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Construction strategies of skin organoids: A review

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Abstract

Skin organoids have evolved into versatile models, including reconstructed epidermis, skin equivalents, and composite organoids with appendages. Despite this progress, a unified framework linking construction approaches with developmental outcomes and experimental value remains needed. This review summarizes key determinants of organoid construction: cell source, assembly methods, extracellular matrix, precise signaling control, and quality assessment. It addresses how these factors shape developmental complexity, reproducibility, and scalability, guiding model selection. Advances in engineering, such as air–liquid interface culture and three-dimensional bioprinting, are highlighted for their potential to improve tissue structure and function. By outlining stage-specific evaluation and practical optimization strategies, this review provides a foundation for standardized protocols and the future development of physiologically relevant skin organoid systems for research and clinical applications.

Keywords: Skin organoid; Skin equivalent; Skin appendage organoid; Organoid construction; Pluripotent stem cell; Adult stem cell; Extracellular vesicle; Bioengineering

1. Introduction

Human skin is a complex and dynamic organ that provides mechanical resilience, metabolic activity, and robust immune surveillance. Its physiological functions depend on the coordinated interplay among epidermal keratinocytes, dermal fibroblasts, adnexal epithelial cells, vascular components, peripheral nerves, melanocytes, adipocytes, extracellular matrix elements, and diverse immune populations.[1–4] This intricate tissue architecture presents distinct challenges for *in vitro* modeling. While conventional monolayer cultures remain valuable for fundamental mechanistic studies, they are inherently limited in recapitulating processes such as barrier formation, appendage development, epithelial and mesenchymal crosstalk, infection, wound healing, and multicellular drug responses.[5–8] The development of reconstructed epidermis and full-thickness skin equivalents marked important

advances, and the emergence of organoid technology has further propelled the field by enabling three-dimensional (3D) models that more faithfully preserve tissue hierarchy, self-organization, and multicellular communication compared to planar cultures.[3,4,9–12]

The term "skin organoidc" now refers to a range of distinct yet conceptually related experimental platforms. One approach involves engineered assemblies, in which keratinocytes and stromal cells are separately expanded and combined on collagen-rich matrices.[10,12–14] Another approach uses self-organizing pluripotent stem cell aggregates to generate epidermal, dermal, sensory, and appendageal elements after extended differentiation.[4,7,15,16] A third strategy employs organoids derived from adult tissue-resident progenitors, which offer shorter construction times, greater phenotypic maturity, and stronger experimental tractability.[5,6,17–19] Additionally, there are hybrid systems at the intersection of organoid biology and tissue engineering, including epithelial and mesenchymal recombination models, follicle germ constructs, spheroid skin units, planarized assemblies, and bioprinted configurations that impose spatial order to support morphogenesis or enhance manufacturing.[20–23] In this review, we adopt a focused definition of skin organoids as selforganizing, stem cellderived 3D structures that contain at least the epidermis and dermis. Reconstructed epidermis and conventional skin equivalents (manual layering) are excluded; follicle germs are included only when formed within organoids; bioprinted tissues are excluded but discussed as engineering tools; skinonchip models are excluded and treated as complementary systems.

The variety of available platforms allows the field to converge around a set of common design principles. In skin biology, key factors to consider include how the model is assembled, how much tissue organization is achieved, and which biological questions the system is intended to address.[9,24] A protocol optimized for hair placode emergence[4,7,15] emphasizes different design choices than a protocol directed toward barrier maturation.[5,11] A platform developed for host–pathogen interactions[5,25] highlights strengths distinct from those of a platform designed for vascular integration.[26] Likewise, bilayer constructs are particularly well-suited for toxicological studies because of their reproducibility and experimental control, whereas inducing skin appendages, such as hair follicles, relies on broader developmental potential.[15,22] Therefore, choosing the right skin organoid model is a precise decision that must balance biological goals with technical feasibility.

Several reviews published between 2021 and 2026 have examined skin organoids and related *in vitro* skin model systems from complementary perspectives, including pluripotent stem cell-derived organoids, bioengineered skin models, appendage-focused systems, skin-on-chip platforms, and microphysiological systems (Table 1). These studies have provided an important foundation for understanding model development, technical advances, and biomedical applications. Building on this literature, the present review organizes the field according to five operational dimensions: starting cell origin and lineage potential, method of tissue integration, scaffold or matrix characteristics, temporal sequence of developmental cues, and assessment criteria for differentiation milestones. Systematic comparison of these parameters across different model types allows researchers to pinpoint essential variables, appropriately align experimental design with biological objectives, and anticipate potential technical hurdles to reproducibility. This conceptual approach thereby informs the selection and adaptation of diverse platforms for specific research questions and translational goals.

This review begins by examining pluripotent stem cellbased systems, which cover the broadest developmental spectrum and have enabled the formation of complex, appendagebearing skin organoids directly from embryonic and induced pluripotent stem cells (iPSCs). Next, we focus on organoids derived from adult stem and progenitor cells, which offer experimentally tractable models for studying mature epidermal and glandular biology.[5,6,17–19] Subsequent sections discuss key signaling principles, engineering strategies that improve fidelity and reproducibility, biomedical applications, and emerging directions, including vascularized and immune competent skin organoids, planar models cultured at an air–liquid interface (ALI), and integration with extracellular vesicle (EV)-based approaches.[26–28]

2. Positioning and practical classification of skin organoid platforms

Skin model platforms differ in biological complexity, experimental controllability, and translational applicability. Positioning skin organoids within this broader landscape is therefore essential for defining their appropriate use. This section first compares skin organoids with classical skin equivalents, skin-on-chip systems, and animal models in terms of predictive capability, ethical value, and translational relevance. It then classifies the principal skin organoid construction strategies according to cell source, mode of tissue assembly, developmental potential, and experimental utility. This framework is intended to support the selection of model systems that are appropriately matched to specific biological questions and translational objectives.

Table 1. Representative reviews of skin organoids and related *in vitro* skin model systems published between 2021 and 2026

Review focus	Representative reviews	Main topics covered
General advances, construction strategies, and biomedical applications of skin organoids	Lee and Koehler[29]; Sun *et al.*[30]; Li *et al.*[31]; Hong *et al.*[9]; Huang *et al.*[8]; Zeng *et al.*[24]; Wang *et al.*[32]	These reviews summarize the conceptual scope, cellular sources, three-dimensional culture methods, self-organization processes, developmental basis, appendage formation, and biomedical applications of skin organoids. More recent reviews further discuss biomaterial scaffolds, bioengineering-assisted approaches, key signaling pathways, the integration of vascular and immune components, and the use of skin organoids in disease modeling, drug screening, and regenerative medicine
Pluripotent stem cell-derived skin organoids	Sandoval *et al.*[33]; Zhang *et al.*[34]	These reviews focus on the directed differentiation and self-organization of embryonic stem cells and induced pluripotent stem cells into skin organoids with appendageal structures. They also discuss applications in skin development research, inherited skin disease modeling, personalized studies, and tissue regeneration
Engineered skin models, skin equivalents, and vascularized three-dimensional skin models	Hosseini *et al.*[35]; Rimal *et al.*[36]; Lakeh and Shafiee[37]	These reviews summarize scaffold-free and scaffold-based biofabrication strategies, reconstructed epidermal models, full-thickness skin equivalents, appendage-containing models, and vascularized three-dimensional skin systems. Particular attention is given to cellular composition, biomaterials, spatial organization, vascular integration, and functional evaluation
Appendage-focused models	de Groot *et al.*[38]; Ji *et al.*[39]; Liu *et al.*[40]; Yao *et al.*[41]	These reviews examine hair follicle development and regeneration, epithelial–mesenchymal interactions, hair follicle organoid construction, sebaceous gland organoid engineering, and appendage-specific functionalization. They also discuss primary cell co-culture, pluripotent stem cell differentiation, biomaterial-assisted construction, incomplete maturation, and standardization challenges
Skin-on-chip and microengineered skin models	Zoio and Oliva[42]; Cho *et al.*[43]; Ismayilzada *et al.*[44]; Teertam *et al.*[45]	These reviews cover epidermis-on-chip, full-thickness skin-on-chip, vascularized skin-on-chip, and disease-specific microfluidic skin models. They discuss the contributions of dynamic perfusion, mechanical stimulation, spatial compartmentalization, and real-time monitoring to barrier assessment, disease modeling, drug screening, toxicological evaluation, and translational development
Integration of organoids, organ-on-chip systems, and skin microphysiological systems	Huang *et al.*[46]; Lee *et al.*[47]	These reviews discuss the convergence of skin organoids, organ-on-chip systems, three-dimensional bioprinting, and microfluidic technologies. Major topics include biomimetic artificial skin, non-animal testing alternatives, complex functional simulation, standardization, validation, automation, and high-content screening

2.1. Comparative predictive, ethical, and translational value of major skin model platforms

Classical reconstructed epidermal models and full-thickness skin equivalents provide reproducible and experimentally controlled systems for barrier assessment, irritation testing, and topical exposure studies. Reconstructed human epidermis models have been incorporated into standardized testing frameworks for skin irritation and serve as important tools for non-animal safety assessment.[48–50] However, these models generally do not fully recapitulate the complex interactions among skin appendages, vascular components, neural elements, and immune cells and therefore remain limited for studies of multicellular coordination and regulation of the tissue microenvironment.

Skin organoids can preserve self-organization and multicellular interactions to varying degrees. Selected systems also support the formation of skin appendages and the incorporation of vascular, neural, or immune components, making them particularly suitable for studying skin development, appendage morphogenesis, host–pathogen interactions, and regenerative responses. Skin-on-chip systems provide another form of experimental control through microfluidic perfusion, spatial compartmentalization, mechanical stimulation, and dynamic medium exchange, thereby offering more stable conditions for dynamic exposure studies and multiparametric evaluation.[51,52] Animal models remain important when the research question involves systemic immune responses, endocrine regulation, vascular responses, or pharmacokinetic processes. However, species-specific differences and ethical constraints limit their ability to fully reproduce human skin biology. These platforms should be regarded as complementary rather than interchangeable, and model selection should be guided by the biological question, required tissue complexity, experimental controllability, and intended translational use. The comparative characteristics of these platforms are summarized in Table 2.

2.2. A practical classification of skin organoids

For experimental design, skin organoids can be grouped into four principal construction strategies. The first

Table 2. Comparative value of major skin model platforms for experimental and translational applications

Model platform	Main predictive strengths	Key limitations	Ethical and translational relevance
Classical reconstructed epidermis and full-thickness skin equivalents	Reproducible barrier architecture; controlled epithelial and stromal composition; compatibility with standardized topical exposure and irritation testing	Limited appendageal, vascular, neural, and immune complexity	Established value in non-animal safety assessment; suitable for standardized screening workflows
Skin organoids	Self-organization; multicellular communication; donor-specific modeling; potential integration of appendageal, vascular, neural, and immune components	Variable maturation; appendage heterogeneity; prolonged culture; limited throughput	Human-derived models may reduce and refine animal use; useful for disease-specific and patient-specific studies, although broader validation remains necessary
Skin-on-chip systems	Dynamic perfusion; spatial compartmentalization; mechanical stimulation; defined exposure conditions; potential for real-time monitoring	Fabrication complexity; platform-specific readouts; incomplete interlaboratory standardization	Suitable for controlled dynamic exposure studies and preclinical testing; may improve human relevance while reducing animal use
Animal models	Whole-organism physiology; systemic immune, endocrine, vascular, and pharmacokinetic responses	Species-specific differences; ethical constraints; limited prediction of some human-specific responses	Remain necessary for selected systemic questions, but should be complemented by human-relevant *in vitro* models

comprises reconstructed skin equivalents derived from pluripotent stem cells. In such models, embryonic or iPSCs are first differentiated into keratinocytes and, depending on the specific protocol, fibroblast-like stromal cells. Subsequently, these cell populations are assembled on dermal scaffolds to generate either stratified epidermal or fullthickness constructs.[10–14,28,53,54] The second approach uses selforganizing composite skin organoids, also derived from pluripotent stem cells. Here, a single cell aggregate is guided through sequential lineage induction and, over a prolonged culture period, selforganizes into tissue bearing skin appendages.[4,7,15,55,56] The third approach involves lineage-restricted organoids generated from adult stem or progenitor cells, such as epidermal, sebaceous gland, or sweat gland organoids. These systems are typically established from tissue-resident progenitors embedded within a basement membrane matrix.[5,6,17–19,57] The fourth approach comprises epithelial and mesenchymal recombination systems, exemplified by dermal papilla spheroids, hair follicle germ constructs, and hybrid models that are specifically designed to recreate the bidirectional signaling between epithelial and mesenchymal compartments.[20–23,58–60]

This classification directly links the cellular source to the developmental potential of each organoid system, thereby offering a practical framework for model selection. Pluripotent stem cellbased systems support the generation of multiple lineages and are particularly well-suited for studying early tissue patterning, appendage morphogenesis, and patientspecific developmental disorders.[4,15,55] In contrast, organoids derived from adult cells possess comparatively limited developmental potential.[5,6,30] Nonetheless, this limited developmental potential often confers distinct benefits—lineage memory is maintained, culture durations are reduced, and mature tissue properties develop more swiftly.[17,19,57] Recombination approaches occupy an intermediate position, allowing the reconstruction of specific morphogenetic interactions with a degree of control that is difficult to achieve through self-organization alone.[20,21,58,60–62] Recognizing these distinctions provides a rational basis for selecting the most appropriate model for defined research objectives.

