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Review

Recent progress in the identification and in vitro culture of skin organoids

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ABSTRACT

An organoid is a cell-based structure that shows organ-specific properties and shares a similar spatial organization as the corresponding organ. Organoids possess powerful capability to reproduce the key functions of the associated organ structures, and their similarity to the organs makes them physiologically relevant systems. The primary challenge associated with the development of skin organoids is the complexity of the human skin architecture, which encompasses the epidermis and the dermis as well as accessory structures, including hair follicles, sweat glands, and sebaceous glands, that perform various functions such as thermoregulation. The ultimate objectives of developing skin organoids are to regenerate the complete skin structure in vitro and reconstruct the skin in vivo. Consequently, safety, reliability, and the fidelity of the tissue interfaces are key considerations in this process. For this purpose, the present article reviews the most recent advances in this field, focusing on the cell sources, culture methods, culture conditions, and biomarkers for identifying the structure and function of skin organoids developed in vitro or in vivo. The subsequent sections summarize the recent applications of skin organoids in related disease diagnosis and treatments, and discuss the future prospects of these organoids in terms of clinical applications. This review of skin organoids can provide an important foundation for studies on human skin development, disease modeling, and reconstructive surgery, with broad utility for promising future opportunities in both biomedical research and clinical practice.

Contents

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1. Introduction

Organoids are multicellular structures created by three-dimensional (3D) culture of isolated adult stem cells (ASCs) or pluripotent stem cells (PSCs) under appropriate conditions [1]. They can maintain the physiological structure and functional traits of their source tissue or organ, and are capable of self-renewal and self-organization. Every organ has its own distinct specialized ASCs, which are frequently found in "niches," offering a particular milieu for stem cell upkeep and operation [2]. Embryonic stem cells (ESCs) and induced PSCs (iPSCs) are examples of PSCs and are also the main cells used to create organoids [3–6]. Due to their scarcity and ethical concerns, ESCs are utilized less frequently [7].

Induced pluripotent stem cells can be produced from any type of somatic cell and are broadly accessible since terminally differentiated somatic cells can be reprogrammed by adding specific transcription factors. Theoretically, iPSCs possess the developmental potential of early ESCs and can differentiate into any adult cell type in vitro when grown under settings that mimic in vivo development. Due to their almost endless capacity for proliferation and differentiation, which can result in an infinite number of cells, including somatic cells, stromal cells, and endothelial cells, iPSCs offer significant potential for tissue regeneration. The groundbreaking success of experiments using iPSCs has opened up a new realm for organoids, and iPSCs have developed into a significant source of cells for generating organoids, as illustrated in Fig. 1.

The "mini liver," which was created by Dr. Takanori Takebe's team in 2013, was the first organoid created from iPSCs. On June 2020, researchers at the University of Pittsburgh School of Medicine created a completely functional micro-liver utilizing human skin cells that had been converted to iPSCs (Fig. 1A) [8]. In 2021, human PSCs were successfully used by a research team from the Austrian Academy of Sciences to create the first in vitro self-organizing heart organoid model in the history of science [9]. In the same year, a team of scientists from the National Eye Institute (NEI) in the United States used patient iPSCs to create a retinal organoid model of dominant CRX-Leber congenital amaurosis (LCA) (Fig. 1B) [10], and Professor Guo-Li Ming and his colleagues from the University of Pennsylvania in the United States developed a technique to produce organoids that resembled the hypothalamus and pituitary

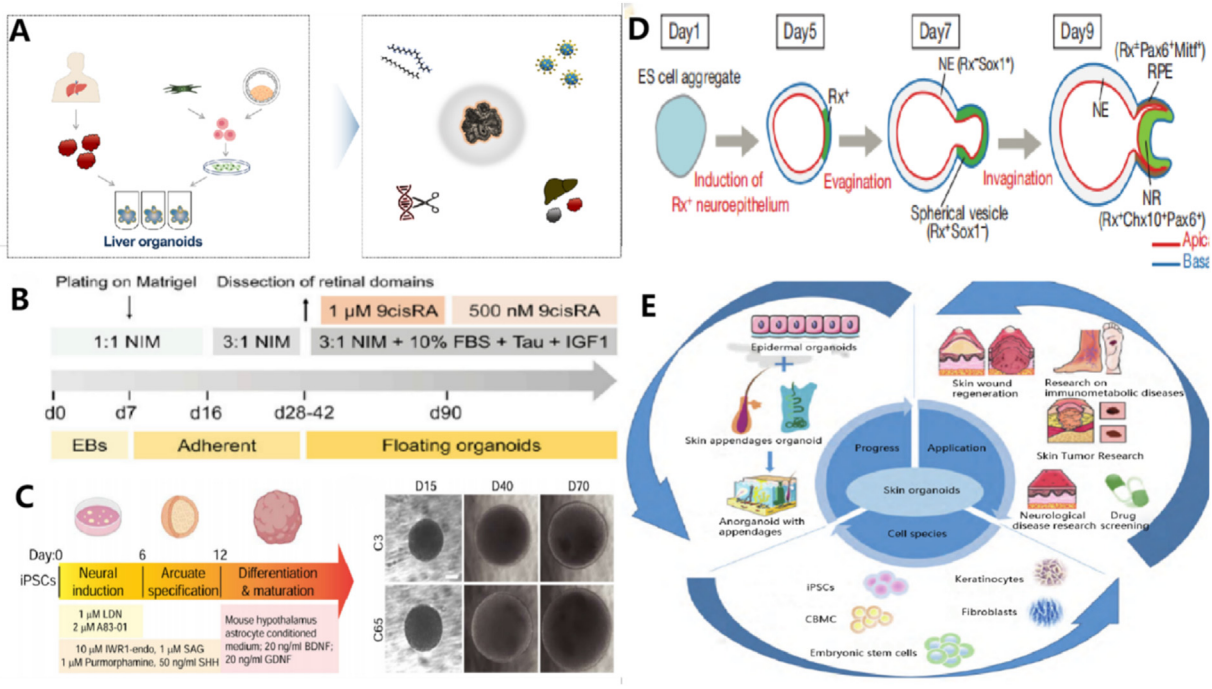

Fig. 1. Cultivation and application of organoids in major research areas. Organoid cultures have been used for various tissues. (A) Generation scheme and characteristics of adult tissue-derived and PSC-derived liver organoids. (B) An overview of the retinal organoid differentiation protocol. (C) Generation and characterization of arcuate organoids from human iPSCs. Sample bright-field images of arcuate organoids at 15, 40, and 70 days in vitro (DIV). (D) Schematic representation of optic-cup self-formation. (E) History of organoid research development.

using human iPSCs (hiPSCs; Fig. 1C) [11]. Additionally, scientists from the University of Dusseldorf in Germany and other organizations reported that iPSCs could be utilized to create brain organoid models that included an optic cup (Fig. 1D) [12].

With the growing scope for the use of organoids in areas like regenerative medicine, precision medicine, drug toxicity and efficacy testing, cell therapy, and organ transplantation as well as the strong potential shown in various research areas, organoid research has garnered significant attention from academics, greatly advancing the field (Fig. 1E).

The skin is the largest organ of the human body, and it serves as a barrier to the outside world, protecting the body from harmful stimuli or substances such as excess heat and chemicals [13]. In addition to forming the physical boundary of the body, the skin also performs various biochemical and sensory functions [14]. Moreover, it helps regulate the body's fluid content and temperature. Additionally, the sensory nerve endings in the skin regulate information related to touch, pain, heat, and cold [15,16]. Skin diseases are common, with 5.4 million cases of skin cancer and around 500,000 patients receiving treatment for burns or other skin injuries each year [17–22]. Moreover, from an ethical standpoint, refinement of skin-related organ technology holds promise for reducing or replacing the widespread use of animals in biomedical research and can be used to simulate genetic diseases [23–25].

Skin organoids can be used to (1) simulate treatment by infective agents (such as human papillomavirus or Ebola virus), inflammatory agents (such as cytokines or chemokines), or various types of drugs acting on skin-related organs [26]; (2) test the toxicity and efficacy of cosmetics [27]; and (3) test the efficacy of transdermal drug absorption [28]. In summary, large-scale generation of human skin with these accessories is expected to be beneficial, but it is challenging. The skin epidermis originates from the ectoderm, while the dermis has different embryonic origins. However, regardless of the dermis origin, all types of skin require interactions between epithelial (epidermal) and mesenchymal (dermal) cells to develop and form appendages [29–32].

In the 1980s, skin equivalents came into prominence on the basis of the pioneering co-culture of fibroblasts and keratinocytes at the air-liquid interface (ALI) proposed by Rheinwald and Green [33]. After 5–6 weeks of cultivation, these skin equivalents could be used for basic research and toxicity testing, thus replacing animal experiments. Although in vitro human skin-cultivation systems for epidermal cells and dermal cells have been used for studies and clinical applications for more than 40 years, cultivation of functional skin appendages in these systems has remained a challenge.

Recent studies have demonstrated that the epidermis and its appendages can be developed from a monolayer of pluripotent ESCs. Embryonic stem cells receive signals from the environment that instruct them to undergo specific differentiation programs and produce stratified epidermis, hair follicles, or sebaceous glands. Lei et al. demonstrated the formation of hair-bearing skin from free cells in in vitro skin organoids [1]. In June 2020, a research team from the United States used hiPSCs to cultivate skin organoids that formed not only the characteristic multilayered tissue of the skin but also hair follicles, sebaceous glands, and neural circuits over a period of 4–5 months, thereby approaching the structure of complete real skin. Lee et al. described the discovery of hair-bearing skin organoids produced from human PSCs (hPSCs). These findings open up fascinating new possibilities for the modeling of skin diseases, regenerative medicine, and developmental research.

