

REVIEW ARTICLE

Advances in skin organoid technology and their applications across biomedical fields: A review

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Abstract

Organoids have revolutionized biomedical research by recapitulating the structural and functional complexity of human tissues. Enabled by advances in cell biology, innovative biomaterials, and three-dimensional (3D) bioprinting technologies, organoids are increasingly being utilized as reliable alternatives to traditional cell culture models and human skin explants. This review presents an overview of the methodologies and culture systems employed in the development of composite skin organoids and single-skin appendage organoids, covering a wide range of technical approaches from directed differentiation of induced pluripotent stem cells to the self-organization of adult stem cells. We also highlight recent progress in organoid generation driven by advanced engineering technologies, such as 3D printing and microfluidic systems. Furthermore, this review evaluates the diverse applications of skin organoids in the fields of developmental biology, disease modeling, regenerative medicine, and clinical translation, while addressing current challenges and future perspectives. By proposing a strategic developmental roadmap, this study aims to provide a translational framework for advancing regenerative dermatology and therapeutic innovation.

Keywords: Skin organoids; Skin appendage organoids; Organoid construction; Application of organoids; Disease modeling; Drug efficacy evaluation

1. Introduction

The term “organoid” has historically referred to organ fragments generated by mechanical or enzymatic dissociation, cultured *in vitro* on top of coverslips.^{1,2} Following widely accepted definitions,³⁻⁵ organoids are defined as three-dimensional (3D) structures derived from tissue-specific stem or progenitor cells, embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), which exhibit sustained self-renewal capacity and spontaneously differentiate into organized, tissue-like architectures

that mirror key physiological functions of their *in vivo* counterparts. A pivotal milestone in the field was achieved through the groundbreaking work of Hans Clevers' laboratory,^{5,6} which demonstrated that single *Lgr5*-positive adult stem cells isolated from mouse intestinal tissue could generate self-renewing cultures capable of recapitulating the crypt-villus architecture and cellular composition, including mature and functional cell types. Since then, continuous research efforts have enabled the establishment of organoid models for a broad range of tissues, including the intestine, retina, brain, liver, pancreas, prostate, lung,

breast, fallopian tube, placenta, and skin, as well as tumor organoids that faithfully replicate the pathological and genetic features of various cancers.⁷⁻⁹ Recognized as the “Method of the Year 2017” by *Nature Methods*,¹⁰ organoid technology has emerged as an experimentally tractable and biologically faithful model system, providing robust platforms for investigating organ development and disease pathogenesis. With expanding applications in therapeutic innovation, organoids are increasingly integrated into both basic and translational research, serving as high-fidelity tools for precision oncology, drug discovery and screening, developmental biology, pharmacodynamics and toxicodynamics studies, and regenerative medicine strategies aimed at tissue repair or replacement.¹¹

Skin organoids stand at the forefront of organoid technology, serving as valuable tools for disease modeling and translational medicine. Skin organoids demonstrate the ability to self-organize into stratified epithelium and generate functional appendages such as hair follicles, sebaceous glands, and sweat gland primordia. This remarkable structural complexity positions them as a premier model system for investigating human skin biology and advancing regenerative medicine. Following transplantation into immunodeficient mice, these organoids reconstitute functional, multilayered human skin, presenting a transformative therapeutic avenue for severe burn injuries and inherited dermatological disorders.¹² Nevertheless, the reliable generation of appendage-bearing organoids remains technically demanding, constrained by extended culture durations, protocol complexity, and variable reproducibility. A further limitation lies in the absence of functional vascular and neural networks within current models, which impedes their scalability, compromises graft integration, and limits their utility in modeling cutaneous neuro-sensory physiology. In this review, we summarize recent advances in skin organoid technology and their applications as experimental models and tools for discovery. Subsequently, we outline the current limitations of existing methodologies and propose actionable strategies for future research directions aimed at further advancing the field of organoid technology.

2. Evolution of skin organoid technology

The technological evolution of skin organoids can be categorized into three major phases marked by advances in cell sourcing and functional complexity, which collectively drive the rapid diversification of skin organoid platforms (Figure 1).

2.1. Xenogeneic co-culture foundation phase (1975–1989)

Early developments in skin tissue engineering relied on cross-species cellular interactions to support epithelial

morphogenesis. In the seminal system established by Rheinwald and Green in 1975,¹³ irradiated and mitotically inactivated mouse fibroblasts were employed as a feeder layer to provide essential growth factors, including fibroblast growth factor (FGF) and epidermal growth factor (EGF), as well as structural extracellular matrix (ECM) components for human keratinocytes. This co-culture approach enabled the formation of a fully stratified squamous epithelium, consisting of distinct basal, spinous, granular, and cornified layers, thereby laying the groundwork for *in vitro* epidermal modeling.¹³ In 1989, Shipley *et al.*¹⁴ reported that supplementing keratinocyte cultures with high concentrations of bovine pituitary extract increased clonal efficiency more than threefold by enriching the secretory milieu that supports epithelial proliferation. This refinement solidified the foundational principle of “stromal cell-supported 3D epithelial differentiation,” setting a standard for subsequent skin reconstruction protocols.¹⁴

2.2. Induced pluripotent stem cell reconstitution phase (2011–2018)

With the maturation of iPSC technology, research efforts shifted toward generating autologous, full-lineage skin models. In 2011, Itoh *et al.*¹⁵ pioneered a strategy combining signaling pathway-directed differentiation with scaffold-based colonization. By applying retinoic acid to activate epidermal commitment and bone morphogenetic protein 4 (BMP4) to suppress mesenchymal lineage specification, they achieved highly efficient differentiation of iPSCs into keratinocytes with purity exceeding 90%. These cells were subsequently seeded onto dermal equivalents containing fibroblasts, resulting in the formation of composite skin tissues featuring a structurally intact basement membrane.¹⁵ A critical advancement followed in 2013, when researchers developed a fully iPSC-derived system: dermal fibroblasts were also generated from iPSCs, replacing both murine and primary human stromal cells. This eliminated xenogeneic or allogeneic immunogenicity risks and opened the possibility for personalized, autologous skin regeneration.¹⁶ A milestone was achieved in 2018 when Lee *et al.*¹⁷ demonstrated that mouse iPSCs could self-organize into bilayered skin organoids with epidermal and dermal components via Wnt/ β -catenin and Notch signaling modulation, entirely without exogenous scaffolds. This represented the first successful recapitulation of an organ-level epithelial–mesenchymal interface in a 3D culture system, marking a significant leap in developmental fidelity.

2.3. Functionalization and application expansion phase (2019–2024)

Recent advancements have emphasized the generation of specialized cutaneous structures and the translation

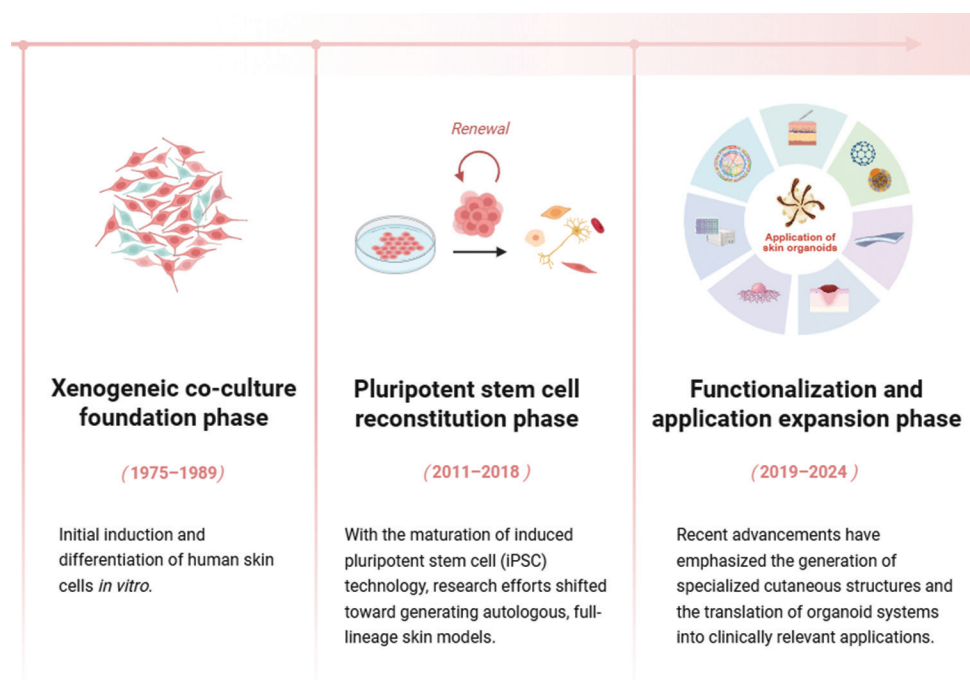


Figure 1. Key milestones in skin organoid development. Created in BioRender. Liu, H. (2025) <https://BioRender.com/n6wk1x7>.

