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Abbreviations: AGE, advanced glycation end product; AME, amniotic epithelial cell; ASC, adipose tissue stem cell; AM, amniotic membrane; BMSC, bone marrow stem cell; DEJ, dermal-epidermal junction; DHA, docosahexaenoic acid; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; EPO, erythropoietin; FGF, fibroblast growth factor; FGF-7, fibroblast growth factor 7; FN, fibronectin; GH, growth hormone; GM-CSF, granulocyte–macrophage colony-stimulating factor; HA, hyaluronic acid; HB-EGF, heparin-binding epidermal growth factor; HGF, hepatocyte growth factor; HGF/SF, hepatocyte growth factor/scatter factor; HMW, high molecular weight; IGF, insulin growth factor; IGFBP-1, insulin-like growth-factor-binding protein-1; IL, interleukin; K, keratin; LMW, low molecular weight; miRNAs, microRNAs; MSC, mesenchymal stem cell; MIP-3α, macrophage inflammatory protein-3α; MMP, matrix metalloproteinase; NGF, nerve growth factor; 3D, three-dimensional; PDGF, platelet-derived growth factor; PRP, platelet-rich plasma; SP, Substance P; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Abstract

Cutaneous wound healing in adult mammals is a complex multi-step process involving overlapping stages of blood clot formation, inflammation, re-epithelialization, granulation tissue formation, neovascularization, and remodelling. Re-epithelialization describes the resurfacing of a wound with new epithelium. The cellular and molecular processes involved in the initiation, maintenance, and completion of epithelialization are essential for successful wound closure. A variety of modulators are involved, including growth factors, cytokines, matrix metalloproteinases, cellular receptors, and extracellular matrix components. Here, we focus on cellular mechanisms underlying keratinocyte migration and proliferation during epidermal closure. Inability to re-epithelialize is a clear indicator of chronic non-healing wounds, which fail to proceed through the normal phases of wound healing in an orderly and timely manner. This review summarizes the current knowledge regarding the management and treatment of acute and chronic wounds, with a focus on re-epithelialization, offering some insights into novel future therapies.

Keywords: Wound healing, Re-epithelialization, Epidermal regeneration, Keratinocyte, Extracellular matrix, Chronic wounds, Therapeutic strategies

1. Introduction

The skin is the largest organ in the body and fulfils a variety of functions. Most importantly, the skin acts as a barrier separating the internal organs of the body from the external environment. The skin's physical robustness, which has evolved to withstand the many mechanical, chemical, and biological insults organisms face from the outside world every day, including heat, friction, radiation, pathogenic microorganisms, and toxic chemicals and materials, is critical to this function. To achieve this, the skin can remodel and adapt to external conditions and quickly repair itself in the event of injury.

The skin is composed of the epidermis, which acts as a barrier against the external environment, and the underlying dermis, to which the epidermis is firmly anchored, conferring elasticity and mechanical resistance [1].

The epidermis is made primarily of keratinocytes and continuously renewed by the proliferation of stem cells and the differentiation of their progeny, which undergo terminal differentiation as they leave the basal layer and move upward toward the surface, where they die and slough off [2]. Basal keratinocytes express keratins K5, K14, and K15, whereas differentiating keratinocytes express K1 and K10 [3]. Basal keratinocytes append the dermal-epidermal junction (DEJ), a cell surface-associated extracellular matrix (ECM) that forms as a concerted action of both epidermal and dermal cells [4].

Wound healing in adult mammals is a complex, multi-step process involving blood clot formation, inflammation, re-epithelialization by keratinocyte migration and proliferation, granulation tissue formation, neovascularization, and tissue contraction, which largely overlap [5,6,7]. These stages are generally grouped into three phases: inflammation, proliferation, and remodelling. The process of foetal wound healing differs from that of adult wounds. During the first two trimesters, foetal skin wounds heal rapidly by regenerating normal epidermis and dermis without forming scar tissue. This ability is lost

in late gestation, when foetal dermal wounds heal with fibrosis and scar formation similar to wounds in adults [8].

In this review, we appraise the cellular mechanisms by which keratinocytes close the wound and describe therapeutic approaches applied when wound re-epithelialization fails. Strategies targeting wound repair and fibrosis have been described elsewhere [9].

2. Wound re-epithelialization

Re-epithelialization is the term used to describe the resurfacing of a skin wound with new epithelium. The excision of full-thickness skin creates a defect that is wider and longer than an incision [10]. In excisional wounds, re-epithelialization progresses from the surrounding wound margins toward the centre, creating a continuum in the regeneration of a differentiated epidermis over the DEJ in reorganization [11,12]. As comprehensively documented recently [13], re-epithelialization of human partial-thickness wounds is thought to occur primarily from stem/progenitor cells in the eccrine sweat glands and pilosebaceous units, and to a lesser extent from basal stem and progenitor cells in the interfollicular epidermis [14,15]. In full-thickness wounds, in which these adnexal structures are destroyed, re-epithelialization must originate from interfollicular epidermal cells at wound margins [15,16]. Thus, the chronology of events during re-epithelialization can be studied with the full spectrum of changes that develop spatially. The multiple cellular and molecular processes involved in the initiation, maintenance, and completion of epithelialization are essential for successful wound closure, including the formation of a provisional wound bed matrix that is formed by an insoluble protein exudate, the migration of epidermal keratinocytes from cut edges, the proliferation of keratinocytes that feed the advancing and migrating epithelial tongue, stratification and differentiation of the neo-epithelium, reformation of an intact basement membrane zone, and the repopulation

of specialized cells that direct sensory functions, pigmentation, and immune parameters [17]. The regeneration of a functional epidermis depends on reconstitution of the DEJ, which anchors the epidermis to the dermis [12,18,19], and on the terminal differentiation of keratinocytes into a protective cornified layer [11,20].

Re-epithelialization starts approximately 16-24 h after injury during the proliferation phase, and continues over the second and third phase of the wound healing process [11,21-26]. Upon acute skin injury, neutrophils, monocytes, and macrophages are recruited to the site of injury when the barrier is disrupted [27]. Subsequently, keratinocytes become activated, a change in their phenotype that is orchestrated by growth factors, chemokines, and cytokines produced by keratinocytes and other cutaneous cells. The activated phenotype is marked by changes in the cytoskeletal network and cell surface receptors. Keratinocytes move into the healing wound by polymerizing cytoskeletal actin fibres in the outgrowth and forming new adhesion complexes. Integrins and syndecans, cell surface receptors that lack enzymatic activity, transmit intracellular signals by interacting with structural and signalling molecules. These events are accompanied by the expression of integrin αvβ5, αvβ6, and α5β1; various proteases, such as plasminogen and matrix metalloproteinases (MMPs); growth factor receptors; cell surface proteoglycans; and ECM components, such as laminin 332 [28-32]. The responding intracellular signalling pathways activate transcription factors that regulate the expression of keratin genes. Migrating keratinocytes exhibit an upregulation of K6, K16, and K17 keratins, which are hypothesized to increase the viscoelastic properties of migrating cells [33-35]. In mice, the induction of K6 and K16 proteins occurs at the wound edge within 6 h, and their subsequent accumulation correlates with a polarized reorganization of keratin filaments in suprabasal keratinocytes, followed by alterations in their shape and cell-cell adhesion [36]. The existence of a keratinocyte

