hanging-drop culture systems were shown to retain key phenotypic markers and exhibit physiological responses to cholinergic stimulation, providing a reliable *in vitro* platform for studying sweat gland physiology.<sup>81</sup>

Given the limited availability of primary sweat gland tissue, hydrogel-guided transdifferentiation of non-glandular cell sources, such as MSCs and epidermal cells, has emerged as an important strategy.<sup>82</sup> In this context, hydrogels serve not only as structural scaffolds but also as delivery platforms for inductive signals. For example, Sun *et al.*<sup>83</sup> developed an induction strategy in which human epidermal keratinocytes overexpressing the *EDA* gene were embedded in Matrigel, successfully directing their conversion into induced sweat gland-like cells and subsequent assembly into organoids. These structures exhibited long-term survival and functional differentiation following transplantation *in vivo*. Similarly, Huang *et al.*<sup>34</sup> utilized 3D bioprinting to engineer an ECM-mimetic microenvironment, successfully inducing epidermal progenitors to differentiate into the sweat gland lineage by incorporating mouse plantar dermal components and EGF within a gelatin-alginate scaffold. Following transplantation

into murine burn paw pads, the substantial restoration of sweat gland secretory function was directly validated via the starch-iodine test.

As efforts move toward clinical translation, constructing regenerative microenvironments capable of precisely controlling cell fate has become a critical aspect of the overall approach. To overcome the limitations of single-component scaffolds, research has increasingly focused on composite hydrogel systems that integrate multiple inductive cues. Yao *et al.*<sup>84</sup> demonstrated that alginate/gelatin composite hydrogels used as bioinks effectively combined biophysical and biochemical signals to spatially regulate cell behavior. Upon implantation, these engineered constructs promoted the differentiation of MSCs into functional sweat glands and significantly accelerated the structural restoration of damaged sweat gland tissue in murine models.

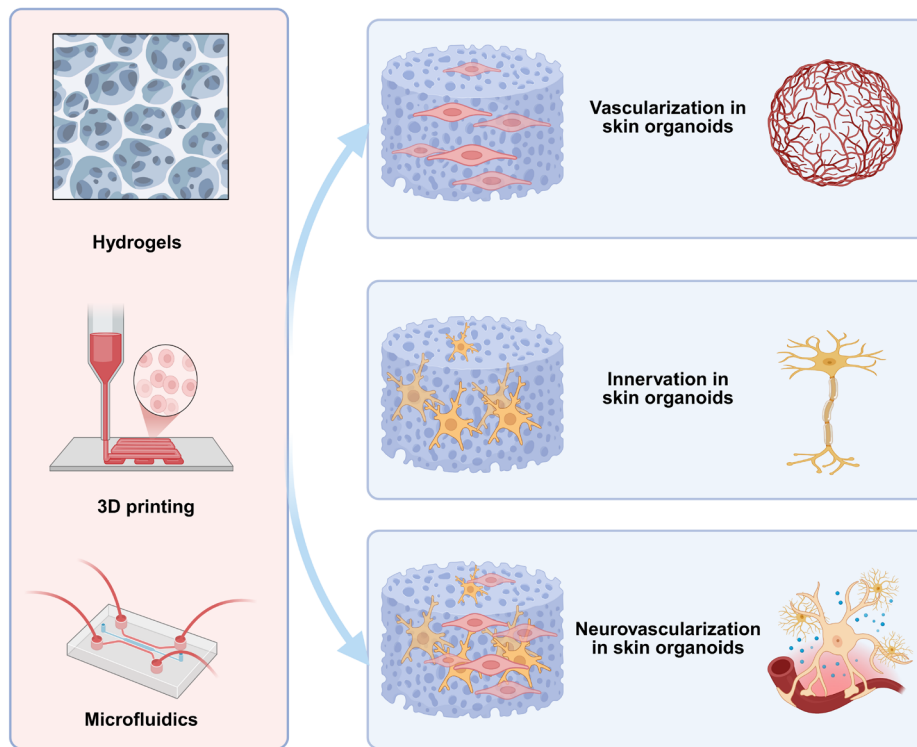
## 4. Hydrogel-assisted engineering of neurovascular integration in skin organoids