of organoid systems into clinically relevant applications. In 2019, two pivotal studies underscored the regenerative power of adult stem cells: one showed that mouse epidermal stem cells form self-renewing, multilineage “skin buds” in 3D culture,¹⁸ while the other demonstrated that single B-lymphocyte-induced nuclear maturation protein 1 (Blimp1)⁺ progenitors generate functional sebaceous gland organoids (SGOs) capable of sebum synthesis, providing a model for lipid-related skin disorders.¹⁹ The year 2020 witnessed a pivotal transition from structural mimicry to functional reconstitution, as human iPSC-derived skin organoids (hiPSC-SOs) containing mature hair follicles were transplanted onto immunodeficient mice and shown to undergo hormonally regulated, cyclic hair growth, a hallmark of physiological functionality.¹² From 2023 to 2024, skin organoid technology expanded into translational domains. On one hand, it was employed as a human-relevant infection model to precisely recapitulate monkeypox virus replication dynamics within native-like epidermal architecture and to evaluate the efficacy of antiviral agents such as tecovirimat.²⁰ On the other hand, a novel “cell-free therapeutic” paradigm emerged through the isolation of extracellular vesicles (EVs) secreted by epidermal organoids, which were applied to diabetic wound models and shown to accelerate healing by promoting angiogenesis and re-epithelialization.²¹ These advances underscore the growing versatility of skin organoids as platforms for disease modeling, drug development, and regenerative medicine.

3. Construction strategy of composite skin organoids

3.1. Pluripotent stem cell-derived skin organoid construction strategy

Beyond observations in adult cell-derived systems, *in vitro* induction of PSC-derived organoids (both ESCs and iPSCs) has similarly demonstrated composite skin formation. Recent advances now enable spatiotemporally controlled differentiation of iPSCs into self-organizing skin organoids that recapitulate key *in vivo* architectures, including a stratified epidermis, dermal compartments with adipocyte populations, and pigmented hair follicles with functional sebaceous glands.¹⁷ This capability epitomizes the current frontier in skin organoid engineering, offering physiologically relevant models to investigate epithelial-mesenchymal interactions and appendage morphogenesis.

The protocol began with a 2-day culture of E8-20Y cells in maintenance medium, followed by supplementation with Y27632 (Rho-kinase inhibitor) to enhance cellular proliferation and aggregate formation. Differentiation was initiated by transitioning to a neural crest induction medium, supplemented with LDN193189 (a BMP inhibitor) and FGF2 on Day 3, to drive cranial neural crest (CNC) specification. By Day 12, the cellular aggregates were transferred to low-adhesion 24-well plates and maintained in maturation medium supplemented with Matrigel and EGF. To prevent organoid fusion, the plates were subjected to orbital shaking within a humidified 5% carbon dioxide incubator. Following a prolonged culture period of 4–5 months, vesicular skin

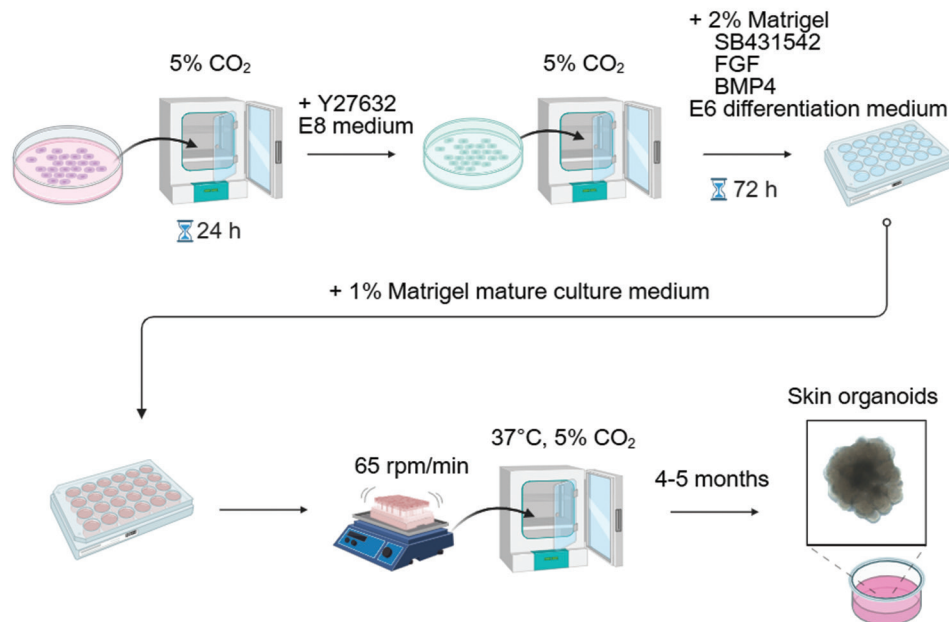


Figure 2. Stem cell-derived skin organoid construction strategy. Created in BioRender. Liu, H. (2025) <https://BioRender.com/n6wk1x7>.

Abbreviations: BMP4: Bone morphogenetic protein 4; FGF: Fibroblast growth factor.

organoids emerged, displaying hallmark features of native skin, including stratified epidermis, adipocyte-enriched dermis, and pigmented hair follicles with functional sebaceous glands (Figure 2). Notably, these organoids also exhibited the integration of sensory neurons and the formation of Schwann cell-like neuroglial bundles.¹² Furthermore, hair follicle-like organoids (HFOs) generated *in vitro* possess most of the characteristic cellular layers found in native hair follicles, except the medulla, indicating the formation of vellus or lanugo-like hairs.²² In later developmental stages, the caudal region of these organoids progressively differentiates into hyaline cartilage containing ACAN⁺ COL9A1-3⁺ (aggrecan with three chains of collagen) chondrogenic progenitor cells. This developmental pattern recapitulates the tissue architecture of native embryonic craniofacial skin, where superficial epithelial tissues and deep mesenchymal structures undergo an inside-out morphogenetic process.¹² However, these organoids currently lack functional sweat gland appendages.

3.2. Tissue cell-derived skin organoid construction strategy

Although the skin appendages and corresponding neural networks in adult stem cell-derived skin organoids are generally less developed compared to those derived from iPSCs, organoids from adult tissues or stem cells offer distinct advantages for *in vitro* tissue transplantation and skin function repair. These include shorter construction time, low cost, and large-scale expansion and culture.²³⁻²⁵ Building upon the dermal-epidermal crosstalk elucidated by

Lei *et al.*,²⁶ skin organoids can be successfully constructed by separately digesting epidermal and dermal cells with trypsin and collagenase I, respectively, and then mixing them in a defined ratio. The resulting primary cell aggregates undergo six morphologically distinct stages: dissociated single cells, cell aggregates, polarized cyst formation, cyst fusion, planar skin structure formation, and ultimately, fully developed skin tissue.^{26,27} Furthermore, researchers have refined the construction methodology by leveraging the *in vitro* self-organization ability of epidermal and mesenchymal cells to engineer hair follicle germ (HFG)-like aggregates,²³ successfully generating cohesive cellular aggregates from both cell types within a significantly shortened timeframe. Upon transplantation into the skin of nude mice, these HFG-like constructs efficiently induced *de novo* hair follicles that progressed through regular growth cycles within weeks. The newly formed follicles expressed characteristic cellular markers representative of both follicular and surrounding dermal tissues, such as stem cell markers cluster of differentiation 34 and SRY-box transcription factor 9.²⁸ The transplanted organoids exhibited robust hair regenerative capacity and sustained hair cycling for at least 10 months. This type of organoid model features a mature hair follicle structure and can serve as a valuable platform for research applications such as hair follicle regeneration (Figure 3).^{23,26,29}

4. Skin appendage organoid construction strategy

Skin appendages, including hair follicles, sebaceous glands, sweat glands, and nails, are critical functional components

