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Review

Bioengineering strategies for regeneration of skin integrity: A literature review☆

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ABSTRACT

Objective: The skin is a complex organ that includes various stem cell populations. Current approaches for non-healing skin defects are sometimes inadequate and many attempts have been made to regenerate skin integrity. The aim of this review is to bridge the gap between basic research and clinical application of skin integrity regeneration.

Methods: A literature search was carried out in PubMed using combinations of the keywords “skin integrity”, “tissue-engineered skin”, “bioengineered skin”, and “skin regeneration”. Articles published from 1968 to 2023 reporting evidence from *in vivo* and *in vitro* skin regeneration experiments were included.

Results: These articles showed that stem cells can be differentiated into normal skin cells, including keratinocytes, and are a significant source of skin organoids, which are useful for investigating skin biology; and that emerging direct reprogramming methods have great potential to regenerate skin from the wounded skin surface.

Conclusion: Recent advances in skin regeneration will facilitate further advancement of both basic and clinical research in skin biology.

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Contents

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1. Introduction

The skin is a large, complex, and multilayered organ with appendages including follicles and glands, which is critical for regulating body temperature, retaining bodily fluids, protecting against external stresses (e.g., infection and electrolyte loss), and mediating touch and pain sensations [1–3]. The skin consists of 3 layers: the epidermis, dermis, and hypodermis [4,5]. Cells of the epidermis include keratinocytes, melanocytes, Langerhans' cells, and Merkel's cells [6], and there are three epidermal appendages: the sweat glands, the pilosebaceous follicles that produce the hair and sebaceous excretions, and the nails that cover the distal phalanges [7]. Sweat glands function as thermoregulators and secrete antimicrobial peptides [8]. The normal turnover rate for the epidermis is about 28 days.

Wounds can be divided into two main categories: acute and chronic, depending on the time and progress of the healing process [9]. Chronic wounds are a major problem in the global aging population [10]; however, acute large skin defects including fresh trauma and skin lesions such as giant congenital melanocytic nevi or keloids are also of concern. There are two common treatment options for wounds: conservative and surgical, with the former including creams and hydrogels [6], and the latter involving skin grafting and flap-plasty [11]. However, patients with non-healing ulcers may not be candidates for surgery due to risks caused by systemic conditions [12], and current techniques are frequently inadequate in cases with absolute epithelial deficiencies such as broad area burns. To overcome these problems, many novel bioengineering methods have been proposed to regenerate skin integrity. These technologies also cover dermatologic research in anti-aging, drug screening, temporal biological barriers, and hair regeneration.

Stem cells emerged as potential sources of bioengineered skin. Pluripotent stem cells can be differentiated into normal skin cells including keratinocytes [13], and skin organoids can generate three-dimensional (3D) *in vitro* models that are useful for investigating development, physiology, and pathology of skin [14]. Finally, emerging direct reprogramming methods have been shown to have potential to regenerate skin from the wounded skin surface [13].

In this review, we introduce basic research on skin integrity and identify the gaps between this research and clinical applications. First, we evaluate recent advances in skin formation in the fields of skin equivalents, stem cells and direct reprogramming. Then, we offer suggestions for future directions and methods to regenerate skin using the latest technologies, including *in vivo* direct reprogramming. Despite tremendous promise, current skin regeneration models still have limitations. Therefore, we also provide an impartial view of the opportunities and challenges in regenerative skin medicine. The article is presented in accordance with the Narrative Review reporting checklist (available at https://sci.amegroups.com/article/view/10.21037/sci-2022-044/rc).

2. Methods

To explicate recent advances and progress in regeneration of skin integrity, we performed a thorough search on the PubMed, Scopus, and Web of Science databases in June 2023. These publicly available and institutionally accessible databases were used to search indexed and published articles from January 1968 to June 2023. Editorials, technical reports and expert opinions were excluded from the literature search. Studies presented at international meetings and conferences, but not published in standard journals in English, were also excluded. Additional records were identified through bibliography review of included articles. Two of the authors (MS and YS) screened, included and excluded the candidate articles (Table 1).

