

Cellular Approaches to Tissue-Engineering of Skin: A Review

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Abstract

The human skin is a complex organ consisting of multiple skin cells that work together to complement each other and provide essential functions such as skin barrier function, skin homeostasis and protection against the harmful ultraviolet radiation. Understanding the roles and paracrine signaling of different skin cells plus the influence of external stimuli on them are crucial towards the design of tissue-engineered skin constructs as these factors regulate the cellular behavior such as cell proliferation, migration and differentiation. Hence, an in-depth understanding of the knowledge on the epithelial-mesenchymal interactions would be valuable towards the design of a tissue-engineered skin construct.

Keywords: Skin; Keratinocytes; Melanocytes; Fibroblasts; Stem cells; Tissue Engineering

Introduction

Human skin, which is the body's largest organ, not only protects us against a plethora of deleterious stressors such as chemical, mechanical and biological insults but also plays a critical role in maintaining the body homeostasis. Wounds are breaches in skin's structure that compromise the skin barrier functions and they can be classified into different categories such as I) epidermal, II) superficial partial-thickness, III) deep partial-thickness or IV) full-thickness wounds based on the depth of injury [1]. In severe cases such as chronic ulcers or extensive burns, the wounds extend deep into the dermis region (full-thickness wounds) and they do not close without intervention. Any full-thickness wound larger than 1 cm in diameter requires the use of a skin graft for proper healing and the current "gold standard" treatment is to apply split-thickness grafts onto the wound site [2]. In the United States, an estimated US \$25 billion is spent on wound treatment annually and this healthcare burden is expected to snowball drastically due to escalating healthcare costs and increasing aging population worldwide [3].

Although the limited availability of suitable skin grafts poses a major challenge, development of tissue-engineered skin substitutes can help to alleviate this problem [4]. At present, tissue-engineered skin substitutes are already a reality due to extensive research in cell biology, wound healing mechanisms and breakthrough in cell culture techniques [5,6]. The advent of skin tissue engineering has brought about a paradigm shift in wound management from the use of passive wound dressings to bioactive skin regeneration scaffolds [7]. Tissue-engineered skin constructs comprising of living cells, biomaterials and biochemical signals aim to expedite wound healing by stimulating the innate capability of the skin for self-regeneration. The gradual understanding of wound healing mechanisms has invoked the development of bioactive skin regeneration scaffolds that promote skin rejuvenation via the incorporation of different skin cells within tissue-engineered scaffolds. As the skin cells themselves are the

primary sources of various extracellular matrix (ECM) molecules that stimulate and coordinate tissue repair, it is important to understand the roles of these cells within the native skin. In this review, the roles of different skin cells and their paracrine signaling are highlighted. In addition, the current progress of tissue-engineered skin substitutes and the potential use of stem cell technology for skin tissue engineering will be discussed.

Cellular Components of Skin

The skin cells found in tissue-engineered skin can be categorized into autologous (from the patient's own cells) or allogeneic (from genetically non-identical donors) cell source. Both autologous and allogeneic skin cells have been used in commercially available skin substitutes and they help to initiate wound healing process [8]. The advantages of utilizing an autologous cell source include the comparatively low requirement for safety testing and a lower risk of immunological rejection by the patients, while the limitations include lengthy cultivation time (~15 to 30 days) and high manufacturing costs arising from having to use separate, independent tissue culture suites. In contrast, allogeneic cell source has the advantages of immediate clinical applications to injured skin and the use of common tissue culture facility for each cell type allows the economies of scale and straightforward logistics. The limitations include more stringent safety-testing, potential risk of immunological rejection and disease transmission. In the following section, the roles of different skin cells in their respective regions and the influence of external factors on cell migration, proliferation and differentiation will be presented and highlighted.