activation cycle in which the cells first become activated by the release of interleukin (IL)-1 has been proposed [33,34]. Subsequently, the activated state is maintained by autocrine production of pro-inflammatory and proliferative signals. K6 and K16 are markers of the active state. Induced by IL-1, tumour necrosis factor (TNF)-α can maintain keratinocytes in an activated state [37]. Signals from the lymphocytes, in the form of interferon-γ, induce the expression of K17 and the contractility of keratinocytes [38]. This enables the keratinocytes to contract the provisional fibronectin (FN)-rich matrix. Signals from fibroblasts, in the form of transforming growth factor (TGF)-β, induce the expression of K5 and K14, reverting the keratinocytes to the basal phenotype and completing the activation cycle. Activated keratinocytes are hyperproliferative, migratory, change their cytoskeleton, have augmented levels of cell surface receptors, and produce components of the DEJ. MMP-9, which is produced by migrating keratinocytes, degrades DEJ components, allowing the keratinocytes to migrate over the wound [39,40]. Activated keratinocytes also produce paracrine signals to alert fibroblasts, endothelial cells, melanocytes, and lymphocytes, and produce autocrine signals targeted at neighbouring keratinocytes. These responses are essential for orchestrating the actions of surrounding cell types in the repair of injured tissue. The affected cell types, in turn, produce their own autocrine and paracrine signals, which modify the actions of activated keratinocytes [34].

2.1 Provisional matrix

In mammals, migrating keratinocytes are in close contact with a provisional ECM that is associated with early wounds and mainly composed of fibrin, plasma FN, vitronectin, and platelets [32,41-43]. The expression of MMPs associated with all of these aspects of wound repair is simultaneously induced, including MMP-1, -3, -7, -9, -10, -14, and -28 [40,44,45]).

2.2 Keratinocyte migration

Keratinocyte migration is an early event in wound re-epithelialization. Microscopic observation of the re-epithelialization of incised superficial skin wounds in humans 1 day post-injury has revealed the presence of a moving cohesive epithelial sheet at the leading edge, migrating towards the centre of the wound and undergoing ruffling undulations of cell membranes [11]. The series of events occurring a few hours to 1 day after injury includes the flattening and elongation of keratinocytes, development of long cytoplasmic extensions (i.e., lamellipodia) and ruffling cytoplasmic projections, loss of cell-cell and cell-matrix contacts, retraction of intracellular tonofilaments, and formation of actin filaments at the end of the cytoplasm [11,46]. Although the cell-cell and cell-ECM junctions are retracted to a perinuclear location, gap junctions appear to be more numerous in migrating keratinocytes [11,46-48].

During the initial re-epithelialization of cutaneous wounds, migrating epidermal cells dissect a pathway between the fibrin clot in the wound space and the collagen-rich dermis [11,49]. Cell migration occurs in the form of a stratified epithelial sheet and, in its early phase, is unaffected by inhibitors of cell mitosis. However, a transient burst of enhanced mitotic activity is necessary to supply the cells required to sustain re-epithelialization and is carried out by a distinct subpopulation of epidermal keratinocytes located behind the migrating epithelium [50,51]. Different mechanisms have been proposed for the process of keratinocyte migration over the wound bed. According to the “leapfrog” or rolling mechanism, the migrating suprabasal cells roll over leading basal cells and dedifferentiate to form new leaders [24,50-53]. In this mechanism, cells located further from the wound edge migrate as a cohesive sheet (Fig. 1A) [24,50-53]. Suprabasal keratinocytes are then thought to undergo a change in cell shape, reduce their

desmosomal attachments, and tumble over basal keratinocytes that remain strongly attached to the basement membrane (Fig. 1A). Paladini et al. proposed that the induction of K16, and possibly K6 or K17, is involved in enabling the competence of differentiating keratinocytes for re-epithelialization, contributing to the reorganization of keratin filaments in wound edge keratinocytes [36]. A study of epithelial cells during corneal wound repair in rabbits and rats contradicted this hypothesis and suggested that superficial cells remain in a superficial position and deep cells in a deep position as they move into the defect [54]. However, a sub-population of suprabasal, post-mitotic keratinocytes appears to be directly involved in re-epithelialization of the epidermis following injury and undergoes significant phenotypic changes, but *in vivo* observations have not conclusively demonstrated the role of the rolling mechanism. The tractor-tread or sliding model, which focuses on the newly exposed leading edge basal keratinocytes, postulates that layered keratinocytes move forward in a cohesive block, whereas the cluster of superficial cells above is passively dragged along (Fig.1B) [17,55-57]. Chick embryo epithelial cells cultured *in vitro* on a glass surface have shown that the spreading of an epithelial cell sheet is directed by the adherent cells located at the free edges [55]. These cells appear to exhibit membrane activity. The cells behind the edge are likely pulled passively along by virtue of their firm adhesion to the cells at the free edge. Occasionally, cells become detached from the body of a sheet and move over the surface of surrounding cells, later becoming leading marginal cells [55]. This model supports the view that epithelial cells move as coherent sheets, and that contact inhibition is the mechanism by which the movements of the constituent cells of a sheet are coordinated. The important role of the marginal cells was confirmed by studies on corneal epithelial repair in rabbits, in which the presence of ruffles filopodia up to 100 μm in length was found on the marginal cells [58]. Epithelial cell movement on the basal lamina appears to depend mainly on the

ruffling and filopodia that project out ahead of the cell edge, contacting the basal lamina and apparently drawing the cells forward into the area of the defect. Normal corneal re-epithelization proceeds with two or three cell layers moving as an orderly sheet over the basal lamina until the defect is closed. Studies of corneal repair in mouse epithelium have corroborated the work of Hanna et al. [54] and further suggested that the function of the marginal cells relates not to locomotion, but to substrate exploration and information gathering [56]. This is consistent with the presence of filopodia, as seen in the rabbit cornea during repair. The marginal cells have been suggested to relay information to other epithelial cells via gap junctions [59]. Intercellular communication gives the signal for the epithelial sheet to advance and for its immediate arrest when the wound is covered. The cells responsible for the motion are mainly the basal cells, which first release their hemidesmosome attachment to the basal lamina, and then the most superficial cells are carried along passively [56]. The sliding model has also been documented during wound closure experiments in the transparent epidermis of *Xenopus laevis* tadpoles [57,60]. The ventral tail fin is transparent, flat, and thin enough to allow direct observation of epidermal cell movement during wound closure. Cells begin migrating within seconds after wounding and spread rapidly and continuously until the wound margins meet, when contact inhibition of movement halts their advance. Cells in the basal layer, both at the margin and within the sheet, spread actively, whereas cells in the upper layer are passive, pulled by the activity of the basal cells [57,60]. An interesting alternative was a model in which suprabasal cells de-differentiate and participate together with the basal cells in reconstituting the new human epithelium (Fig. 1C) [61]. Their study revealed that, in response to injury, suprabasal keratinocytes undergo dramatic and adaptive changes, such as the induction of K16, downregulation of K10, and up-regulation of K14, and acquire a migratory phenotype.