2.1. Differentiation of stem cells into skin cells

In the late 20th century, novel stem cell-based technology emerged. Pluripotent stem cells that are present in later embryogenesis have the potential to differentiate into all three germ layers, but are unable to differentiate into placenta [15]. Embryonic stem cells (ESCs) were first described in 1998, and subsequently, stem cell therapies based on ESCs became the gold standard due to their limitless capacity for differentiation and self-renewal. However, research and development for ESCs and induced pluripotent stem cells (iPSCs) are limited by ethical and legal issues associated with the protection of human rights. Thus, adult stem cells are now favored due to their immunocompatibility and freedom from ethical concerns. Among a wide variety of adult stem cells, mesenchymal stem cells (MSCs) and one of their subsets, adipose-derived stem cells (ADSCs), have attracted attention [16,17]. At the start of the 21st century, therapeutic strategies using these stem cells were introduced for skin regeneration [18].

2.1.1. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)

ESCs are pluripotent stem cell lines derived from the inner cell mass of human blastocyst-stage embryos. These cells can give rise to all somatic cell types in humans, and thus, provide an ideal model system to study human embryogenesis [19]. Serum, retinoic acid or dual inhibition of transforming growth factor β (TGF-β) and bone morphogenetic protein (BMP) pathways can initiate differentiation of ESCs into keratinocytes [20–22]. Inhibition of BMP, fibroblast growth factor (FGF), or NOTCH is effective to drive epidermal cell fate [22–24]. Enrichment and expansion of derived keratinocytes requires a low-calcium keratinocyte medium [25]. Keratinocyte differentiation from human ESCs is achieved in defined conditions, including temporal and combinatory actions of TGF-β, BMP4, WNT and NOTCH signaling *in vitro*; however, none of these methods have succeeded for 3D skin equivalents [23–28]. Fibroblasts can also be derived from ESCs, and several studies have developed methodology to define the mechanisms that regulate fibroblast differentiation *in vitro* from ESCs [29,30]. Applications of ESCs for skin regenerative medicine seem to be very potent, but are complicated by ethical issues due to the requirement for human embryos.

In 2009, reprogramming of human foreskin fibroblasts (HFFs) to iPSCs was first shown using four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc: OSKM) under ESC culture conditions [31,32]. This result suggested that multiple differentiated adult cell varieties have the potential for reprogramming into a pluripotent state [33,34]. For proper human skin formation, mutual interactions between epidermal and dermal cells are required. Nakajima et al. [35,36] described a novel protocol to derive dermal fibroblasts from iPSCs in chick and mouse embryos undergoing somitogenesis. The somitic mesoderm was induced via inhibition of TGF-β and BMP and activation of WNT. Subsequently, dermal fibroblasts were generated by activation of BMP and WNT, resulting in expression of collagen I and hyaluronic acid. Another attempt for driving fibroblasts from iPSCs was reported by Itoh et al. [37] Culturing iPSCs in medium including TGF-β2 and ascorbic acid led to formation of embryoid bodies, resulting in development of fibroblast-like cells. Dermal fibroblast markers including CD10, CD44, CD73 and CD90 were expressed on these cells. The iPSC-derived fibroblasts with normal human keratinocytes were also grafted on the back of SCID mice, resulting in generation of *in vitro* 3D skin equivalents with a basement membrane expressing epidermal differentiation markers, including

Table 1
Summary of the search strategy.

Items	Specification
Date of research	30 June 2023
Databases and other sources researched	NIH National Library of medicine PubMed, MEDLINE database
Search terms used	Skin integrity; tissue-engineered skin; Bio-engineered skin; skin regeneration
Timeframe	January 1968 to June 2023
Inclusion and exclusion criteria	Only in English

keratin 1 and loricrin. Shamis et al. [38] reported that ascorbic acid enhanced expression of collagen genes, secretion of soluble collagen, and extracellular deposition of type I collagen from iPSCs. Thus, iPSCs have the potential to differentiate into layers of dermal fibroblasts by modulation of certain signaling pathways. In addition, iPSCs are the main source of skin organoids, which are novel 3D *in vitro* models that can self-assemble to form an organized skin-like structure [3,39,40].