Keratinocytes

Keratinocytes found in epidermis (the outermost layer of the skin) form an impermeable barrier to pathogens and play an important role in cell signaling within the extracellular matrix. The epidermis measures about 0.2 mm thick and epidermal keratinocytes with different degrees of differentiation are arranged into 4 layers within the epidermis as depicted in Figure 1 (namely *stratum corneum*,

stratum granulosum, stratum spinosum and stratum basale). The outermost *stratum corneum* is a cornified layer of 15-30 sheets of terminally-differentiated keratinocytes (corneocytes). The next inner layer (*stratum granulosum*) consists of 3-5 sheets of granular layer of non-dividing keratinocytes, which flatten as the dividing cells below progressively push them to the skin surface. The following layer (*stratum spinosum*) consists of 8-10 sheets of keratinocytes with limited capacity for cell division. The basal or dividing layer of the epidermis (*stratum basale*) houses the differentiating keratinocytes and keratinocyte stem cells. Every keratinocyte in the basal layer would eventually mature and differentiate into corneocyte, followed by organizing into “bricks-and-mortar” arrays that provide the important skin barrier function [9].

Autologous keratinocytes are required for the development of a permanent skin substitute as allogeneic keratinocytes are usually rejected by the host [10]. The major challenge encountered was the lengthy cultivation period for autologous keratinocytes from skin biopsies prior to obtaining sufficient amount of keratinocytes for clinical application. Strategies that were proposed to alleviate this problem include the use of low calcium medium to enhance the proliferation rate of keratinocytes, the use of sub-confluent keratinocytes on functionalized plasma treated surfaces or fibrin glue and chimeric composition of keratinocytes (a mixture of allogeneic and autologous keratinocytes) [11-17]. Interestingly, allogeneic neonatal foreskin-derived keratinocytes do not elicit immune rejection due to undeveloped human leukocyte antigen (HLA) tissue markers and they also demonstrate high proliferation and differentiation capabilities [18,19]. The proliferation of keratinocytes is highly dependent on the epithelial-mesenchymal interactions [10]; the keratinocytes cease to proliferate after 1 week and differentiate into discontinuous epithelium in the absence of fibroblasts.

Upon reaching confluency, these proliferating keratinocytes would undergo a sequential differentiation process which is regulated by a calcium gradient across the epidermal region to form fully stratified keratinocyte layers that provide the epidermal barrier function [20]. In another study, both calcium and vitamin D were shown to activate the phospholipase C (PLC) and protein kinase C (PKC) signaling pathways that are involved in the regulation of keratinocyte differentiation [21]. A recent study also highlighted the synergistic effect of multifactorial external stimuli (calcium, confluence, serum and lower incubation temperature) on optimal differentiation of *in vitro* keratinocytes [22]. The proliferation and differentiation of the keratinocytes are regulated by a variety of biological factors; the change in protein phosphorylation is one of the major mechanisms that alter the keratinocyte's response to extracellular signals. This protein phosphorylation process involves the binding of ligands to receptors which subsequently leads to the activation of several transduction pathways in keratinocytes. These signal transduction pathways include protein tyrosine kinases (PTK), protein kinase A (PKA), protein kinase C (PKC), mitogen-activated protein kinase (MAPK), casein kinase II, phospholipases and cytokine receptor superfamily [23]. The activation of the tyrosine kinase activities and tyrosine phosphorylation are required for keratinocyte differentiation; which is closely associated with epithelial growth factor receptor (EGFR), calcium concentration (Ca^{2+}) and 12-O-tetradecanoylphorbol-13-acetate (TPA). It was also shown that PKC activation is essential for keratinocyte terminal differentiation [24] and this activation is regulated by Ca^{2+} , diacylglycerol (DAG) or phorbol ester. PKC is an important transduction pathway that regulates the sequential differentiation of keratinocytes from the spinous to

granular region in the epidermal skin. Keratinocytes in a reduced Ca^{2+} concentration (0.05mM) demonstrates a basal-cell like morphology, whereas keratinocytes cultured in higher Ca^{2+} concentration (0.12 mM) leads to sequential induction of early differentiation markers (in the spinous cells) such as keratins 1 (K1) and 10 (K10) and late markers (in the granular cells) that include involucrin, profilaggrin and loricrin [20]. More comprehensive reviews on the signal transduction pathways in keratinocytes were already covered [23,25,26].