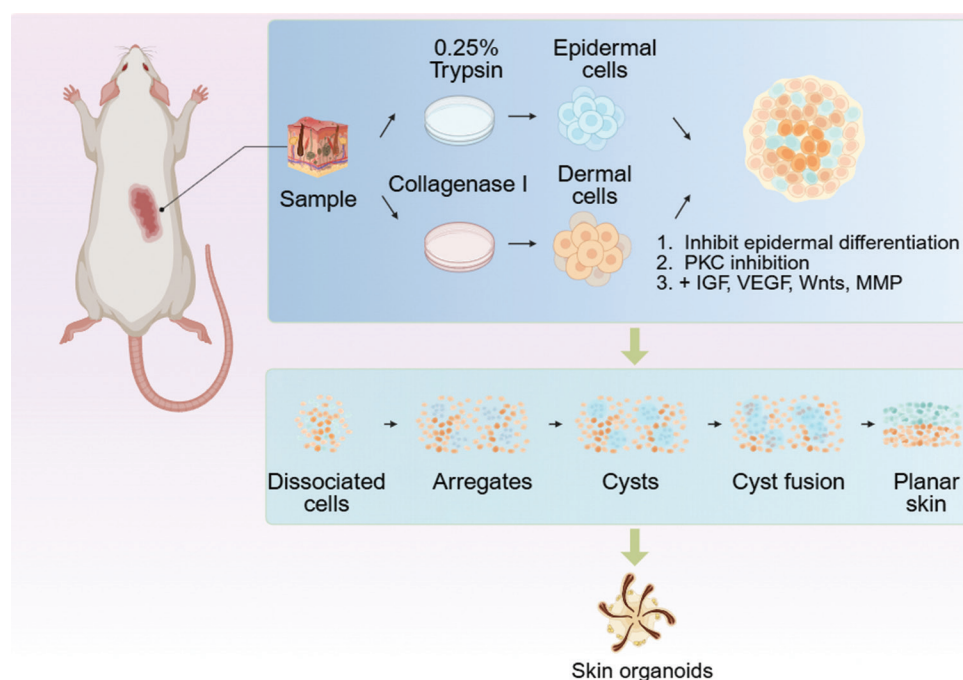


Figure 3. Tissue cell-derived skin organoid construction strategy. Created in BioRender. Liu, H. (2025) <https://BioRender.com/n6wk1x7>. Abbreviations: IGF: Insulin-like growth factor; MMP: Matrix metalloproteinase; PKC: Protein kinase C; VEGF: Vascular endothelial growth factor.

of the integumentary system. However, most current skin-related organoid models often lack these complex structures (Figure 4), which significantly limits their utility for elucidating the pathogenesis of adnexal disorders and hampers their potential in regenerative medicine. Conversely, appendage-specific organoids, such as hair follicle-only models, offer simplified yet highly specialized platforms. These systems enable targeted investigation of diseases affecting discrete adnexal structures, including hidradenitis suppurativa and sebaceous gland dysfunction. Detailed methodologies for constructing skin organoids are summarized in Table 1.

4.1. Hair follicle organoids

The hair shaft is generated by the hair follicle, a mini organ comprising sebaceous glands, apocrine glands, and arrector pili muscles.⁴⁴ Hair follicles continuously undergo phases of degeneration (catagen) and regeneration (anagen) throughout life, a process orchestrated by intrinsic molecular oscillators.⁴⁵ During the anagen phase, matrix keratinocytes undergo robust proliferation followed by lineage-specific differentiation, characterized by the sequential expression of stage-specific markers.^{46,47} This autonomous hair cycle clock is distinct from extrinsic dermal signals that modulate cycle progression, potentially inducing pathological quiescence or hyperactivation of follicular regeneration.⁴⁸ Thus, HFOs, which recapitulate follicular architecture without other skin appendages, represent the most promising approach for elucidating

the intrinsic hair cycle clock when combined with mouse genetics and hair cycle transcriptomic mapping.

The foundation of current hair follicle reconstitution systems was pioneered by Rogers *et al.*,³⁵ who demonstrated *de novo* hair shaft generation by recombining isolated murine follicular cells with dermal counterparts in collagen matrices upon transplantation. Subsequent protocols leveraging iPSC differentiation enabled the self-organization of epidermal–dermal chimeras, yielding skin organoids with hair follicles, sebaceous glands, and adipocytes within 30 days.¹⁷ Further advancing human models, Weber *et al.*³⁶ developed a droplet-based system using neonatal keratinocytes and scalp dermal cells to generate the first *in vitro* differentiated human hair follicle organoids, albeit with limited dermal expansion capacity. Su *et al.*³⁷ developed a novel suspension culture-based platform that facilitates the efficient expansion of HFOs within 24 h by co-culturing fetal scalp-derived dermal progenitor cells with adult foreskin-derived epidermal stem cells, demonstrating high scalability. Subsequently, Gupta *et al.*³⁸ further advanced organoid technology by employing sericin hydrogel for the 3D encapsulation of dermal papilla cells, keratinocytes, and stem cells. The spheroid structures generated through this approach not only recapitulate the morphological features and intercellular interactions of native hair follicles but also effectively mimic the *in vivo* hypoxic microenvironment, thereby providing critical physiological conditions conducive to organoid formation.

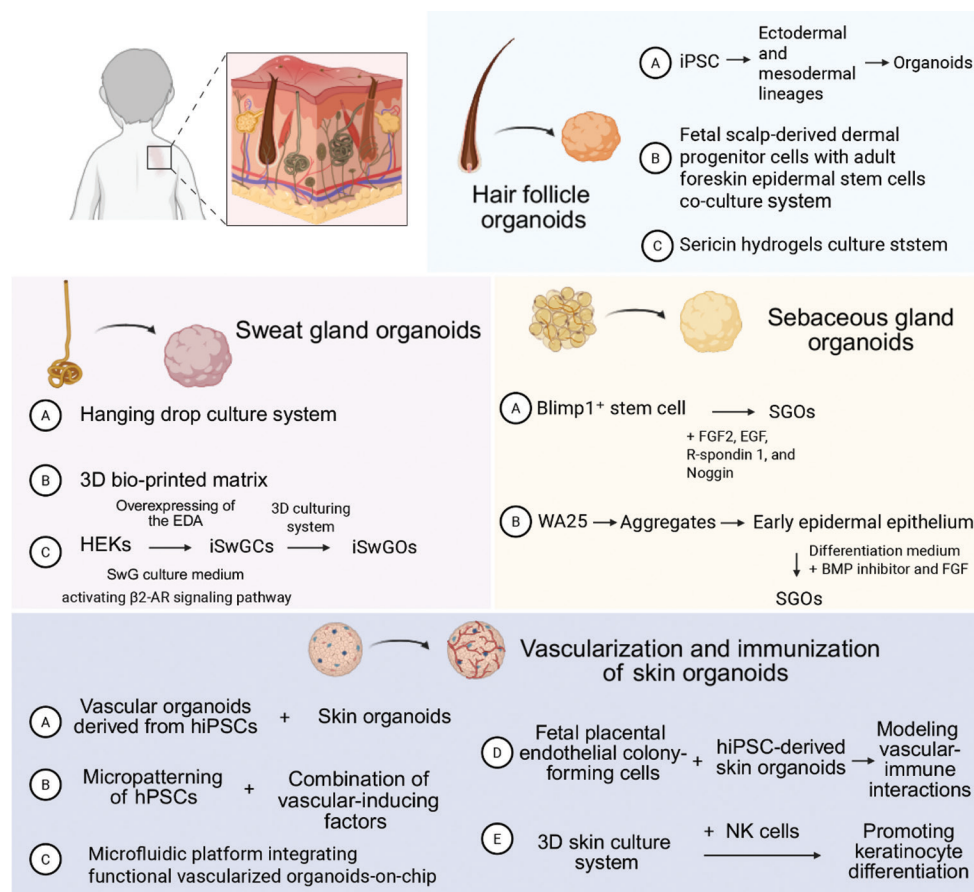


Figure 4. Classification and construction paradigms of skin appendage organoids. Created in BioRender. Liu, H. (2025) <https://BioRender.com/n6wk1x7>. Abbreviations: 3D: Three-dimensional; BMP: Bone morphogenetic protein; EDA: Ectodysplasin A; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; h: Human; iPSC: Induced pluripotent stem cell; NK: Natural killer; SGO: Sebaceous gland organoid; SwGC: Sweat gland cells; SwGO: Sweat gland organoid.

4.2. Sebaceous gland organoids

Pilosebaceous units, consisting of hair follicles and sebaceous glands, are epicenters of lipid metabolism and barrier homeostasis within the skin.⁴⁹ Anatomically, each sebaceous gland possesses a dual-component functional architecture:

- Acinar lobules, functioning as lipid synthesis centers, are composed of multilayered sebocytes that operate as “lipid factories,” continuously biosynthesizing fatty acids and triglycerides while accumulating sebum through regulated metabolic processes.⁵⁰
- The ductal system, lined by stratified squamous epithelium lacking granular and cornified layers, exhibits a distinct “decornified” morphology that supports its specialized role as a dedicated conduit for sebum transport.⁵¹

Functionally, the sebaceous duct acts as both a physical conduit and a directional transport system for lipid matrices and metabolic byproducts, enabling their gradient delivery to the skin surface to support hydrolipidic film barrier

formation.⁵² The sebaceous gland regenerates cyclically in synchrony with the hair cycle. Under homeostasis, basal progenitor cells initiate a “migration–differentiation” cascade: they migrate inward, activate lipid synthesis genes, and mature into lipid-laden sebocytes, where terminally differentiated sebocytes disintegrate and release their contents via the duct to form the skin surface sebaceous film.³⁹ This “proliferation–differentiation–disintegration–renewal” cycle underscores the inherent self-renewal capacity of cutaneous appendages and reveals the dynamic regulation of sebaceous gland homeostasis. Such a framework offers critical insight into the pathophysiology of sebaceous gland-related diseases, including acne.