This framework emphasizes the complementary strengths of engineered assembly and self-organizing strategies. Engineered assembly offers reliable control over cellular composition, spatial organization, and experimental reproducibility.[14,58,62–64] In contrast, self-organizing systems excel at uncovering emergent developmental processes and multicellular pattern formation. Taken together, engineered assembly and self-organization define a continuum from biological plasticity to manufacturing precision, with each approach providing distinct advantages for developmental biology, disease modeling, and regenerative medicine (Table 3; Figure 1).

3. Pluripotent stem cell-derived skin organoids

Skin organoids derived from pluripotent stem cells offer the most extensive developmental potential, as a single starting population can give rise to epidermal, dermal, pigmentary, neural crest, and appendage-supportive lineages. Current

Table 3. Practical comparison of the main skin organoids

Organoid	Starting material	Core construction	Typical time to informative findings	Best-fit applications
Pluripotent reconstructed skin equivalents.	ESCs or iPSCs differentiated into keratinocytes and fibroblast-like stromal cells	Sequential lineage induction followed by deliberate epithelial–stromal assembly on collagen-rich dermal supports	2–6 weeks for keratinocyte differentiation; longer for full-thickness assembly	Barrier studies, disease modeling, transplantation-oriented engineering, and gene editing
Self-organizing composite skin organoids	hESCs or hiPSCs aggregated as uniform embryoid bodies	Timed ectoderm/neural crest patterning plus prolonged low-attachment maturation with diluted ECM support	Early cystic checkpoints within ~1 week; appendage-bearing features commonly appear after 2–3 months	Developmental biology, appendage morphogenesis, infection, regenerative modeling
Adult epidermal organoids	Basal-enriched adult epidermal keratinocytes	Matrix embedding with a stemness-preserving medium that still permits structured differentiation	Days to 1–2 weeks for early spheres; multi-week expansion	Barrier biology, host–microbe interaction, donor-specific perturbation studies
Hair follicle organoids/follicle germs	Epithelial cells plus dermal papilla or dermal condensate-like mesenchyme	Reconstitution of epithelial–mesenchymal reciprocity through spheroids, Janus microtissues, peg-like aggregates, or printed constructs	Days to weeks for aggregate organization; transplantation is often needed for robust hair output	Hair inductivity, folliculogenesis, regenerative hair engineering
Sebaceous gland organoids	Sebaceous lineage-enriched progenitors (for example, Blimp1+ populations)	Basement membrane matrix culture with a balance between proliferative outer cells and inward sebocyte differentiation	1–2 weeks for organized gland-like units	Sebocyte specification, lipid metabolism, and acne-related biology
Sweat gland organoids	Primary sweat gland epithelial cells or reprogrammed epidermal keratinocytes	Matrix-supported glandular morphogenesis with EGF/FGF/EDA/BMP- and cAMP-related tuning	Days to weeks, depending on the source and reprogramming burden	Wound repair, appendage regeneration, gland-specific modeling

Abbreviations: BMP: Bone morphogenetic protein; cAMP: Cyclic adenosine monophosphate; ECM: Extracellular matrix; EDA: Ectodysplasin A; EGF: Epidermal growth factor; ESCs: Embryonic stem cells; FGF: Fibroblast growth factor; hiPSCs: Human induced pluripotent stem cells; hESCs: Human embryonic stem cells; iPSCs: Induced pluripotent stem cells.

approaches with pluripotent systems have developed along three main strategies. The first uses stepwise differentiation to produce reconstructed epidermal or full-thickness skin equivalents. The second involves self-organizing composite skin organoids that can form appendages. The third applies secondary engineering, in which organoid-derived skin cells or their precursors are redeployed for disease modeling or regenerative purposes. While these strategies share core early-signaling requirements, they differ in construction sequence, time frames, scalability, and common technical challenges.

3.1. Embryonic stem cell-derived reconstructed skin models

Embryonic stem cell (ESC)-based skin engineering established two principles that later shaped iPSC organoids. First, pluripotent cells can be directed toward epithelial and mesenchymal skin-supportive states through staged differentiation. Second, functional skin tissue can be constructed from predefined epithelial and stromal modules, where self-organization operates within a controlled framework. Shamis *et al.*[12] demonstrated that fibroblasts derived from human ESCs support the development and repair of 3D human skin equivalents, confirming that pluripotent cells can generate an instructive dermal compartment. Kidwai *et al.*[10] then developed an autogenic strategy to convert human ESCs into clinically relevant keratinocytes. Petrova *et al.*[11] subsequently generated epidermal equivalents from hESCs and iPSCs that reproduced epidermal architecture and formed a functional permeability barrier.

Embryonic stem cell-derived reconstructed models are generated through a staged sequence. The starting point is a high-quality pluripotent colony state with little spontaneous differentiation and strong viability.[10] Colonies are then directed toward the surface ectoderm, commonly via bone morphogenetic protein (BMP)-associated epithelial induction, together with retinoid signaling or related cues

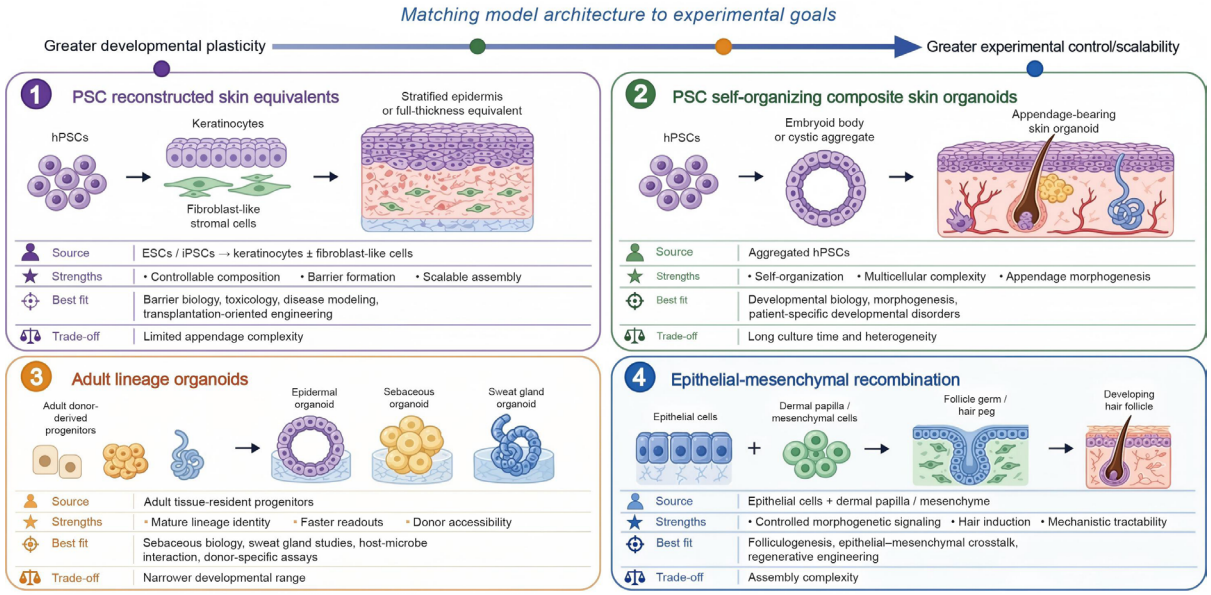

Figure 1. Practical selection and comparison of major skin organoids. Created with BioRender.com. Miao Zhen, Peng Lei (2026). https://BioRender.com/dg2qzw0

Abbreviations: BMP: Bone morphogenetic protein; ECM: Extracellular matrix; ESCs: Embryonic stem cells; hPSCs: Human pluripotent stem cells; iPSCs: Induced pluripotent stem cells; PSC: Pluripotent stem cell.

that promote keratinocyte differentiation. A TP63-positive, keratin 14 (KRT14)-positive keratinocyte progenitor state is expanded before terminal stratification is promoted by calcium shift, matrix exposure, or ALI culture. In parallel, fibroblast-like stromal cells are generated from ESCs or supplied exogenously.[12] Final assembly is usually performed on a collagen-rich dermal matrix or related support.

In this configuration, ESC-derived reconstructed skin is characterized by precision and modularity. This design allows epithelial and stromal compartments to be manipulated separately, genes to be edited before assembly, and barrier competence, differentiation, or stromal support to be examined within clearly defined experimental boundaries. These strengths make the system especially valuable for studies of epidermal commitment, barrier formation, matrix-supported maturation, and clinically oriented skin reconstruction. Within the broader skin organoid landscape, ESC-derived assembly remains a rigorous and conceptually important approach for focused epithelial–stromal engineering.

3.2. Induced pluripotent stem cell-derived reconstructed skin equivalents

The rise of iPSC technology has transformed skin engineering by enabling patient-specific modeling and disease-specific regenerative design.[65,66] Early studies focused on the directed differentiation of iPSCs into keratinocytes.[65,67,68] Itoh *et al.*[65] derived keratinocytes from iPSCs of patients with normal and recessive dystrophic epidermolysis bullosa, demonstrating that patient-specific epidermal cells could be generated from pluripotent stem cells. Kogut *et al.*[67] later described a practical differentiation framework in which retinoic acid and BMP4 served as core cues for epidermal lineage commitment. Together, these studies established keratinocyte derivation as a reproducible upstream step for downstream skin reconstruction.

Once keratinocyte derivation became reliable enough, the field moved toward full-thickness reconstruction. Itoh *et al.*[13] showed that iPSC-derived keratinocytes and iPSC-derived fibroblasts could be combined to generate skin equivalents comprising a stratified epidermis overlying a stromal compartment. Kim *et al.*[14] extended this strategy by employing a layered co-culture of iPSC-derived keratinocytes and fibroblasts, thereby establishing a more elaborate engineered skin structure with translational potential. Takagi *et al.*[22] further advanced the concept by bioengineering a 3D integumentary organ system from iPSCs *in vivo*, showing that pluripotent cell-based reconstruction could support not only skin architecture but also appendage-containing organogenesis following transplantation.

Keratinocyte differentiation proceeds through

ectodermal commitment and epidermal stabilization, typically involving retinoid and BMP exposure, after which the cells are matured and expanded under keratinocyte-permissive conditions.[67–69] Stromal support cells are derived in parallel or introduced from matched lines.[70,71] Assembly is generally performed by seeding fibroblasts into a collagen I-rich dermal matrix, allowing the matrix to contract or stabilize, and then layering keratinocytes onto the surface. Stratification is reinforced by calcium exposure and, in many protocols, by transfer to an ALI.[14,53,68]

The defining strength of iPSC systems is personalization. Disease-associated genotypes can be carried forward into organoids, corrected by gene editing, or compared isogenically before and after manipulation.[66,70,72] This feature is particularly valuable for inherited blistering disorders and models of fibrotic or inflammatory skin disease.[70,72,73] iPSCderived reconstructed equivalents further inherit the advantages of engineered assembly, such as high structural reproducibility and precise control over epithelial and stromal composition.[74] With the addition of developmental design, these systems can be effectively linked to more complex platforms that support appendage formation.[22,53,75] Taken together, iPSC-derived reconstructed skin equivalents are especially well-suited to barrier biology, disease modeling, and regenerative reconstruction.