The substrate stiffness also plays a critical role in regulating the proliferation, migration and differentiation of the keratinocytes; a stiffer surface is preferred for keratinocyte migration and proliferation, whereas a softer surface is favorable for keratinocyte differentiation [27]. Furthermore, the widely-accepted practice of culturing confluent keratinocytes at the air-liquid interface is another critical step in the formation of a fully-stratified epidermal layer [28,29]. Understanding these mechanisms that regulate the proliferation and differentiation process of keratinocytes will aid in the development of techniques and procedures for accelerated maturation of a fully functional epidermal layer.

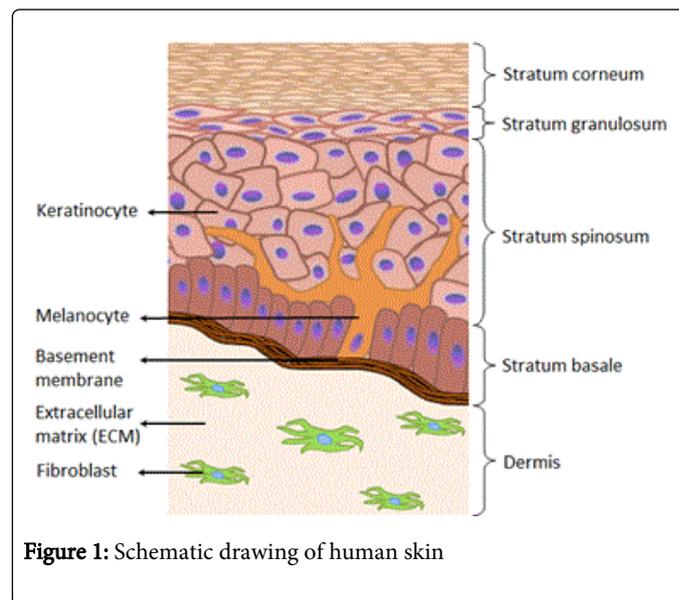


Figure 1: Schematic drawing of human skin

Melanocytes

Melanocytes found on the basement membrane at the epidermal-dermal junction, synthesize the pigment melanin which provides our distinct skin colour and protects us against the ultraviolet radiation (UV-R). The melanin are synthesized and stored within melanosomes, which are then transferred to the overlying suprabasal keratinocytes via elongated dendrites. These melanins can be categorized into eumelanin (black to brown colour), pheomelanin (yellow to reddish-brown colour), neuromelanin (brown/black colour) and mixed melanin pigment (contains both eu- and pheomelanin) [30]. The variation in skin colour is attributed to the density, size and distribution of melanosomes to the cluster of surrounding keratinocytes. Although every individual has a distinctive skin colour, the melanocyte density in a specified area is identical for all skin types [31]. The ratio of epidermal melanocytes to basal keratinocytes in the human skin is approximately 1:20 and it is imperative to note that a minimum density of 1.0×10^4 melanocytes/cm² is required to restore

the skin pigmentation completely [32]. The main components of a basement membrane (BM) consist of collagen type IV, collagen VII and laminins and the presence of a basement membrane is essential for the positional orientation of the melanocytes [33]. In the absence of BM, these melanocytes migrate to the superficial keratinocyte layers and undergo spontaneous pigmentation. In an elegant study, it was demonstrated that the dynamic epithelial-mesenchymal interactions required for basement membrane formation are predominantly regulated by diffusible factors without the need for direct keratinocyte-fibroblast contact [34].