As skin organoids increase in structural and cellular complexity, the establishment of functional vascular and neural networks becomes critical for tissue viability, maturation, and physiological relevance. Vascularization ensures adequate nutrient transport and metabolic exchange, while innervation contributes to sensory function and dynamic tissue regulation. Hydrogel-based platforms, combined with biofabrication technologies such as 3D printing and microfluidics, offer unique opportunities to spatially organize vascular and neural components within developing skin organoids. These strategies collectively aim to promote coordinated neurovascular integration, advancing skin organoids toward more faithful *in vitro* models of native skin (Figure 4).

### 4.1. Vascular network engineering in skin organoids

Establishing skin organoids with functional vascular networks is essential for improving graft survival and enabling the healing of large-area wounds, yet it remains a key barrier to organoid-based grafts.<sup>85</sup> Hydrogels, owing to their biocompatibility and tunable processability, are widely used to recreate angiogenic microenvironments.<sup>86</sup> Current strategies mainly include endothelial cell assembly through hydrogel-based co-culture and the fabrication of pre-vascularized architectures via 3D bioprinting.

In co-culture-based approaches, natural hydrogels such as collagen and fibrin are commonly used to encapsulate endothelial cells alone or together with supporting cells, thereby mimicking physiological vasculogenesis.<sup>87</sup> Strobel *et al.*<sup>88</sup> co-cultured adipose-derived microvascular fragments with MSC-derived adipocytes in a 3D collagen matrix, generating vascularized structures resembling native tissue organization.



**Figure 4.** Functional network integration in skin organoids. Advanced biofabrication tools are leveraged to construct vascularized and innervated systems, ultimately achieving integrated neurovascularization for enhanced model fidelity. Image created by the authors.

To address the limited scalability of stochastic vascular self-assembly and the associated nutrient transport constraints, engineered hydrogels with defined geometries and preformed perfusable channels have attracted growing interest. Microscale control over endothelial spatial distribution and matrix porosity can improve network connectivity and vascular maturation. Zhou *et al.*<sup>89</sup> developed a digital light processing–based printing strategy to precisely position endothelial cell clusters, generating functional living skin that promoted efficient regeneration and robust neovascularization. In parallel, Bertassoni *et al.* employed bioprinting to create microchannel networks within hydrogels, improving mass transport and guiding endothelial lining formation to produce perfusable vascularized constructs.<sup>35</sup>

Beyond the direct fabrication of vascular networks, engineering microenvironments that induce host angiogenesis represents a potent alternative strategy. As previously noted, Kwak *et al.*<sup>41</sup> demonstrated that extracellular vesicles secreted by iPSC-derived epidermal organoids effectively accelerate angiogenesis and wound healing. In terms of material selection, photosensitive multi-component hydrogels, such as GelMA/HAMA, have demonstrated superior carrier functionality. Studies have confirmed that encapsulating epidermal stem cells

and SKPs within 3D-printed GelMA/HAMA constructs not only facilitates the regeneration of skin appendages post-transplantation but also successfully drives neovascularization within the dermal layer.<sup>90</sup> Similarly, laser-assisted bioprinting, utilized to deposit fibroblasts and keratinocytes onto stable matrices, has been validated *in vivo* to support the formation and ingrowth of new blood vessels.<sup>91</sup> These advancements suggest that optimizing the physical architecture and bioactive composition of hydrogels offers a robust solution to the vascularization bottleneck in skin organoid engineering.

#### 4.2. Neural and neurovascular network engineering

Innervation is essential for cutaneous sensation and also exerts key regulatory effects on wound healing and skin homeostasis.<sup>92</sup> Although skin organoids have advanced substantially in recapitulating layered architecture and appendage regeneration, generating constructs with functional neural networks remains a major challenge. Unlike highly proliferative fibroblasts or keratinocytes, neurons are post-mitotic and highly sensitive to their surrounding physical and biochemical microenvironments. Consequently, hydrogel design must be carefully tailored to support neurite outgrowth, directional guidance, and interactions with glial cells.