Re-epithelialization mechanisms have also been studied in a tissue-engineered wound-healing model composed of human skin keratinocytes and fibroblasts (Fig.1D) [62]. Reconstructed human skin [63] was wounded with a 6-mm biopsy punch. Examination of the model 3 days after injury revealed that two independent and complementary mechanisms were involved in wound re-epithelialization. The first mechanism, consistent with observations in the cornea [56], involves passive displacement of the superficial layers near the wound margins, possibly due to their passive sliding as a coherent sheet over the living layers of keratinocytes. The pushing force coming from the adjacent unwounded epidermis likely originates from the mitotic pressure (Fig. 1D). The second mechanism occurs in the migrating tongue, where keratinocytes migrate over each other individually, as in the leapfrog model of migration [24,53,64]. Another model of the generation of mechanical forces implied in the migratory process involves pressure from the cells proliferating behind the wound edge [65].

Regardless of the mechanism, the events that occur during wound re-epithelialization are reminiscent of the developmental process for the epithelial-mesenchymal transition (EMT). EMT is a dramatic phenotypic alteration characterized by the transformation of anchored epithelial cells into individualized migratory fibroblast-like cells. EMT involves complete dissociation of intercellular adhesion structures (adherens junctions and desmosomes), cell elongation, and reorganization of the cytoskeleton [66-69]. During re-epithelialization, migrating keratinocytes retain some intercellular junctions and remain part of a cohesive cell sheet. The transcription factor Slug modulates some EMT-like events that occur during re-epithelialization [70]. Slug expression and keratinocyte migration at wound margins can occur in the absence of keratinocyte proliferation [71]; thus, wound re-epithelialization represents a partial and reversible form of EMT.

The characterization of a three-dimensional (3D) organotypic full-thickness *in vitro* skin wound model comprising keratinocytes and fibroblasts by two-step time-lag fluorescence staining provides a novel theoretical wound closure model based on a shield extension mechanism (Fig.1E) [72]. Collectively migrating cells continuously build a multilayered epithelium in which suprabasal cells never contact the ECM. An immediate burst in proliferation occurs in a concentric pattern around the wound, travelling with former and newly produced cells as a wave in the direction of the wound. Two recent studies in mice revealed the existence of two concentric and spatially distinct compartments of epidermal cells around the wound edge. Immediately around the wound is a migrating and non-proliferative leading edge followed by a highly proliferative hub (Fig. 1F-G) [73,74]. The molecular signature associated with the migrating leading edge was uncovered and its molecular profiling not only revealed its singularity and specificity from the distinctive proliferation zone (Fig. 1F) but also brought to light a number of regulated genes and proteins either previously reported (i.e. α5- and α6-integrins, MMPs, fibrinolytic proteins, fibronectin, laminin 332) or newly identified [74]. Photolabelling and live imaging cells at different distances from the wound edge revealed that the proliferative and migratory zones overlap, as this area is the major source of surface expansion (Fig. 1G) [16,73]. In addition, Aragona et al. [74] revealed that repair of the skin epidermis involves increased proliferation of a small population of stem cells derived from the upper follicle that give rise to progenitors upon asymmetric division, generating larger clones than those arising from the interfollicular epidermis. Donati et al [75] showed that, during wound healing, cells of the sebaceous duct positive for transcription factor Gata6 migrate from the sebaceous duct into the interfollicular epidermis and dedifferentiate, acquiring the ability to undergo long-term self-renewal and differentiate into a wider range of epidermal lineages than undamaged tissue.

In the final stages of re-epithelialization, reversion to the differentiated epithelial phenotype occurs with the formation of stable intercellular and cell-substrate contacts [5,76,77]. Migration ceases, probably as a result of contact inhibition, and keratinocytes reattach to the underlying substratum before resuming the process of terminal differentiation to generate a stratified epidermis [78]. Fusion of the opposing epithelia is realized by degradation of the actin fibres in filopodia, which are replaced by intercellular adherence contacts to finally close the wound like a zipper [79]. When the epithelium has covered the wound bed, the proteins of the DEJ reappear to be sequentially ordered from the margins to the centre of the wound [80]. Basal keratinocytes then re-adopt a stationary phenotype with apical polarity, firmly anchored to the DEJ through reconstituted hemidesmosomes on the basal surface.

Cell-cell and cell-ECM interactions are crucial during re-epithelialization, and the collective, coordinated cell migration event is a highly stimulating topic of interest [81], as it is found in both embryonic development [82] and tumour cell invasion [83]. The distinction between leader cells (located at the wound edge) and follower cells (located in the cell layer) is a typical feature of cell-sheet movement. Both leader and follower cells have been observed to develop lamellipodia to coordinate collective migration [84]. The mechanically robust and dynamic coupling of cells to one another and the substrate is accomplished via adherens junction proteins, desmosomal proteins, and integrins [83,85]. Wounding an epithelial monolayer induces directional migration of a cell sheet, which maintains tight intercellular adhesion [86,87]. Adhesive junctions maintain tissue consistency, generate tension between cells, and entail the entire group of cells. The protein merlin has been shown to coordinate collective cell migration by acting as a mechanochemical transducer [88]. The level of adhesion between the cell and substrate, moderated by integrins, controls the speed of wound closure [89]. The effects of substrate

stiffness on cell traction forces have been quantified for epithelial cells and fibroblasts; cell movement is modulated by changing the stiffness of the substrate [90,91]. In turn, the rigidity of the substrate regulates the migration of cells through the activation of myosin II [92]. The cell response to the rigidity of the substrate depends on specific integrins [93-95].