The epidermal melanin unit (EMU) is a functional complex unit found within the epidermis that consists of both keratinocytes and melanocytes. Once melanosomes are transferred to the keratinocytes, the melanin granules accumulate above the nuclei and absorb the harmful UV-R. The keratinocyte-melanocyte complex reacts spontaneously to a wide range of external stimuli in both autocrine and paracrine signaling [35]. Upon stimulation, increased expression of proopiomelanocortin (POMC, precursor of melanocyte-stimulating hormone) and its receptor melanocortin 1 receptor (MC1-R) by melanocytes was observed. Furthermore, production of keratinocyte-derived factors such as adrenocorticotropic hormone (ACTH), alpha-melanocyte stimulating hormone (α -MSH), basic fibroblast growth factor (bFGF), nerve growth factor (NGF) and endothelins was detected. Fibroblasts also respond to UV-R by producing melanogenic factors such as hepatocyte growth factor (HGF), stem cell factor (SCF) and bFGF.

One of the main challenges in cultivation of melanocytes is due to drastic reduction in number of melanocytes after serial sub-cultivation and cryopreservation [36]. Conventionally, melanocytes are isolated from the primary culture consisting of both keratinocytes and melanocytes. This leads to low initial density and subsequently, low proliferative capacity in the first passage. In a recent study, the melanocytes were co-cultured with keratinocytes present in the primary culture until sufficient melanocytes are obtained [37] and the presence of keratinocyte-derived growth factors enhance the differentiation, migration and proliferation of the melanocytes. It was also reported that phospholipase A2, a component of bee venom, helps to stimulate melanocyte dendricity and pigmentation [38]. Extensive research was conducted to induce the differentiation of different stem cells into melanocytes [39-42] and this provides alternative sources of melanocytes which could overcome the above limitation.

Recent works on reconstruction of tissue-engineered skin containing melanocytes clearly emphasize the poor reliability of animal models [43]. Reconstructed pigmented skin substitutes transplanted on the full-thickness defects created on the back of the nude rats showed darker pigmentation as compared to the donor melanocytes [44]. This observation is corroborated by an earlier study [45] that murine fibroblasts were shown to secrete more soluble factors that increase the pigmentation. Markedly, it was shown in another study that the skin colour of the reconstructed pigmented skin transplanted onto full-thickness defects on the backs of nu/nu rats was similar to the donor skin, independent of the melanocyte to keratinocyte ratio used [46]. The surgical steel rings that were sutured to the full-thickness skin defects prevent wound closure with the surrounding rat skin; this might negate the influence of murine fibroblast on the skin pigmentation [33]. To the best of our knowledge, no work on the use of allogeneic melanocytes that closely matches the patient's skin colour for skin repigmentation has been reported.

Fibroblasts

Fibroblasts residing in the dermal region produce collagen, growth factors, glycosaminoglycans (GAGs), and fibronectin to initiate wound healing process. The dermis is predominantly extracellular matrix with low fibroblast density [47] and this dermal region can be further divided into an upper 'papillary' and a lower 'reticular' region. The papillary dermis is characterized by thin, randomly orientated collagen fiber bundles (with a high ratio of type III to type I collagen) that are arranged into ridge-like structures, while the reticular dermis consists of numerous thick, orderly-orientated fiber bundles (low ratio of type III to type I collagen) as illustrated in Figure 2. The dermal fibroblasts are heterogeneous and the papillary and reticular fibroblasts are known as superficial and deep fibroblasts respectively [48]. Recent studies on evaluation of the fibroblast heterogeneity on wound healing have shown that incorporation of superficial dermal fibroblasts within cultured skin substitutes demonstrated reduced hypertrophic scarring and improved basement membrane and epidermal barrier formation [49,50].

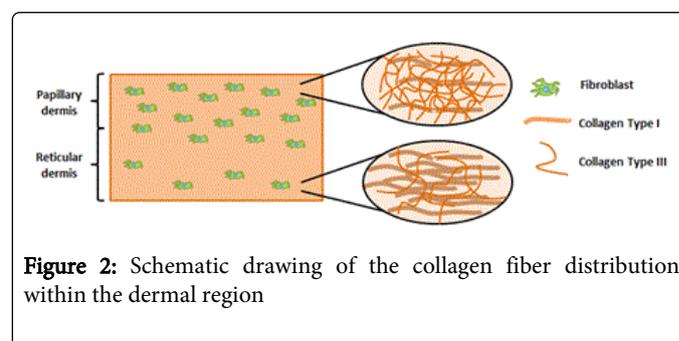


Figure 2: Schematic drawing of the collagen fiber distribution within the dermal region