The complete skin mainly consists of the following cell subpopulations: keratinocytes, fibroblasts, vascular and lymphatic endothelial cells, pericytes and vascular smooth muscle cells, myeloid cells, lymphocytes, melanocytes, and various types of nerve-related cells [28]. Over the past few decades, a substantial amount of research has been devoted to the development of in vitro skin-equivalent models composed of primary human skin cells and extracellular matrix (ECM) components [17,29–32]. Although tissue-engineered skin substitutes (ESS) are now available clinically to replace missing skin, they do not possess the complete functionality of skin due to the lack of hair follicles, other skin appendages [29–32], and skin-related cells such as dermal adipocytes and sensory neurons [34,35]. These limitations have a major impact on the long-term quality of life of the patients [36]. These major limitations have accelerated the demand for improving the existing in vitro human skin-generation models. Additionally, the need for effective treatments for skin injuries has indirectly stimulated the rapid development of skin organ engineering in recent years, yielding significant progress.

Existing in vitro skin research models mainly consist of two-dimensional (2D) skin cell cultures and 3D skin cell models, both of which show substantial gaps in interlayer interaction and real physiological status. The use of 3D skin organoids differentiated from umbilical cord stem cells and PSCs for in vitro environmental simulation can improve the limitations of existing 2D and 3D models, such as cultivation of a single cell type, lack of interaction between the cells and the ECM, and the inability to self-organize and differentiate in vitro, thereby reducing the differences in comparison with multicellular tissue and their physiological functions in vivo (Table 1) [37–39].

The advent of 3D culture systems (or organoids) in culture dishes has radically changed the approach used for investigating human biology [40]. The use of stem/progenitor cell-derived 3D organoid cultures has become a common technique in biomedical research and regenerative medicine [40–43]. Organoid-based approaches can also be combined with other cutting-edge technologies like gene editing, organ-on-a-chip, and single-cell RNA sequencing (scRNA-seq) to overcome the limitations of conventional models. The information obtained from such approaches can be useful for disease modeling, drug discovery, and precision medicine at the organ level. Organoids can also be cryopreserved as living biobanks for high-throughput screening.

Table 1
Advantages and limitations of various skin cell models.

	2D skin cell model	3D skin cell model	3D skin organoids
Advantages	Simple and easy to operate; short experimental cycle; high reproducibility; low cost; easy to control growth conditions	Multiple cell co-culture; mimics in vivo cell communication; three-dimensional growth; high stability; high drug sensitivity	In vitro self-organizing differentiation; presence of cell response and cell homeostasis; intercellular signal transmission; close to in vivo physiological conditions; stronger stress response; can intuitively simulate drug effects
Limitations	Single culture type; uneven physiological environment; complex cell environment; large difference from in vivo physiological conditions; not adequately representative at present	Strict culture conditions, tedious; high requirement for operational skills; large differences from the real physiological state in interlayer interactions	Inconsistent detection standards; varying growth cycles; poor consistency in finished products; differences between different batches of matrix gel

Therefore, in this review, we aim to summarize the current culture methods and the progress in in vitro characterization of mature skin organoids, as well as the latest technologies being used in this field. In addition, we discuss the origins of skin organoid culture systems and provide feasible suggestions for the future development of ideal skin organoids. Despite their great promise, the current models still have limitations. In this review, we also aim to provide a fair perspective on the opportunities and challenges associated with the use of skin organoids. This will help readers better understand the current models, thereby promoting the development of skin organoids and improving the existing understanding of human skin biology and skin-related diseases.

2. Construction of skin organoids

The epidermal layer of the skin produces hair and glands, with keratinocytes being the main cell type that contributes to temperature regulation and barrier formation. The dermal layer of the skin encompasses other structures such as blood vessels and nerves, with fibroblasts being the main cell type that produces ECM and helps initiate and cycle hair follicles. Therefore, on the basis of the physiological structure of the skin, directing the culture of cells from varied sources or inducing their differentiation can facilitate the construction of in vitro skin tissue systems and skin appendage organoid models. Such systems can yield insights into the complex interactions among different cell types and molecular signaling pathways during development and homeostasis.

Stem cells are the basis of all mammalian life and are the primary cell source for constructing organoids. Stem cells can be primarily categorized into ASCs and hPSCs. The former are specialized for individual organs due to their potential for self-renewal, high proliferation, and multi-directional differentiation. The latter include human ESCs (hESCs) and human iPSCs (hiPSCs). Kim et al. [44,45] recently showed that cord blood mononuclear cells (CBMCs) could also serve as an alternative source of stem cells.

As a type of PSCs, iPSCs have the characteristics of unlimited self-renewal and proliferation, and can differentiate into mature cell types of all three germ layers: ectoderm, mesoderm, and endoderm [46]. The pluripotency of iPSCs, as well as their genetic characteristics specific to certain patients, allow them to potentially treat a variety of diseases [47]. However, some challenges in differentiating iPSCs into specific cell types remain unresolved. Currently, the most researched approach involves inducing hPSCs to differentiate into any cell or tissue type in the human body under specific induction conditions and generating organoids in vitro.

To better understand the basic requirements for skin cell regeneration and the development of functional skin, the key environmental factors that enable hair formation in adult mouse cells and ultimately achieve this in human cells need to be identified. Basic research studies have shown that a mixture of isolated newborn mouse epidermal and dermal cells can be reconstituted in vivo to form new hair follicles (Fig. 2A) [48–51]. These grafts form reconstructed, organized skin with oriented hair follicles that respond to injury and regenerate through cycles [51]. This culture method uses a combination of epidermal and dermal cells (in a 1:9 ratio), and this cell mixture is inserted onto a cell culture medium to form an ALI in a 3D culture system using Transwell. The self-organizing process in this system is characterized by six consecutive stages: 1. Cell isolation; 2. Aggregation: aggregates of different sizes form on the first day. 3. Vesicle formation: polarized aggregates of approximately 350 cells, surrounded by 2–3 dermal cells, form a spherical layer of the dermis on the second day. The polarized aggregates become vesicular on the third day and are filled with keratin. 4. Merging of vesicles: on approximately the fourth to fifth day, epidermal cells bridge the vesicles and fuse to form an epidermal plane. 5. Planar skin formation: from the 5.5th day to 7th day, smaller epidermal planes further merge to form a large plane. The large plane forms a bilaterally symmetrical bilayer epidermal structure. 6. Hair follicle induction: hair follicle-like structures are induced on the tenth and eleventh days.

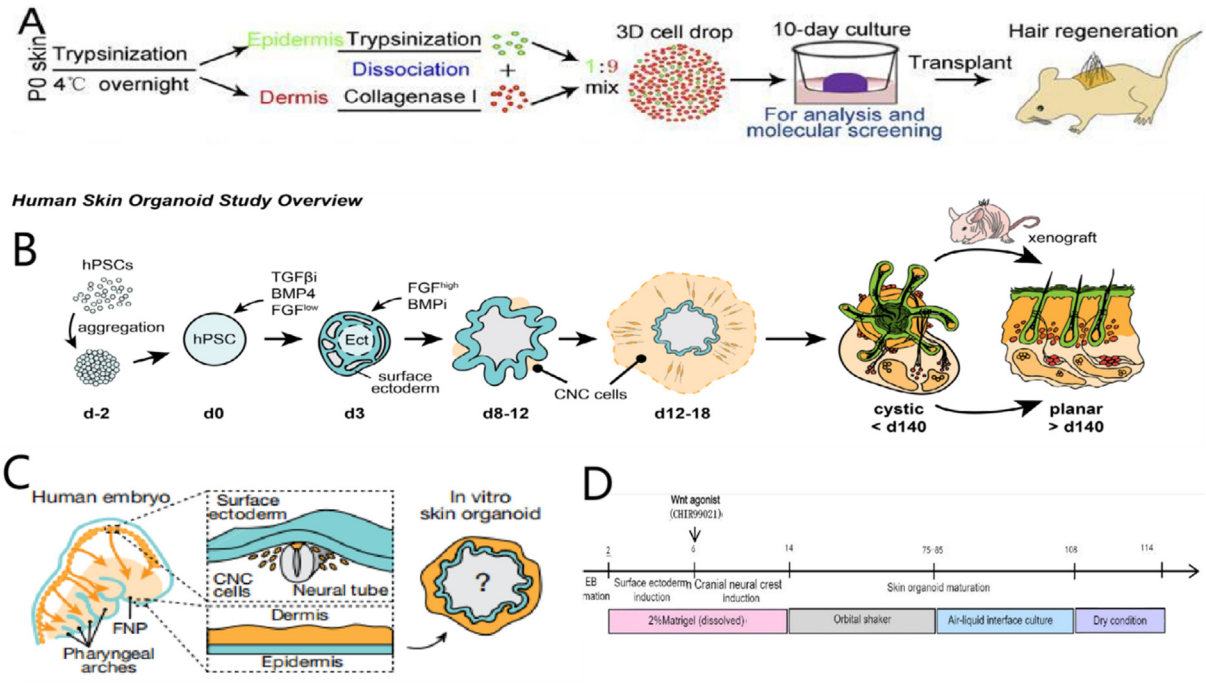

Fig. 2. Culturing methods for skin organoids. (A) Phase transition from isolated skin progenitor cells to flat hairy skin morphology. (B) Pattern of induced differentiation of hPSCs into skin organoids. (C) Induced differentiation of human ESCs into skin organoids. Surface ectodermal and mesenchymal cells were co-induced from human PSCs. (D) Generation of hiPSC-derived skin organoids with activation of the Wnt signaling pathway. Schematic overview and timeline of the culture protocol for generating skin organoids from hiPSCs. Day 0 refers to the time point when hiPSC colonies are detached to form EBs.