2.3 Keratinocyte proliferation

In cutaneous wound healing, significant proliferation occurs 1 day after wounding and appears to be restricted to cells distal from the leading edge of the wound [17,24]. To generate more cells to cover the wound, epidermal stem cells from the interfollicular epidermis at the edges of the wound, nearby hair follicle bulge, or sebaceous glands start proliferating approximately 2–3 days after injury [96-103]. Only the basal keratinocytes have the ability to proliferate, as the terminally differentiated keratinocytes in the suprabasal layer lose this ability [104]. A recent spatiotemporal study of regenerating cells in a wound generated with a 3-mm punch biopsy in the tail skin of adult mice revealed that cells at the leading edge of the epidermal sheet do not actively proliferate, but migrate to the centre of the wound [74]. In that study, the size of the non-proliferating leading edge zone progressively decreased after reaching its maximum size 4 days after wounding, suggesting that the specific phenotype of leading edge cells occurs only during the early stage of wound healing. After day 14, the wound edges fused at the midline and proliferation resumed at the centre of the wound region [74]. Interestingly, blocking cell proliferation did not impact the fate of the leading edge cells, confirming previous studies of wounded tissues, *ex vivo* skin explants, and *in vitro* models that hypothesized the existence of a cell division-independent migrating tongue [11,72,105]. Aragona et al. further identified a proliferative zone distal to the leading edge for which the distance from

the wound centre and width progressively decreased with time [74]. Lineage tracing and quantitative clonal analysis revealed that this proliferative hub is composed of interfollicular and hair follicle-derived stem cells giving rise to new progenitors that expand and repair the wound. The authors found no evidence that suprabasal cells can revert back to a progenitor-like state, suggesting that de-differentiation of differentiated suprabasal cells does not contribute to wound healing in the tail epidermis [74]. The role of epidermal stem cells in wound healing, as well as the mechanism that orchestrates their function, has been studied predominantly in mouse models, pointing to the need for confirmatory evidence in human skin.

The synergy between growth factors, the ECM, and integrins plays a pivotal role in the regulation of keratinocyte proliferation during re-epithelialization [40]. Re-epithelialization can be stimulated by a variety of wound–related signals, such as nitric oxide, which is mainly synthesized by macrophages [106], as well as cytokines and growth factors, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs) including FGF-7, heparin-binding epidermal growth factor (HB-EGF), TGF-β, TGF-α, insulin growth factor (IGF)-1, nerve growth factor (NGF), and epidermal granulocyte–macrophage colony-stimulating factor (GM-CSF), secreted from multiple cell types in the wounds in a spatio-temporal manner [27,45,107-109]. IL-1 is produced immediately by keratinocytes in response to injury and acts in a paracrine and autocrine fashion by influencing keratinocyte migration and proliferation and the expression of an activation-specific set of genes. The release of pre-stored IL-1 by keratinocytes constitutes the first signal alerting surrounding cells to barrier damage [27,40,110]. IL-1 is also produced by neutrophils, monocytes, and macrophages. The genes activated by IL-1 include growth factors and cytokines, such as GM-CSF, TNF-α, TGF-α, amphiregulin, additional IL-1, K6, and K16. Though IL-1 is responsible for the

initiation of keratinocyte activation, other signals (e.g., TNF-α) are required for maintenance of the activation state. There is ample evidence that keratinocytes stimulate fibroblasts to synthesize growth factors, which in turn stimulate keratinocyte proliferation in a double paracrine manner [111]. MMPs work via their proteolytic activity to release growth factors from the wound matrix and can digest latent forms of growth factors, such as IGF-1, converting them to their active forms [112]. The ECM can also engage integrins to modulate growth factor receptor pathways, leading to increased growth factor activity [113]. A better understanding of the impaired epithelialization process may provide insights into new therapeutic approaches to enhance re-epithelialization and accelerate wound closure.

3. Re-epithelialization in chronic wounds: when problems arise

Chronic wounds are the result of multifactorial components within the wound healing process being compromised and can be defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner [114]. Pathologically, one of the clearest indicators of a chronic wound is the failure to re-epithelialize [115]. The aging population includes those most burdened with chronic wounds and low keratinocyte cell turn-over, which is a factor affecting their ability to re-epithelialize [116]. A chronic wound may enter a persistent inflammatory state and perpetual non-healing state characterized by chronicity and frequent relapse. A number of pathophysiological factors can cause a failure of normal wound healing, including inflammation, infection, malnutrition, age, diabetes, tissue maceration, pressure necrosis, and renal impairment [117-119]. An impaired re-epithelialization process may occur in various pathological conditions, including diabetes, trauma, burns, and numerous other conditions [120,121],

and may be due to bacterial infection, tissue hypoxia, local ischaemia, exudates, and excessive levels of inflammatory cytokines that create a continuous state of inflammation. Despite differences in aetiology at the molecular level, chronic wounds share certain common features, including excessive levels of pro-inflammatory cytokines, proteases, reactive oxygen species, and senescent cells, as well as the existence of persistent infection and a deficiency of stem cells, which are often also dysfunctional [122]. In chronic wounds, protease levels exceed that of their respective inhibitors, leading to destruction of the ECM and degradation of growth factors and their receptors [123-125]. Plasmin activity may also be impaired in chronic wounds [126]. The proteolytic destruction of the ECM not only prevents the wound from moving forward into the proliferative phase, but also attracts more inflammatory cells, amplifying the inflammation cycle.

Keratinocytes are dysfunctional in chronic wounds. Keratinocytes at the non-healing edges of chronic wounds continuously proliferate as a result of c-myc activation and overexpression [127] and undergo division throughout suprabasal layers. Parakeratosis and hyperkeratosis are characteristic features of chronic wound keratinocytes [45,128]. For example, chronic venous ulcers have been shown to have a loss of stem cell niche signalling and subsequent deregulation and depletion of stem cells, which possibly contribute to the hyperproliferative epidermis [129]. Although keratinocytes are hyperproliferative, they are unable to migrate and close the wound, and they undergo abnormal epidermal differentiation [130,131]. The poor migratory ability is attributed to altered integrin expression [132] and degraded ECM components. Differentiation anomalies include suppression of K1/K10 and a subset of small proline-rich proteins, along with the late differentiation marker filaggrin, whereas late differentiation markers involucrin and transglutaminase 1 are induced in venous ulcers [128]. Desmosomal and tight junction components are also deregulated and keratinocyte activation markers

K6/K16/K17 induced. Furthermore, ß-catenin is present in the nuclei of chronic wound keratinocytes [127,130].

Contributing to poor epithelialization is the overall excessive inflammatory tissue microenvironment, which inhibits the migration of fibroblasts and synthesis of new ECM in the granulation tissue. This excessive and dysregulated protease activity may cause the degradation of adhesion proteins, preventing the cell adhesion necessary for normal wound healing [133,134]. For example, fibronectin is found degraded in wound fluids of chronic ulcers, and fibronectin supplementation into these non-healing ulcers seems to improve re-epithelialization [135]. Degradation of vitronectin and tenascin-C is also increased, whereas the presence of the precursor form of laminin-332 is reduced [131,133,136]. The expression and processing of the C-terminal LG45 module of the laminin α3 chain has been shown to be enhanced by keratinocytes after infection and in chronic wounds [137]. Increased MMP activity may also cause the degradation of growth factors important for re-epithelialization [125], and changes in growth factor expression have been detected in chronic wounds [138,139].