Although ESC-derived and iPSC-derived systems share broad pluripotency and overlapping differentiation principles, their use involves distinct practical considerations. ESC-derived models remain valuable reference platforms for controlled studies of epidermal and stromal differentiation, whereas iPSC-derived models offer greater flexibility for donor-specific disease modeling and isogenic comparison.[10–12] ESC derivation raises ethical considerations related to the use of embryo-derived materials. Reprogramming somatic cells into iPSCs avoids these embryo-specific concerns, although informed consent, donor privacy, data governance, and the permitted scope of downstream use remain relevant.[76] Genomic quality control is required for both cell sources. In iPSC-based systems, additional variability may arise from reprogramming methods, donor background, clone selection, prolonged culture, and residual epigenetic memory, which can influence differentiation efficiency and batch-to-batch reproducibility.[77–79] The relative suitability of ESC-derived and iPSC-derived systems should therefore be assessed according to the intended experimental application rather than treated as a fixed hierarchy. These considerations are summarized in Table 4.

Table 4. Practical comparison of ESC-derived and iPSC-derived skin organoid systems

Consideration	ESC-derived systems	iPSC-derived systems
Cell source and ethical considerations	ESCs are derived from early-stage embryos. Their use, therefore, requires careful ethical review and compliance with national and institutional regulations concerning embryo-derived materials	iPSCs are generated by reprogramming somatic cells and avoid the embryo-specific ethical concerns associated with ESC derivation. However, informed consent, donor privacy, data governance, and the permitted scope of downstream use remain important consideration[76]
Genomic stability	Established ESC lines provide experimentally tractable reference platforms, but prolonged culture and selection pressure may still result in genetic or epigenetic alterations. Routine genomic quality control remains necessary	iPSCs require additional attention to genomic integrity because reprogramming, clone selection, donor background, and prolonged culture can introduce or enrich genetic and epigenetic abnormalities. The reprogramming method should be reported, and genomic quality control should be performed before differentiation
Differentiation efficiency	Established ESC lines can provide a relatively consistent starting point for directed epidermal and stromal differentiation. However, differentiation efficiency remains dependent on cell-line quality, passage history, and protocol conditions	iPSCs support patient-specific and disease-specific modeling, but differentiation efficiency may vary across donors and clones. Residual epigenetic memory and differences in pluripotent cell states can influence lineage commitment [78]
Batch-to-batch reproducibility	Reproducibility can be improved by using validated reference lines, standardized passage ranges, and defined quality-control criteria. Batch effects may still arise from cell-state variation, culture handling, and matrix conditions	Batch-to-batch and line-to-line variability may be more prominent because donor background, reprogramming method, clone selection, and pluripotent-state heterogeneity can affect downstream differentiation. Standardized cell preparation, aggregate formation, and early morphological checkpoints are particularly important
Best-fit applications	Mechanistic studies of epithelial commitment, dermal support, barrier formation, and controlled tissue reconstruction	Patient-specific disease modeling, isogenic gene editing studies, personalized drug testing, and regenerative applications
Practical interpretation	ESC-derived models remain useful as controlled reference systems	iPSC-derived models provide greater personalization but require more extensive characterization before comparative or translational use

Abbreviations: ESC: Embryonic stem cell; iPSC: Induced pluripotent stem cell.

3.3. Self-organizing appendage-bearing composite skin organoids

The most developmentally expansive skin organoids are self-organizing pluripotent composite organoids that recapitulate key aspects of fetal skin development. Unlike systems that begin with separately matured epithelial and mesenchymal compartments, these organoids start from a single pluripotent aggregate. This aggregate is directed through sequential developmental states, allowing epidermal and dermal lineages, along with pigmentary and neural elements, to emerge within a single morphogenetic program. Lee *et al.*[55] first established this approach using mouse pluripotent stem cell-derived skin organoids. They later reported the breakthrough that hair-bearing human skin generated entirely from pluripotent stem cells.[4] These human organoids contained stratified epidermis, fat-rich dermis, pigmented hair follicles, sebaceous glands, Merkel cells, and sensory neuron-associated structures. The accompanying protocol translated this advance into a step-by-step method, and Ahmed *et al.*[7] later improved aggregate consistency across human iPSC (hiPSC) lines by adopting direct embryoid body formation in differentiation medium.

These protocols follow a tightly staged sequence. Pluripotent cells are first aggregated under conditions that maximize survival and reduce line-to-line variability; inhibition of Rho-associated coiled-coil kinase (ROCK) supports aggregate formation during the earliest phase. In the optimized Ahmed protocol[7], hiPSCs are aggregated directly into the differentiation medium, thereby simplifying the transition to induction conditions. Early lineage induction is then performed in Essential 6 medium supplemented with growth-factor-reduced Matrigel, SB431542, low-dose fibroblast growth factor (FGF) 2, and BMP4 to drive non-neural ectoderm, followed by LDN193189 and higher FGF2 to support cranial neural crest-related states. These staged transitions establish both epidermal competence and mesenchymal or neural crest-related supportive lineages within the same aggregate.[7,15,16]

After early patterning, maturation conditions become equally important. In Ahmed's optimized protocol[7], organoids are transferred at day 12 into maturation medium that initially contains low Matrigel and later becomes matrix-free. Early morphology is a practical checkpoint: successful differentiation produces a transparent epithelial cyst-like structure during the first 6–12 days, whereas rough-edged or irregular aggregates usually fail later.[7] Over the following weeks, organoids enlarge, stratify, build a dermal compartment, and eventually form hair placodes and hair follicles; in many lines, visible follicular protrusions emerge by about 2–3 months. The long culture period reflects the fetal-like developmental timeline captured by these organoids.

This self-organizing approach continues to evolve. Shafiee *et al.*[56] reported the generation of physiologically relevant skin organoids from hiPSC lines derived from human skin fibroblasts or placental $CD34^+$ cells. They demonstrated that direct embryoid body formation can yield complex organoids containing stratified skin layers, pigmented hair follicles, sebaceous glands, Merkel cells, and eccrine sweat glands. Sun *et al.*[27] then showed that moving day-12 organoids to a Transwell ALI culture improved hair follicle number and maturation compared with floating culture. Mostina *et al.*[26] further extended the platform by combining vascular organoids with hiPSC-derived skin organoids to generate vascularized skin organoids that also contained immune cell populations. Recent mechanistic work further showed that hypoxia-driven metabolic adaptation contributes to planarization: hypoxia-inducible factor 1A-dependent glycolysis helps position epidermal cells, lysosomal hydrolases remove suprabasal keratin debris, and fibroblasts next to the basal epidermis acquire papillary-like features with enhanced retinoid metabolism.[80] Together, these studies show that the original floating composite organoid has become a starting architecture that can be refined to achieve greater physiological relevance.

3.4. Organoid-sourced skin cells and secondary engineering

A particularly important conceptual shift is the move from viewing organoids as final experimental products to using them as cell reservoirs. Garriga-Cerda *et al.*[70] used iPSC-derived organoid-derived skin cells to construct functional 3D models of recessive dystrophic epidermolysis bullosa, thereby demonstrating that organoid-generated cell populations can be harvested, reassembled, and deployed as modular building blocks. This strategy combines the developmental richness of organoid differentiation with the experimental control of engineered tissue assembly. It is especially attractive in settings where organoid-derived lineage diversity is needed, but where batch heterogeneity or the long timelines required to maintain full composite organoids may limit experimental consistency.

Work on localized scleroderma and SARS-CoV-2 skin infection further illustrates the flexibility of pluripotent skin organoid platforms.[25] In the SARS-CoV-2 model, hiPSC-derived skin organoids provided a more physiologically informative infection context than monolayer cultures. In localized scleroderma, iPSC-derived epithelial–mesenchymal organoids were used therapeutically, resulting in reduced fibrosis and the regeneration of epidermis, sweat glands, and vasculature.[73] These examples underscore an emerging principle: pluripotent skin organoids are not only developmental models but also increasingly adaptable

modules for disease modeling, regenerative intervention, and cell-free biological outputs, including EV production.[28]

4. Adult stem/progenitor-derived skin organoids

Adult stem/progenitor-derived skin organoids offer speed, lineage fidelity, and close access to mature tissue behavior. These cultures begin with progenitors that already possess strong positional and lineage memory, making them particularly well-suited to focused questions such as epidermal homeostasis, dermatophyte infection, sebocyte differentiation, glandular regeneration, hair induction, or wound-related epithelial plasticity. Their targeted scope often enhances reproducibility and interpretability, especially in experiments centered on a single epithelial lineage or appendage program.[5,6,8,9]

4.1. Epidermal organoids

Epidermal organoids provide one of the clearest demonstrations that adult skin stem cells can be expanded long-term in three dimensions under feeder-free, serum-free, or chemically defined conditions while retaining their differentiation capacity.[5,6] Boonekamp *et al.*[6] established a murine epidermal organoid culture system in which adult epidermal stem cells were embedded in basement membrane extract and expanded for at least six months, while maintaining basal and apical organization. The expansion medium combined cyclic adenosine monophosphate (cAMP) activation, FGF signaling support, R-spondin pathway activation, and BMP inhibition. This work demonstrated that adult epidermal organoids require an environment that preserves stemness while still permitting tissue architecture and controlled differentiation.[6]

Wang *et al.*[5] extended this concept to human primary epidermal organoids, advancing the field toward a chemically defined human system. These organoids were derived from epidermal cells isolated from foreskin and maintained under defined conditions that recapitulated the morphological, molecular, and functional features of human epidermis for several weeks. The culture was permissive to *Trichophyton rubrum* infection, rendering it useful for modeling host–fungal interactions. The protocol began with epidermal cell isolation, followed by attachment in low-calcium, serum-free EpiLife medium. Optimization experiments identified a critical contribution of A83-01, and organoids could be generated from integrin subunit alpha 6-high, basal-enriched cells, including from single cells.[5]

Together, these studies define a practical approach to generating epidermal organoids. First, basal-competent keratinocytes are enriched, either by anatomical source and enzymatic processing or by marker-based selection such as high integrin subunit alpha 6 expression. Second, the cells are embedded in a basement membrane-type matrix (e.g., Matrigel, basement membrane extract, or Cultrex), where 3D epithelial spheres expand into organoid structures. Third, the medium composition is optimized to preserve clonogenicity while permitting organized differentiation. In murine cultures, this includes Noggin, R-spondin, forskolin, and FGF family factors; in human cultures, chemically defined media and modulation of the transforming growth factor-beta (TGF-β) pathway are particularly important. Fourth, organoids are monitored for size, luminal organization, retention of basal markers, and stratification status.[5,6,81]

Epidermal organoids offer several experimental advantages. They are relatively fast to establish, far easier to passage than complex composite organoids, and well-suited for medium-scale perturbation studies. Their compact nature simplifies infection assays, barrier analysis, and lineage-focused transcriptional profiling. Moreover, because they can be initiated directly from adult tissue, epidermal organoids are useful for modeling donor-specific biology, thereby streamlining donor-linked experimental designs. Their primary strength lies in their focus on epidermal biology, although broader dermal, vascular, neural, or appendageal features can be incorporated when a more integrated tissue context is required.[5,6,8,24,46,82]

4.2. Hair follicle organoids, follicle germs, and epithelial–mesenchymal recombination

Hair follicle organoid construction is governed by one principle above all others: epithelial–mesenchymal reciprocity must be recreated with sufficient spatial intimacy and inductive fidelity to initiate folliculogenesis.[83] For this reason, the hair field encompasses a wide range of models, from dermal papilla spheroids and epithelial–mesenchymal aggregates to bioengineered hair follicle germs, self-organizing hair peg-like structures, and hiPSC-derived ectodermal precursor-based follicle organoids.[20,21,84–87] Several of these systems fall outside the strictest definition of an organoid, but they are central to skin organoid methodology because they demonstrate how appendage morphogenesis can be experimentally reconstructed.

A key observation in the hair field is that dermal papilla cells lose their inductive capacity in routine monolayer culture but regain key features in 3D. Gupta *et al.*[20] established an *in vitro* dermal papilla organoid model composed of 3D dermal papilla spheroids, with or without a silk–gelatin hydrogel microenvironment. They showed improved dermal papilla gene expression, extracellular matrix production, and epithelial crosstalk relative to monolayer culture. When these mesenchymal modules were combined with hair follicle keratinocytes and stem

cells, the resulting constructs more closely reproduced features of the follicle niche.[20]

Weber *et al.*[21] approached the problem from another angle by generating self-organizing hair peg-like structures from dissociated skin progenitor cells. Their work showed that early follicular organization can emerge from suitable progenitor mixtures under asymmetric morphogenetic conditions, offering insight into how pattern formation and hair peg morphogenesis can be reproduced *in vitro*.[21] Lei *et al.*[88], working with newborn skin organoids, similarly showed that dissociated skin cells can self-organize into structures bearing hair primordia, and that the self-organization process itself reveals stage-specific molecular and biophysical requirements. These models are valuable not only as regenerative tools but also as systems for dissecting the early events that govern follicle initiation.