When the explant is transplanted to the back of a nude mouse, the regenerated hair follicles are derived from the donor cells and have a normal structure. Epidermal cells become relatively quiescent again from the 9th to 10th days. The study also observed dermal cell behavior by observing PdgfraEGFP mouse cells [52]. Six major stages, equivalent to the epidermal cell stages, were observed, and the entire process can be summarized as the progression from dissociated cells to aggregates, polarized cysts, merged cysts, and finally, flat, hairy skin.

Interestingly, although skin cells from newborn and adult mice have the same genome, adult cells lose this regenerative capacity. Skin reconstructed from mature cells can indeed form hair, but it is not as robust as skin reconstructed from newborn mouse cells. Cells from neonatal mice exhibit greater self-organization and tissue-regeneration capabilities than those from aged mice, and this self-organizing ability of cells is essential for future tissue regeneration.

Another basic study demonstrated that epidermal and dermal clusters from the back skin of young mice have the ability to self-organize and induce hair follicle formation in 3D culture. This ability is lost in mature skin but can be restored by adding matrix metalloproteinases and other cytokines to the culture. In that study, the researchers also uniquely investigated which dermal cell populations could promote the formation of flat skin, and in the K14-GFP/Lef1RFP mouse reconstruction experiment, Lef1RFP + cells belonging to the dermal papilla were observed near the epidermal plane. These dermal cells can form alkaline phosphatase-positive dermal condensate-like structures. The authors believe that this may be one of the main reasons why cells lose their hair-regeneration and terminal-differentiation capabilities.

Ebner-Peking et al. reported the 3D self-assembly of adult and iPSC-derived fibroblasts, keratin-forming cells, and endothelial progenitor cells into novel floating spherical skin organoids (FSOs). The generated keratin-forming cells and fibroblasts resembled the parent cell line in terms of their traits [7]. The precise induction of self-organized differentiation into ectodermal structures possessing epidermis and dermis by major signaling pathways (transforming growth factor [TGF], bone morphogenetic protein 4 [BMP4], and basal fibroblast growth factor [bFGF]) on the basement membrane leads to the co-development of a skin-like organ, which forms an organized skin-like structure resembling fetal skin, consisting of primitive skin cells and hair follicles. This is accomplished using cell suspension transplantation combined with human platelet factor administration. The drawback of this strategy is that it can deprive the cell layers of the connections they need to form some of the accessory structures.

Jie Ma et al. utilized hiPSCs to generate epithelial and mesenchymal (EM) organoids in a 3D culture model. After aggregating hiPSCs in a U-bottom 96-well plate, the aggregates were cultured in Matrigel supplemented with BMP4, TGF-β inhibitor, and bFGF. After 3 days of differentiation, the organoids were treated with LDN-193189 and FGF on a shaker [53]. Another study used hiPSCs cultured in mouse embryonic fibroblast (MEF) medium and hESC medium (containing 80 % Dulbecco's modified Eagle medium [DMEM]/F12 medium (1:1), 20 % serum substitute, 100 mM nonessential amino acids, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 10–15 ng/mL bFGF) to induce differentiation. The study demonstrated that BMP4-induced hiPSC-derived multipotent keratinocytes could allow the reconstruction of normal skin and its appendages in a mouse model. The hiPSCs were dissociated into single cells and formed embryoid bodies (EBs) in low-adherent U-bottom 96-well culture dishes. Surface ectoderm differentiation was promoted using SMAD inhibitors (SB431542), FGF2, and BMP4. On the sixth day of differentiation, the organoids were treated with CHIR to activate the Wnt signaling pathway, resulting in significant enlargement of the organoids. CHIR treatment inhibited the development of transparent cartilage in skin organoids [54]. This finding provides a solution for eliminating or reducing unwanted cell types in skin organoids, and is an important step toward culturing and maturing skin tissue similar to human skin.

Gonzalez Malagon et al. found that a GSK3β inhibitor (CHIR99021) could activate the Wnt signaling pathway to promote organoid growth and inhibit the migration of cranial neural crest cells (CNCCs) to prevent cartilage formation [55].

Lee et al.'s latest study used hESCs from the WA25 cell line (Fig. 2C), which were dissociated into single cells and plated on U-bottom 96-well plates to generate uniform cell aggregates. The aggregates were then transferred to a new plate containing a differentiation medium that promoted epidermal induction by key factors (BMP4 and TGF-β inhibitor). The TGF-β inhibitor promoted the induction of PSCs into the ectoderm, while BMP4 promoted induction into the neuroectoderm [56–59]. This differentiation protocol ultimately produced uniform epithelial cysts with a diameter of approximately 500–1000 μm, which could be reproduced using the DSP-GFP hiPSC line [60].

Combined treatment with the alkaline FGF and the BMP inhibitor LDN-193189 has been shown to promote induction of CNC-like cells, thereby producing mesenchymal cells. By the 16th day, the LDN-193189- and FGF-treated organoids exhibited an inner layer of TFAP2A+ECAD+ epithelial cells surrounded by migrating outer TFAP2A+ CNC-like cells, and by the 18th day, the WA25 and DSP-GFP organoids gradually became bipolar, with one end being the epithelial cysts and the other end being an opaque cell mass. After approximately 50 days of culture, the epithelial cells stratified into a keratin (KRT)5+ KRT15+ basal layer, a KRT5 basal layer, and a KRT15+ suprabasal layer, and the skin morphology became more mature.

At the 70th day, the skin organoids reached the hair growth stage, and at the 120th day, the researchers observed the production of hair follicles. The organoid hair follicles appeared periodically in the epidermis, indicating that the appendage-patterning mechanism is preserved in the organoids (Fig. 2B) [61]. After approximately 150 days of culture, the researchers observed accumulation of squamous cells and abnormal hair follicle morphology at the core of the skin organoids, suggesting that this time point may represent the upper limit of the skin organoid culture [50].

The two-dimensional culture method for the skin organoids uses an ALI culture method [62], which employs a Transwell permeable support. The basal surface of the cells is in contact with the liquid culture medium, while the apical surface is exposed to the air to increase oxygenation. At around 85 days, the Loricrin+ and Filaggrin+ mature epidermis begins to differentiate. This culture is performed on a collagen-coated Transwell permeable support, with the dermis facing downward and placed on an insert coated with cell collagen, exposing the epidermis to the air. This form of culture not only reduces differences between individual organs but also promotes the maturation of the epidermis and the growth of hair follicles.

The skin organoids are cultured at 37 °C and 5 % CO_2 under dry conditions, which promotes the maturation of the cornified cells of the skin organoids. Skin organoids cultured by the ALI method can be cultured for 6 days under dry conditions to differentiate into mature cornified cells, similar to adult skin, and develop hair follicles. The ALI culture method represents an improvement on the conventional skin organoid model. In this method, closed circular aggregates are transformed into flat and open skin organoid models, with skin organoids growing on top of the Transwell culture insert and exposing the epidermis to the air (Fig. 2D) [63].

3. Identification methods of skin organoids in vitro

3.1. Histological observation of skin organoids

Transplanted mature collagen fibers produced by hiPSC-FBs showed morphology consistent with the human epidermis and dermis. Under polarized light microscopy, the cell aggregates stopped growing and transformation when the outer epidermal cells were crescent-shaped. At this time, the epidermal cyst was surrounded by two or three layers of dermal cells, presenting dynamic and random movements [64]. Inverted microscopic analysis of differentiated and mature skin organoids allowed observation of pigmented hair follicles and revealed the development of hair bulbs, germinal buds, and spikes [20]. Confocal microscopy can be used to observe biomarkers of epithelial cells, stromal cells, blood vessels, sweat glands, and fibrotic features after immunofluorescence imaging.

3.2. Histological examination

Histological examination usually assesses the dermal quality of the grafted tissue on the basis of the density of fibroblasts, matrix production, vascularity, hemorrhage, and collagen fiber production. Williams et al. observed the epithelial structure of skin organoids cultured by the ALI method and found that they formed a mature stratified epithelial layer with a basket-weave pattern characteristic of the keratinized layer on the surface during development [65]. In basic research studies, when the skin organoids were transplanted to the back of mice and evaluated with histological analysis and Ki67 staining, the morphology of the tissue surrounding the mice graft was found to be consistent with that of human epidermis and dermis. New keratinizing cells forming hair follicles and a multilayered epidermis are similar to those of humans; at the end of the culture, the skin basal keratinocytes in the organoid differentiate and stratify into all skin layers: spinous, granular, and cornified. The entire skin produced villi similar to those of human mid-pregnancy fetuses [13]. Histological quantification showed embedded specific histone 14, pan-histone, CD31, and the waveform protein wave in FSO sections and indicated that the skin organoids had a dense dermal core structure and were covered by closely spaced epidermal layers, suggesting that the grafts exhibited clear signs of epidermal thickening and hyperkeratinization [7].