Accumulation of advanced glycation end products (AGEs) in diabetic wounds has deleterious effects on wound healing [140]. Glycated proteins act through receptors of AGEs activating pathways, the NF-kB-mediated inflammation pathway, and ERK- and PI3K/Akt signalling [141]. The link between carbohydrates and epithelial repair suggests that glycation events may have deleterious effects [142]. Studies have revealed that more AGEs are concentrated, the less keratinocytes will be activated. AGEs hamper two key stages of the cell cycle and inhibit keratinocyte proliferation [143].

4. Ongoing treatments and therapeutic perspectives (Table 2)

Epithelialization is an essential component of wound healing that is used as a defining parameter of its success. In the absence of re-epithelialization, a wound cannot be considered healed. Epidermal barrier rupture is a risk for wound infection. A failure of keratinocytes to maintain the barrier may contribute to wound reoccurrence, which is another significant clinical problem. A better understanding of the process of epithelialization may provide insights into new therapeutic approaches to accelerate wound closure, particularly in chronic wounds. No product is currently on the market that specifically targets epithelialization. However, healthy granulation tissue, free of scab or infection, is essential for efficient re-epithelialization. Products that promote granulation tissue formation promote restoration of the epidermis. The management of re-epithelialization differs depending on the wound surface, type (e.g., burn, ulcer, acute or chronic wound), and depth (e.g., epidermal, superficial partial-thickness, deep partial-thickness, and full-thickness wounds), the patient's health (e.g., diabetes and persistent infections), and the level of the exudate [144]. This chapter will focus on strategies that promote wound epithelialization and will not address other aspects of wound management.

4.1 Moist and interactive dressings

A moist wound environment is considered to promote re-epithelialization [21,145,146]. Wounds exposed to the air lose water, the upper dermis dries, and healing occurs beneath a dry scab. Covering a wound with an occlusive dressing prevents scab formation and radically alters the pattern of epidermal wound healing. When the wound is kept moist and desiccation prevented by occlusion or semi-permeable membranes, such as thin, transparent adhesives made of polyurethane, these wounds will re-epithelialize faster than wounds that are allowed to desiccate [21,147,148]. This effect

seems to be somewhat limited, as a window of opportunity appears to exist for the favourable use of moist occlusion of a wound [149]. Modern dressings were developed to create and maintain a warm, moist environment, providing optimal conditions for improved healing [150,151], whereas traditional dressings may absorb a large amount of exudate, drying the wound bed. Modern dressings are created from natural or synthetic polymers or a combination of both, are available as thin films, foams, or gels, and can be classified as hydrocolloid dressings, alginate dressings, or non-alginate dressings [150,152,153].

In superficial wounds, damage is generally limited to the epidermis, and moisture retentive dressings (occlusive or semi-occlusive) that help promote re-epithelialization are used. The main dressings for superficial wounds are hydrocolloids, interfaces, greasy dressings, hydrocellular dressings, metalloprotease inhibitors, and hyaluronic acid (HA) dressings. Superficial wounds, including thin burns, catheter sites, partial-thickness wounds, and epidermal skin graft harvest sites, often require a basic practical dressing. One option is a film dressing made of transparent polyurethane, providing a barrier against bacterial invasion that is gas permeable and suitable for delicate and minimally exudative wounds. Another option is hydrocolloid dressings composed of gelatine, pectin, and/or carboxymethylcellulose, which can be occlusive or semi-occlusive dressings. Dressings based on a silicon dioxide/polyvinylpyrrolidone composite were recently shown to accelerate the healing of full-thickness dermal wounds in a murine model by enhancing wound contraction and re-epithelialization [154]. Histological examination of the wounds revealed that the composite improved epidermal differentiation and the multi-stratified layer structure, which would be a consequence of improved vascularization of the regenerated tissue. Another example was the use of silver-impregnated dressing on deep burns in humans [155]. Silver is an antiseptic agent. The study revealed that silver

exposure to a meshed skin graft significantly increased the rate of mesh closure compared to a standard antibiotic solution. However, the mechanism is unknown, as the pro-healing effect was unrelated to silver's antimicrobial properties. The meshed skin graft provides an interesting model for the study of re-epithelialization, as the open area between the bridges of epithelium and dermis in the graft are constant in each placed graft [156].

4.2 Scaffolds and bioactive wound dressings

Bioactive dressings are produced from biomaterials, which play an important role in the healing process. The biomaterials are made from various components of the ECM and are conceived to improve healing by providing a structural scaffold and the signals important for complex cellular interactions during healing. These dressings are known for their biocompatibility, biodegradability, and non-toxic nature and are derived generally from natural tissues or artificial sources, such as collagen [157], HA [158], chitosan [159], alginate, and elastin. Polymers of these materials are used alone or in combination depending on the type of wound. Dermal substitutes are generally preferred for full-thickness lesions and have been shown to minimize hypertrophic scarring and contractures and increase scar elasticity in acute burn wounds. They are also known to promote the formation of granulation tissue and accelerate endothelial cell migration, and may consequently provide favourable conditions for re-epithelialization. Biological dressings are sometimes incorporated with growth factors and antimicrobials to enhance the wound healing process. HA is an important biomaterial for wound healing, as it possesses various biological activities [160,161]. A number of animal studies using wound models assessed the usefulness of HA-based scaffolds [162]. These scaffolds promote granulation tissue formation and epidermal closure [162,163].

HA is a non-sulphated linear glycosaminoglycan consisting of repeating units of disaccharides composed of D-glucuronic acid and D-N-acetylglucosamine linked via alternating β-1,4 glycosidic and β-1,3 glycosidic bonds. High molecular weight (HMW) HA is present in most living tissues and in large amounts in the skin, brain, and central nervous system [164,165], constituting an important structural element of the ECM. Once secreted, HA size is regulated primarily by cleavage via hyaluronidases [166], and low molecular weight HA promotes inflammation, angiogenesis, and cell proliferation [167,168].

Interest in HA initially came from observations that differences between foetal and adult wound healing may be linked to the wound microenvironment, and that amniotic fluid may be essential to the regenerative properties of foetal wounds. Studies have revealed that amniotic fluid favours both epithelial and dermal regeneration of adult skin [169-171], an effect that has been linked to its high HA concentration or HA-stimulating activity [172-176]. The enhanced re-epithelialization obtained in an *ex vivo* human skin wound model supplemented with amniotic fluid was suppressed when hyaluronidase was added, suggesting that HA played a key role in the epidermal regeneration event [177]. Furthermore, the prolonged presence of HA during foetal wound healing was associated with the scarless foetal tissue repair [161,175,176,178]. Foetal skin contains high levels of HMW-HA, which is thought to induce healing without fibrosis and scar formation [179], an effect proposed to be supported by its capacity to inhibit myofibroblast differentiation and the subsequent expression of TGF-β1 [180]. In addition, HA is found in relatively high concentrations in the basal layer of the epidermis [181,182], where it is involved in keratinocyte proliferation and migration [183].