More recent work has focused on increasing scalability and translational control. Kageyama *et al.*[84] developed hair follicle germs containing vascular endothelial cells, arguing that endothelial support can enhance hair inductivity, and subsequently reported the generation of hair follicle organoids from human iPSC-derived ectodermal precursor cells for regenerative medicine applications.[84,85] Sugiyama *et al.*[60] introduced a microfluidic method for the large-scale preparation of hair follicle germs using Janus-structured collagen microbeads containing epithelial and mesenchymal cells; within three days, the beads contracted into dense follicle germs that regenerated hair efficiently after transplantation. Catarino *et al.*[62] applied similar engineering principles to 3D bioprinting by printing dermal papilla cells and endothelial cell spheroids within a pregelled dermal layer, after which keratinocytes and melanocytes migrated and organized around them, generating hair follicle-like structures in engineered skin tissue.

Despite their diversity, successful hair organoid methods share recurrent technical requirements. First, both the epithelial and mesenchymal populations must be of high quality prior to recombination. Mesenchymal cells, particularly dermal papilla cells, must be maintained in an inductive-competent state, often achieved through spheroidization or the use of supportive biomaterials.[20,84,89,90] Second, spatial apposition is critical: the closer and more stable the interface between epithelial and mesenchymal modules, the more robust the reciprocal signaling.[58,84,91] Third, matrix mechanics also matter; collagen-rich or hybrid hydrogels can preserve tissue architecture while allowing compaction. Finally, evaluation should be staged: early condensation, peg-like morphology, and inductive marker expression are not equivalent to mature follicle formation. Conflating these endpoints can lead to overinterpretation.

As a category, hair follicle organoids are indispensable for appendage biology, but they are also among the most technically fragile skin organoid systems. Minor changes in the starting cell phenotype, passage number, matrix composition, or geometry can dramatically alter the outcome. This sensitivity is the price of modeling one of the most signaling-intensive morphogenetic events in skin biology. When successful, these systems provide unmatched access to follicular induction, epithelial–mesenchymal communication, hair-regeneration strategies, and the testing of hair-growth-promoting or hair-inhibitory cues.

4.3. Sebaceous gland organoids

Sebaceous gland organoids are focused systems, yet they are highly informative, capturing a glandular differentiation program that remains underrepresented in many broader skin models. Current studies have established a strong foundation for future work on functional maturation and integration with neighboring skin compartments.[19,40,92,93] Feldman *et al.*[19] demonstrated that single Blimp1-positive cells isolated from mouse skin could generate functional sebaceous gland organoids *in vitro*. These organoids developed an architecture in which proliferative cells occupied the outer layer, then moved inward and differentiated into lipid-filled sebocytes, closely mirroring the directional organization of sebaceous gland homeostasis *in vivo*. Marker analysis and lipidomics further showed that the organoids reproduced a sebaceous-like molecular and metabolic profile.[19]

The method for generating sebaceous organoids is instructive, as it illustrates how enriching for lineage-committed starting cells can simplify downstream culture design. The key preparative step is enrichment for gland-associated progenitors, in this case, Blimp1-positive cells from the sebaceous gland base. Once isolated, cells are placed in 3D culture with appropriate growth-factor support, and organoid formation can be followed over roughly 1–2 weeks. The critical endpoints to assess include the emergence of an outer proliferative layer, inward cell migration, lipid accumulation, and the acquisition of sebocyte markers together with a sebaceous lipid profile.

Sebaceous organoids are especially valuable as they reveal gland-specific features with unusual clarity. Feldman *et al.*[19] used them to identify a role for c-Myc in sebocyte proliferation and to model early features of acne vulgaris, including androgen-responsive changes and responses to retinoid derivatives. This close alignment between construction strategy and gland biology makes these organoids highly relevant to research on sebaceous diseases.

Sebaceous organoids also show that organoid maturity should be judged in a manner appropriate to the tissue being modeled. A sebaceous organoid does not require

a full stratified epidermis, a vascular network, or neural components to be considered informative; rather, it should exhibit the spatial and metabolic hallmarks of glandular function.[93] This more focused standard of success helps explain why lineage-restricted organoids can be highly productive experimental systems, even when they are not intended to recreate the entire organ.

4.4. Sweat gland organoids

Sweat gland organoids are notable for both their biological importance and their potential in regenerative medicine.[17,30,94,95] Diao *et al.*[17] generated sweat gland organoids from adult sweat gland epithelial cells and showed that these organoids contributed to cutaneous wound healing and sweat gland regeneration. This study established a solid experimental foundation for the application of appendage organoids in tissue repair.

A second strategy emerged through lineage reprogramming. Sun *et al.*[30] reported that epidermal keratinocytes could be reprogrammed to form sweat gland organoids that functionally recapitulated the structure and function of damaged skin. This study expanded the field in two important ways. First, it created a practical path forward when native sweat gland cells are scarce. Second, it established sweat gland organoid construction as a cell-state engineering strategy with clear relevance to burn injury and wound repair studies.[30,96]

A practical approach to generating sweat gland organoids thus begins with a choice between two starting cell sources: direct gland-derived epithelial cells, which preserve lineage commitment but require tissue access[17,94], or reprogrammed epidermal keratinocytes, which are more accessible but require robust fate conversion.[30,97] In both cases, cells are embedded in a gland-supportive 3D matrix and expanded under conditions that maintain sweat gland specification.[97,98] Successful cultures are judged by gland-related marker expression, epithelial architecture, regenerative function, and, where relevant, transplantation or wound-repair evidence.[99] Generic epithelial spheroid growth is not enough; the system should demonstrate gland-specific differentiation and function.

Sweat gland organoids are particularly well aligned with regenerative dermatology, especially burn reconstruction, wound healing, and appendage restoration. Their derivation from either tissueresident progenitors or reprogrammed cells underscores their potential for personalized therapies. These studies further demonstrate that appendage organoids can be generated from accessible donor cells, thereby providing a valuable foundation for future work in skin regeneration and repair. In this regard, the sweat gland field serves as a useful model for developing additional appendagefocused organoids from practical donor sources (Figure 2).

5. Signaling principles that support skin organoid construction

Across model categories, successful skin organoid construction depends less on any single factor than on the temporal choreography of a relatively small set of pathways. WNT signaling underpins epidermal competence, placode initiation, and multiple aspects of follicular morphogenesis.[4,15,100–103] BMP signaling is equally central but exquisitely context-dependent: it helps specify non-neural ectoderm early, yet later modulation or inhibition may be required to permit neural crest emergence, maintain organoid-forming competence, or support appendage programs.[4,7,15,104] FGF signaling repeatedly appears as a proliferative and stabilizing input, particularly during early pluripotent differentiation and in adult epithelial cultures.[4,15] Inhibition of the TGF-β pathway, achieved with agents such as SB431542 or A83-01, is a recurring strategy to support epithelial induction or to preserve organoid-forming epithelial states.[5,7] ROCK inhibition is mainly an early survival tool, most useful during aggregate formation or immediately after passaging dissociated pluripotent cells.[15,56] cAMP activation, often with forskolin, is a common feature of adult epithelial organoid media[5,6], whereas Sonic hedgehog (SHH) and ectodysplasin A (EDA) become especially relevant when the desired endpoint includes appendageal morphogenesis.[101,105,106]

The clearest illustration of a signaling sequence comes from modern hiPSC skin organoid protocols. In these systems, the first step establishes a non-neural ectoderm-competent aggregate by combining BMP4 with TGF-β inhibition and low FGF activity in the presence of low matrix support. This is followed by a second step that introduces BMP inhibition and higher FGF input to promote cranial neural crest-related competence, thereby seeding lineages that later contribute to dermal and appendage-supportive compartments.[4,7,15,104] Only after these competence states are established can maturation media sustain cyst growth, stratification, and eventual appendage formation. These protocols are therefore strongly dependent on signal order, with the same pathway producing distinct outcomes at different developmental stages.[4,15,27]

Appendage morphogenesis further illustrates why these pathways should not be considered as independent inputs. During early hair follicle development, epidermal WNT/β-catenin activity is required for placode initiation and contributes to periodic follicle spacing through interactions with WNT inhibitors such as Dickkopf.[100,107] WNT signaling also induces EDA receptor (*EDAR*) expression and is required for downstream nuclear factor kappa B activation, establishing reciprocal coupling between WNT and EDA/EDAR signaling during follicle induction.[101] EDA/EDAR signaling subsequently suppresses BMP activity and promotes SHH expression, thereby permitting

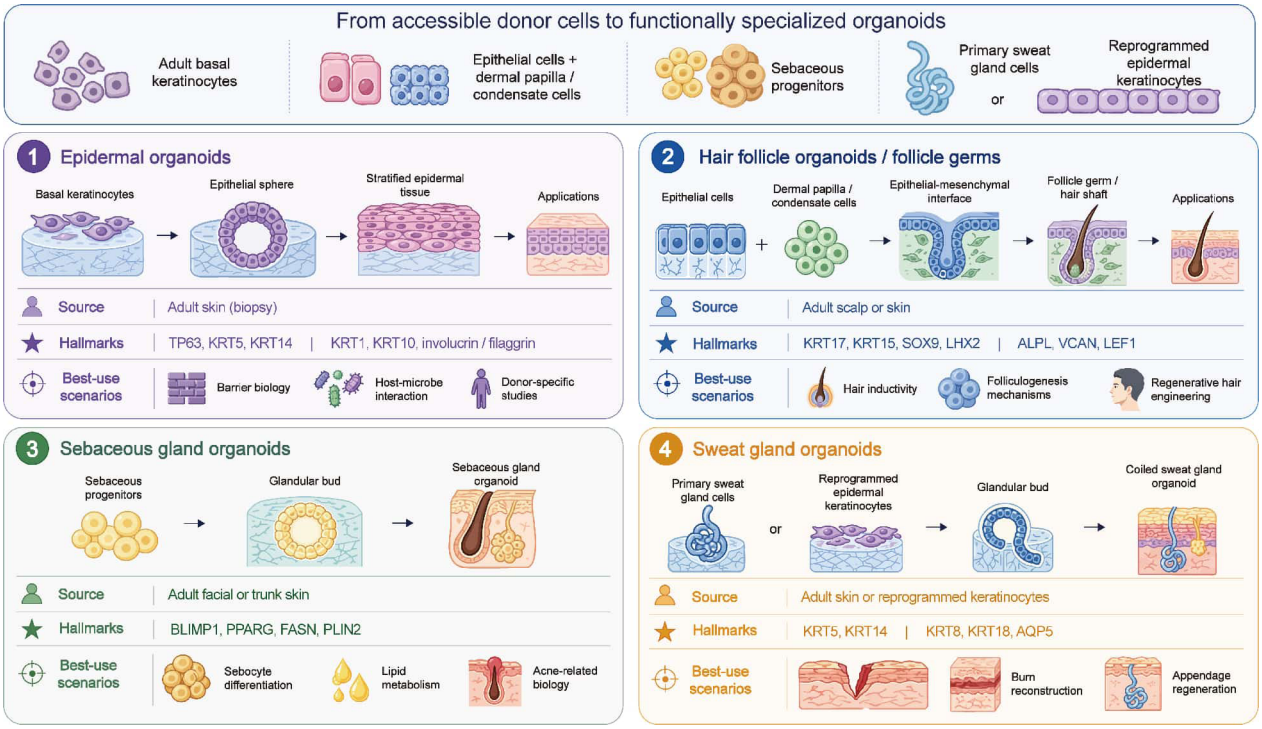

Figure 2. Adult lineage and appendage-focused skin organoids. Created with BioRender.com. Miao Zhen, Shiyu Li, and Zhenning Dai (2026). https://BioRender.com/nt1iktr

Abbreviations: APLP: Alkaline phosphatase; AQP5: Aquaporin 5; BLIMP1: B lymphocyte-induced maturation protein 1; FASN: Fatty acid synthase; KRT: Keratin; LEF1: Lymphoid enhancer-binding factor 1; LHX2: LIM homeobox 2; PLIN2: Perilipin 2; PPARG: Peroxisome proliferator-activated receptor gamma; SOX9: SRY-box transcription factor 9; TP63: Tumor protein p63; VCAN: Versican.

placode progression rather than uniform epidermal differentiation.[108] At the epithelial–mesenchymal interface, placode-derived FGF20 provides an additional temporal signal that is required for primary and secondary dermal condensate formation.[109] FGF20 primes dermal cells for cell-cycle exit, directional movement, and aggregation, linking epithelial specification to mesenchymal condensation.[110] Although the precise timing and magnitude of these interactions remain incompletely defined in human skin organoids, this developmental sequence provides a useful basis for interpreting appendage formation and for refining stage-specific culture conditions.