3.3. Biological characteristics of skin organoids

Many studies have examined the biological characteristics of skin organoids by diverse methods, including staining, immunostaining analysis, scRNA-seq, flow cytometry, fluorescent nanotracking and DNA molecular hybridization techniques.

3.4. Functional measurements of skin organoids

In one study, researchers established mature skin organoids in culture for 140 days and analyzed their proteomics profile. The proteins identified in these organs were mainly enriched in epidermal cells, nerve cells, matrix cells, fibroblasts, muscle cells, and vascular-related cells. Biological process analysis showed that the identified proteins were mainly enriched in skin development processes, including epidermal development, nervous system development, and stem cell division. The study also found that typical and atypical Wnt, epidermal growth factor receptor, fibroblast growth factor, TGF-β, and TGF-γ, which are closely related to skin development, were enriched in the skin organoids. In particular, proteins related to the establishment of the skin barrier and epidermal development (e.g., keratin [KRT]1 and KRT16) and epithelial cell differentiation (e.g., KRT14 and KRT4) were identified. In addition, the skin organoids showed an abundance of processes related to bone tissue, including ECM and cytoskeletal organization and adhesion, as well as lipid and energy metabolism-related processes. A total of 111 ECM proteins, including 15 collagen proteins, 31 glycoproteins, and 10 proteoglycans, as well as 16 ECM regulatory proteins, 18 ECM-related proteins, and 21 secreted factors, were identified. These results are related to the various structural and cellular functions of skin organoids and indicate that they are similar to human skin tissue physiology [66]. Quantitative real-time polymerase chain reaction (PCR) can detect the expression of sebocyte markers, including KRT 7, peroxisome proliferator-activated receptor alpha (PPAR-α or PPARA), and lipoprotein lipase (LPL), in sebocyte-like cells in comparison with mature human sebocytes.

3.5. Other technologies

Flow cytometry can be employed to verify the origin and purity of cells, while clonality assessment can be used to determine their stem/progenitor cell potential. This technique can be utilized to confirm the presence of pluripotent markers in skin-derived organoids. In a study utilizing floating spheroids composed of fibroblasts, keratinocytes, and endothelial progenitor cells—induced by human platelet-derived growth factor under specific culture conditions—the researchers employed nanoparticle cell labeling to monitor tissue-formation processes [7]. Video imaging techniques were used to visualize the continuous assembly of FSOs, followed by the alignment of endothelial colony-forming cells (ECFCs) and the anchoring of keratinocytes (KCs) to the surface [7].

3.6. Functions and applications of skin organoids

With the widespread research and application of synthetic organs, the application of skin organoids has expanded from building laboratory research models to post-injury healing, skin cancer and inflammatory disease treatment, skin nerve ending research, and drug testing.

3.7. Applications of skin organoids in injury healing

Over the past decade, the role of stem cells in skin wound healing and tissue regeneration has emerged as a significant research focus [67]. Artificial skin, which is most widely used in skin grafting, can be derived from non-cellular materials as well as autologous, allogeneic, xenogeneic, or artificially synthesized sources [68]. Despite the development of various commercial artificial skin products for treatment, limitations persist, including reduced vascularization, poor mechanical integrity, inability to integrate, scarring, and immune rejection. The construction of skin organoids offers potential solutions to these challenges, particularly the inability of most artificial skin products to produce deep dermis.

Toyoshima et al. tested whether skin organoids could be integrated into endogenous skin in a mouse model. On the 140th day, a WA25 skin organoid with hair was implanted into a small incision in the back skin of a nude mouse after removing the cartilage tail with a tungsten needle (Fig. 3A) [50]. The authors observed that 55 % of the xenografts grew 2–5 mm of hair, with no ulceration or uncontrolled tumor-like growth in any of the grafts. Histological examination of the xenografts showing hair growth confirmed fusion of the organ epidermis with the host epidermis and perpendicular orientation of the hair shafts to the skin surface, resembling authentic human skin tissue. These results demonstrated that cystic skin organoids could unfold and integrate into

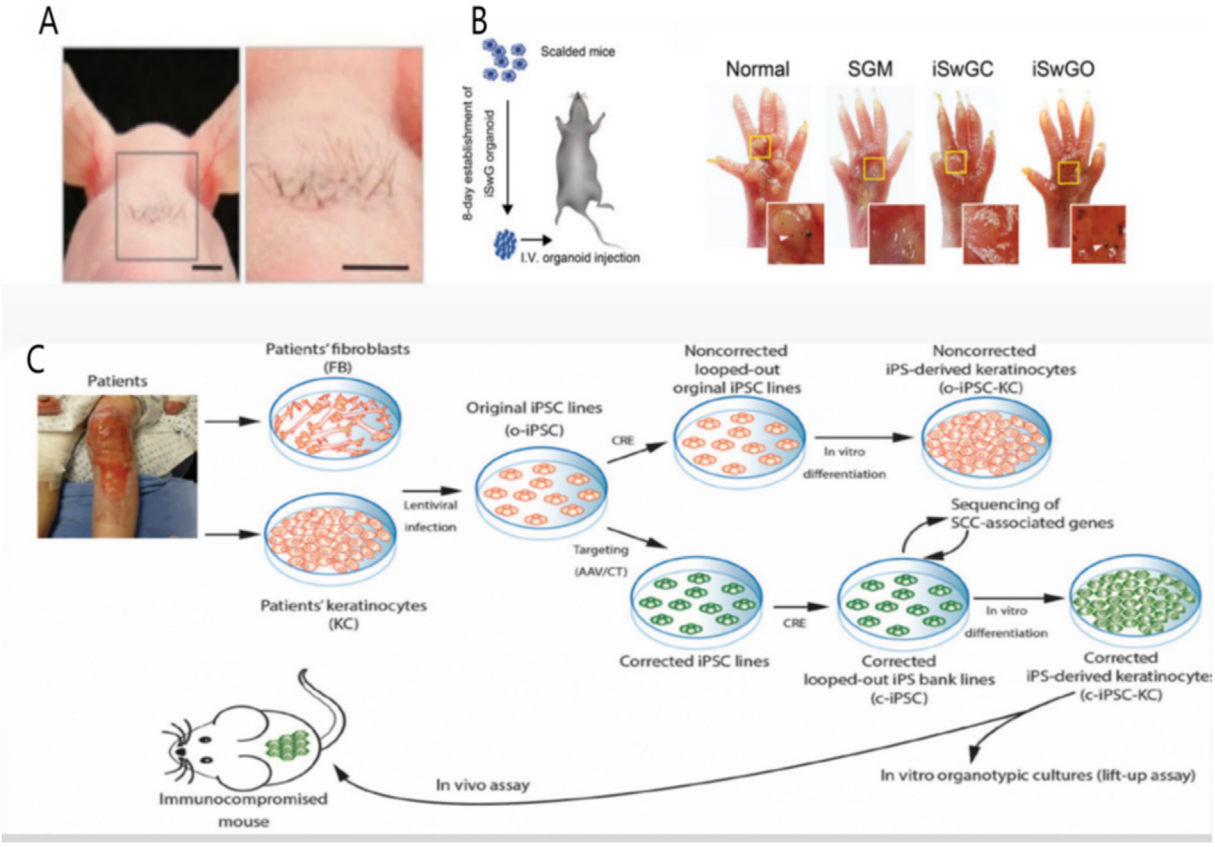

Fig. 3. Application of skin organoids. A) High-density intracutaneous transplantation of bioengineered follicle germs. A total of 28 independent bioengineered hair follicle germs were transplanted into the cervical skin of nude mice, and the mice showed high-density hair growth at 21 days after transplantation. Scale bars, 5 mm. B) Starch–iodine sweat tests on the paw skin of thermal-injured mice showed that only the paws of iSwGO-treated mice showed indigo-black dots at day 21 after transplantation. C) Schematic overview of the study protocol for derivation and characterization of iPSCs from patients with RDEB. Fibroblasts and keratinocytes were derived and cultured from a skin biopsy, and iPSCs were established from both cell types. The iPSCs then underwent correction of the COL7A1 loci by adeno-associated virus (AAV) or conventional targeting and were differentiated in vitro into keratinocytes (c-iPS-KC), or were left uncorrected and directly differentiated into keratinocytes (o-iPS-KC). In vitro-derived keratinocytes (from corrected and noncorrected iPSCs) were used for organotypic cultures and for in vivo skin reconstitution assays in immunocompromised mice. The red cells are the uncorrected cells, while the green cells are genetically corrected cells.

flat skin at the wound site. SCD1+ sebaceous glands were detected in all xenografted hair follicles [60]. The hair follicles of the skin organoids achieved a maturity level approximately equivalent to that of mid-fetal hair and possessed the cellular components necessary for further maturation.

Takagi et al. [69] transplanted 3D human skin organoids with hair follicles and sebaceous glands into nude mice, and the skin organoids functioned normally and correctly connected with host tissues such as the epidermis, arrector pili muscle, and nerve fibers without tumor formation. Although combining biomaterials can enhance the stability and functionality of skin transplantation, the high manufacturing costs and lack of skin appendages (such as glands and hair) limit the therapeutic application of these products [70].