Because of its unique hygroscopic, rheological, and viscoelastic properties, HA creates an excellent wound healing environment. Its potential in epidermal regeneration

applications was shown in a study in which a preparation of HA obtained from excised human umbilical cord and other sources was able to induce the healing of human epithelial cells *in vitro* [178,184-188]. In addition, HA has been shown to induce cell migration [189,190] and been used in *in vivo* wound models, where it was shown to facilitate re-epithelialization, improve granulation tissue formation, increase microvascular density [187,191-196], and reduce inflammation [197,198]. HA has been used in several human studies [162,163]. A positive effect of HA in the healing of chronic wound ulcers of various aetiologies, burns, and epithelial surgical wounds has been observed regardless of the form in which HA is delivered, showing that HA derivatives favour the healing process [186,199]. Experiments in animals have shown that HA promotes corneal epithelial wound healing by stimulating the migration, adhesion, and proliferation of corneal epithelium [200-202]. The skin re-epithelialization potential of HA was shown in a small sample of volunteers in which skin lesions were produced by erbium-YAG laser [203]. A multicentre, retrospective, national survey revealed that the use of HA-based dermal substitute in deep partial- and full-thickness burns improved re-epithelialization [204].

In the early stages of wound healing, HA plays several architectural roles in ECM organization by binding cells and other components through specific and non-specific links. HA specifically interacts with several proteins and proteoglycans through HA-binding motifs and organizes the networks of fibrin, FN, and collagen [160,205,206]. The hydrophilic properties of HA make the fibrin clot softer and easier for the cells to colonize and create an environment that loosens cell anchorage to the ECM, facilitating cell migration and division [186]. HA has been shown to regulate a number of keratinocyte activities through its interaction with cellular receptor CD44, triggering a number of signalling pathways leading to migration and proliferation [161,207-210]. An analysis of

gene expression in *in vitro* wound closure revealed the ability of HA to upregulate MMP-2 and -9, facilitating keratinocyte migration [188].

The process by which HA fragments promote granulation tissue formation is associated with higher collagen deposition and protection from the deleterious effects of oxygen free radicals [167,211,212]. In addition, HA has been shown to activate the host innate immune response [213], control the angiogenic process, and stimulate collagen production in endothelial cells [194-196].

Other materials have had interesting effects on re-epithelialization. For example, a chitosan-based dressing at split skin graft donor sites has demonstrated rapid wound re-epithelialization [214] and reduced scar formation [215].

4.3 Growth factors, cytokines, hormones, and related products

Because of their involvement in all steps of wound healing, growth factors and cytokines have been examined for their potential as wound therapeutics. Early studies revealed the importance of fibroblast and endothelial cell-derived growth factors in re-epithelialization [6,27,107]. For example, the conditioned medium from fibroblasts embedded in a fibrin matrix supports the viability and migration of keratinocytes *in vitro,* supporting the idea that such supplementation could be beneficial to keratinocyte transplantation with compromised attachment, spread, and cell proliferation [216]. These properties can be obtained following fibroblast growth in a collagen gel [217]. The wound-healing effect of secretory factors released by human embryonic stem cell-derived endothelial precursor cells has been tested in cutaneous excisional wounds in mice, revealing global improvement of wound healing, including re-epithelialization [218]. Another study revealed that a human embryonic stem cell line producing elevated levels of HGF supports re-epithelialization of wounds generated in a 3D skin model [219]. Lentiviral

shRNA-mediated knockdown of HGF dramatically decreases HGF secretion from these cells, leading to a marked reduction in their ability to promote keratinocyte proliferation and re-epithelialization of cutaneous wounds. Grafting in mice excisional wounds, an engineered skin construct incorporating bone marrow-derived mesenchymal stem cells (MSCs) loaded in EGF-containing microspheres, accelerates healing with increased re-epithelialization rates and less skin contraction [220]. Due to its outstanding function in the acceleration of epidermal regeneration, EGF has been widely investigated as a wound healing agent for the treatment of surgical wounds, burns, and diabetic ulcers. Its release stimulates keratinocyte migration and proliferation [221], two events that were recently shown to depend on the involvement of cytoskeleton protein kindlin-1, contributing to re-epithelialization [222]. In pigs, the transplantation of tissue-engineered dermis containing microencapsulated human umbilical cord MSCs expressing vascular endothelial growth factor (VEGF) in full-thickness wounds has been shown to improve vascularization and re-epithelialization [223]. In contrast, the prolonged release of growth factors from fibrin-based nanoparticles loaded with platelet-rich plasma (PRP) promotes faster wound closure in mice by enhancing keratinocyte migration [224]. PRP is defined as the portion of the plasma fraction with a high concentration of autologous platelets. After activation and degranulation by thrombin, platelets release the α-granule contents, including potent mitogenic and chemotactic factors important in wound healing, such as coagulation factors, fibrinogen, platelet thromboplastin, thrombospondin, PDGF, TGF-β, VEGF, EGF, IGF, calcium, serotonin, histamine, and hydrolytic enzymes [225]. Therefore, administration of autologous PRP gel on wound sites has been proposed as a novel strategy to promote the wound-healing cascade and tissue regeneration in chronic and non-healing wounds, as well as soft tissue ulcerations [226].

TGF-β signalling is important for re-epithelialization, inflammation, angiogenesis, and granulation tissue formation during wound healing. All three isoforms (TGF-β1, 2, and 3) participate in wound healing and re-epithelialization. TGF-β1, which is known to control skin homeostasis through inhibition of keratinocyte proliferation and regulation of differentiation, also contributes to keratinocyte functions during wound re-epithelialization. The implication of TGF-β signalling in acute and complex wounds was reviewed recently, highlighting its promising regenerative therapeutic potential [227]. A large number of studies have analysed the impact of each individual growth factor or cytokine on various parameters of wound healing in diverse wound healing models. Some of these studies are shown in Table 1, indicating the potential impact of the tested molecules or products on wound re-epithelialization. Most studies have been performed on full-thickness wounds, and a direct effect on epithelialization is often difficult to evaluate because other wound closure parameters, such as tissue granulation formation, vascularization, and contraction, are also improved most of the time. Although most of these studies have revealed interesting results, a tremendous amount of work remains to be done to challenge these technologies in human wounds, particularly those that exhibit re-epithelialization defects, such as chronic wounds or burns, in which many factors are deficient and dysregulated. A number of growth factors have already reached clinical trials and had interesting outcomes [228,229]. Nevertheless, the development of efficient delivery systems will be relevant in the future to ensure the controlled release of growth factors and protection against proteolysis [228], a particularly elevated constraint of highly inflammatory environments, such as chronic wounds [230].