Adult organoids show the same principle in a more compact form. Murine epidermal organoids rely on high calcium plus activation of cAMP, FGF, and R-spondin pathways, together with BMP inhibition.[6] In contrast, human primary epidermal organoids require careful control of the timing of TGF-β inhibition under chemically defined conditions.[5] Sweat gland organoids use another variation on the same theme, combining proliferative support with appendage-specific EDA/BMP signaling patterns.[17,30,105,106] Even sebaceous organoids, though less discussed in signaling terms, illustrate the idea that a successful medium must preserve an outer proliferative compartment while allowing inner sebocyte differentiation, rather than driving the culture toward uniform expansion.[19]

The repeated use of developmental pathways across skin organoid systems reflects the staged nature of cutaneous tissue formation. A helpful way to think about protocol design is to divide it into four phases: survival, competence, morphogenesis, and maturation. ROCK inhibition mainly supports survival. BMP/TGF-β/FGF combinations chiefly define competence. WNT, SHH, and EDA become more prominent during morphogenesis, especially in appendage-bearing models. During maturation, factors such as ALI exposure, matrix withdrawal or modulation, increased medium volume, and mechanical conditioning often contribute most strongly. Thus, successful organoid formation depends not only on which pathways are manipulated but also on when and in what sequence they are applied, and on how they interact across epithelial and mesenchymal compartments, as summarized in Figure 3.

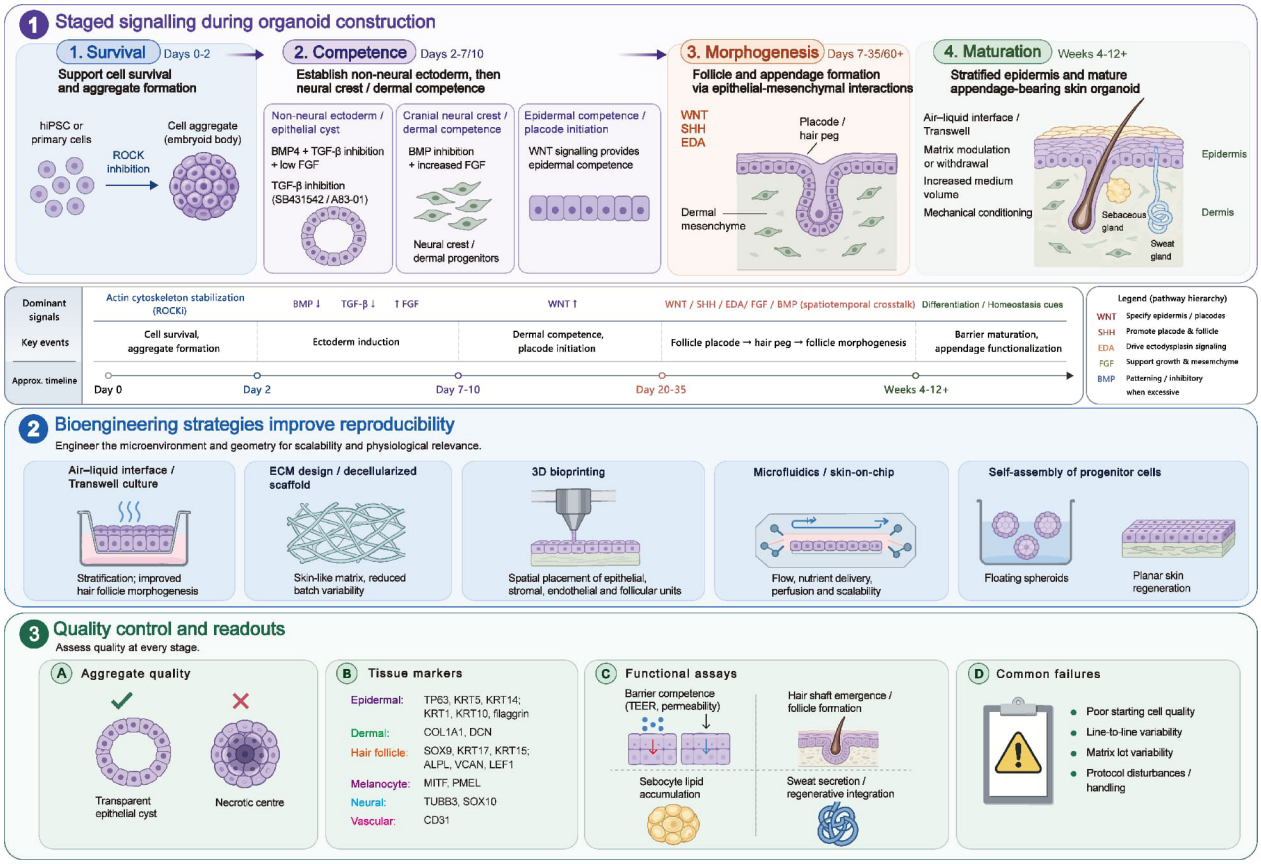

Figure 3. Recurrent supportive signals, engineering refinements, and stage-specific evaluation in skin organoid construction. Created with BioRender.com. Miao Zhen, Shiyu Li, and Zhenning Dai (2026). https://BioRender.com/kz9bit2

Abbreviations: BMP: Bone morphogenetic protein; ECM: Extracellular matrix; EDA: Ectodysplasin A; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; KRT: Keratin; LEF1: Lymphoid enhancer-binding factor 1; MTFP: Melanocyte-inducing transcription factor; ROCK: Rho-associated coiled-coil-containing protein kinase; SHH: Sonic hedgehog; SOX: SRY-box transcription factor; TEER: Trans-epithelial electrical resistance; TGF-β: Transforming growth factor beta; TP63: Tumor protein p63; TUBB3: Tubulin beta 3 class III; VCAN: Versican.

6. Bioengineering strategies that improve skin organoid reproducibility

As skin organoids have become increasingly ambitious in their biological scope, engineering has transitioned from a supportive function to a defining one. The core challenge is that skin serves simultaneously as a stratified epithelial barrier, a mechanically active connective tissue, a vascularized and innervated interface, and a habitat for appendages, each of which requires spatially organized epithelial–mesenchymal niches. While purely spontaneous self-organization recapitulates aspects of this complexity, engineering now largely determines whether a model is reproducible, scalable, and biologically interpretable.

A growing range of bioengineering approaches has therefore been introduced to improve organoid construction, maturation, and experimental consistency. These include ALI culture, extracellular matrix optimization, mechanical conditioning, microfluidic assembly, perfusion systems, and 3D bioprinting. Because performance metrics remain model-specific and are not directly comparable across platforms, Table 5 summarizes representative quantitative measures reported in bioengineering-assisted skin organoid and related skin model studies. These measures include appendage number and length, epidermal and basement membrane maturation, barrier resistance, production efficiency, regenerative output, perfusion parameters, and spatially controlled tissue organization.

Table 5. Representative quantitative performance measures for bioengineering-assisted skin organoid and related skin model platforms

Engineering strategy	Quantitative performance measures	Representative study and model
Air–liquid interface culture and Transwell construction	Skin organoids reached approximately 4 mm in diameter by day 115. Hair follicles elongated to approximately 1 mm. Hair placode numbers were significantly higher than those in floating culture on days 72 and 92 across three hiPSC lines ($p < 0.05$)	Sun *et al.*[27]; human induced pluripotent stem cell-derived composite skin organoids
Mechanical conditioning	Five days of uniaxial cyclic stretch increased epidermal thickness from 27.2 ± 5.94 μm to 46.8 ± 12.4 μm ($p < 0.05$). Laminin 5, collagen IV, and collagen VII deposition increased to 1.45 ± 0.09-fold, 1.56 ± 0.38-fold, and 1.41 ± 0.24-fold of control values, respectively (all $p < 0.01$)	Tokuyama *et al.*[111]; human skin equivalents
Microfluidic hair follicle germ assembly	Janus collagen microbeads contracted into dense hair follicle germs within three days. Three weeks after transplantation, microfluidically generated hair follicle germs produced more than twice as many hair shafts as self-sorting controls	Sugiyama *et al.*[60]; microfluidically generated hair follicle germs
Perfused skin-on-chip culture	Stable perfusion was maintained with an inlet flow rate of 10 μL/min and an outlet flow rate of 12 μL/min. The chip holder enabled the parallel culture of six chips in a single six-well plate	Rhee *et al.*[51]; full-thickness perfused skin-on-chip model
Barrier-function evaluation in engineered skin models	After 12 days of air–liquid interface culture, transepithelial electrical resistance reached 11,424 ± 6,033 Ω·cm^2 in reconstructed human epidermal models and 953 ± 398 Ω·cm^2 in full-thickness skin models	Zoio *et al.*[112]; reconstructed human epidermal and full-thickness skin models
Three-dimensional bioprinting with appendage-supportive modules	Printed dermal papilla and endothelial spheroids supported the formation of hair follicle-like structures after tissue maturation. Standardized metrics for follicle density, barrier resistance, and vascular network were not consistently reported	Catarino *et al.*[62]; bioprinted human skin containing dermal papilla and endothelial spheroids

6.1. Air–liquid interface and Transwell construction

Air–liquid interface culture has long been central to epidermal reconstruction because it promotes stratification and terminal differentiation.[113] However, recent work indicates that ALI culture can also improve composite skin organoids that contain appendages.[27,114] In a Transwell-based study, day-12 hiPSC-derived skin organoids were transferred either to floating culture or to Transwell inserts under ALI conditions. Both conditions yielded mature skin organoids, but Transwell culture increased hair follicle number and supported improved morphogenesis compared to floating culture. Hair follicles generated under Transwell conditions were also longer and more structurally developed than those formed in floating organoids.[27] This result demonstrates that self-organizing composite organoids remain highly responsive to interface engineering, even after early fate specification is complete.[27]

Air–liquid interface-related planarization is likely to become more important. Fully spherical organoids are useful for self-organization, but they are less convenient for imaging, topical exposure, and barrier testing.[114,115] Transwell-based ALI culture, self-assembled spheroid skin units, and printed hair-containing skin all point toward flatter configurations that are easier to interrogate and more compatible with translational use.[27,59,62,114,115]

Mechanical conditioning represents another model-dependent variable during skin tissue maturation. ALI culture changes the exposure conditions at the epithelial surface and the route of nutrient delivery, whereas controlled stretch can directly influence epidermal architecture and basement membrane organization. In human skin equivalents, Tokuyama *et al.*[111] applied uniaxial cyclic stretch for five days using a 10% strain rate, a stretch-and-return speed of 10% per second, a 30-second hold time, and a 30-second interval between loading cycles. Stretch increased epidermal thickness from 27.2 ± 5.94 μm to 46.8 ± 12.4 μm ($p < 0.05$). Laminin 5, collagen IV, and collagen VII deposition in the basal layer increased to 1.45 ± 0.09-fold, 1.56 ± 0.38-fold, and 1.41 ± 0.24-fold of the respective control values (all $p < 0.01$). The number of hemidesmosomes per 100 μm of the dermal–epidermal interface also increased from 18.01 ± 2.26 to 39.01 ± 5.76 ($p < 0.01$), accompanied by a longer lamina densa.[111]

Direct evidence for mechanical conditioning in complex appendage-bearing skin organoids remains limited. Stretch, perfusion, and shear stress should therefore be considered model-specific engineering variables rather than mandatory components of every skin organoid protocol. When these strategies are applied, studies should report the loading mode, strain amplitude, frequency, duration, initiation time, flow rate, and whether conditioning is continuous or intermittent. Such reporting would improve comparisons across reconstructed skin equivalents, organoid-based platforms, and microfluidic skin models.[42,116]

6.2. Extracellular matrix composition and mechanical properties

Most skin organoid systems still rely on Matrigel or related basement membrane extracts at specific steps, especially during early aggregate stabilization or lineage-restricted organoid growth.[5,6,15,16,117] However, matrix composition actively influences polarity, aggregate cohesion, epithelial stability, mesenchymal support, and tissue mechanics.[118,119] In optimized pluripotent protocols, small amounts of growth-factor-reduced Matrigel are used during early patterning and initial maturation, and its subsequent withdrawal can support further development.[7,15] In adult lineage-restricted organoids, a persistent basement membrane-like environment often remains essential for stable growth and differentiation.[5,6,120]

Matrix selection should therefore consider both biochemical composition and mechanical behavior. Evidence from other organoid systems indicates that stiffness and degradability can exert stage-dependent effects: a mechanically supportive matrix may promote early stem cell expansion, whereas a more remodelable environment may become necessary for subsequent differentiation and organoid formation.[121] Viscoelasticity represents a related but distinct property. Hydrogels with comparable initial elastic moduli can differ in stress-relaxation behavior, which influences cell spreading, proliferation, and matrix remodeling.[122] Although these principles have not yet been systematically defined in skin organoids, they provide a useful basis for optimizing matrices according to the developmental stage and intended tissue complexity.