At the material level, the mechanical stiffness and biochemical composition of the ECM play crucial roles in axonal elongation. Neurons generally favor softer matrices that allow matrix remodeling and physical penetration. In addition, incorporating bioactive motifs into synthetic hydrogels can further enhance neuronal behavior. For example, laminin-derived peptides have been widely used to functionalize hydrogels, significantly promoting neurite extension and neuronal survival compared with unmodified matrices.

Beyond physical and biochemical cues, engineered architectures can provide spatial guidance for axonal growth. Hydrogels integrated with microfluidic scaffolds and axon-guiding microchannels have shown particular promise in directing neurite extension. For instance, researchers have developed a microfluidic chip incorporating a “Slope-ALI” design. By filling microchannels with acellular dermal matrix hydrogels and harnessing microchannel geometry-mediated physical guidance and compartmentalized microfluidic design, this system precisely guides dorsal root ganglion sensory axons to traverse the matrix and penetrate vertically through a porous membrane into the keratinocyte layer. This model successfully recapitulates the anatomical trajectory of free nerve endings in human skin as they cross the dermal–epidermal junction to reach the epidermal compartment, although deep epidermal innervation remained incomplete under the reported culture conditions. Moreover, the resulting innervated epidermal unit exhibits functional neuro-epidermal crosstalk, evidenced by calcium influx responses upon stimulation with chemical agents such as capsaicin.<sup>93</sup>

Another important strategy toward physiologically relevant models involves the integration of human-derived neural components. Incorporating human induced pluripotent stem cell (hiPSC)–derived sensory neurons into 3D skin equivalents has therefore emerged as a key approach. Muller *et al.*<sup>94</sup> utilized collagen sponges as dermal scaffolds to engineer full-thickness skin models comprising fibroblasts and iPSC-derived sensory neurons. Their study underscored the critical role of the microenvironment in neurite outgrowth, revealing that neuronal seeding alone is insufficient for long-distance axonal extension. The incorporation of Schwann cells proved indispensable for inducing neurites to navigate the dense dermal collagen matrix and ultimately innervate the epidermis. This complex co-culture system—encompassing neurons, Schwann cells, and keratinocytes—not only established a dense, dermis-penetrating neural network but also retained physiological functions, including the secretion of neuropeptides such as substance P and calcitonin gene-related peptide. Thus, it provides a standardized *in vitro* platform for investigating human-specific neuro-cutaneous interactions.<sup>94</sup>

Emerging material innovations are further expanding

the possibilities for neural integration. Electroconductive hydrogels have recently been introduced into skin tissue engineering as dynamic matrices capable of providing electrical cues that facilitate neuronal maturation and electrophysiological activity in 3D constructs. In parallel, functional neural integration also requires innervation of skin appendages. Recent studies have begun exploring the spatial co-culture of neural crest–derived cells with hair follicle organoids within permissive hydrogel niches, with the long-term goal of reconstructing specialized sensory structures such as the lanceolate nerve endings that surround native hair follicles.

Because cutaneous nerves and vascular networks are anatomically coupled and reciprocally regulated, neurovascular units (NVUs) reconstruction is considered critical for functional skin regeneration. A hydrogel-based co-culture system integrating porous P34HB microspheres has been developed to co-seed human umbilical vein endothelial cells (HUVECs) with neural crest cells, thereby assembling NVU-like structures. This platform recapitulated key features of angiogenic sprouting *in vitro*, with evidence suggesting neural–vascular crosstalk potentially mediated through autophagy-related pathways. The resulting dermal neurovascularized constructs provide a minimally invasive route for *in situ* neurovascular formation and a useful tool for evaluating drug effects on the cutaneous neurovascular system.<sup>95</sup>

Despite these advances, skin neural organoid research remains relatively underdeveloped. While current hydrogel strategies enable localized innervation and NVU formation, establishing long-range, functional neural circuits and achieving robust integration with host neural systems remain major unmet needs.