Amniotic membrane (AM) was first used for therapeutic purposes by Davis in 1910 for skin transplantation [231]. It is the innermost layer of the placenta, a thin membrane composed of a human amniotic epithelial cell monolayer aligned on a basal membrane

and the underlying stroma enclosing human amniotic membrane stromal cells. Preserved AM has been used for decades in various clinical fields, including ophthalmology and wound care for burns and non-healing ulcers [232]. In these applications, AM serves as an ECM and delivers growth factors important for wound healing [233], including TGF-β, EGF, FGFs, and platelet-derived growth factors (PDGFs), without causing immunological rejection after transplantation. Placental membrane also contains neonatal fibroblasts, epithelial cells, and MSCs that release soluble factors that stimulate the proliferation and migration of the predominant cell types in the wound. MSCs also have anti-scarring functions via paracrine release of VEGF and HGF. Fibroblasts facilitate the production of ECM, as well as growth factors, supplying the ideal environment for epidermis formation and wound closure. Cryopreserved or dehydrated forms of AM are commercialized and used in clinical practice for the management of all subtypes of chronic wounds, with proven efficacy in all wound healing parameters and a notable stimulatory effect on re-epithelialization [234-236]. Interestingly, a recent analysis of the cellular and molecular mechanisms underlying the influence of AM on re-epithelialization revealed the ability of AM to stimulate the dynamics and turnover of adhesion structures expressed by keratinocytes at the leading edge of epithelializing wounds [237]. Thus, the impact of AM on the cytoskeleton-related machinery that contributes to enhanced migration of keratinocytes not only offers insights into the mechanisms governing wound closure, but provides opportunities for the development of improved therapies and procedures.

In addition to increasing red blood cell production, the glycoprotein hormone erythropoietin (EPO) has a tissue protective effect in several other organs. Numerous investigations have shown that EPO is therapeutically beneficial when used to treat acute and chronic skin wounds and burns in healthy and diabetic animals. The administration of EPO during skin wound healing is most likely based on its cytoprotective,

proangiogenic, anti-apoptotic, and anti-inflammatory effects [238]. Re-epithelialization with hair follicle development at the injury site has also been observed in full-thickness burns upon local or systemic EPO administration [239-241].

Insulin is a peptide hormone and growth factor that regulates blood glucose levels and is able to promote the healing of damaged skin by stimulating cellular migration. Therefore, exogenous insulin application has been investigated for nearly a century as a potential therapeutic intervention to improve wound recovery [242]. A recent meta-analysis aimed at providing a complete review of the impact of exogenous insulin in wounds revealed that animal wound treatment with topical insulin favours granulation formation and vascularization, faster wound contraction, and re-epithelialization [243]. Although the effect of insulin on re-epithelialization has been assigned to its property of inducing proliferation, a capacity to stimulate keratinocyte migration through the PI3K-Akt-Rac1 pathway has been demonstrated [244]. Insulin-accelerated epithelialization results from increased expression of integrin α3β1 and laminin 332 in keratinocytes, leading to stronger attachment of the epidermis to the dermis and increased numbers of skin appendages and epidermal reticular ridges [244]. Studies of wound healing in Drosophila larvae, which is now recognised as an attractive model for studying epidermal closure, have revealed an epidermal-specific and cell-autonomous requirement for insulin signalling for efficient actomyosin cable formation and wound healing [245].

Substance P (SP) is an 11-amino-acid peptide that acts as a neurotransmitter in the central nervous system and is associated with pain sensation. SP also acts as an immune modulator and injury messenger in various peripheral tissues [246]. In addition, SP mobilizes MSCs and endothelial progenitor cells in the bone marrow, inducing them to migrate into the injured peripheral tissues, where they are involved in regeneration. Furthermore, SP has been shown to accelerate the normal acute and chronic wound

healing processes and to play an important role in re-epithelialization in corneal repair [246].

Androgen receptors expressed on keratinocytes and dermal fibroblasts seem to play a major and opposite role in re-epithelialization [247]. Opioid antagonists, which enhance epithelial cell division, are good candidates for promoting wound closure. Their use in wound healing models not only accelerates re-epithelialization, but reduces wound contraction rates [248].

4.4 Gene and small interfering RNA therapies

The skin possesses qualities that make it suitable for gene therapy, which uses a vector to introduce genetic material into cells to either alter gene expression to negate a pathological process or insert a desired gene to allow expression of a missing component. This can be accomplished with a variety of viral vectors or non-viral means [249]. The skin is an ideal candidate for genetic manipulations; it is easily accessible, rendering it easy to monitor for adverse reactions and easy to transfect. The epidermis has a high turnover, which is ideal for most gene transfer methods. Fibroblasts and keratinocytes are easily harvested and cultured, allowing for testing *in vitro* and the use of skin cells as vehicles in gene transfer. This has led to increasing interest in the use of gene therapy in skin diseases, especially wound healing [250,251]. As a superficial organ that is easy to manipulate and observe, the epidermis was one of the first targets for cell isolation, *in vitro* tissue engineering, and *in vivo* experimental gene transfer [252]. A number of skin diseases, particularly inherited skin disorders, have benefitted from advances in molecular genetics and biotechnology, and subsequently in gene therapy approaches [253]. Successful and extremely promising results have been achieved with autologous transgenic keratinocyte cultures, regenerating an entire, fully functional epidermis in a 7-

year-old child suffering from a devastating, life-threatening form of junctional epidermolysis bullosa [254]. Gene therapy for wound healing is currently designed to boost factors that are known to assist with the wound-healing process. In order to accelerate wound closure, genes encoding growth factors or cytokines have shown the most potential. For example, keratinocytes treated with a plasmid encoding EGF have demonstrated increased wound healing in a porcine model [255]. The majority of gene delivery systems are based on viral transfection, naked DNA application, high pressure injection, or liposomal vectors. A number of examples are presented in Table 1, but numerous other strategies have been and are currently being tested to improve the delivery method and stability of gene expression *in vivo* [249,251,256,257] and combine gene and stem cell therapies [258]. Slow-release matrices and gene-delivering gel/matrix products allow for prolonged transgenic expression [257]. Recent progress includes the gene transfer of multiple genes to improve the wound healing process. For example, combined FGF-7 and IGF-I cDNA therapy accelerates re-epithelialization, increases proliferation, and decreases skin cell apoptosis in a rat burn model [259]. These new techniques are promising but require further studies to define the efficacy and clinical applicability. More studies are also needed to define growth factor levels in different phases of wound healing and to elucidate the precise timing of gene expression or downregulation required to better augment wound healing and the control of scar formation.