2.1.2. Mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs)

In 1967, the presence of nonhematopoietic stem cells was first observed in bone marrow and these cells were isolated and cultured *in vitro* in 1968 [41,42]. After introduction of the term "mesenchymal stem cells" in 1991 [16], many studies have identified bone marrow as a possible source of fibroblasts that deposit collagen fibers as part of the normal process of wound repair in rodents and humans, and have suggested the potential of MSCs as a therapeutic agent in regenerative medicine [43,44]. In addition, multilineage cells isolated from processed adipose tissue were discovered in 2001 and later called "adipose-derived stem cells", which are fundamentally different cell types from MSCs [45]. ADSCs were shown to differentiate into the adipogenic lineage, similarly to MSCs; however, these cells have a higher potential than other MSCs to maintain stem cell characteristics [46].

MSCs are mainly applied to promote wound healing. These cells induce generation of an M2-like phenotype, a type of macrophage capable of suppressing both innate and adaptive immune responses [47]. MSCs secrete interleukin-1 receptor antagonist (IL1RA), resulting in polarization of macrophages toward an M2-like phenotype and inhibition of B cell differentiation *in vivo* [48]. T-helper 17 (Th17) cell differentiation is accelerated by interleukin-1 (IL-1), such that IL1RA leads to an increase in the anti-inflammatory effects of the cells [49]. IL1RA released from MSCs significantly blocks production and activity of IL-1 and TNF-α, which are pro-inflammatory cytokines [50]. In the proliferative phase, MSC-derived cultured media (MSCs-CM), extracellular matrix (ECM), and MSC-derived exosomes (MSC-EXOs) are emerging for treatment of injuries in diverse tissues. MSCs manipulate macrophages to release EGF and TGF-α to stimulate migration and proliferation of keratinocytes, which interact with fibroblasts via EGF, fibronectin, and keratinocyte growth factor (KGF) [51]. MSCs-CM release soluble signaling factors for dermal fibroblast responses to cutaneous injury [52]. MSC-seeded hydrogels increase VEGF expression and result in enhanced angiogenesis compared to MSCs injected only into the wounded skin area [53]. MSC-pretreated exosomes from neonatal mouse serum have also been shown to improve wound healing by inducing angiogenesis [54].

Similar to MSCs, ADSCs promote wound healing via greater secretion of protective factors [55–63]. These include pro-angiogenic factors such as hVEGF, interleukin-6, and CXCL5 cytokine, resulting in regulation of angiogenesis and related reactions to promote wound healing [58,59,62,63]. ADSCs also have stronger differentiation [45] and proliferation [55] abilities compared to MSCs. In 2009, the potential of ADSCs to differentiate into keratinocytes was first reported [60]. ADSCs can also differentiate into endothelial cells, cardiomyocytes, and vascular smooth muscle cells, and promote peripheral nerve regeneration [64–69]. A wide variety of uses of ADSCs have been investigated in treatment of skin ulcers [70–72], radiated skin damage [73], peripheral nerve damage [68,74], and ischemic diseases [75]. Combinational use of exosomes with ADSCs has also developed rapidly. ADSC-derived exosomes (ADSC-EXOs) have important paracrine components that are released from ADSCs [76]. These components promote mechanical repair for cell survival, migration, proliferation, and neovascularization [77,78]. ADSC-EXOs have a better biosafety profile with lower immunogenicity than MSC-EXOs, and also have the potential to be applied to skin regeneration [79].