Sebaceous gland organoids have been successfully established using Blimp1⁺ stem cells located in the basal layer of the mouse sebaceous gland.¹⁹ These Blimp1⁺ cells were initially isolated from murine skin by fluorescence-activated cell sorting and subsequently seeded in Matrigel with the addition of FGF2, EGF, R-spondin 1, and Noggin. Then, periodic replenishment of medium and matrix can yield mouse-derived SGOs.

Table 1. Methods for constructing skin organoids

Type	Cell source	Induction time	Method	Main cell types	References
Composite skin organoids	Human PSCs	>75 days	(i) Day -3-0: 10 μ M Y27632 + E8 medium. (ii) Day 1-3: E6 medium + 2% Matrigel, 10 μ M SB431542, 4 ng/mL basic-FGF, and 2.5 ng/mL BMP4. (iii) Day 3-11: 200 ng/mL LDN, and 50 μ g/mL of FGF. (iv) Day 12-18: Centrifuged at a speed of 65 rpm in the 37°C incubator with 5% CO ₂ ; OMM medium with 1% Matrigel. OMM is composed of advanced DMEM/F12 and neurobasal media at a 1:1 ratio, 1 $\times$ GlutaMax, 0.5 $\times$ B27 minus vitamin A, 0.5 $\times$ N ₂ supplements, 0.1 mM 2-mercaptoethanol, and 100 μ g/mL normocin. (v) Starting from day 18, half-medium was changed every three days (from day 18-45) or every other day (from day 45-150 or longer) with fresh OMM without Matrigel	Head-tail structures, including epidermis, HF outer root sheath, DC, DP, Merkel cells, and melanocytes.	12
			(i) ESCs (R1, kindly provided by Andras Nagy, and Atoh1/nGFP) and iPSCs (C57BL/6J) were maintained in feeder-free conditions using LIF2i medium. (ii) Day 0: PSCs were dissociated with 1 $\times$ TrypLE Express enzyme or Accutase cell dissociation reagent, resuspended in ectodermal differentiation medium. (iii) Day 3: 25 μ L of fresh ectodermal differentiation medium was added to each well, which makes final concentrations of 10 ng/mL BMP4 and 1 μ M SB431542. (iv) Day 4: 25 μ L of fresh ectodermal differentiation medium (without Matrigel) containing 6 μ M LDN and 150 ng/mL FGF2 was added to each well, which makes final concentrations of 1 μ M LDN and 25 ng/mL FGF2. (v) Day 8: Each cell aggregate was transferred to individual wells on 24-well low-cell-adhesion plates in 500 μ L of maturation medium containing 1% (v/v) Matrigel. (vi) Day 10: Half of the spent medium (250 μ L) was removed and replenished with 250 μ L of fresh maturation medium (without Matrigel) every other day up to day 30.		
Tissue cell-derived skin organoid	Mouse	20 days	(i) Initial formation (3 days): Epithelial and mesenchymal cells were suspended in medium with 2% Matrigel and seeded in a non-adhesive 96-well plate. The plate was cooled at 4°C for 30 min and then incubated at 37°C for gelation. Half of the medium was changed every two days. (ii) Long-term culture (20 days): After 3 days, individual follicles were collected, embedded in 100% Matrigel in a dish, and incubated for 30 min for gelation. They were then cultured for an additional 20 days, with half-medium changes every two days.	Including bulges, sub-bulge, dermal papillae, melanosomes, adipose tissues, and cuticles.	23,32,33
			(i) Dissociated cells $\rightarrow$ aggregate $\rightarrow$ polarized cysts $\rightarrow$ coalescing cysts $\rightarrow$ planar hair-bearing skin. (ii) IGF2/VEGF, WNT3A, WNT10B, and MMP14 recombinant proteins + PKC inhibitors.		
Hair follicle organoids	Mouse	7 days	(i) Purified follicles were cultured in a type 1 collagen matrix using medium 199 and 8% fetal calf serum as the basic nutrient. (ii) Culture incorporated thymidine into DNA and methionine into proteins for at least 7 days. (iii) Follicles isolated from the collagen matrix after seven days formed a haired skin when recombined with dermal fibroblasts and grafted to a skin site on nude mice.	A minority of follicles.	35

(Cont'd...)

Table 1. (Continued)

Type	Cell source	Induction time	Method	Main cell types	References
	Mouse PSCs	30 days	(i) Day 0: PSCs were dissociated with 1× TrypLE Express Enzyme or Accutase cell dissociation reagent, resuspended in ectodermal differentiation medium. (ii) Day 1: Replenishing with half of the fresh ectodermal differentiation medium containing 4% (v/v) Matrigel. (iii) Day 3: Culturing in final concentrations of 10 ng/mL BMP4 and 1 μM SB431542 per well. (iv) Day 4: Final concentrations of 1 μM LDN and 25 ng/mL FGF2 in the final volume of 150 μL per well. (v) Day 8: Each cell aggregate was transferred to individual wells on 24-well low-cell-adhesion plates in 500 μL of maturation medium containing 1% (v/v) Matrigel. (vi) Starting from Day 10, half of the spent medium was removed and replenished with another half of fresh maturation medium (without Matrigel) every other day up to Day 30.	1. HF, including the HF matrix, inner root, outer root, and dermal sheath layers. 2. p75 ⁺ SOX10 ⁺ neural crest-like cells in the mesenchymal layer. 3. Melanocytes were visible around the epidermis as early as Day 21. 4. Merkel cells were present in the early stage.	17
	Neonatal foreskin keratinocytes and fetal scalp dermal cells	8 weeks	Epidermal and dermal cells were isolated from neonatal foreskin and second-trimester fetal scalp, respectively. The cells were suspended in F12: DMEM (1:1) medium and incubated in droplet form at 37°C with 5% CO ₂ for 4–7 days, with daily supplementation of growth factors. Subsequently, the cell suspension was injected into the deep dermal layer of hairless nude mice. Subcutaneous nodules exhibiting hair follicle formation were harvested after 8 weeks.	Primitive hair follicle-like structures.	36
	Scalp-derived dermal progenitor cells mixed with foreskin-derived epidermal stem cells	Co-cultured for 24 h before transplantation	Dermo-epidermal aggregates	Hair peg-like formation.	37
	Human HF DP cells, HF keratinocytes, and human HF stem cells	7 days	Employing sericin hydrogel for the 3D encapsulation of dermal papilla cells, keratinocytes, and stem cells	A part of the HF niche consisting of HF keratinocytes, stem cells, and many other cell types.	38
Sebaceous gland organoids	B6.Cg-Tg (Prdm1-EYFP) 1Mnz/J mice	Within 12 days	(i) Cell culture and expansion: • αα ⁺ /Sca1 ⁺ /Blimp ⁺ epidermal stem cells were first cultured for 4–6 passages on a J2 feeder layer in a specialized keratinocyte stem cell medium to stabilize clonal growth. • Cells were then transitioned to feeder-free conditions. (ii) Organoid generation: • Approximately 5000 cells were embedded in 50 μL of Matrigel. • After the gel solidified, it was overlaid with a 3D culture medium supplemented with key factors (including hFGE, mEGF, hR-spondin 1, mNoggin, and B27).	Blimp ⁺ cell-derived organoids encompass lipid-producing cells in the inner mass.	19
	Human PSCs	120 days	(i) Day –2: Dissociate hPSC colonies into single cells and resuspend in E8 medium supplemented with Y27632. Seed 3,500 cells per well (100 μL volume) in a 96-well U-bottom plate, followed by centrifugation to promote cell aggregation. (ii) Day –1: Spherical aggregates had formed. Fresh E8 medium was supplemented in each well to dilute Y27632 and remove dead cells, yielding aggregates with clean surfaces.	1. Day 3: Epithelial layer: ECAD ⁺ , NCAD ⁺ ; Neural tube: NCAD ⁺ ; 2. Days 6–9: Epithelial layer: ECAD ⁺ ; 3. Days 12–21: Epithelial cyst: ECAD ⁺ , TFAP2A ⁺ ; NC cells:	39

(Contd...)