A study on cell adaptation to a physiologically relevant ECM with varying viscoelastic properties demonstrates contrasting effects, whereby the fibroblasts migrate faster ($0.81 \mu\text{m}/\text{min}$) on softer substrates (95Pa) while proliferating preferentially on the stiffer substrates (4270Pa) [51]. Notably, the proliferation of fibroblasts demonstrated high dependency on the substrate hardness with a dimensionality-specific response [52]. In contrast to higher proliferation rate on a 2D microenvironment with increasing substrate stiffness, the fibroblasts demonstrated slower proliferation rate within a 3D microenvironment with increasing substrate stiffness. Although the knowledge on fibroblast biology within a 3D microenvironment is integral towards our understanding of the cell-matrix interactions and development of improved skin substitutes; it is beyond the scope of this review. Detailed reviews on the fibroblast mechanics and biology within 3D collagen matrices could be found elsewhere [53,54]. Other factors that affect the fibroblast proliferation include passage number, donor's age and fibroblast phenotype [55]. Furthermore, the presence of vitamin C and antioxidant (coenzyme Q10) were shown to increase migration and proliferation of fibroblasts [55].

In contrast to allogeneic keratinocytes, numerous reports advocated the hypothesis that allogeneic fibroblasts can be tolerated by the host and long-term grafting of these fibroblasts for up to 2 months was demonstrated [10,56-60]. Notably, the allogeneic neonatal fibroblasts are more responsive to mitogens as compared to allogeneic adult fibroblasts [61]; they migrate faster and have a shorter cell population doubling time [62]. The allogeneic fibroblasts in the cultured skin substitutes are gradually replaced by the host cells over time [21]; hence the use of autologous fibroblasts in dermal substitutes is required for permanent engraftment and was shown to result in better

restoration and minimal scar formation as compared to allogeneic dermal substitute [63,64].

The feeder cell-culture system is an exemplar which clearly depicts the importance of epithelial-mesenchymal interactions. The keratinocyte-derived interleukin-1 (IL-1) stimulate the fibroblasts to synthesize and secrete growth factors and cytokines such as keratinocyte growth factor (KGF) / fibroblast growth factor -7 (FGF7), interleukin 6 (IL-6), and granulocyte macrophage colony-stimulating factor (GM-CSF) [65], which in turn regulates keratinocyte proliferation and differentiation in the keratinocyte-fibroblast cocultures [66]. This elucidates the existence of a double paracrine loop and highlights the significance of epithelial-mesenchymal interactions. Nevertheless, it is important to note that the presence of fibroblasts in the tissue-engineered skin induced a reduction in melanocyte pigmentation as compared to tissue constructs without fibroblasts [45] and this observation is corroborated by another study which highlights that the presence of fibroblast-secreted proteins, Dickkopf 1 (DKK1), suppress the melanocyte function and proliferation [67]. As such, it is desirable to have a low fibroblast density within the tissue-engineered skin constructs which closely mimics the native dermal region.

Current Progress of Tissue-engineered Skin Substitutes

The demand for improved healing outcomes has invoked the development of skin substitutes that actively promote wound regeneration. Over the last three decades, several tissue-engineered skin substitutes have been developed and they have emerged as important therapeutic products for treatment of chronic wounds or extensive burns. These novel skin substitutes can be classified into epidermal, dermal and epidermal-dermal (composite) tissue-engineered constructs. Nevertheless, some of the limitations include more stringent safety-testing, potential risk of immunological rejection and disease transmission. In the subsequent sections, the types of skin substitutes and their respective roles will be briefly covered.