2.2. Skin organoids

Skin organoids are emerging for use in modeling of organogenesis and developmental disorders of skin [18]. In 2009, Sato et al. [79] proposed the concept of organoids and established an intestinal organoid culture system. Organoids are derived from iPSCs and can self-assemble to form organized target tissues. Recent studies have shown that skin organoids of complex hair-bearing human skin tissue can be generated from stem cells. Skin organoids are made from two components: embryoids and specific skin precursor cell spheroids. Embryoids are derived from ESCs or iPSCs, resulting in differentiation into fibroblasts and keratinocytes [80]. Specific skin precursor cell spheroids are generated from adult stem cells whose "niches" provide distinct microenvironments for stem cell maintenance and function [81]. Adult stem cells are obtained from a wide variety of sources, including the epidermis, hair follicles, dermal MSCs, melanocytes, endothelial cells, and hematopoietic stem cells [82]. Different skin precursor cells are then fused to produce assembloids, and finally, embryoids and assembloids form multifunctional skin organoids. The terminologies used differ among studies, but "organoids," "assembloids," and "spheroids" are generally described as complex 3D cell models created through an assembly strategy [83]. These structures allow for detailed study and replication of cellular environments.

There have been many attempts to generate appendage-bearing skin tissue [84–88]. Tsuji et al. reported a novel bioengineered 3D integumentary organ system including hair follicles and sebaceous glands for cosmetics and quasi-drug testing [89,90]. Kim et al. [91] generated cord blood mononuclear cell-derived human iPSCs (CBMC-iPSCs) using Sendai virus. Epidermal and dermal layers were developed using CBMC-iPSCs, and a humanized mouse model was made by transplanting the 3D skin organoids [46]. Lee et al. [3] generated iPSC-derived skin organoids in mice to study hair follicle development, *in vitro* drug testing, and specific skin disease models. The same group also established methods to generate hair-bearing skin organoids by co-induction of surface ectoderm and cranial neural crest (CNC) cells using key factors including Matrigel, BMP4, and a TGFβ inhibitor [44,92]. Kageyama et al. [93] later developed an *in vitro* hair follicle model using microenvironmental

reprogramming of skin organoids. Notably, a stratified epidermis and a dermis with sebaceous glands and neural innervation were equipped with these skin organoids (Table 2).

2.3. Direct reprogramming

Direct reprogramming is a novel approach in which one specialized cell type is converted into another lineage without the need for a pluripotent intermediate [94]. In 1987, a classic example of direct reconversion was described based on overexpression of a key transcription factor, MyoD, which led to reprogramming of fibroblasts into myoblasts [95]. This method has fewer risks than use of iPSCs, including genetic abnormalities, tumorigenicity, and immunogenicity in the transplanted cells [96]. This is because direct reconversion is a faster process due to bypassing the pluripotent cell state, and this offers great potential for clinical and therapeutic applications [97]. From the 2010s, direct reprogramming technology has been shown to be applicable to skin regeneration (Fig. 1, Table 3). Chen et al. examined six candidate transcription factors that are critical for epidermal fate determination and differentiation: PRDM1, DNP63A, KLF4, OVOL1, ZNF750, and GRHL3 [98–106]. Among these, KLF4 and DNP63A were found to be sufficient to convert human dermal fibroblasts to an induced keratinocyte phenotype (iKC) *in vitro* with retroviral induction of keratinocyte-specific genes, KRT14 and GJB2 [107]. Iacovides et al. [108] reported direct conversion of somatic cells into functional keratinocytes in mice using retroviral vectors expressing Oct4, Sox2, and Klf4 pluripotency-associated factors to initiate reprogramming. Zheng et al. [109] showed that iKCs can be generated from human urine cells by retroviral transduction of two lineage-specific transcription factors, BMI1 and DNP63, which can be cultured for a long period and can self-assemble to form stratified skin equivalents *in vitro*. Kurita et al. [110] investigated direct reprogramming of wound-resident mesenchymal cells into skin epithelial tissue using four transcription factors: DNP63A, GRHL2, TFAP2A, and cMYC (DGTM factors) *in vivo*. Regarding sweat glands, Zhao et al. [111] showed direct reconversion of human fibroblasts into sweat gland-like cells by introducing NF-κB and Lef-1 using plasmid vectors. Similarly, Yao et al. [112] showed that overexpression of FoxC1 induced BMP5 upregulation, which led to reprogramming of epidermal cells into induced functional sweat gland-like cells. For melanocytes, Yang et al. [113] found that a combination of three factors, MITF, SOX10, and PAX3, is sufficient to reprogram mouse and human fibroblasts into human melanocytes. Fehrenbach et al. [114] also showed that retroviral induction of MITF, PAX3, SOX2, and SOX9 can be used for direct reprogramming of keratinocytes into a melanocytic lineage.