Table 1. (Continued)

Type	Cell source	Induction time	Method	Main cell types	References
Sweat gland organoids	Human skin biopsies	7 days	(iii) Day 0: All aggregates were collected and thoroughly washed to remove residual E8 medium. The aggregates were then transferred to a new 96-well U-bottom plate, with each well supplemented with E6 medium containing SB431542, BMP4, and a low concentration of bFGF to induce surface ectoderm formation.	ECAD ⁺ , TEAP2A ⁺ , PDGFRα ⁺ , P75 ⁺ , SOX10 ⁺ ; 4. Days 35: Basal layer: KRT5 ⁺ , KRT17 ⁺ , TEAP2A ⁺ , CD49f ⁺ ; Intermediate layer: KRT10 ⁺ , TFAP2A ⁺ ; 5. Day 45: Periderm layer: KRT15 ⁺ , KRT17 ⁺ ; 6. Days 55–75: Hair placodes and germs: PCAD ⁺ , LHX2 ⁺ , EDAR ⁺ ; Dermal condensates/papillae: SOX2 ⁺ .	40
			(iv) Day 3: A thin epithelial layer forms at the periphery of the aggregates. The culture medium was replaced with an E6 medium supplemented with LDN and a high concentration of bFGF to induce neural crest fate.		
			(v) Day 6 and Day 9: A half-medium change was performed using fresh E6 medium to sustain nutrient supply. During this period, the aggregates became cystic with mesenchymal cells migrating outward from the core.		
			(vi) Day 12: Collect all aggregates and perform thorough washing. Transfer individual aggregates separately into a 24-well plate and conduct suspension culture using OMM medium supplemented with 1% M antibiotic on an orbital shaker to facilitate further self-organization. Replace OMM medium periodically thereafter.		
			(i) Tissue digestion: Skin tissue fragments were digested in DMEM with 0.5% collagenase V and 0.25 mg/mL thermolysin at 37°C and 5% CO ₂ for 3–5 h.	Simplified sweat gland.	
			(ii) Sweat gland isolation and seeding: Intact sweat glands were isolated using capillary micropipettes and seeded into type I collagen-coated culture vessels.		
			(iii) Primary culture: Glands were cultured in DMEM/F12 medium supplemented with 30% Ham's F12, 10% serum substitute, 10 ng/mL EGF, and antibiotics at 37°C and 5% CO ₂ . The medium was refreshed every 2–3 days, and migrating cells were passaged.		
			(iv) Spheroid formation: For 3D culture, passaged cells were seeded at 10,000–50,000 cells per drop in hanging-drop plates and cultured for up to 7 days. The resulting spheroids were collected using gravity-capture plates.		
	Dorsal skin of E12.5 embryonic mice C57BL/6-Tg (ACTB-EGFP)10sb/J	28 days	(i) Cell isolation and culture.	Sweat gland cells.	41
			(ii) Bioink preparation.		
			(iii) 3D Porous construct fabrication.		
			(iv) <i>In vitro</i> differentiation and tissue formation.		
	Human skin biopsies	7 days	(i) Initial seeding: Seed isolated cells at 1,000 cells/cm ² in Mammocult medium on ultra-low attachment plates. Add 2% Matrigel if required.	1. Luminal cells and basal cells of the ductal compartment. 2. Secretory luminal cells and myoepithelial cells.	42
			(ii) Passaging: Harvest spheroids, digest with trypsin/dispase, and filter. Reseed the single cells at the same density with 2% Matrigel.		
			(iii) Clonal culture: Plate single cells in 96-well plates at one cell/well with 2% Matrigel-supplemented medium.		
			First, Reprogramming of HEKs into SwG cells by combining the stimulation of β2-AR, forced transgenic expression of EDA, and SGM culture, and the organoids were transplanted into mouse models to regenerate functional SwG <i>in vivo</i> .		
	Human epidermal keratinocytes	Within 4 weeks		iSwGOs retained the typical cyto-organization and histological features closely resembling the native SwG bulk <i>in vivo</i> .	43

(Cont'd...)

Table 1. (Continued)

Type	Cell source	Induction time	Method	Main cell types	References
	Mouse	7–10 days	(i) Isolation of mouse SGCs. (ii) Cell culture (complete sweat gland medium was prepared based on adDF12). (iii) Reconstruction of tissue engineering epidermis <i>in vitro</i> .	Pseudostratified epithelium with epidermal markers and also with sweat gland markers (sweat-gland-specific markers [CK19, CK18, and αSMA], and functional markers [AQP5, αATP]).	24

Abbreviations: 3D: Three-dimensional; ACTB: Actin B; ADDF12: Advanced Dulbecco's Modified Eagle Medium; AQP5: Aquaporin 5; ATP: Adenosine triphosphate; Blimp: B lymphocyte-induced maturation protein 1; BMP4: Bone morphogenetic protein 4; CK: Cytokeratin; CNC: Cranial neural crest; DC: Dendritic cells; DP: Dermal papillae; ECAD: E-cadherin; EDA: Ectodysplasin A; EGF: Epidermal growth factor; ESCs: Embryonic stem cells; FGF: Fibroblast growth factor; HFG: Hair follicle germ; hPSC-SOs: Human induced pluripotent stem cell-derived skin organoids; hPSCs: Human pluripotent stem cells; IGF2: Insulin-like growth factor 2; iPSCs: Induced pluripotent stem cells; KRT: Keratin; MMP14: Matrix metalloproteinase 14; mNoggin: Mouse Noggin; MSCs: Mesenchymal stem cells; OMM: Organoid maturation medium; ORS: Outer root sheath; PDGFRα: Platelet-derived growth factor receptor alpha; PKC: Protein kinase C; SGCs: Sweat gland cells; SwG: Sweat gland; SwGOs: Sweat gland organoids; TGF-β: Transforming growth factor beta; VEGF: Vascular endothelial growth factor.

Human SGOs were generated from the WA25 human ESC line.^{12,39} Before induction, the cells were dissociated into single cells and seeded into U-bottom 96-well plates to form uniform cell aggregates. These aggregates were transferred to differentiation medium supplemented with ECM components. Early epidermal epithelium was induced using BMP4 and SB431542. Subsequently, the epithelium was further patterned into surface ectoderm in a different differentiation medium containing a BMP inhibitor and FGF. Concurrently, the addition of FGF2 and LDN193189 facilitated the encapsulation of the surface ectoderm by CNC-like cells. By day 120, hair follicle structures associated with the sebaceous glands can be observed within the skin organoids.¹²

4.3. Sweat gland organoids

As a critical component of the human exocrine system, sweat glands play a central role in thermoregulation by secreting sweat, a process that depends on the efficient evaporative cooling of the skin surface. The development of this complex structure is governed by a tightly regulated embryonic morphogenetic program, involving precise spatiotemporal signaling across multiple developmental stages.⁴⁰ Human sweat gland morphogenesis begins during the mid-trimester of gestation (approximately weeks 12–13), initially occurring in anatomically restricted regions such as extremities. This developmental process originates from multipotent stem cells located in the basal layer of the epidermis. These stem cells, which reside in regions destined to become high-density sweat gland areas such as the palms, are selectively activated and undergo proliferation to form columns of epithelial progenitor cells that migrate in a directed manner toward the dermis.

As development proceeds, these epithelial columns undergo substantial morphological remodeling: the proximal segment gradually elongates and stratifies, eventually forming a straight ductal system that spans the epidermis and dermis; concurrently, the distal segment differentiates into a coiled tubular structure, progressively developing into a glandular unit with secretory function.⁴¹ This polarized differentiation process ultimately results in the formation of a fully functional sweat gland unit, a composite organ composed of a secretory portion, responsible for producing an electrolyte-rich fluid, and a ductal portion, which facilitates the directional transport of sweat to the skin surface.

In patients with extensive burns or severe skin trauma, structural regeneration and functional recovery of sweat glands often fail to occur through the natural proliferation and differentiation of residual sweat gland cells following wound healing. There is no effective treatment for some patients with severe sweat gland development gene disease

and abnormal sweat gland function. Therefore, the basic research to achieve burn wound healing and sweat gland regeneration represents a critical and yet unresolved challenge in skin tissue engineering and burn rehabilitation.

Current strategies for constructing sweat gland organoids (SwGO) are primarily based on rodent-derived sources. Klaka *et al.*⁴⁰ established an organotypic sweat gland model using a hanging drop culture system, which successfully recapitulated the physiological regulation of sweat secretion and provided significant assistance in investigating mechanisms underlying sweat gland function. Liu *et al.*⁴¹ utilized a 3D bio-printed matrix to guide the self-organization of sweat glands, achieving structural features that closely resemble native sweat gland development. Kurata *et al.*⁴² attempted to use stem cells derived from sweat gland myoepithelial cells to generate spheroidal SwGOs, but the efficiency of organoid formation was only approximately 2.5%. Sun *et al.*⁴³ enhanced the stemness of keratinocytes by activating the β 2-adrenergic receptor signaling pathway and overexpressing the ectodysplasin A (EDA) gene, thereby reprogramming HEK cells into sweat gland-like lineages. These engineered cells were further cultured using organoid-based methods to achieve functional regeneration of sweat gland structures.⁴³ Diao *et al.*²⁴ developed a culture medium that can effectively induce the formation of SwGOs in the long term, composed of NIC, N-acetylcysteine, and B27. After adding growth factors EGF, basic FGF, and EDA, the formation of SwGOs was significantly improved.

4.4. Vascularization and immunization of skin organoids

Hair regeneration is orchestrated by intrinsic factors within hair follicles and extrinsic cues from the niche microenvironment.⁵³ The perifollicular niche comprises both macroenvironmental and microenvironmental components.⁵⁴⁻⁵⁶ Macroenvironmental influences, including aging, circadian rhythms, hormonal fluctuations, light exposure, seasonal changes, psychological stress, and temperature, exert significant effects on the hair regeneration process.⁵⁴ Local microenvironmental elements within the skin, such as dermal adipose tissue, arrector pili muscles, blood vessels, immune cells, lymphatic structures, neural networks, and neighboring follicles, fine-tune the regenerative process at the tissue level.^{55,57,58} Among these regulatory components, the vascular system and the immune system are particularly prominent.