Epidermal Skin Substitutes

Epidermal skin substitutes containing autologous keratinocytes are often cultivated on top of irradiated murine fibroblast feeder layer. These autologous keratinocytes from patient's skin biopsy are usually cultivated and expanded in laboratories over a period of approximately 3 weeks to obtain stratified keratinocyte cell sheets, which are also known as cultured epithelial autografts (CEAs). Over the last three decades, confluent CEAs have been utilized for treatment of extensive burns and temporary wound dressings are required due to the lengthy cultivation period for CEAs. Furthermore, these CEAs (which typically range between 2 to 8 cell layers thick) do not result in satisfactory healing outcomes [68] and meticulous handling of the fragile cell sheets is required.

The use of synthetic carrier templates such as petrolatum gauze backings and silicone membranes provides mechanical support to the fragile cell sheets. Intriguingly, an acid-functionalized silicone membrane was reported to facilitate attachment, proliferation and easy transfer of keratinocytes [69]. However, these carrier templates are non-biodegradable, and as such it is necessary to subsequently remove them from the wound site. The use of natural biomaterials such as fibrin [70,71] and hyaluronic acid [72] as delivery system for cultured keratinocytes provides a suitable microenvironment for migration, proliferation and differentiation of keratinocytes and also improves graft adherence. Furthermore, they can undergo enzymatic

degradation *in vivo* which eliminate the hassle for subsequent removal from the wound sites. The missing dermal component in these skin substitutes often results in unsatisfactory healing outcomes, as the degree of epithelial attachment and scarring is closely dependent on the condition of the underlying dermal wound bed [73]. To improve the clinical outcomes, another type of skin substitute with the dermal component was developed.

Dermal Skin Substitutes

The biomaterials in dermal skin substitutes not only aid in wound bed preparation, but also provide temporary scaffolds for cell attachment and proliferation [73]. Fibroblasts found in the dermis layer of human skin produce collagen, growth factors, glycosaminoglycans (GAGs), and fibronectin to initiate wound healing. Generally, a two-step process is required for the reconstruction of deep wounds (as shown in Figure 2). The dermal skin substitute is first placed over the wound site for wound bed preparation, followed by the application of an epidermal layer over a well-vascularized dermal layer.

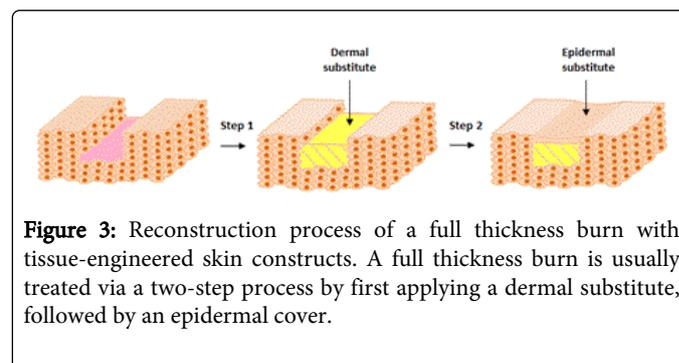


Figure 3: Reconstruction process of a full thickness burn with tissue-engineered skin constructs. A full thickness burn is usually treated via a two-step process by first applying a dermal substitute, followed by an epidermal cover.

These dermal skin substitutes can be further classified into cell-seeded scaffolds [74] or acellular scaffolds [75-78]. Most of these commercially available dermal skin substitutes are acellular scaffolds, which mainly function as temporary scaffolds for cellular infiltration and attachment. This could be due to lower manufacturing costs and straightforward logistics and storage [79]. It was highlighted that acellular dermal substitutes could be repopulated by autologous fibroblasts *in vivo* from day 3 onwards [80]. In contrast, the allogeneic human neonatal fibroblasts present in the cellular dermal skin substitutes die within a few weeks after implantation [10]; hence the cellular dermal skin substitutes only function as a delivery system for temporary production of growth factors and ECM proteins.

Epidermal-Dermal Skin Substitutes

Epidermal-dermal skin substitutes comprising both epidermal and dermal layers is currently the most sophisticated tissue-engineered skin product that closely resembles the structure of native human skin. The presence of both keratinocytes and fibroblasts within the epidermal-dermal skin substitutes leads to the production of a variety of growth factors and cytokines which expedite wound healing [81-83], highlighting the importance of epithelial-mesenchymal interactions. These epidermal-dermal skin substitutes have been utilized for treatment of chronic wounds and ulcers with higher incidences of wound closure were reported [84].