Research by Ruifeng Yang et al. established a method for obtaining scalable human epithelial stem cells (hEpSCs) with key hEpSC features, opening new avenues for studying regenerative therapies for hair loss, wound healing, and skin aging. Ebner-Peking et al. reported similar findings, demonstrating that co-transplantation of human endothelial cells with fibroblasts and keratinocytes in vitro or in vivo resulted in the self-assembly of a complex mixture of platelet-derived growth factors in human platelet lysate (HPL) in a dose-dependent manner, promoting angiogenesis [71]. This process effectively mimics wound repair, and the addition of HPL to transplanted cell suspensions may enhance wound repair with fewer local cells [72].

In current clinical practice, vacuum sealing drainage (VSD) plays an unprecedented role in repairing soft tissue defects of the skin. The three-stage VSD treatment involves autologous skin grafting, which is widely used in the treatment of large-area burns and skin defects. Restoration of glandular function is a crucial consideration in skin repair. Programmed sweat gland organoids have been shown to promote the repair of damaged sweat glands in mice and have demonstrated better healing than the sweat gland medium injection group (mSwG-M) and the sweat gland-like cell transplantation group (iSwGs) along with a shorter healing time (Fig. 3B) [6].

In the future, when autologous skin cannot meet transplantation demands for large-area skin wounds, the combination of autologous PSC-induced skin organoids and VSD technology may mitigate rejection reactions, accelerate wound healing, and facilitate the treatment of large-area skin injuries. This represents a significant step toward developing cell-based therapies for hair loss and other skin disorders.

3.8. Applications of skin organoids in skin cancer research

Organoids serve as powerful models for studying skin cancer, including basal cell carcinoma, squamous cell carcinoma, and melanoma [73–78]. Skin organoids contain precursor cells of basal keratinocytes, squamous cells, and melanocytes [79], providing a foundation for researchers to investigate cancer initiation

mechanisms [80]. These organoids can be readily generated from patient-derived cells and can also be used to simulate genetic diseases, such as epidermolysis bullosa [5,81–83].

3.9. Applications of skin organoids in the treatment of inflammatory diseases

Skin organoids, which can be simulated using primary cells, effectively demonstrate the multilayered structure and gene expression patterns of human skin, enabling the use of relevant primary cells to study targeted molecular mechanisms in complex disease models. Elias et al. [84] established a skin organoid model using fibroblasts from healthy individuals and siRNA-mediated gene knockdown. They found that filaggrin deficiency may trigger atopic dermatitis. Sriram et al. [85] induced an atopic dermatitis-like skin model using a fibrous protein dermal matrix and serum-free culture conditions under the influence of cytokines such as interleukin (IL)-4. Wang et al. [86] embedded sorted ITGA6-high basal cells in Matrigel® matrix gel and covered them with culture medium to culture and expand the cells. They ultimately obtained a human primary hair follicle-like organ that could simulate a skin fungal infection model caused by *Trichophyton rubrum*. Kitisin et al. [87] established an in vitro infection model using dermatophytes and high-throughput screening to identify drugs targeting various antifungal-resistant pathogens, potentially contributing to future targeted therapies. Keratinocytes derived from iPSCs have shown great potential in regenerative medicine as an infinite cell source for rapid tissue replacement therapy in skin diseases. Human keratinocytes can trigger or exacerbate immune responses against scabies infection by secreting IL-1β, tumor necrosis factor (TNF)-α, and matrix metalloproteinases. The discovery of iPSCs and genome editing has yielded the possibility of providing definitive genetic therapy through corrected autologous tissues (Fig. 3C) [81]. Ishibashi et al. [88] showed that during *Propionibacterium acnes* infection, increased IL-5 production by human keratinocytes plays a crucial role in skin inflammation and allergic reactions. Thus, the development of corresponding skin organoid models may facilitate better research and treatment of inflammatory diseases.

3.10. Applications of skin organoids in studies of cutaneous nerve endings

Skin organoids can also serve as models of skin nerve endings. Lee et al. described a basic mechanical sensory complex-like structure formed at the hair follicle bulge—nerve interface and observed the formation of nerve endings [89]. Sensory neurons in skin organoids form ganglia and fascicular axons, mimicking the structure of the dorsal root or trigeminal ganglia/nerves. These nerves can be incorporated into existing skin-equivalent cultures, but the extent to which these models replicate the neural innervation patterns and nerve endings of natural skin is uncertain due to the limited descriptions of nerve endings in skin organoids [90]. Skin organoid models provide an excellent method for studying the mechanisms of skin nerve innervation. This model can be used to better understand how sensory neuron diversity emerges during development. Future research can use skin organoids to further investigate skin nerve innervation as well as brainstem or spinal nerve innervation [91], or the effects of diseases/drugs on peripheral neurons [92].

3.11. Applications of skin organoids in drug detection

Drug development and screening in preclinical trials and disease treatment require high-throughput, large-scale, rapid, and low-cost drug-screening models. Traditional skin disease research mainly relies on 2D and 3D skin cell models, which lack wound microenvironments and heterogeneity and cannot accurately reflect the mechanisms underlying disease occurrence, development, and drug response. Multicellular drug resistance can occur when cells form 3D cell clusters or spheres [93]. Organoid models can maximize the reproduction of in vivo physiological characteristics and reduce heterogeneity, making them suitable for efficient and low-cost screening of a large number of candidate drugs in the early stages of drug development before animal experiments [94]. Floating skin organoids are characterized by simplicity, repeatability, and scalability. In related model systems, 3D skin organoids composed of fibroblasts and HaCaT keratinocytes have been successfully used to study the invasion of melanoma cell lines and for drug-response tests [79]. Ebner-Peking et al. used healthy adult hiPSC-derived progenitor cells that self-organized under non-malignant conditions to form FSOs for high-content drug testing and regenerative therapy. Xiaoyan Sun et al. cultured induced sweat gland cells (iSwGCs) in a special 3D culturing system to obtain sweat gland organoids (iSwGOs) that exhibited structural and biological features characteristic of native sweat glands. These iSwGOs were successfully transplanted into a mouse skin damage model and developed into fully functioning sweat glands in vivo [66]. As a model and method, skin organoids can be used to establish accurate drug-screening systems for targeted treatment of individual symptoms while also identifying inappropriate drugs at an earlier stage. Thus, they offer broad application prospects in skin disease research and treatment fields in both laboratory and preclinical studies.

3.12. Challenges

With advancements in cell culture techniques, in vitro skin organ culture technology has become increasingly mature. Typical skin organoid models are cultured from ASCs and hiPSCs, and their ability to form in vivo and in vitro has been confirmed. However, in vitro culture methods have limitations, and culture conditions require further refinement. The main limitation of skin organoid models derived from hiPSCs is their structure. Skin organoids form a spherical cyst under 3D printing, with hair shafts growing inward and being filled with fluid in the core, and hair follicles protruding outward from the surface of the cyst [24,95]. In normal human skin, the outermost layer of the stratum corneum serves as a physical barrier and is shed as cells age and die. However, in skin organoids, the cystic structure limits shedding, and the stratum corneum tissue accumulates in the core [96].

In addition, the differentiation process of skin organoids is influenced by various regulatory factors, and the mechanisms underlying their interactions are extremely complex. Thus, additional research is required to evaluate the impact of these factors on in vitro cultures. Another limitation is the absence of key normal human skin cell populations in skin organoid models [35]. For example, current models of organoids lack sweat glands, blood vessels, arrector pili muscles (rarely observed), and immune cells, which may prevent their complete maturation and limit the use of these cells and tissues to specific applications. Therefore, further optimization is needed to produce fully mature skin organoids, including all the components of the entire niche. In addition, the hair follicles produced in skin organoids do not contain the medulla layer. The medulla is the innermost layer of hair and is a unique feature of adult terminal hair that is influenced by hormones. Another major challenge of iPSC therapy is safety and the potential for formation of teratomas. Tissue-specific stem cells retain their regenerative potential even after maturation. One of the important functions of regeneration is to repair injured tissue and maintain tissue integrity and function. However, the regenerative capacity of

humans after birth is limited to prevent excessive regeneration that can lead to tumor formation. Considering the possibility of tumor formation due to the powerful regenerative ability of stem cells, gene editing technology (such as CRISPR/Cas9) can be used to prevent specific mutations and knock out target genes known to be involved in tumor formation or skin-related cancer induction factors [97,98]. Scar formation and loss of skin appendages in adult skin can cause irreversible tissue damage. Recent studies have shown that reduction of scar formation and regeneration of hair follicles can be achieved through interactions between stem cells and their surrounding niche. Although complete regeneration of fully functional skin under current conditions still presents challenges, a deeper understanding of ASCs and their niche environment will provide better directions for future skin regeneration.

Currently, in the preparation of in vitro models that simulate tumors in vivo, the methods used for generating skin organoids include 2D cell culture systems, 3D sphere cultures, and adult stem cell transplantation methods, but all of these methods have certain limitations, such as lacking heterogeneity, unstable genetic features, simple structure, and no immune response, indicating changes in the biology of organoids during cultivation. Preserving early passages and primary tissue biobanks for organoid recovery can help standardize experimental conditions and increase sample sizes. The method of establishing organoids from the same tissue differs due to differences in cell-dissociation techniques and the use of growth factors, which may result in differences in the maturity of cultured cells. The most ideal and standardized conditions for organoid culture still need to be discovered [99].

4. Conclusions and future perspectives

The cultivation of skin organoids remains an active area of research requiring further refinement. Future investigations could leverage skin organoids to explore cutaneous nerve innervation, brainstem or spinal nerve connections (utilizing assembly techniques), and the impact of various pathologies or pharmacological agents on peripheral neurons. A critical avenue for research is elucidating the mechanisms by which skin organ cells transition from sac-like structures to planar conformations at sites of injury, and determining the involvement of organoid structures in established wound-healing processes during in vitro injury models. Such studies may yield valuable insights into the crucial developmental stages, cellular populations, and signaling cascades integral to skin regeneration.