4.4.1. Engineering functional three-dimensional bioprinted vascular networks within skin organoids

Blood vessels deliver oxygen and nutrients to hair follicles via the dermal papillae, thereby supporting hair growth and regeneration. With aging, vascular function declines due

to impaired vasoconstriction, inflammation, and elevated blood pressure.⁵⁹ Similarly, cyclical vascular remodeling occurs periodically around hair follicles during the skin hair cycle.⁶⁰ A critical limitation of current skin organoids is the lack of functional vascularization, which restricts metabolic exchange and impedes long-term growth. To address this, Mostina *et al.*⁶¹ advanced skin organoid vascularization by demonstrating that integrating hiPSC-derived vascular organoids leads to the formation of complex vasculature and the successful interchange of endothelial, mural, hematopoietic, and mesenchymal cells, establishing a chimeric system. Complementary strategies, including small molecule induction, endogenous gene-driven vascular differentiation, microfluidic chips, and 3D bioprinting, have also established nutrient-waste exchange in cardiac, liver, and other organoids. For example, Abilez *et al.*⁶² applied combinatorial vascular induction factors to generate both cardiac and liver organoids with spatially organized vascular networks. In parallel, engineering-based approaches have advanced significantly. As early as 2010, studies showed that seeding endothelial progenitor cells with fibroblasts in collagen gels or other biomaterials could successfully generate microvascular beds in dermal analogs.⁶³

In recent years, advances in 3D bioprinting have enabled the use of endothelial cells as bioinks to fabricate vascularized 3D tissue constructs within organoid systems.⁶³ A bio-printed vascularized bilayer model composed of fibroblasts, endothelial cells, pericytes, and type I collagen in the dermal layer, overlaid with keratinocytes, successfully recapitulated key features of skin architecture.⁶⁴ Complementing this approach, microfluidic platforms provide dynamic perfusion and real-time control of biochemical and physical parameters (pH, gases, ions, and temperature), significantly extending organoid culture longevity.⁶⁵ Based on this principle, Quintard *et al.*⁶⁶ developed a microfluidic platform sustaining endothelial network formation around mesenchymal and islet spheroids, as well as PSC-derived vascular organoids, for up to 30 days. While intra-organoid vascular networks are known to support oxygenation, nutrient delivery, and long-term growth, they remain poorly developed in skin organoids, hindering physiological relevance and translational utility. Nevertheless, vascularization strategies that have succeeded in other organoid systems provide a clear roadmap for overcoming this barrier in skin models.

4.4.2. Modeling vascular-immune interactions in skin organoids

Human skin consists of diverse cell types and features intricate vascular-immune interactions, prominently involving the “perivascular emigration unit,” which facilitates neutrophil extravasation during microbial invasion.⁶⁷ Perivascular macrophages produce neutrophil-

recruiting chemokines, including C-X-C motif chemokine ligands 1 and 2, supporting transendothelial migration,⁶⁷ whereas their absence markedly impairs neutrophil infiltration into the skin.⁶⁷ In addition, T-cell trafficking is critically regulated by postcapillary venules.⁶⁸ During cutaneous inflammation, T cells, dendritic cells, and macrophages aggregate around these vessels to form perivascular cellular clusters.⁶⁸ These structures, termed inducible skin-associated lymphoid tissue, serve as localized sites for antigen presentation and adaptive immune responses. This vascular-immune complexity poses significant challenges for accurate *in vitro* modeling.

Recent advances have increasingly focused on recapitulating immune cell populations within skin organoids. Mostina *et al.*⁶¹ integrated human fetal placental endothelial colony-forming cells into hiPSC-SOs, generating endothelialized constructs with capillary-like networks and coordinated co-development of macrophages, CD207⁺ immune cells, and neutrophils.⁶¹ Arjmand *et al.*⁶⁹ demonstrated that the incorporation of natural killer cells into a 3D skin culture system enhanced keratinocyte differentiation and modulated inflammatory responses in dermatopathological conditions such as psoriasis.⁶⁹ However, natural killer cell engraftment and sustained presence within the organoid parenchyma were not achieved in this model.

Although recent advances have enabled partial vascularization, folliculogenesis, and immune cell integration in 3D skin models, a fully endogenous differentiation strategy that yields vascularized, hairy human skin substitutes with a complete and functional immune repertoire remains to be realized.⁷⁰⁻⁷²

5. Applications of skin organoids

5.1. Applications of skin organoids in developmental biology research

Organoids serve as powerful platforms for developmental biology research at the tissue and organ levels. Recently, several laboratories have published single-cell omics technologies to explore the advantages and disadvantages of various organoid culture systems. These studies include organoid models simulating the central nervous system,⁷³ the gastric tract,⁷⁴ the intestinal tract,⁷⁵ and gastrulation processes,⁷⁶ with skin organoids constituting an established paradigm.⁷⁷ Due to ethical or technical limitations, our understanding of the early lineage specification and morphogenesis of human epidermis, dermis, and skin appendages is currently limited to basic histological observations.⁷⁸⁻⁸⁰ Both murine and human-derived *in vitro* skin organoid systems provide appropriate research platforms for this purpose.

Lee and Koehler⁸¹ provided comprehensive single-

cell RNA sequencing datasets spanning three key stages, covering the entire approximately 150-day period from skin organoid induction to maturation. They expanded these analyses to cover additional time points spanning the full 4–5-month culture period, offering a resource for investigating fate decisions of stem and progenitor cells during human skin development.⁸¹ Another study investigated the transcriptomes of three distinct skin organoid models and confirmed the presence of relevant cell types analogous to their *in vivo* counterparts. However, organoid-specific alterations were also observed, including basal cell program expansion, impaired terminal differentiation, and abnormal epithelial-mesenchymal transition features, particularly predominant in organoids derived from fibroblast-embedded matrix gel systems. In addition, xenotransplantation of skin organoids into immunodeficient mice demonstrated robust regenerative capacity under hypoxic conditions, effectively repairing various skin defects.⁸² Furthermore, bioinformatics tools combining cell lineage and spatial relationships were developed, which can be applied to map precise lineages, transcriptomes, and final spatial positioning of cells during wound healing in skin injuries.⁸³⁻⁸⁶

Organoid technology has not only established itself as a powerful platform for investigating environmental signal transduction but has also demonstrated indispensable value in elucidating the mechanisms underlying mechanical force-regulated organogenesis. Research indicates that compressive stress from excessive proliferation in the embryonic murine epidermis inhibits stem cell division via the Hippo/YAP pathway, thereby contributing to the maintenance of tissue homeostasis.^{87,88} In the context of human hair loss disorders, hair follicle miniaturization alters the mechanical niche and triggers Piezo1-Ca²⁺-tumor necrosis factor alpha signaling, leading to stem cell exhaustion.⁸⁹ Conversely, moderate tensile force promotes epidermal and follicular regeneration by activating Hippo and EGFR/Ras/mitogen-activated protein kinase pathways, highlighting the therapeutic potential of mechano-intervention.^{90,91} This technology has enabled the modeling of biomechanical processes that are highly dependent on the physical microenvironment, such as cerebral cortical folding, intestinal villus morphogenesis, and mammary gland branching development.^{92,93}

In the field of skin organoid research, the regulatory mechanisms governing mechanical force-mediated cell fate specification are becoming increasingly well understood. For example, Wang *et al.*²⁹ demonstrated that genetic activation of the mechanosensitive ion channel Piezo1 in epidermal basal cells within organoids dynamically modulates dermal-epidermal interactions by downregulating the expression of dermal cell adhesion molecules. This study represents the first *in vitro* recapitulation of endogenous mechanical

contraction forces that regulate morphogenesis, providing crucial insights into the mechanotransduction mechanisms involved in skin development. The integration of bioengineering with organoid models represents a research frontier. Advanced microfluidic systems now enable precise regulation of mechanical cues and real-time analysis of dynamic processes, including tissue curvature, strain distribution, and branching morphogenesis. These high-precision platforms are poised to elucidate how mechanical forces guide skin development and regeneration.⁹⁴⁻⁹⁸

5.2. Regenerative applications of skin organoids

Skin wound healing constitutes a finely orchestrated regenerative program involving multiple cell types and progressing through four temporally overlapping phases: Hemostasis, inflammation, proliferation, and remodeling.^{99,100} Epidermal and dermal cells engage in dynamic crosstalk to reconstruct tissue, supported by angiogenesis that delivers oxygen and nutrients. Concurrently, the ECM undergoes intelligent structural reorganization, and plasma-derived proteins act as crucial signaling mediators and provisional scaffolding components. Collectively, these coordinated mechanisms restore skin integrity and function, achieving near-complete regeneration. Upon injury, dermal cells actively remodel the ECM to facilitate regeneration. Fibroblasts, the primary cellular component of the dermis, drive this process by synthesizing and remodeling the ECM, notably through the production of type I collagen. In addition, they also interact with lysyl oxidases and their inhibitors, which collectively contribute to the extension of new epithelium. During this process, redundant vessels, fibroblasts, and inflammatory cells are systematically eliminated via apoptosis or immune-mediated clearance, leading to scar maturation.^{97,99}