## 5. Conclusions and future perspectives

Hydrogels, as core materials for recapitulating the skin ECM, are fundamentally reshaping the paradigm of skin organoid engineering. Across epidermal stratification, dermal mechanical support, full-thickness skin assembly, induction of skin appendages, and the initial integration of vascular and neural networks, hydrogels function not merely as passive scaffolds but as active regulators of cell fate through programmable mechanics, biochemical cue presentation, and spatial architectural design.<sup>96,97</sup> Current models can achieve basic barrier function, partial appendage formation, and pre-vascularization, enabling early applications in disease modeling, drug screening, and wound repair.<sup>40,90,95</sup> However, these constructs remain far from recapitulating the structural integrity and functional coordination of native human skin.<sup>85,98</sup>

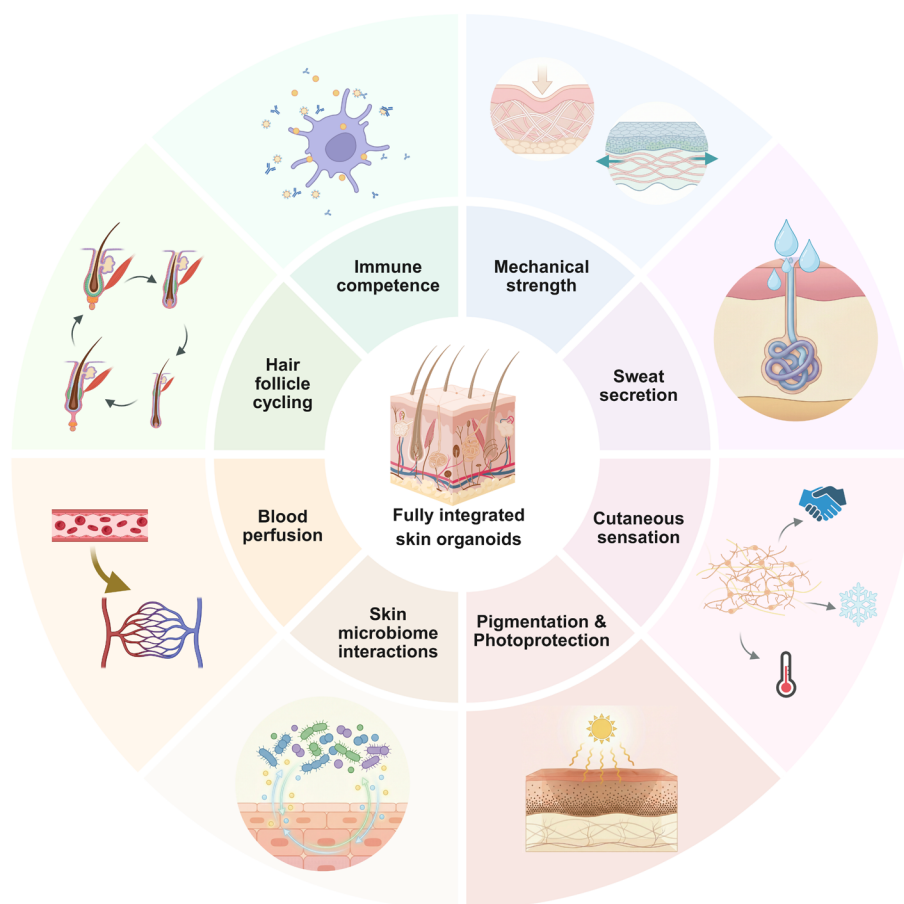
The central limitation lies in the lack of synchronized maturation and long-term homeostasis across multiple