Skin organoids have demonstrated potential for reconstructing damaged integument with appendages in clinical applications, although precise control over off-target cell lineages remains a challenge. The limitations associated with 3D-cultured skin organ spheroids, particularly abnormal metabolic shedding, may be mitigated through the application of microfluidic technologies or ALI platforms to facilitate planar growth of skin organs [13].

Current obstacles include the heterogeneous populations of chondrocytes and myoblasts derived from the neural crest lineage in skin organoids. Minimizing the generation of undesired cell types will require further optimization of early signal-induction protocols. Moreover, the skin organoid culture system requires continued refinement, with future advancements anticipated through the integration of gene therapy approaches.

Standardization of organoid culture conditions remains a significant challenge that has impeded consistent production. Furthermore, the existing organoid models cannot fully recapitulate in vivo physiological conditions, such as lipid metabolism and circulatory processes. To meet the requirements for practical applications, future research should focus on developing scalable and reproducible technologies for organoid production. Investigators should also consider factors such as inter-individual variability and the impact of passage number, since the declining organoid growth rates over time documented in previous studies are indicative of biological alterations during extended culture periods [100].

The ultimate objective is to regenerate a comprehensive skin substitute that incorporates all functional components, including hair follicles, sweat glands, and nerves. Rapid advancements in the fields of skin development, wound repair, stem cell biology, and tissue bioengineering offer promising avenues for realizing this goal. Future research should also focus on reconstructing missing skin features such as sweat glands, blood vessels [98–102], and immune cells [43], while minimizing off-target cells to enhance the complexity and accuracy of these models. The ideal skin organoid model should exhibit safety, clinical efficacy, ease of production, and straightforward management [103,104].

Authorship contribution statement

Yanan Huang, Qing Ye, and Jiyuan Wang contributed equally to this work as co-first authors, and were responsible for designing and writing the manuscript. Meihua Shen and Xiaoping Jiang acted as co-corresponding authors who contributed equally to this work, and participated in revising and finalizing the manuscript. Kaimin Zhu and Haojie Yang prepared the figures.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

References

[1] Lei M, Schumacher LJ, Lai YC, Juan WT, Yeh CY, Wu P, et al. Self-organization process in newborn skin organoid formation inspires strategy to restore hair regeneration of adult cells. Proc Natl Acad Sci U S A 2017;114(34):E7101–10. https://doi.org/10.1073/pnas.17004751143.
[2] Mahmoudi S, Brunet A. Aging and reprogramming: a two-way street. Curr Opin Cell Biol 2012;24(6):744–56. https://doi.org/10.1016/j.ceb.2012.10.0044.
[3] Mora C, Serzanti M, Consiglio A, Memo M, Dell'Era P. Clinical potentials of human pluripotent stem cells. Cell Biol Toxicol 2017;33(4):351–60. https://doi.org/10.1007/s10565-017-9384-y6.
[4] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663–76. https://doi.org/10.1016/j.cell.2006.07.0245.
[5] Wenzel D, Bayerl J, Nyström A, Bruckner-Tuderman L, Meixner A, Penninger JM. Genetically corrected iPSCs as cell therapy for recessive dystrophic epidermolysis bullosa. Sci Transl Med 2014;6(264):264ra165. https://doi.org/10.1126/scitranslmed.3010083102.
[6] Sun X, Xiang J, Chen R, Geng Z, Wang L, Liu Y, et al. Sweat gland organoids originating from reprogrammed epidermal keratinocytes functionally recapitulated damaged skin. Adv Sci 2021;8(22):e2103079. https://doi.org/10.1002/advs.202103079136.
[7] Ebner-Peking P, Krisch L, Wolf M, Hochmann S, Hoog A, Vári B, et al. Self-assembly of differentiated progenitor cells facilitates spheroid human skin organoid formation and planar skin regeneration. Theranostics 2021;11(17):8430–47. https://doi.org/10.7150/thno.5966118.
[8] Takeishi K, Collin de l'Hortet A, Wang Y, Handa K, Guzman-Lepe J, Matsubara K, et al. Assembly and function of a bioengineered human liver for transplantation generated solely from induced pluripotent stem cells. Cell Rep 2020;31(9):107711. https://doi.org/10.1016/j.celrep.2020.10771119.
[9] Hofbauer P, Jahnel SM, Papai N, Giesshammer M, Deyett A, Schmidt C, et al. Cardioids reveal self-organizing principles of human cardiogenesis. Cell 2021;184(12):3299–3317.e3222. https://doi.org/10.1016/j.cell.2021.04.03420.
[10] Kruczek K, Qu Z, Gentry J, Fadl BR, Gieser L, Hiriyanna S, et al. Gene therapy of dominant CRX-leber congenital amaurosis using patient stem cell-derived retinal organoids. Stem Cell Rep 2021;16(2):252–63. https://doi.org/10.1016/j.stemcr.2020.12.01821.