Clinically, skin layer defects exceeding 4 cm in diameter require implantation-based therapy.^{100,101} While artificial skin grafts are widely used, they are often hampered by poor vascularization, suboptimal integration, scarring, and the absence of deep dermal structures. Skin organoids present a promising alternative. Research demonstrates that transplanted skin organoids can integrate into host skin in nude mice, where they guide follicle formation and support hair growth.¹⁰² Notably, 3D human skin organoids containing hair follicles and sebaceous glands can form functional interfaces with host epidermis, erector pili muscles, and nerve fibers, without inducing rejection or aberrant growth.¹⁰³ Cotransplantation of epidermal and dermal cells further enhances wound repair and folliculogenesis.^{26,81,103} For severe skin defects where autografts are inadequate, iPSC technology has pioneered the generation of functional skin organoids. These iPSC-SOs recapitulate native skin complexity with hair follicle competence and ensure immune compatibility

due to their autologous origin.¹² Upon transplantation, such bioengineered organoids integrate into wound sites, markedly accelerating re-epithelialization, restoring barrier function, and even enabling the regeneration of hair coverage.¹² This approach represents a clinically promising therapeutic option that combines personalized medicine with substantial translational potential.

Wound healing and skin regeneration remain significant challenges in the field of regenerative medicine. Recent developments in biomaterials, including the 3D bio-printed skin organoid, novel nanoparticle, liposomal delivery carriers, and hydrogel scaffolds, have opened advanced avenues for promoting wound healing, tissue regeneration, and mitigating aging-associated morphological decline.^{104,105} Existing studies have shown that skin organoids derived from iPSCs can secrete abundant exosomes, which contain high concentrations of the angiogenic growth factor vascular endothelial growth factor and a range of regenerative microRNAs. These exosomal contents facilitate key biological processes such as cellular proliferation, migration, differentiation, and angiogenesis.²¹ Combined with new materials such as nanoparticle materials, liposomal delivery carriers, and hydrogel scaffold structures, skin organoids hold great potential for applications in the field of skin regeneration (Figure 5).

5.3. Disease models of skin organoids

Skin organoids represent a transformative platform for disease modeling, enabling detailed investigation into the pathogenesis and progression of infections, inflammatory conditions, and skin cancers. Furthermore, these 3D models provide a valuable system for evaluating novel drug candidates. Their application signifies a paradigm shift in methodologies for both mechanistic disease research and therapeutic development.

5.3.1. Skin infection models

Infectious skin diseases are caused by pathogenic invasion of the skin and, in severe cases, may progress to systemic disorders affecting multiple organ systems. In the context of relevant organoid models, Ishibashi *et al.*¹⁰⁶ demonstrated that infection of human keratinocytes with *Malassezia* spp. induces *Malassezia globosa*-mediated secretion of interleukin (IL)-5, IL-10, and IL-13, whereas suppressing IL-4 production. This suggests a synergistic role in AD pathogenesis through Th2-polarized immune activation during *Malassezia* colonization.¹⁰⁶ During the COVID-19 pandemic, Ma *et al.*¹⁰⁷ identified *KRT17*⁺ hair follicle cells as targets of SARS-CoV-2 infection, which disrupted follicular and epidermal morphogenesis and caused neuronal death via neurotropic viral dissemination. Similarly, Li *et al.*²⁰ reported that iPSC-SOs are permissive to Mpox virus infection and sustain productive viral

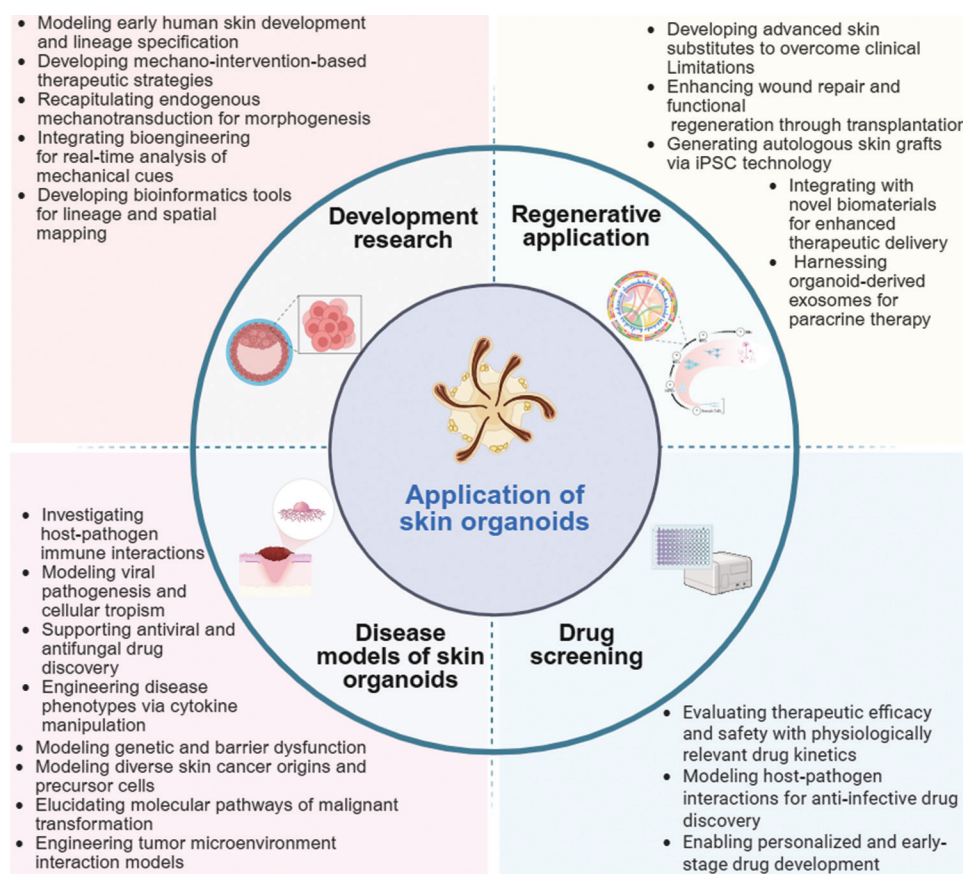


Figure 5. Applications of skin organoids. Created in BioRender. Liu, H. (2025) <https://BioRender.com/n6wk1x7>.

replication, providing a platform for investigating poxvirus-host interactions. Kitisin *et al.*¹⁰⁸ established a *Candida*-infected skin organoid model for high-throughput antifungal drug screening, identifying compounds effective against multidrug-resistant fungal pathogens. Collectively, these advances underscore the utility of skin organoids in dissecting infection mechanisms and accelerating therapeutic development for microbial-driven dermatoses.

5.3.2. Research on inflammatory skin diseases

Atopic dermatitis (AD), also known as atopic eczema, is a chronic, relapsing inflammatory skin disease characterized by intense itching, eczematous lesions, and epidermal barrier dysfunction. Its pathogenesis involves a complex interplay of genetic predisposition, dysregulated immune responses, and environmental factors.¹⁰⁹ Skin organoids have emerged as a promising *in vitro* model by recapitulating the stratified architecture and *in vivo*-like gene expression profiles of human skin, thereby offering a novel platform for investigating the molecular mechanisms underlying complex dermatological conditions such as AD. A pivotal study by Sriram *et al.*¹¹⁰ demonstrated the successful development of an AD-mimicking organoid model using a serum-free culture system supplemented with Th2-

polarizing cytokines, particularly IL-4, in combination with a fibrin-based dermal matrix. This model effectively replicates key histopathological features of AD, including epidermal hyperplasia and immune cell infiltration, while also mirroring disease-specific inflammatory signaling pathways and transcriptomic signatures at the molecular level. As such, it provides a highly biomimetic and experimentally tractable system for mechanistic studies and therapeutic development in AD.¹¹⁰

Elias *et al.*¹¹¹ constructed a fully humanized 3D skin organoid model by combining fibroblasts from healthy individuals with small interfering RNA gene knockdown. Their model featured a stratified, terminally differentiated epidermis and an underlying dermal compartment, and revealed that filaggrin deficiency may be a potential contributor to AD. By employing mouse psoriasis and skin organoid models, Jiang *et al.*¹¹² discovered that *LGALS9* secreted by DCs interacts with CD44⁺ dermal fibroblasts. This establishes a novel mechano-chemical circuit that drives increased ECM expression, which further promotes basal epidermal cell hyperproliferation and ultimately leads to a stiffer dermal environment characteristic of psoriasis. In summary, the development of skin organoid models specifically engineered for

inflammatory skin diseases provides powerful platforms to advance mechanistic investigations and accelerate therapeutic discovery.