2.4. Clinical application of regenerative strategies

This review provides current viewpoints on regenerative strategies for skin integrity. There are many skin equivalents, and recent products show higher efficiency with a low rate of rejection. Stem cell-based technology using MSCs, ADSCs, and iPSCs has been established as a basis of cell therapy, and direct reprogramming has emerged as a novel strategy to modify the cell status. In the very near future, it will be possible to manipulate the skin structure at will, as Liu et al. [115] have developed a novel lentiviral transduction system using PPARG and small molecules in reprogramming human epidermal keratinocytes isolated from adult foreskin into induced sebaceous gland cells.

Regarding direct reprogramming, the efficiency of this approach is not reported to be high. Current research in direct reprogramming seeks to identify and understand the factors that impede efficiency, such as chromatin remodelers and regulatory transcription factors [116]. It will be crucial to apply these insights to improve the effectiveness of direct reprogramming. Additionally, it is important to recognize that factors reducing reprogramming efficiency are often related to the preservation of cellular identity, which must be carefully balanced with efforts to increase the proportion of rejuvenated cells. Moreover, the durability of the rejuvenation effects achieved through direct reprogramming warrants further exploration. Thus, there is a need to determine whether cells maintain their rejuvenated state post-reprogramming, revert to their original state while losing all rejuvenation characteristics, or undergo an accelerated decline compared to normal cells. This makes the longevity of rejuvenation effects a key area of ongoing research. Safety concerns also persist around direct reprogramming, particularly when considering its translation into clinical therapies. These concerns emphasize the need for extensive research to map out the processes involved in direct reprogramming.

Recent technological advances are promising, but there are still major difficulties and challenges in translation to clinical applications. The risk of mutagenesis in changing cell fate is the most evident problem. As we have shown, viral vectors and longer cell culture cannot eliminate this risk; however, novel non-viral techniques including CRISPR/Cas9 may provide safer regenerative cells [117,118]. Recently, several studies have shown genetic modification

Table 2
Overview of current skin organoids.

References	Organoid identity	Originating species	Starting cells	Extracellular matrix	Growth factors and inhibitors	Protein expressed
[81,82]	Integumentary organ systems (IOS)	Mouse	iPSC	CDB	A mitomycin C–treated SNLP feeder layer Wnt10b a novel *in vivo* transplantation model	Sox2, Sox17, dNp63
[83,84]	Induced pluripotent stem cell-derived skin organoid	Human	iPSCs	KCs: Col IV Organoids: Col I	RA, BMP4, EGF	dNp63, K5, K14, loricrin
[85]	PSC-derived skin organoids	Mouse	ESCs iPSCs	Matrigel	A TGFβ inhibitor, BMP4, FGF-2, a BMP inhibitor	K5, K10, loricrin, filaggrin
[86,87]	Hair-bearing human skin organoids	Human	ESCs iPSCs	Matrigel	A TGFβ inhibitor, BMP4	D49f, K10, K15, loricrin, LHX2
[98]	Hair follicloids	Mouse	Embryonic epithelial and mesenchymal cells	Matrigel	An NF-κB inhibitor, a PI3K inhibitor	α-SMA, Sox9, CD34, K5, Ki67, versican, gp100, TRP1, c-Kit

α-SMA: α-smooth muscle actin; BMP: bone morphogenetic protein; CDB: clustering-dependent embryoid body; Col: collagen; EGF: epidermal growth factor; ESCs: embryonic stem cells; FGF: fibroblast growth factor; iPSCs: induced pluripotent stem cells; KCs: keratinocytes; K5: keratin 5; K10: keratin 10; K14: keratin 14; K15: keratin 15; LHX2: LIM/homeobox protein2; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: phosphatidylinositol 3-kinases; PSCs: pluripotent stem cells; RA: retinoic acid; TGF-β: transforming growth factor β.