The epidermal-dermal skin substitutes are around 2.5mm thickness on average and delayed vascularization in such thick tissue-engineered

skin construct remains a critical bottleneck in skin tissue engineering [85]. Integration of a vascularized skin construct with the host vasculature is vital for efficient diffusion of oxygen, nutrients and waste products, as such most cells reside close to the blood capillaries as the oxygen and nutrient diffusion limit is approximately 0.1-0.2mm [86]. Different engineering approaches have been proposed to induce the formation of blood vessels *in vitro* [87]. A combination of cell-based [88], biomaterial-based [89,90] and micro-fabrication approaches [91,92] could possibly alleviate the problem of delayed vascularization. In the cell-based approach, endothelial cell cocultures, growth factor-producing cells or stem/progenitor cells were utilized to accelerate the formation of new blood vessels. Studies have also shown that incorporation of biomaterials such as fibrin [89] or hyaluronic acid [90] within the tissue-engineered constructs can help to improve angiogenesis. In the micro-fabrication approach, miniature channels were fabricated to enhance the oxygen and nutrient diffusion. Detailed reviews on the different vascularization strategies were already covered elsewhere [88,93,94].

Furthermore, the aesthetic outcomes of these skin substitutes remain unsatisfactory [4]. The lack of pigmentation within the skin substitutes leads to the formation of white patches resulting vitiligo, which may result in a negative impact on the patient's social life. A spray-on cell suspension, ReCell[®], which comprises of non-cultured autologous keratinocytes, melanocytes and fibroblasts, was studied for treatment of vitiligo. In one study, repigmentation time takes approximately 3-5 weeks but clinical results demonstrated good colour match and high extent of repigmentation [95]. Conversely, the results from another study showed that repigmentation of transplanted skin after 4months is highly dependent on the patient's age, whereby unsatisfactory results (less than 65%) were obtained for patients above 30 years old [96].

Stem Cell Technology

Although the use of tissue-engineered skin substitutes have demonstrated some success in the treatment of chronic wounds, current technologies only remain partially effective in mimicking the native human skin (eg. lack of skin appendages, skin pigmentation and extensive network of blood vessels). Extensive stem cell research is being carried out due to their high differentiation and proliferation capacity. The use of stem cells is gaining attention due to their potential to overcome these limitations. In this section, recent studies on the potential use of stem cells in skin tissue engineering will be highlighted and discussed.

Mesenchymal Stem Cells (Mscs)

Human mesenchymal stem cells (MSCs) are adult stem cells which can be isolated from tissues such as bone marrow [97,98] and adipose tissue [99,100]. Bone marrow MSCs (BM-MSCs) were shown to differentiate into multiple skin cell types which aid in wound healing [97,100]. It was also demonstrated that BM-MSCs can differentiate into sweat gland cells *in vitro* [102]. Notably, the BM-MSCs enhance angiogenesis [97,99], which is crucial for wound healing. Although BM-MSCs have many beneficial outcomes, the use of allogeneic MSCs does induce slight immunoreactivity [103].

Human adipose-derived stem cells (ADSCs) could be harvested easily from liposuction of human adipose tissue in a minimally invasive manner, providing an abundant source of autologous cells [104]. An approximately 40-fold yield of MSCs could be obtained from

the adipose tissue as compared to the bone marrow [105]. Automatic cell sorters such as Celution[™] system provided a cost-effective and efficient method of isolating and concentrating these stem cells automatically from adipose tissue [106]. A study showed that conditioned medium of ADSCs stimulated collagen synthesis and migration of human dermal fibroblasts (HDFs) [100], thus indicating that ADSCs secreted growth factors which accelerated wound healing. Intriguingly, increased secretion of growth factors such as vascular endothelial growth factors (VEGFs) and basic fibroblast growth factors (bFGFs) is observed under hypoxic conditions [107]. Another study has shown that ADSCs secreted angiogenic cytokines increase neovascularization [108]. Markedly, Chan, et al. have developed vascularized skin constructs from autologous ADSCs, which further differentiated into different mesenchymal cell types by varying the type of hydrogels used in the bilayered construct [99]. Lin, et al. [109] have demonstrated that wound closure rates by ADSC sheets were significantly higher than non-treated control group during the first 18 days. Trottier et al. produced a tri-layered skin substitute using ADSCs, this result elucidated that ADSCs could be used as a potential replacement for fibroblasts in skin reconstruction [110].