[11] Huang WK, Wong SZH, Pather SR, Nguyen PTT, Zhang F, Zhang DY, et al. Generation of hypothalamic arcuate organoids from human induced pluripotent stem cells. Cell Stem Cell 2021;28(9):1657–1670.e1610. https://doi.org/10.1016/j.stem.2021.04.00622.
[12] Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 2011;472(7341):51–6. https://doi.org/10.1038/nature0994123.
[13] Lee J, van der Valk WH, Serdy SA, Deakin C, Kim J, Le AP, et al. Generation and characterization of hair-bearing skin organoids from human pluripotent stem cells. Nat Protoc 2022;17(5):1266–305. https://doi.org/10.1038/s41596-022-00681-y24.
[14] Dąbrowska AK, Spano F, Derler S, Adlhart C, Spencer ND, Rossi RM. The relationship between skin function, barrier properties, and body-dependent factors. Skin Res Technol 2018;24(2):165–74. https://doi.org/10.1111/srt.1242425.
[15] Woo SH, Lumpkin EA, Patapoutian A. Merkel cells and neurons keep in touch. Trends Cell Biol 2015;25(2):74–81. https://doi.org/10.1016/j.tcb.2014.10.00326.
[16] Zimmerman A, Bai L, Ginty DD. The gentle touch receptors of mammalian skin. Science 2014;346(6212):950–4. https://doi.org/10.1126/science.125422927.
[17] Sun BK, Siprashvili Z, Khavari PA. Advances in skin grafting and treatment of cutaneous wounds. Science 2014;346(6212):941–5. https://doi.org/10.1126/science.125383628.
[18] Karimkhani C, Dellavalle RP, Coffeng LE, Flohr C, Hay RJ, Langan SM, et al. Global skin disease morbidity and mortality: an update from the global burden of disease study 2013. JAMA Dermatol 2017;153(5):406–12. https://doi.org/10.1001/jamadermatol.2016.553829.
[19] He Z, Marrone G, Ou A, Liu H, Ma L, Huang Y, et al. Factors affecting health-related quality of life in patients with skin disease: cross-sectional results from 8,789 patients with 16 skin diseases. Health Qual Life Outcome 2020;18(1):298. https://doi.org/10.1186/s12955-020-01542-630.
[20] Laughter MR, Maymone MBC, Karimkhani C, Rundle C, Hu S, Wolfe S, et al. The burden of skin and subcutaneous diseases in the United States from 1990 to 2017. JAMA Dermatol 2020;156(8):874–81. https://doi.org/10.1001/jamadermatol.2020.157331.
[21] Laughter MR, Maymone MBC, Mashayekhi S, Arents BWM, Karimkhani C, Langan SM, et al. The global burden of atopic dermatitis: lessons from the Global Burden of Disease Study 1990-2017. Br J Dermatol 2021;184(2):304–9. https://doi.org/10.1111/bjd.1958032.
[22] Mehrmal S, Uppal P, Nedley N, Giesey RL, Delost GR. The global, regional, and national burden of psoriasis in 195 countries and territories, 1990 to 2017: a systematic analysis from the Global Burden of Disease Study 2017. J Am Acad Dermatol 2021;84(1):46–52. https://doi.org/10.1016/j.jaad.2020.04.13933.
[23] Letsiou S. Tracing skin aging process: a mini- review of in vitro approaches. Biogerontology 2021;22(3):261–72. https://doi.org/10.1007/s10522-021-09916-z34.
[24] Hennies HC, Poumay Y. Skin disease models in vitro and inflammatory mechanisms: predictability for drug development. Handb Exp Pharmacol 2021;265:187–218. https://doi.org/10.1007/164_2020_42835.
[25] Lee J, Koehler KR. Skin organoids: a new human model for developmental and translational research. Exp Dermatol 2021;30(4):613–20. https://doi.org/10.1111/exd.1429237.
[26] Niehues H, Bouwstra JA, El Ghalbzouri A, Brandner JM, Zeeuwen P, van den Bogaard EH. 3D skin models for 3R research: the potential of 3D reconstructed skin models to study skin barrier function. Exp Dermatol 2018;27(5):501–11. https://doi.org/10.1111/exd.1353138.
[27] Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, et al. Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch Toxicol 2011;85(5):367–485. https://doi.org/10.1007/s00204-011-0693-239.
[28] Abd E, Yousef SA, Pastore MN, Telaprolu K, Mohammed YH, Namjoshi S, et al. Skin models for the testing of transdermal drugs. Clin Pharmacol 2016;8:163–76. https://doi.org/10.2147/cpaa.S6478840.
[29] Itoh M, Umegaki-Arao N, Guo Z, Liu L, Higgins CA, Christiano AM. Generation of 3D skin equivalents fully reconstituted from human induced pluripotent stem cells (iPSCs). PLoS One 2013;8(10):e77673. https://doi.org/10.1371/journal.pone.007767347.
[30] Yang R, Zheng Y, Burrows M, Liu S, Wei Z, Nace A, et al. Generation of folliculogenic human epithelial stem cells from induced pluripotent stem cells. Nat Commun 2014;5:3071. https://doi.org/10.1038/ncomms407143.
[31] Gledhill K, Guo Z, Umegaki-Arao N, Higgins CA, Itoh M, Christiano AM. Melanin transfer in human 3D skin equivalents generated exclusively from induced pluripotent stem cells. PLoS One 2015;10(8):e0136713. https://doi.org/10.1371/journal.pone.013671344.
[32] Abaci HE, Coffman A, Doucet Y, Chen J, Jacków J, Wang E, et al. Tissue engineering of human hair follicles using a biomimetic developmental approach. Nat Commun 2018;9(1):5301. https://doi.org/10.1038/s41467-018-07579-y45.
[33] Kim D, Chung KB, Kim TG. Application of single-cell RNA sequencing on human skin: technical evolution and challenges. J Dermatol Sci 2020;99(2):74–81. https://doi.org/10.1016/j.jdermsci.2020.06.00246.
[34] Tjin MS, Chua AWC, Tryggvason K. Chemically defined and xenogeneic-free culture method for human epidermal keratinocytes on laminin-based matrices. Nat Protoc 2020;15(2):694–711. https://doi.org/10.1038/s41596-019-0270-348.
[35] Weng T, Wu P, Zhang W, Zheng Y, Li Q, Jin R, et al. Regeneration of skin appendages and nerves: current status and further challenges. J Transl Med 2020;18(1):53. https://doi.org/10.1186/s12967-020-02248-549.
[36] Castro AR, Logarinho E. Tissue engineering strategies for human hair follicle regeneration: how far from a hairy goal? Stem Cells Transl Med 2020;9(3):342–50. https://doi.org/10.1002/sctm.19-030150.
[37] Antoni D, Burckel H, Josset E, Noel G. Three-dimensional cell culture: a breakthrough in vivo. Int J Mol Sci 2015;16(3):5517–27. https://doi.org/10.3390/ijms1603551751.
[38] Souza AG, Silva IBB, Campos-Fernandez E, Barcelos LS, Souza JB, Marangoni K, et al. Comparative assay of 2D and 3D cell culture models: proliferation, gene expression and anticancer drug response. Curr Pharmaceut Des 2018;24(15):1689–94. https://doi.org/10.2174/1381612824666180404152305 2.
[39] Imamura Y, Mukohara T, Shimono Y, Funakoshi Y, Chayahara N, Toyoda M, et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 2015;33(4):1837–43. https://doi.org/10.3892/or.2015.376753.
[40] Kaluthantrige Don F, Huch M. Organoids, where we stand and where we go. Trends Mol Med 2021;27(5):416–8. https://doi.org/10.1016/j.molmed.2021.03.0019.
[41] Laurent J, Blin G, Chatelain F, Vanneaux V, Fuchs A, Larghero J, et al. Convergence of microengineering and cellular self-organization towards functional tissue manufacturing. Nat Biomed Eng 2017;1(12):939–56. https://doi.org/10.1038/s41551-017-0166-x7.
[42] Shahbazi MN, Siggia ED, Zernicka-Goetz M. Self-organization of stem cells into embryos: a window on early mammalian development. Science 2019;364(6444):948–51. https://doi.org/10.1126/science.aax01648.
[43] Bar-Ephraim YE, Kretzschmar K, Clevers H. Organoids in immunological research. Nat Rev Immunol 2020;20(5):279–93. https://doi.org/10.1038/s41577-019-0248-y16.
[44] Kim Y, Ju JH. Generation of 3D skin organoid from cord blood-derived induced pluripotent stem cells. J Vis Exp 2019;146. https://doi.org/10.3791/5929759.
[45] Kim Y, Park N, Rim YA, Nam Y, Jung H, Lee K, et al. Establishment of a complex skin structure via layered co-culture of keratinocytes and fibroblasts derived from induced pluripotent stem cells. Stem Cell Res Ther 2018;9(1):217. https://doi.org/10.1186/s13287-018-0958-260.
[46] Yilmaz A, Braverman-Gross C, Bialer-Tsypin A, Peretz M, Benvenisty N. Mapping gene circuits essential for germ layer differentiation via loss-of-function screens in haploid human embryonic stem cells. Cell Stem Cell 2020;27(4):679–691.e676. https://doi.org/10.1016/j.stem.2020.06.02361.
[47] Cherry AB, Daley GQ. Reprogrammed cells for disease modeling and regenerative medicine. Annu Rev Med 2013;64:277–90. https://doi.org/10.1146/annurev-med-050311-16332462.
[48] Lichti U, Weinberg WC, Goodman L, Ledbetter S, Dooley T, Morgan D, et al. In vivo regulation of murine hair growth: insights from grafting defined cell populations onto nude mice. J Invest Dermatol 1993;101(1 Suppl):124s–9s. https://doi.org/10.1111/1523-1747.ep1236316563.
[49] Zheng Y, Du X, Wang W, Boucher M, Parimoo S, Stenn K. Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. J Invest Dermatol 2005;124(5):867–76. https://doi.org/10.1111/j.0022-202X.2005.23716.x64.
[50] Toyoshima KE, Asakawa K, Ishibashi N, Toki H, Ogawa M, Hasegawa T, et al. Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. Nat Commun 2012;3:784. https://doi.org/10.1038/ncomms178465.
[51] Lee LF, Jiang TX, Garner W, Chuong CM. A simplified procedure to reconstitute hair-producing skin. Tissue Eng C Methods 2011;17(4):391–400. https://doi.org/10.1089/ten.TEC.2010.047766.
[52] Collins CA, Kretzschmar K, Watt FM. Reprogramming adult dermis to a neonatal state through epidermal activation of β-catenin. Development 2011;138(23):5189–99. https://doi.org/10.1242/dev.06459271.
[53] Ma J, Li W, Cao R, Gao D, Zhang Q, Li X, et al. Application of an iPSC-derived organoid model for localized scleroderma therapy. Adv Sci 2022;9(16):e2106075. https://doi.org/10.1002/advs.20210607572.
[54] Lim X, Nusse R. Wnt signaling in skin development, homeostasis, and disease. Cold Spring Harbor Perspect Biol 2013;5(2). https://doi.org/10.1101/cshperspect.a00802973.
[55] Gonzalez Malagon SG, Lopez Muñoz AM, Doro D, Bolger TG, Poon E, Tucker ER, et al. Glycogen synthase kinase 3 controls migration of the neural crest lineage in mouse and Xenopus. Nat Commun 2018;9(1):1126. https://doi.org/10.1038/s41467-018-03512-574.
[56] Koehler KR, Nie J, Longworth-Mills E, Liu XP, Lee J, Holt JR, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. Nat Biotechnol 2017;35(6):583–9. https://doi.org/10.1038/nbt.384075.
[57] Koehler KR, Mikosz AM, Molosh AI, Patel D, Hashino E. Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. Nature 2013;500(7461):217–21. https://doi.org/10.1038/nature1229876.
[58] Tchieu J, Zimmer B, Fattahi F, Amin S, Zeltner N, Chen S, et al. A modular platform for differentiation of human PSCs into all major ectodermal