Defined synthetic hydrogels offer a complementary strategy for reducing reliance on Matrigel. Compared with basement membrane extracts, synthetic matrices provide greater control over composition and enable independent adjustment of mechanical and biochemical properties. Modular systems can be designed by varying polymer concentration, stiffness, adhesive-ligand density, and protease-sensitive crosslinking, allowing the matrix environment to be adapted to different stages of organoid development.[117,121] For example, fully defined polyethylene glycol-based hydrogels have supported human organoid generation without Matrigel encapsulation while retaining control over cell adhesion and matrix degradability[117]. Recombinant-protein and peptide-based materials provide additional options for developing more defined culture systems.[123,124] However, synthetic matrices may not fully reproduce the biochemical complexity of the native skin extracellular matrix, and their optimal properties are likely to vary with cell source, developmental stage, and intended application. Direct validation in complex skin organoid platforms, therefore, remains necessary.

Alongside the development of defined synthetic matrices, another important direction is the replacement of generic matrices with more instructive, skin-like environments.[118,120,125,126] Decellularized extracellular matrix (dECM), tailored collagen systems, and hybrid biomaterials can deliver biochemical cues and mechanical properties that more closely match those of native dermis or appendageal niches.[118,125,127,128] Ligand composition is also relevant to model design. Basement membrane-associated components, including laminins and collagen IV, support epidermal organization and dermal–epidermal junction formation, whereas collagen I-rich matrices, fibronectin-containing materials, and skin-derived dECM provide a more dermis-like environment for stromal support and tissue remodeling.[120,125] Although fully mature skin-specific dECM protocols are not yet standard across the field, adjacent organoid work and skin engineering studies strongly support this direction.[125,129] The anticipated benefits include not only improved maturation but also reduced batch variability and enhanced compatibility with translational manufacturing.

Matrix standardization remains equally important. Matrigel and related basement membrane extracts have incompletely defined compositions and may vary across production lots, limiting experimental reproducibility and complicating cross-study comparisons.[123] Variability can also arise in dECM-derived hydrogels because tissue source and processing conditions affect biochemical composition and mechanical performance.[119] Studies should therefore report the matrix supplier, product formulation, lot number, concentration or dilution ratio, growth factor-reduced status, gelation conditions, and timing of matrix addition or withdrawal. For dECM and composite hydrogels, reporting the tissue source, decellularization method, residual DNA content, major matrix components, stiffness, degradation behavior, and stress-relaxation properties would further improve interlaboratory comparability.

6.3. Three-dimensional bioprinting and imposed topology

Bioprinting addresses a different limitation: geometry. Self-organizing systems are powerful but only partly controllable. Bioprinting allows investigators to place epithelial, stromal, vascular, and appendage-supportive modules in deliberate spatial relationships from the outset.[62,130] Catarino *et al.*[62] provided a clear example by incorporating hair follicle units into bioprinted human skin, printing dermal papilla and endothelial spheroids within a fibroblast-containing dermal layer, and then allowing keratinocytes and melanocytes to populate the follicle-like structures. This work shows how bioprinting can bridge organoid-like units and a skin-equivalent architecture.[62] However, quantitative performance measures remain relatively underdeveloped

in many bioprinted skin systems. Although follicle-like structures can be generated through spatially controlled incorporation of dermal papilla and endothelial spheroids, standardized metrics such as follicle density, barrier resistance, vascular-network formation, and long-term functional performance are still insufficiently reported across studies.[62]

The broader implication is that bioprinting may help address one of the field's main translational challenges: how to preserve biological richness while achieving spatial consistency, higher throughput, and manufacturing compatibility.[131,132] In skin organoid research, those gains are particularly valuable because appendage placement, epithelial–mesenchymal distance, and tissue geometry often shape downstream biological behavior as strongly as medium composition or signaling input.[62,82,95,130,131]

6.4. Microfluidics, follicle-germ engineering, and skin-on-chip convergence

Microfluidic systems provide an additional layer of control by regulating fluid handling, nutrient delivery, and scalable tissue assembly.[51,60,133] Sugiyama *et al.*[60] demonstrated that hair follicle germs can be produced at scale using a flow-focusing microfluidic device that generates Janus collagen beads containing epithelial and mesenchymal cells. After three days of culture, these beads contract into dense follicle germs. Quantitative transplantation data further showed that microfluidically generated hair follicle germs produced more than twice as many hair shafts as self-sorting hair follicle germ controls three weeks after transplantation.[60,132,134] This example illustrates how microfluidics can solve manufacturing challenges while preserving biological function.

At the whole-skin level, microfluidic culture naturally aligns with skin-on-chip design.[51,52] Perfusion, controlled shear, defined medium exchange, and compartmentalization make it easier to model vascular and inflammatory processes and to prolong tissue viability.[51,52,133,135] Recent full-thickness skin-on-chip studies have further demonstrated the feasibility of stable long-term perfusion and parallelized culture formats, supporting more standardized and scalable experimental workflows.[51] This convergence is likely to be especially important for vascularized, immune-containing, and drug-testing-oriented skin models, where static culture increasingly becomes a limiting factor.

6.5. Self-assembly as an engineering strategy

Engineering in this field also includes strategies that harness self-assembly. Ebner-Peking *et al.*[59] showed that differentiated progenitor cells can self-assemble into floating spheroid skin organoids that support planar skin regeneration. This work highlights a valuable design principle: engineering can orchestrate combinations of cells and environmental constraints to guide tissue-level self-assembly while preserving biological plasticity. In skin organoid research, this balance between directed design and spontaneous self-organization is likely to define many future systems with substantial experimental utility.

7. Quality control, evaluation, and common failures

As skin organoids become more complex, integrating quality control into experimental design becomes increasingly important. A useful quality-control framework follows the same four-phase sequence described earlier: survival, competence, morphogenesis, and maturation. Each phase contributes its own checkpoints, and coordinated assessment across phases provides the clearest view of organoid fitness and developmental progress.

In pluripotent protocols, the first checkpoint is the quality of the input cells. Colonies should be compact, minimally differentiated, and appropriately confluent before aggregation.[7,15,136] The second checkpoint is aggregate morphology: after centrifugation and early culture, embryoid bodies should have dense centers and smooth, defined edges.[7,137] In optimized skin organoid protocols, the appearance of a transparent epithelial cyst during the first week to 10 days is a practical predictor of eventual success.[7,8] Irregular edges, premature dense cores, or abnormal cyst-like defects at day 0 usually foreshadow poor differentiation.[7,138]

Marker selection should align with the tissue compartment under evaluation.[136,139] In reconstructed skin, the epidermal panel includes TP63, KRT5, and KRT14 for basal keratinocytes, along with stratification markers such as KRT1, KRT10, involucrin, loricrin, and filaggrin.[140,141] A convincing dermal compartment shows fibroblast markers such as vimentin, collagen type 1 alpha 1 chain, collagen type III alpha 1 chain, and decorin, while laminin and collagen IV are key indicators of epidermal–dermal junction formation.[140,142,143] In self-organizing skin organoids, the marker panel expands to include appendageal, pigmentary, neural, and vascular markers, enabling a comprehensive assessment of tissue complexity.[4,26]

For hair-bearing systems, follicular epithelium is commonly supported by KRT17, KRT15, SRY-box transcription factor (SOX) 9, and LIM homeobox 2[144–146], whereas dermal papilla identity is supported by alkaline phosphatase, biomineralization-associated, versican, and lymphoid enhancer-binding factor 1.[20,147,148] Melanocytes are best confirmed using microphthalmia-associated transcription factor, premelanosome protein, or tyrosinase-related protein 1[149,150]; Merkel cells by KRT20 and SOX2[151,152]; and neural elements by tubulin beta 3 class III, neurofilament heavy polypeptide, SOX10,

or S100B.[4,29] Sebaceous organoids should show an outer proliferative compartment together with B-lymphocyte-induced maturation protein-1, peroxisome proliferator-activated receptor gamma, fatty acid synthase, perilipin 2, or other sebocyte-associated lipid markers.[19,40,153,154] Sweat gland organoids should distinguish basal or myoepithelial and luminal compartments, for example, using KRT5/KRT14 and KRT8/KRT18/aquaporin 5, and functional claims are strengthened by evidence of secretion or regenerative integration.[17,18,96,155] When vascular or immune incorporation is claimed, CD31 or CDH5 for endothelium, platelet-derived growth factor receptor beta or actin alpha 2 for mural cells, and CD45, together with lineage-resolved immune markers such as CD207, should be included.[26,156–158]

Functional assays are crucial because morphology alone can be misleading.[136,139] Barrier competence can be evaluated by transepithelial electrical resistance, dye penetration, or permeability measurements in epidermal and reconstructed models.[159–161] Systems designed to generate hair follicles should be evaluated by placode or follicle formation, shaft emergence, and, when applicable, transplantation outcomes.[4,162,163] Sebaceous organoids should show lipid accumulation and gland-appropriate metabolic features, whereas sweat gland organoids are strengthened by secretory or regenerative evidence.[19,40,154] Matching the functional assessment to the tissue category under study is essential for a convincing interpretation of organoid maturity.[136,139]

Long-term stability should also be distinguished from short-term maturation. For prolonged culture and aging-related applications, evaluation should extend beyond terminal differentiation to include the maintenance of tissue architecture, cell viability, proliferative capacity, barrier function, and extracellular matrix organization over time. When aging-related phenotypes are of interest, additional readouts may include senescence-associated β-galactosidase activity, p16 and p21 expression, senescence-associated secretory factors, collagen homeostasis, and matrix metalloproteinase activity.[164,165] Longitudinal assessment of these parameters would help distinguish stable maturation from progressive deterioration during extended culture.

Several technical bottlenecks become more apparent as organoids mature and increase in size. In the absence of functional circulation, oxygen and nutrient delivery depend largely on passive diffusion. Because the effective diffusion distance in mammalian tissues is generally limited to approximately 100–200 μm, larger organoids are more susceptible to central stress, hypoxic injury, and necrosis.[166] These limitations may also contribute to regional differences in epithelial stratification and appendage maturation. Appendage formation remains heterogeneous across individual organoids and hiPSC lines. Aggregate size, early morphology, matrix exposure, and the timing of developmental cues can influence whether hair placodes and follicles emerge consistently.[7,56] The same biological plasticity that makes self-organizing systems valuable for developmental studies also limits experimental throughput. Prolonged maturation, manual organoid selection, and organoid-to-organoid variability complicate scale-up and drug screening. Transwell culture, planarization, perfusion, microfluidic assembly, and bioreactor-based production may alleviate specific bottlenecks, although no single strategy currently resolves all of these limitations.[27,60,133,167]

Against this background, transparent reporting of common technical failures is essential for improving interlaboratory reproducibility. Several technical problems recur across studies. First, poor starting cell quality remains the most common hidden source of batch failure.[7,15,136] Second, line-to-line variability in hiPSCs can shift aggregate growth dynamics enough to alter differentiation outcomes; aggregate standardization is therefore critical.[7,79,137,168] Third, overreliance on gross morphology can lead to false optimism, especially in hair systems, where peg-like structures are sometimes mistaken for mature follicles.[21,162] Fourth, matrix lot variability remains a substantial issue when using Matrigel-like substrates.[169–172] Fifth, protocol disturbances matter more than many investigators expect: temperature fluctuations, evaporative edge effects, and rough handling during transfer can disrupt sensitive early patterning stages.[7,173,174] Reporting these variables transparently, together with organoid size, culture duration, signs of central stress, appendage yield, and selection criteria, will be essential for improving interlaboratory reproducibility and enabling more scalable applications (Table 6).