Nestin-Positive Stem Cells

Nestin-positive stem cells are adult stem cells, which can be isolated from the human skin appendages [111-114]. They maintained high proliferation and differentiation capacity during propagation [115]. Markedly, it was demonstrated that nestin-positive stem cells improve wound healing via formation of microvasculature networks [116-118]. Amoh et al. have demonstrated that the incorporation of nestin-expressing hair follicle cells induced the formation of an extensively branched network of blood vessels that anastomose with host vessels in the nude mice models [116].

Amniotic Fluid-Derived Stem Cells

Human amniotic fluid-derived stem (AFS) cells belong to a new type of stem cells intermediate between embryonic and adult stem cells [119]. Notably, AFS cells can be isolated easily and they demonstrated high proliferation rates without the need of any supportive feeder layer [120]. Furthermore, AFS cells demonstrated high expansion capacity (more than 250 passages) and chromosomal stability [120,121], allowing effective cell banking for off-the-shelf applications. AFS cells do not induce immune rejection [122] and they were shown to secrete cytokines and chemokines essential for accelerated wound healing [123]. Intriguingly, a novel study has demonstrated the ability of AFS cells to support crucial cross-talk between mesenchyme and epithelia needed for epidermal stratification [124]. This finding suggested that AFS cells could be used as an alternative for fibroblasts. A histological study by Skardal et al. [125] elucidated an increased micro-vessel density and capillary diameters in AFS cell-treated wounds as compared to the MSC-treated wounds. Furthermore, proteomic analysis showed that AFS secreted a higher concentration of growth factors than those of MSCs. These results corroborated that bioprinted AFS cells in a mouse model resulted in accelerated healing of large skin wounds. Hence, the use of AFS cells is attractive for skin tissue engineering.

Conclusion

Over the last three decades, the field of skin tissue engineering has been developing rapidly. This is evident with the development of

several skin substitutes for treatment of chronic wound and severe burns. They can be categorized into epidermal, dermal and epidermal-dermal skin substitutes. The epidermal skin substitutes often result in poor healing outcomes as pre-conditioning of the underlying wound bed is important for epithelial attachment and reduced scarring. This leads to the development of dermal substitutes which function as temporary scaffolds for cellular attachment and infiltration. A two-step reconstruction process is required for full-thickness wound; a dermal substitute helps to condition the wound bed prior to the application of an epidermal substitute on top of the dermal substitute. To eliminate the hassle of two-step reconstruction process, an epidermal-dermal substitute that closely resembles the native skin was developed. The involvement of paracrine signaling from both keratinocytes and fibroblasts accelerate the wound healing process via the secretion of a wide variety of growth factors as highlighted in earlier sections. Despite the advancement in skin tissue engineering, limitations such as delayed vascularization and lack of skin pigmentation remain unsolved.

The purpose of this review is to highlight the paracrine signaling of different skin cells and different external factors that influence the cell migration, proliferation and differentiation. A relatively mature understanding of this knowledge is critical towards the development of improved skin substitutes. The stem cells not only have high proliferation and differentiation capacity but they can also be isolated and propagated easily, hence they could be a potential source of cells for wound healing applications. Nevertheless, it is still important to conduct more preclinical studies to evaluate the potential risks of malignant teratoma formation, amount of cell-dosage administration and long-term adverse effects of these stem cells. These valuable insights would provide significant benefits to patients with chronic wounds or severe burns and the vast potential of skin substitutes has yet to be fully discovered.

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