lineages. Cell Stem Cell 2017;21(3):399–410.e397. https://doi.org/10.1016/j.stem.2017.08.01577.
[59] Wilson PA, Hemmati-Brivanlou A. Induction of epidermis and inhibition of neural fate by Bmp-4. Nature 1995;376(6538):331–3. https://doi.org/10.1038/376331a078.
[60] Lee J, Rabbani CC, Gao H, Steinhart MR, Woodruff BM, Pflum ZE, et al. Hair-bearing human skin generated entirely from pluripotent stem cells. Nature 2020;582(7812):399–404. https://doi.org/10.1038/s41586-020-2352-358.
[61] Chuong CM, Yeh CY, Jiang TX, Widelitz R. Module-based complexity formation: periodic patterning in feathers and hairs. Wiley Interdiscip Rev Dev Biol 2013;2(1):97–112. https://doi.org/10.1002/wdev.7479.
[62] Pruniéras M, Régnier M, Woodley D. Methods for cultivation of keratinocytes with an air-liquid interface. J Invest Dermatol 1983;81(1 Suppl):28s–33s. https://doi.org/10.1111/1523-1747.ep1254032480.
[63] Jung SY, You HJ, Kim MJ, Ko G, Lee S, Kang KS. Wnt-activating human skin organoid model of atopic dermatitis induced by Staphylococcus aureus and its protective effects by Cutibacterium acnes. iScience 2022;25(10):105150. https://doi.org/10.1016/j.isci.2022.10515081.
[64] Yang B, Brazile B, Jan NJ, Hua Y, Wei J, Sigal IA. Structured polarized light microscopy for collagen fiber structure and orientation quantification in thick ocular tissues. J Biomed Opt 2018;23(10):1–10. https://doi.org/10.1117/1.Jbo.23.10.10600183.
[65] Williams ML, Elias PM. From basket weave to barrier. Unifying concepts for the pathogenesis of the disorders of cornification. Arch Dermatol 1993;129(5):626–9. https://doi.org/10.1001/archderm.129.5.62682.
[66] Ma J, Liu J, Gao D, Li X, Zhang Q, Lv L, et al. Establishment of human pluripotent stem cell-derived skin organoids enabled pathophysiological model of SARS-CoV-2 infection. Adv Sci 2022;9(7):e2104192. https://doi.org/10.1002/advs.20210419287.
[67] Blanpain C. Stem cells: skin regeneration and repair. Nature 2010;464(7289):686–7. https://doi.org/10.1038/464686a88.
[68] MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007;445(7130):874–80. https://doi.org/10.1038/nature0566489.
[69] Takagi R, Ishimaru J, Sugawara A, Toyoshima KE, Ishida K, Ogawa M, et al. Bioengineering a 3D integumentary organ system from iPS cells using an in vivo transplantation model. Sci Adv 2016;2(4):e1500887. https://doi.org/10.1126/sciadv.150088790.
[70] Brockmann I, Ehrenpfordt J, Sturmheit T, Brandenburger M, Kruse C, Zille M, et al. Skin-derived stem cells for wound treatment using cultured epidermal autografts: clinical applications and challenges. Stem Cell Int 2018;2018:4623615. https://doi.org/10.1155/2018/462361591.
[71] Burnouf T, Strunk D, Koh MB, Schallmoser K. Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials 2016;76:371–87. https://doi.org/10.1016/j.biomaterials.2015.10.06592.
[72] Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: eleven years of clinical experience. Burns 2006;32(5):538–44. https://doi.org/10.1016/j.burns.2006.02.02593.
[73] Condorelli AG, Dellambra E, Logli E, Zambruno G, Castiglia D. Epidermolysis bullosa-associated squamous cell carcinoma: from pathogenesis to therapeutic perspectives. Int J Mol Sci 2019;20(22). https://doi.org/10.3390/ijms2022570794.
[74] Kim DP, Kus KJB, Ruiz E. Basal cell carcinoma review. Hematol Oncol Clin N Am 2019;33(1):13–24. https://doi.org/10.1016/j.hoc.2018.09.00495.
[75] Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther 2019;20(11):1366–79. https://doi.org/10.1080/15384047.2019.164003296.
[76] Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: incidence, risk factors, diagnosis, and staging. J Am Acad Dermatol 2018;78(2):237–47. https://doi.org/10.1016/j.jaad.2017.08.05997.
[77] Waldman A, Schmults C. Cutaneous squamous cell carcinoma. Hematol Oncol Clin N Am 2019;33(1):1–12. https://doi.org/10.1016/j.hoc.2018.08.00198.
[78] Hogue L, Harvey VM. Basal cell carcinoma, squamous cell carcinoma, and cutaneous melanoma in skin of color patients. Dermatol Clin 2019;37(4):519–26. https://doi.org/10.1016/j.det.2019.05.00999.
[79] Klicks J, Maßlo C, Kluth A, Rudolf R, Hafner M. A novel spheroid-based co-culture model mimics loss of keratinocyte differentiation, melanoma cell invasion, and drug-induced selection of ABCB5-expressing cells. BMC Cancer 2019;19(1):402. https://doi.org/10.1186/s12885-019-5606-4118.
[80] Yum MK, Han S, Fink J, Wu SS, Dabrowska C, Trendafilova T, et al. Tracing oncogene-driven remodelling of the intestinal stem cell niche. Nature 2021;594(7863):442–7. https://doi.org/10.1038/s41586-021-03605-0101.
[81] Sebastiano V, Zhen HH, Haddad B, Bashkirova E, Melo SP, Wang P, et al. Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. Sci Transl Med 2014;6(264):264ra163. https://doi.org/10.1126/scitranslmed.3009540103.
[82] Tolar J, Xia L, Riddle MJ, Lees CJ, Eide CR, McElmurry RT, et al. Induced pluripotent stem cells from individuals with recessive dystrophic epidermolysis bullosa. J Invest Dermatol 2011;131(4):848–56. https://doi.org/10.1038/jid.2010.346104.
[83] Itoh M, Kiuru M, Cairo MS, Christiano AM. Generation of keratinocytes from normal and recessive dystrophic epidermolysis bullosa-induced pluripotent stem cells. Proc Natl Acad Sci U S A 2011;108(21):8797–802. https://doi.org/10.1073/pnas.1100332108105.
[84] Elias MS, Wright SC, Nicholson WV, Morrison KD, Prescott AR, Ten Have S, et al. Functional and proteomic analysis of a full thickness filaggrin-deficient skin organoid model. Wellcome Open Res 2019;4:134. https://doi.org/10.12688/wellcomeopenres.15405.2106.
[85] Sriram G, Bigliardi PL, Bigliardi-Qi M. Full-Thickness human skin equivalent models of atopic dermatitis. Methods Mol Biol 2019;1879:367–83. https://doi.org/10.1007/7651_2018_163107.
[86] Wang X, Wang S, Guo B, Su Y, Tan Z, Chang M, et al. Human primary epidermal organoids enable modeling of dermatophyte infections. Cell Death Dis 2021;12(1):35. https://doi.org/10.1038/s41419-020-03330-y108.
[87] Kitisin T, Muangkaew W, Ampawong S, Sukphopetch P. Utilization of an in vitro biofabricated 3D skin as a pathological model of cutaneous candidiasis. New Microbiol 2020;43(4):171–9. 109.
[88] Ishibashi Y, Sugita T, Nishikawa A. Cytokine secretion profile of human keratinocytes exposed to Malassezia yeasts. FEMS Immunol Med Microbiol 2006;48(3):400–9. https://doi.org/10.1111/j.1574-695X.2006.00163.x110.
[89] Meltzer S, Santiago C, Sharma N, Ginty DD. The cellular and molecular basis of somatosensory neuron development. Neuron 2021;109(23):3736–57. https://doi.org/10.1016/j.neuron.2021.09.004111.
[90] Vidal Yucha SE, Tamamoto KA, Nguyen H, Cairns DM, Kaplan DL. Human skin equivalents demonstrate need for neuro-immuno-cutaneous system. Adv Biosyst 2019;3(1):e1800283. https://doi.org/10.1002/adbi.201800283113.
[91] Andersen J, Revah O, Miura Y, Thom N, Amin ND, Kelley KW, et al. Generation of functional human 3D cortico-motor assembloids. Cell 2020;183(7):1913–1929.e1926. https://doi.org/10.1016/j.cell.2020.11.017114.
[92] Wainger BJ, Buttermore ED, Oliveira JT, Mellin C, Lee S, Saber WA, et al. Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. Nat Neurosci 2015;18(1):17–24. https://doi.org/10.1038/nn.3886115.
[93] Desoize B, Jardillier J. Multicellular resistance: a paradigm for clinical resistance? Crit Rev Oncol Hematol 2000;36(2–3):193–207. https://doi.org/10.1016/s1040-8428(00)00086-x116.
[94] Kondo J, Inoue M. Application of cancer organoid model for drug screening and personalized therapy. Cells 2019;8(5). https://doi.org/10.3390/cells8050470117.
[95] Lee J, Böscke R, Tang PC, Hartman BH, Heller S, Koehler KR. Hair follicle development in mouse pluripotent stem cell-derived skin organoids. Cell Rep 2018;22(1):242–54. https://doi.org/10.1016/j.celrep.2017.12.00754.
[96] Ramovs V, Janssen H, Fuentes I, Pitaval A, Rachidi W, Chuva de Sousa Lopes SM, et al. Characterization of the epidermal-dermal junction in hiPSC-derived skin organoids. Stem Cell Rep 2022;17(6):1279–88. https://doi.org/10.1016/j.stemcr.2022.04.008120.
[97] Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med 2013;19(7):939–45. https://doi.org/10.1038/nm.3201122.
[98] Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 2013;13(6):653–8. https://doi.org/10.1016/j.stem.2013.11.002123.
[99] Shiota J, Samuelson LC, Razumilava N. Hepatobiliary organoids and their applications for studies of liver health and disease: are we there yet? Hepatology 2021;74(4):2251–63. https://doi.org/10.1002/hep.31772124.
[100] Hu H, Gehart H, Artegiani B, Löpez-Iglesias C, Dekkers F, Basak O, et al. Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell 2018;175(6):1591–1606.e1519. https://doi.org/10.1016/j.cell.2018.11.013126.
[101] Lu C, Fuchs E. Sweat gland progenitors in development, homeostasis, and wound repair. Cold Spring Harb Perspect Med 2014;4(2). https://doi.org/10.1101/cshperspect.a015222127.
[102] Lu CP, Polak L, Keyes BE, Fuchs E. Spatiotemporal antagonism in mesenchymal-epithelial signaling in sweat versus hair fate decision. Science 2016;354(6319). https://doi.org/10.1126/science.aah6102128.
[103] Zhao X, Xu Z, Xiao L, Shi T, Xiao H, Wang Y, et al. Review on the vascularization of organoids and organoids-on-a-chip. Front Bioeng Biotechnol 2021;9:637048. https://doi.org/10.3389/fbioe.2021.637048129.
[104] Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol 2009;10(3):165–77. https://doi.org/10.1038/nrm2639130.