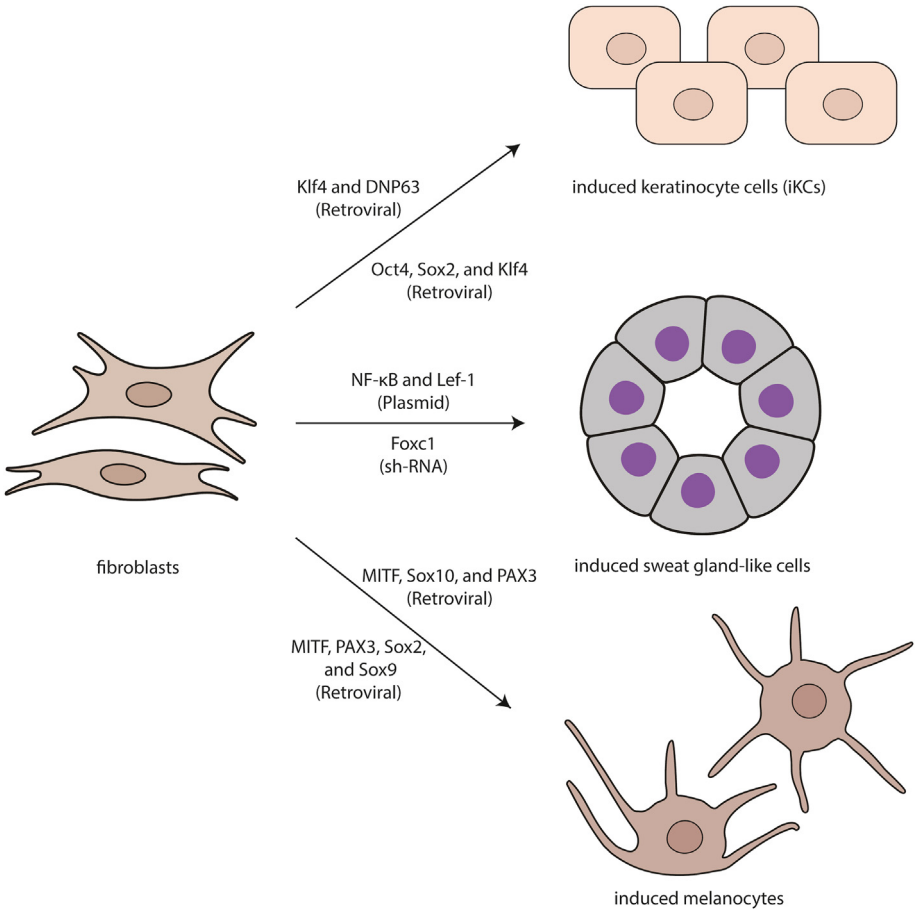

Fig. 1. Direct reprogramming from fibroblasts into skin cells. The figure refers to Chen et al. [107], Iacovides et al. [108], Zhao et al. [111], Yao et al. [112], Yang et al. [113], and Fehrenbach et al. [114]. The image was created with BioRender (https://www.biorender.com/). Foxc1: forkhead box C1; GRHL2: grainyhead like transcription factor 2; KLF4: Kruppel-like factor 4; Lef-1: lymphoid-enhancer-binding factor 1; MITF: melanocyte inducing transcription factor; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; Oct4: octamer-binding transcription factor 4; PAX3: paired box gene 3; shRNA: short hairpin RNA; Sox2: SRY-box transcription factor 2; Sox9: SRY-box transcription factor 9; Sox10: SRY-box transcription factor 10.

Table 3
Recent reports of direct reprogramming of skin cells.