tissue hierarchies. Epidermal layers can undergo stratified differentiation but lack immune components and sensory innervation, limiting relevance to inflammatory and sensory pathologies. Dermal compartments support fibroblast activity yet frequently collapse due to uneven matrix deposition or insufficient mechanical stability. Skin appendages can be initiated within permissive niches, but ductal continuity, innervation, and functional secretion remain largely absent. Vascular networks formed by self-assembly or bioprinting typically lack pericyte coverage and hierarchical organization, preventing physiological perfusion. Neural integration remains largely at a proof-of-concept stage, constrained by inadequate trophic support and the difficulty of achieving precise targeting within static matrices. Collectively, these shortcomings reflect the inability of current systems to reproduce the dynamically coupled spatiotemporal microenvironment of skin development, including sustained paracrine signaling, progressive matrix remodeling, and coordinated mechano-biochemical signal integration.

Another major translational hurdle in current skin organoid research is the variability and lack of

reproducibility across studies. Many existing models rely heavily on animal-derived matrices, such as Matrigel. These natural hydrogels suffer from inherent batch-to-batch compositional variations and poorly defined biochemical profiles, which severely compromise the reproducibility of organoid size, appendage yield, and architectural consistency.<sup>99</sup> Consequently, the important task for the field is the transition toward chemically defined, synthetic, or humanized recombinant hydrogels. Materials such as tunable polyethylene glycol-based matrices, gelatin methacryloyl, or recombinant human collagen offer precise, decoupled control over biophysical and biochemical properties, ensuring the standardization required for rigorous mechanistic studies and clinical manufacturing.<sup>22,97,100</sup>

To transcend mere morphological resemblance and achieve true functional equivalence, we propose a conceptual roadmap centered on the systematic integration of the eight core physiological elements of native skin, as illustrated in Figure 5. Addressing these elements requires overcoming specific material and biological gaps. Reproducing the physiological mechanical strength



**Figure 5.** The eight core elements of an idealized skin organoid, encompassing mechanical strength, immune competence, blood perfusion, sensory perception, appendage functions, and skin microbiome interactions. Image created by the authors.

of skin will depend on the development of hierarchically structured composite hydrogels that better recapitulate the nonlinear elasticity of the native reticular dermis.<sup>101,102</sup> Sustaining thick, multilayered organoids also hinges on the establishment of stable vascular perfusion, potentially enabled by hydrogel-based microfluidic networks and more mature vascular structures.<sup>103-105</sup> Beyond structural and vascular requirements, functional skin models must also incorporate immune competence, which calls for biomaterial architectures that permit the infiltration and long-term residency of immune cells such as Langerhans cells and macrophages.<sup>106-108</sup> Dynamic appendage behavior represents another important dimension. For example, supporting hair follicle cycling may require remodelable microenvironments capable of accommodating the extensive tissue reorganization that occurs during hair growth cycles, while functional sweat secretion would likely require biofabrication strategies that preserve stable epithelial conduits for polarized fluid transport.<sup>78,84,96,109,110</sup> In parallel, restoring cutaneous sensation will rely on approaches that promote deeper neuronal integration, such as electroconductive materials or axon-guiding biochemical cues.<sup>111-114</sup> Accurate pigmentation and photoprotection may further benefit from reconstructing dermal-epidermal microtopography to better recapitulate the native epidermal niche and support physiologically relevant melanocyte distribution.<sup>115-117</sup> Finally, an emerging frontier lies in modeling host-microbiome interactions, which may benefit from air-liquid interface skin models combined with hydrogel systems engineered to better withstand microbial enzymatic degradation while preserving epidermal barrier integrity.<sup>118,119</sup> In conclusion, closing these critical gaps through the convergent innovation of materials science, advanced biofabrication, and stem cell biology will dictate the future trajectory of skin organoid engineering. By meticulously addressing these prioritized directions, next-generation skin organoids will fully unlock their transformative potential in personalized medicine, predictive toxicology, and the treatment of complex, full-thickness skin defects.

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## Conflict of interest

The authors declare that they have no competing interests.

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