8. Biomedical applications linked to organoids

Applications become most evident when they align with the construction logic of each system. For example, reconstructed epidermal and fullthickness skin equivalents are well-suited for barrier biology, toxicology, genecorrected epithelial replacement, and diseases in which epidermal architecture plays a central role.[11,49,66,70] Highlighting these specific systems keeps the connection between construction strategy and application explicit.

Self-organizing pluripotent composite organoids are especially powerful for developmental biology, appendage morphogenesis, sensory integration, host–pathogen interactions, and multicompartmental regenerative design.[15,25,175,176] Their ability to combine epidermis, dermis, appendages, and neural elements into a single system underscores their distinctive experimental value, making them particularly attractive to researchers.[4,26,56]

Table 6. Key variables, early checkpoints, and practical corrective actions

Model or stage	Variables that most strongly shape the outcome	Marker	Early quality control	Common problems and corrective action
hiPSC aggregate formation	Single-cell quality, aggregate size, timing of ROCK inhibitor exposure, and low-attachment geometry	POU5F1 (OCT4), SOX2, NANOG	Uniform round embryoid bodies with smooth contour and minimal debris	Irregular aggregates usually predict poor downstream induction; refresh the starting culture and re-standardize the seeding density
Early skin organoid induction	Balance among TGF-β inhibition, FGF activation, BMP modulation, and diluted matrix supplementation	TFAP2A, EPCAM, KRT8, KRT18; SOX10, PAX3	Transparent epithelial cysts and absence of extensive dark necrotic centers	Cloudy or collapsing aggregates often indicate failed patterning or poor matrix handling.
Long-term self-organizing skin organoid maturation	Medium-change cadence, orbital motion, oxygen and nutrient diffusion, ECM concentration, organoid selection	KRT14, KRT10, COL1A1, DCN; KRT17, KRT15, SOX9; MITF, PMEL	Stable growth, expanding epithelial architecture, and visible appendage primordia	Overgrowth and central stress can be reduced by culling poor organoids and adjusting the medium volume/change schedule.
Pluripotent reconstructed skin equivalents	Keratinocyte and stromal-cell differentiation quality, dermal scaffold integrity, and the timing of calcium exposure or air–liquid interface transfer	TP63, KRT14; KRT1, KRT10, filaggrin; VIM, COL1A1, DCN	Continuous basal epithelium over a stable stromal matrix and orderly stratification during maturation	Poor stratification or epithelial delamination usually reflects weak stromal support, immature keratinocytes, or premature differentiation.
Adult epidermal organoids	Basal-cell enrichment, low-calcium attachment phase, timing of A83-01 or comparable pathway modulation, basement membrane quality	TP63, KRT5, KRT14; KRT1, KRT10, involucrin, loricrin, filaggrin	Compact epithelial spheres retaining basal markers and orderly growth	Early spreading into a planar sheet suggests insufficient 3D support or overly differentiating conditions
Hair follicle germ engineering	Epithelial: mesenchymal ratio, dermal papilla competence, forced proximity, ECM mechanics, aggregate geometry	KRT17, KRT15, SOX9, LHX2; ALPL, VCAN, LEF1	Condensed epithelial–mesenchymal interface and maintenance of DP-like signatures.	Loss of inductivity usually reflects DP drift; restore through 3D aggregation or fresh mesenchymal preparation.
Sebaceous gland organoids	Purity of sebaceous progenitors, three-dimensional matrix support, and the balance between proliferative outer cells and inward sebocyte differentiation	BLIMP1, PPARG, FASN, PLIN2	Appearance of an outer proliferative compartment with inward lipid-rich sebocyte differentiation	Mixed differentiation or weak lipid accumulation often improves after tighter progenitor enrichment and better stage-specific medium control.
Sweat gland organoids	Primary gland-cell quality or keratinocyte reprogramming state, EGF/FGF/EDA/BMP/cAMP tuning, and a gland-supportive matrix	KRT5, KRT14; KRT8, KRT18, AQP5	Appearance of gland-like epithelial structures with distinct basal and luminal identity	Loss of gland identity often reflects incomplete lineage conversion or weak gland-specific trophic support.

Abbreviations: ALPL: Alkaline phosphatase; AQP5: Aquaporin 5; BLIMP1: B lymphocyte-induced maturation protein 1 (PRDM1); BMP: Bone morphogenetic protein; cAMP: Cyclic adenosine monophosphate; COL1A1: Collagen type I alpha 1 chain; DCN: Decorin; DP: Dermal papilla; ECM: Extracellular matrix; EDA: Ectodysplasin A; EGF: Epidermal growth factor; EPCAM: Epithelial cell adhesion molecule; FASN: Fatty acid synthase; FGF: Fibroblast growth factor; hiPSC: Human induced pluripotent stem cell; KRT: Keratin; LEF1: Lymphoid enhancer-binding factor 1; LHX2: LIM homeobox 2; MITF: Microphthalmia-associated transcription factor; NANOG: Nanog homeobox; OCT4: Octamer-binding transcription factor 4; PAX3: Paired box 3; PLIN2: Perilipin 2; PMEL: Premelanosome protein; POU5F1: POU class 5 homeobox 1; PPARG: Peroxisome proliferator-activated receptor gamma; ROCK: Rho-associated coiled-coil-containing protein kinase; SOX: SRY-box transcription factor; TGF-β: Transforming growth factor beta; TFAP2A: Transcription factor AP-2 alpha; TP63: Tumor protein p63; VCAN: Versican; VIM: Vimentin.

Adult stem/progenitor-derived organoids excel when the target biology is lineage-focused. Epidermal organoids are useful for infection, barrier, and donor-specific epithelial studies.[5,81,177] Sebaceous organoids support mechanistic investigations of sebocyte differentiation, lipid metabolism, and acne-relevant biology.[19,40] Sweat gland organoids are especially attractive for regenerative studies in burns and wound repair.[17,18,178] Hair follicle organoids and follicle germs are particularly valuable for appendage induction, epithelial–mesenchymal communication, and regenerative hair engineering.[60,62,84,85] Taken together, these systems demonstrate that a narrower biological scope can be a strength when the underlying research question is correspondingly focused (Figure 4).

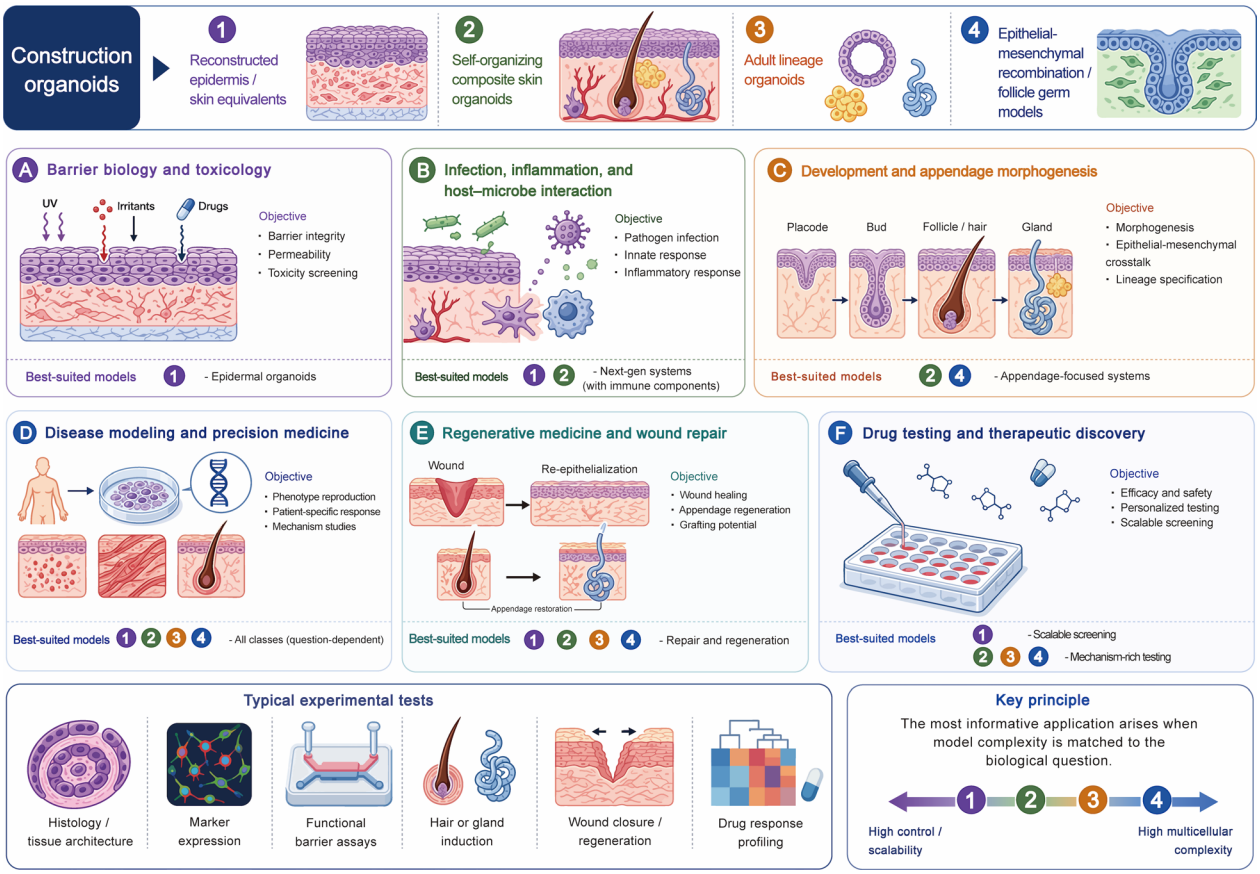

Figure 4. Applications of skin organoids. Created with BioRender.com. Miao Zhen, Shiyu Li, and Zhenning Dai (2026). https://BioRender.com/xg8v0nj

9. Emerging directions in next-generation skin organoids

The next phase of skin organoid research is defined by integration: integration of cell types, engineering configurations, and organoids with acellular instructive systems such as EVs. Progress in this phase will be measured less by whether a model can form skin-like tissue at all, and more by how much controllable physiological complexity it can sustain.

9.1. Vascularized and immune-containing skin organoids

Vascular integration represents an important step toward improving the physiological relevance of skin organoids. Mostina *et al.*[26] compared two incorporation strategies. The addition of human placental endothelial colony-forming cells to hiPSC-derived skin organoids generated capillary-like structures but did not establish a complete vascular network and could interfere with epidermal stratification and hair follicle morphogenesis. In contrast, the combination of hiPSC-derived vascular organoids with skin organoids produced more complex vascular structures and enabled the transfer of endothelial, mural, hematopoietic, and mesenchymal components into the skin organoid context.[26,133] These findings suggest that multicellular vascular modules may be more suitable than endothelial supplementation alone when the intended model requires greater vascular complexity and tissue-level integration.