References	Originating cells	Introduced factors	Protein expressed	Outcomes
[107]	Human fibroblasts	Retroviral induction of Klf4 and p63	K1, filaggrin	Induced keratinocyte like cells
[108]	Mouse embryonic fibroblasts	Retroviral induction of Oct4, Sox2, and Klf4	Vim, K14, K10, loricrin	Induced keratinocyte
[110]	Mouse wound-resident mesenchymal cells	Adeno-associated viral induction of DNP63A, GRHL2, TFAP2A, and cMyc	K14, K13, K10, loricrin	Induced stratified epithelial progenitors
[111]	Human fibroblasts	Transfection of NF-κB and Lef-1	K19, K14, K7, CEA	Induced sweat gland-like cells
[112]	Human fibroblasts	Transfection of Foxc1	K5, K14, K8, CK18	Induced sweat gland-like cells
[113]	Mouse and human fibroblasts	Retroviral induction of MITF, Sox10, and PAX3	TYR, TYRP1, DCT, SILV, S100, Melanin-A	Induced melanocytes
[114]	Mouse and human fibroblasts	Retroviral induction of MITF, PAX3, Sox2, and Sox9	TYR, TYRP1, DCT, SILV, S100, Melanin-A	Induced melanocytes
[115]	Human epidermal keratinocytes	Lentiviral induction of PPARG, and small molecules	FASN, K7, MUC1	Induced sebaceous gland-like cells

AQP5: aquaporin 5; α-SMA: α-smooth muscle actin; CEA: carcinoembryonic antigen; DCT: dopachrome tautomerase; FASN: fatty acid synthase; Foxc1: forkhead box C1; Foxc1: forkhead box C1; GRHL2: grainyhead like transcription factor 2; Klf4: Kruppel-like factor 4; Lef-1: lymphoid-enhancer-binding factor 1; K1: keratin 1; K5: keratin 5; K7: keratin 7; K8: keratin 8; K10: keratin 10; K13: keratin 13; K14: keratin 14; K5: keratin 5; K7: keratin 7; K14: keratin 14; K18: keratin 18; K19: keratin 19; MITF: melanocyte inducing transcription factor; MUC1: mucin1; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; Oct4: octamer-binding transcription factor 4; Pan-CK: pan-cytokeratin; PAX3: paired box gene 3; PPARG: peroxisome proliferator-activated receptor γ; p63: tumor protein P63: SILV: silver locus protein homolog; Sox2: SRY-box transcription factor 2; Sox9: SRY-box transcription factor 9; Sox10: SRY-box transcription factor 10; TFAP2A: transcription factor AP-2 Alpha; TYR: tyrosinase; TYRP1: tyrosinase-related protein-1.

by CRISPR/Cas9 has potential in reprogramming, resulting in curing of skin diseases including epidermolysis bullosa simplex [119–121]. However, efficiency in generating the desired cells raises another concern, since a longer period for regeneration may limit clinical application. Thus, there is a need to focus on improvement of the efficiency and yield of the target cells. Other problems that need to be addressed include heterogeneity among cell lines and differences among cells. Ethical considerations also require further discussion. There is currently no skin regeneration technique available in a clinical setting, but several clinical trials are ongoing for treatments at sites other than the skin. Phase II clinical trials have been completed for treatment of cardiac ischemia by intramyocardial injection of ADSCs [122,123], while a phase I/II clinical trial (NCT06279741) has assessed the effect of MSCs for preventing bronchopulmonary dysplasia (BPD) in preterm newborns [124]. If these problems can be overcome, it is likely that future findings will revolutionize skin regeneration.

3. Conclusion

Research in skin regeneration has undergone remarkable developments. A wide variety of skin equivalents are used clinically and are being developed. Stem cells are potential sources of skin cell differentiation, while skin organoids set the stage for gaining a more in-depth understanding of human skin development and formation in culture that could be applied to cell therapy. Direct reprogramming is a novel method for changing cell fates, and has the potential to regenerate skin directly from wounded skin. However, there are still technical considerations and barriers to clinical applications. Further studies are needed to overcome these issues, but recent advances in skin integrity research have shown the potential for application in clinical fields in the near future.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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None.

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