5.3.3. Skin tumor models

Skin organoids, as 3D models with multiple cell types and biomimetic structures, have emerged as a valuable platform for studying the mechanisms of skin tumorigenesis. Studies confirm that these organoids can harbor key precursor cells of various cutaneous malignancies, enabling the faithful recapitulation of carcinogenesis *in vitro*. These precursor cells and their related tumor types mainly include: (i) Melanoma, which originates from melanocytes or their precursors, commonly driven by mutations in *BRAF* and telomerase reverse transcriptase promoter;¹¹³ (ii) squamous cell carcinoma, resulting aberrant keratinocyte proliferation and differentiation under the long-term influence of carcinogenic factors such as ultraviolet radiation and human papillomavirus infection;¹¹⁴ (iii) basal cell carcinoma, commonly found in the basal layer of the epidermis, usually resulting from the malignant transformation of hair follicle stem cells and closely related to abnormal Hedgehog signaling pathways;¹¹⁵ and (iv) neuroendocrine tumors, such as Merkel cell carcinoma, which is believed to possibly originate from Merkel precursor cells and is associated with Merkel cell polyomavirus (MCPyV) infection or ultraviolet-induced gene mutations.¹¹⁶

The utility of skin organoids in tumor disease modeling is evident in their ability to trace oncogenesis, delineate transformation pathways, and serve as experimental platforms for targeted treatment strategies. This utility is exemplified by Commandeur *et al.*,¹¹³ who developed a 3D skin model with integrated squamous cell carcinoma cells to enable high-throughput drug screening. In another study, Kim *et al.*¹¹⁶ identified that (-)-4-hydroxysalicetol, a compound isolated from marine microbial metabolites, significantly suppressed melanin production in artificial melanoma spheroids, demonstrating its anti-pigmentation potential. In addition, Loke *et al.*¹¹⁷ engineered a Merkel cell carcinoma-like lesion model by embedding MCPyV⁺ carcinoma cells within a collagen-based matrix layered between epidermal (keratinocyte) and dermal (fibroblast) equivalents. This physiologically relevant system enables the mechanistic dissection of MCPyV T-antigen-driven oncogenesis, the identification of pro-tumorigenic factors, and the screening of potential therapeutic agents. Collectively, these advances highlight the transformative potential of skin organoids in elucidating tumor biology and accelerating precision oncology.

5.4. Drug screening

Cutaneous organoid models have demonstrated significant advantages over conventional two-dimensional (2D) cell

culture systems in drug screening applications, owing to their ability to faithfully recapitulate the 3D architecture and physiological as well as pathological characteristics of native skin tissues. In contrast to 2D cultures, where cells grow as homogeneous monolayers that lack the multicellular heterogeneity and tissue-specific structural organization observed *in vivo*, organoids maintain a more physiologically relevant microenvironment. Prolonged *in vitro* expansion in 2D systems may also lead to genetic drift or phenotypic alterations, which can significantly compromise the reliability of drug response assessments. Organoids, by contrast, preserve key features of native tissue, including 3D cellular organization, cell-cell interactions, and dynamic ECM contacts. These attributes give rise to gradients of oxygen, nutrients, and therapeutic agents within the organoid structure, closely mimicking the conditions found in real tissues. As a result, the diffusion and internalization kinetics of drugs are prolonged, enabling the detection of spatially heterogeneous responses across different cell populations or tissue regions, an essential capability for evaluating therapeutic efficacy, mechanisms of drug resistance, and potential cytotoxic effects.^{118,119}

In a mouse primary epidermal organoid model infected with *Staphylococcus aureus*, vancomycin restored cell viability and suppressed bacterial internalization in a concentration-dependent manner.¹²⁰ This dual-readout capability, simultaneously quantifying pathogen invasion and cell recovery, exceeds the functional complexity achievable in conventional 2D cultures. Li *et al.*¹²¹ employed human induced hiPSC-SOs as an Enterovirus A71 (EV-A71) infection model. These results revealed that EV-A71 induces epidermal damage via autophagy and the integrin/Hippo-YAP/TAZ signaling pathway, leading to progenitor cell hyperproliferation. Based on this mechanism, they identified an autophagy-related protein as a therapeutic target and discovered an EV-A71 replication inhibitor with potential clinical relevance for treating hand, foot, and mouth disease. Skin organoid-based platforms enable personalized drug screening tailored to individual pathological features, while also facilitating early-stage drug discovery before clinical trials. Collectively, these advances highlight the potential of skin organoids to drive innovation in dermatological research and therapeutic development.

6. Current limitations of skin organoids

Despite their remarkable progress, skin organoids remain limited in their ability to fully recapitulate the *in vivo* skin system due to the nascent stage of key accessory system structures, including neural networks, immune responses, and vascularization. Although hair follicles can be generated *in vitro* through iPSC-directed differentiation or co-culture systems involving hair follicle keratinocytes,

dermal papilla cells, and stem cells, the successful induction of SGOs and SwGOs remains challenging, requiring their development as separate functional units. Moreover, these skin appendage organoid culture systems (e.g., sebaceous or sweat glands) exhibit incomplete structural maturation compared to native tissues and cannot fully recapitulate their physiological functions.

Current skin organoid models face limitations due to structural homogeneity and the absence of critical native skin components, including neurons and immune cells. This impedes the reconstruction of complex immunopathological and neurotropic viral infection models, such as systemic lupus erythematosus, psoriasis, and herpes zoster. In addition, existing models fail to achieve comprehensive integration of epidermal, dermal, and adnexal components, such as sweat glands, and lack functional vascular networks necessary for efficient nutrient exchange and metabolic waste clearance. These limitations restrict their utility in modeling chronic inflammatory dermatoses, latent viral infections with prolonged incubation periods, and the homeostatic regulatory networks between skin appendages. To overcome these barriers, substantial advancements in multicellular integration and vascularization are required to bridge these gaps.

Skin organoid models face persistent limitations in clinical translation, including low engraftment efficiency, incomplete tissue regeneration, inadequate disease phenocopying, and inconsistent drug screening outcomes. In the context of transplantation medicine, autografts using patient-derived healthy skin remain the clinical gold standard for wound repair. When autologous donor sites are inadequate, allografts from genetically compatible donors are prioritized due to their superior structural integrity post-transplantation compared to organoid-based constructs. For drug discovery, the widespread adoption of skin organoids is impeded by two major barriers: (i) the structural and functional immaturity of current organoid systems, which compromises their ability to reliably identify stable therapeutic candidates; (ii) high inter-patient heterogeneity in donor-derived organoids, necessitating stringent standardization protocols to ensure screening reproducibility.

7. Conclusion

Current drug discovery pipelines rely on 2D cell models, organoids, and animal experiments, yet each platform suffers from inherent limitations hindering clinical translation. Future progress demands scalable, reproducible, and biomimetic engineering strategies. To address structural oversimplification, Su *et al.*¹⁰⁵ employed extrusion-based bioprinting with skin organoid-derived bioinks and dual-light crosslinking to fabricate anatomically precise scaffold systems that facilitate spatial organization and promote organoid maturation. Concurrently,

engineered microvascular networks incorporating novel nanomaterials have been developed to enhance metabolic exchange and sustain long-term organoid expansion.^{119,120} Meanwhile, Stanford University recently developed cardiac vascularized organoids from human PSCs through micropatterning and small-molecule induction. These cardiac vascularized organoids contain cardiomyocytes, endothelial cells, and smooth muscle cells. Remarkably, the team further applied this platform to generate hepatic vascularized organoids, providing an exciting blueprint for organoid vascularization.⁶²

To overcome the limited neural-immune crosstalk in current models, integrated hybrid systems are being developed that combine organ-on-a-chip and microfluidic technologies to establish functional multicellular communication networks. In parallel, 3D bioprinting enables the precise spatial patterning of multiple cell types, replicating native tissue architecture and simulating region-specific biomechanical and biochemical cues to generate sophisticated skin organoids with physiologically relevant cellular interactions. Together, these engineered platforms recapitulate key physiological features such as biomechanical forces, systemic responses, and neuro-immune-endocrine crosstalk.

The clinical translation of organoid and iPSC technologies also faces dual technical and ethical challenges. Technically, issues including tumorigenic risk, batch-to-batch variability, and functional immaturity must be addressed to meet clinical safety and efficacy standards. Ethically, concerns range from informed consent for human biological materials to the moral status of advanced models, particularly neural organoids with potential sentient traits. Resolving these challenges will require the coordinated development of robust technical protocols and ethically informed regulatory frameworks.

Overall, improving organoid transplantation efficacy demands advancements in structural integrity, functional diversity, and culture stability. In the context of drug screening, these technological advancements must be complemented by standardized protocols for organoid-based therapeutic evaluation and rigorous pharmacodynamic validation frameworks. The rapid advancement of organoid technology has provided a powerful research tool for scientific exploration. However, despite numerous challenges, the technology still holds great application potential.

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Conflict of interest

The authors declare that they have no competing interests.

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