Immune-cell incorporation is closely linked to this vascularization process. Skin contains diverse resident and recruited immune populations that contribute to tissue homeostasis, host defense, and inflammatory responses.[179,180] In vascularized skin organoids, $CD45^+$ immune cells migrated from the vascular organoid compartment into the skin tissue. By day 115, most

CD45⁺ cells expressed the macrophage-associated marker CD68. CD207⁺ cells were detected near hair follicles, consistent with Langerhans cell-like or dermal dendritic cell-like populations, while CD66b⁺ neutrophil-like cells were also observed in the dermal compartment.[26] The coordinated emergence of these cell populations indicates that vascular organoid incorporation can support a more complex immune microenvironment than skin organoids alone. However, their long-term stability, functional responsiveness, and relative contributions to resident and recruited immune states remain to be determined.[179–181]

These advances broaden the potential applications of skin organoid platforms. Vascularized and immune-containing systems may provide more informative models for studying inflammatory responses, host–pathogen interactions, immune-mediated tissue injury, and vascular remodeling. Related platforms have already been applied to viral infection modeling, including vascularized immune-competent skin-on-chip systems for herpes simplex virus infection and skin organoid models for Mpox and hand, foot, and mouth disease.[52,175,176] Their use in autoimmune disorders and immunotherapy screening remains an emerging direction rather than an established application. Related work in localized scleroderma has demonstrated the relevance of iPSC-derived epithelial–mesenchymal organoids to fibrotic and inflammatory skin pathology, although further immune-cell integration will be required to reproduce disease-specific immune mechanisms more faithfully.[73]

9.2. Neural integration and assembloid perspectives

The skin is also a sensory interface, and appendage-bearing organoids that include neurites, Schwann cell-associated elements, and Merkel cells already offer a glimpse at this dimension.[4,15,29] More explicit neural integration represents the next step. Insights from assembloid biology, including neuro-mesodermal assembloids and other modular organoid systems, suggest that coupling skin organoids with peripheral neural modules could provide tractable platforms for studying sensory pathology, neurocutaneous interactions, and innervation-dependent maturation.[182–185] Such models would be particularly valuable for pain biology, itch, neuropathic skin diseases, and wound healing, where nerves act as active, instructive participants in tissue function and repair.[185–189]

9.3. Extracellular vesicle-enabled organoid construction and organoid-derived extracellular vesicles

Extracellular vesicles occupy a special place in the next-generation skin organoid landscape, as they can serve both as products and as tools.[28,190] Kwak *et al.*[28] demonstrated that iPSC-derived epidermal organoids can generate EVs with regenerative activity during skin repair, providing clear proof of principle that a tissue-organized epidermal state can produce bioactive vesicles with therapeutic relevance.[28] In a cartilage organoid study, decellularized cartilage extracellular matrix was infused with EVs from SOX9-overexpressing bone marrow-derived stem cells to generate an EV-rich hydrogel, which then served as the matrix for microfluidic fabrication of gradient-structured organoids. The EV-enriched matrix supported chondrocyte expansion, preserved key matrix components, enabled sustained release of protective factors, and enhanced resistance to mechanical stress, demonstrating how EVs can be incorporated into organoid-supporting matrices.[191]

Furthermore, epidermal organoid-derived EVs may help stabilize epidermal differentiation programs, whereas appendage-associated organoid EVs may prove useful for hair, glandular, or wound-related maturation. At present, the strongest direct experimental evidence in skin is for epidermal organoid-derived EVs, providing a clear starting point for broader exploration of skin organoid EV categories.[28,190]

9.4. Interactions between different organoids

Another frontier is the deliberate interaction of skin organoids with other organoid systems. Skin–vascular combinations are already beginning to mature.[23,26] Skin–neural combinations are biologically compelling for the reasons outlined above.[4,182,185] Cartilage and musculoskeletal organoids may be useful for modeling tissue interfaces or mechanically complex anatomical sites, while adipose or immune organoids could help recapitulate deeper skin microenvironments and inflammatory responses. The broader point is that skin does not function in isolation *in vivo*, and multi-organoid systems may become the most faithful way to model chronic wound microenvironments, fibrosis, innervation, vascular compromise, or systemic inflammatory skin disease.

9.5. Planarization, standardization, and translational manufacturing

Finally, the field is converging on a translational design principle: preserve sufficient self-organization to retain biological fidelity while introducing sufficient control to support reproducibility, scalability, and application compatibility.[136,192] Planar hair-bearing organoids, printed follicle-containing skin, microfluidic follicle-germ production, and self-assembled spheroid skin units all point in this direction.[23,59,114] A key opportunity now is to standardize thoughtfully while maintaining biological richness.[136,192,193] Modular platforms that allow investigators to tune complexity to the question at hand are especially well-positioned to lead this next phase.[183]

Clinical translation will require a second level of standardization beyond experimental reproducibility. Manufacturing workflows should increasingly adopt Good Manufacturing Practice-compatible procedures, chemically defined or xeno-free culture conditions, traceable raw materials, and predefined in-process controls.[194–196] The use of animal-derived materials, including serum, basement membrane extracts, and feeder components, can introduce concerns related to adventitious agents, lot-to-lot variability, and incomplete material characterization.[195,197] Their replacement or careful qualification should therefore be considered early in translational development. Automation may further reduce operator-dependent variation through standardized cell seeding, aggregate formation, medium exchange, image-based monitoring, and batch documentation.[196,198] Regulatory planning should proceed in parallel with technical development because the applicable pathway depends on the intended use, degree of manipulation, product composition, and jurisdiction.[197,199] For products intended for clinical use, release criteria will need to address identity, purity, viability, potency, sterility, genomic stability, and consistency across manufacturing batches[194,195,197] (Figure 5).

10. Opportunities and future priorities

Three strategic priorities now stand out for the field. The first is reproducibility. Many skin organoid protocols remain highly sensitive to stem cell quality, aggregate size, matrix lot, handling, and the incubator microenvironment.[7,136,200,201] Recent manufacturing-focused analyses make the same point from an engineering perspective and emphasize standardized cell preparation, defined materials, process control, and application-linked release criteria.[192,193,200] These variables are manageable within experienced laboratories but pose challenges for broader adoption and interlaboratory transfer. Overcoming diffusion constraints, appendage heterogeneity, and the limited throughput of prolonged self-organizing culture will require coordinated use of planarization, perfusion, automated monitoring, and scalable bioengineering platforms.[27,60,166,167,202] Future research should focus on developing standardized protocols, robust quality controls, and detailed reporting guidelines to facilitate reproducibility across diverse settings.[136,192,193] Such efforts will be crucial for translating skin organoid

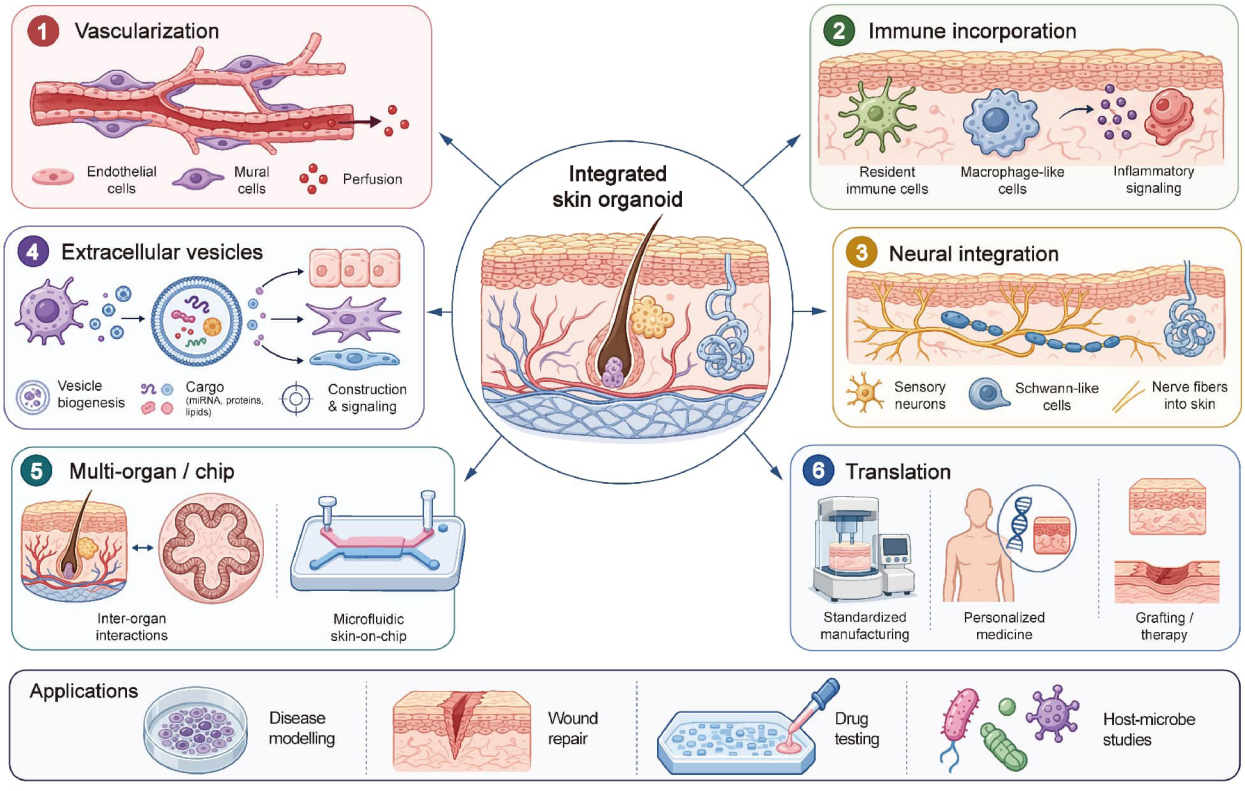

Figure 5. Emerging directions in next-generation skin organoids. Created with BioRender.com. Miao Zhen and Peng Lei (2026). https://BioRender.com/t4kvvbn

research into clinical and industrial applications.

The second priority is matched complexity, which encourages thoughtful research design. Each biological question benefits from a level of model complexity proportional to the mechanism under study.[24,201,203] Future protocol development can therefore be organized as a set of practical decisions: adult epidermal organoids for focused epithelial biology, reconstructed equivalents for standardized barrier studies, composite skin organoids for development and appendage morphogenesis, and vascularized or assembloid platforms for intercompartmental physiology.[4,5,11,26,182] This type of matching will strengthen both experimental clarity and translational relevance.

The third priority is functional maturity. Many current organoids resemble fetal or early postnatal skin more than adult tissue.[4,8,181] That developmental state is useful for morphogenesis studies, but it limits chronic disease modeling, long-term testing, and some nonclinical applications.[47,201,204] Recent work in skin microphysiological systems further underscores the need for mature barrier, vascular, and immune performance when these platforms are used for preclinical evaluation.[47] Strategies to improve maturity will likely combine vascularization, innervation, mechanical conditioning, optimized matrix mechanics, prolonged perfusion, and endocrine or immune cues.[26,129,201,205,206] Importantly, maturity should be measured both functionally and structurally.[136,139,193] An organoid with adult-like barrier performance or regenerative response may be more useful than one with more cell types but weaker functional coherence.[136,163,177]

Closely linked to functional maturity is the need to model long-term stability and aging-related phenotypes. Most current skin organoid platforms are optimized for development, regeneration, or short-term functional assessment rather than for reproducing progressive tissue aging. Emerging approaches include the use of aged donor cells, experimentally induced cellular senescence, photoaging-related stressors, and age-associated extracellular matrix environments. In particular, human dermal dECM hydrogels preserve key components of the native dermal microenvironment and have been proposed as scaffolds for fibroblast-based skin aging-on-chip models.[125] Combining age-relevant matrices with long-term culture, perfusion, and longitudinal evaluation of senescence and barrier function may provide more informative platforms for studying chronic skin aging and testing preventive or therapeutic interventions.

Beyond these model-centered improvements, another opportunity lies in the convergence of organoid systems with cell-free therapeutics and biomaterials.[28,129,190] Organoid-derived EVs, tissue-specific dECM, and smart scaffolds may allow investigators to capture key elements of the biological richness of complex skin while using configurations that are more adaptable to specific applications.[28,35,129,191] Organoids can also serve as a source of higher-fidelity, biologically informative materials than standard two-dimensional culture.[190] This two-way exchange between organoid systems and acellular technologies may prove especially important for clinically realistic wound-healing and graft-design applications.

The field's long-term future is likely to take the form of an interoperable toolkit of skin organoid strategies rather than a single ideal platform.[192,201,203] Some questions will require streamlined epidermal or glandular organoids; others will benefit from appendage-bearing composite systems, vascularized organoids, or multi-organoid assemblies.[4,5,17,19,26,182] The most influential future studies will likely be those that match biological question, construction strategy, and evaluation criteria with unusual precision.

11. Conclusion

Skin organoid research has matured into a field organized around multiple construction strategies, each with distinct biological strengths, technical priorities, and optimal applications. Reconstructed pluripotent skin equivalents, self-organizing appendage-bearing organoids, adult lineage-focused organoids, and epithelial–mesenchymal recombination systems should be viewed as complementary components of a broader experimental toolkit rather than as competing platforms.

The next advances in the field will come through integration: the incorporation of vascular, immune, and neural elements; the combination of self-organization with bioprinting, Transwell culture, and microfluidics; and the convergence of organoid technology with EV and biomaterial engineering. In this light, skin organoids represent modular experimental systems whose value increases when their construction principles are aligned with biological objectives. This perspective is poised to guide the next generation of dermatological modeling, regenerative medicine, and advanced therapeutic development.

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Conflict of interest

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