MicroRNAs (miRNAs) are ~22-nt noncoding RNAs that have been recognized as important gene regulators during the last decade [260]. In humans, miRNAs are proposed to regulate ~60% of all protein-coding genes [261] and are involved in most biological processes investigated (e.g., development, organogenesis, apoptosis, and cell proliferation). The possible involvement of miRNAs in skin wound healing has been

illuminated by several expression profiling studies that have found differential expression of many miRNAs in wounded skin [262,263]. The list of miRNAs, their targets, and their implication in the inflammation, proliferation, or remodelling phases were summarized recently [264-266]. Among these miRNAs, miR-21, -31, -99, -132, -155, -184, -198, -203, -205, -210, and -483-3p work to regulate the production, differentiation, and migration of keratinocytes [265,267]. It is not surprising that aberrant expression of miRNAs contributes to the pathogenesis of major wound complications, such as pathological scars and chronic non-healing wounds, and the list of miRNAs involved in wound complications, such as hypertrophic scars, keloids, and chronic wounds, was reported previously [264]. Plasmid DNA coding for specific miRNAs to upregulate expression or antagomirs to silence expression is used to introduce miRNA into cells. Numerous genetic delivery systems are currently utilized in research, with viral vectors being the most efficient. Examples of non-viral options include cationic polymers, liposomes, and peptides, which exhibit the ability to not only package genetic cargo, but also deliver it to the cell nucleus [266].

MiRNAs are powerful gene regulators that often target several genes within the same gene network. With the identification of miRNAs important for diseases and the development of methods modulating miRNA function *in vivo*, several miRNA-based treatments have entered clinical trials and exhibit very promising therapeutic effects. To date, increasing numbers of miRNAs have been identified that are critical for skin wound repair, and it is important to evaluate the therapeutic potential for skin wounds. If successful, a novel therapeutic approach can be developed for wound complications, which are currently difficult to treat.

4.5 Skin grafts, epidermal substitutes, and stem cells

Autologous skin grafting is an important modality for wound coverage of burns and chronic wounds and varies based on the thickness of the harvested skin. Full-thickness skin grafts consist of the epidermis and entire dermis of the skin, whereas split-thickness skin grafts involve excision of the epidermis and part of the dermis [268]. In both situations, the chances of wound repair and epidermal regeneration are high except when unfavourable factors inherent to the patient hinder the graft take. Small split-thickness skin grafts can be created in some cases and implanted to provide “islands of epithelialization” in, for example, a small- or moderate-sized chronic wound with good granulation tissue. Once harvested, a split-thickness skin graft may also be meshed, allowing expansion of the graft surface area up to 9-times the donor site surface area [268,269]. Epithelialization then starts from the edges of the free graft implanted into a granulating wound surface. Thus, the speed of the epithelialization depends on the total length of the borders of the skin and is inversely proportional to the distance between the starting points of epithelial spread. This technique is indicated when insufficient donor skin is available for large wounds, as in major burns, or when the recipient site is irregularly contoured. An alternative to meshed split skin in deep partial-thickness burn wounds is the cell-spray grafting methods recently introduced to provide fast re-epithelialization, reducing the healing time and minimizing complications [270,271].

Wound epithelialization can be achieved with an autologous epidermal graft, which involves harvesting only the epidermal layer of the skin from the donor site by applying continuous negative pressure on the normal skin to raise a blister [272-274]. The roof of the blister, which is the epidermis, is then excised and transferred onto the wound. Basal keratinocytes, melanocytes, and components of the basement membrane are contained within the harvested epidermis. Automated and minimally invasive tools for harvesting autologous epidermal micrografts for use as skin grafts have been developed to leave

sensory organs and hair follicles undisturbed [275]. The advantages of epidermal grafts include minimal scarring of the donor site and no risk of graft rejection.

Recently, there has been significant interest in the use of stem cells for skin regeneration and extensive efforts have been made to regenerate epidermis by transplanting cultured epithelial cell sheets or using tissue-engineered skin grafts with an epidermal layer. In 1975, Rheinwald and Green described a reliable method of tissue culturing consisting of the expansion of a small piece of epidermis into a very large, viable, transplantable autologous-epidermal cell layer. Subsequently, cultured autologous epidermal sheet transplants became available to complement autologous split-thickness skin grafts in the treatment of major burns or other large wounds [276-280]. Transplantation of autologous cultured epithelial cells is well-established for the treatment of extensive and deep wounds, particularly when using fibrin matrices as vehicles to facilitate the transplantation of epidermal stem cells [281-283]. The use of cultured epithelial allografts to treat chronic skin ulcers or deep dermal burns has also been reported [283]. To facilitate the handling and transfer of cultured epidermal sheets to a wound and assist in their application, a number of membrane delivery systems have been proposed, including collagen gels or matrices, fibrin glue, HA, acellular porcine or human dermis, and even synthetic polymers [13,284]. Enriching these epidermal sheets with epidermal stem cells will contribute to restoring the complete function of the epidermis and provide long-term renewal [285]. The *in vitro*-cultured and constructed dermo-epidermal autologous transplants currently used in clinics or experimentally have been described exhaustively in published reviews [13,286,287].

Interestingly, cell suspension transplantation techniques have been reported to support an accelerated re-epithelialization process in extensive and deep wounds [281,286]. These non-confluent autologous keratinocyte suspensions may be extracted from the

epidermis or hair follicles and sprayed over the wound with or without prior *in vitro* amplification. They can be delivered on their own or in combination with a number of explorative tissue-engineered carriers [286]. Strategies aimed at culturing autologous human keratinocytes on microspheres are currently being developed to produce 3D cell clusters that would potentially enhance cell survival and mimic the micrograft technology to promote re-epithelialization [287].

More complex cell delivery techniques consist of creating bioengineered skin substitutes including both keratinocytes and fibroblasts. The first autologous dermo-epidermal skin substitutes were created by combining the patient's own fibroblasts and keratinocytes on a collagen-glycosaminoglycan scaffold and were clinically tested for the closure of extensive, full-thickness burns [288-290]. Although promising clinical trials in terms of graft take, re-epithelialization, and scar appearance have been reported, these fully autologous skin substitutes are still not available for commercial use. However, tissue-engineered skin substitutes for wound healing have evolved tremendously over the last few years and new advances have been made towards developing skin substitutes made up of artificial and natural materials [291-294].

Hair follicles are considered to be promoters of re-epithelialization, as wound epithelialization can be achieved from grafted hair follicles [295-296]. Numerous types of cells with multiple differentiation potentials have been identified in hair follicles and their contribution to re-epithelialization determined [102], suggesting that these cells may be an important source of novel therapeutic approaches in wound management. Most interestingly, recent studies in mice revealed that hair follicles are necessary to establish adipocyte precursors [297]. The authors uncovered that adipocyte regeneration originates from myofibroblasts reprogramming through bone morphogenetic protein signalling.

Studies have shown that the regenerative potential of multipotent stem cells may be applicable to the treatment of chronic wounds [298,299]. Bone marrow is used as a source of cellular therapy because it contains inflammatory cell progenitors important in wound healing, as well as MSCs and other multipotent stem cells [300,301]. MSC transplants promote wound healing and angiogenesis by producing paracrine angiogenic cytokines and possibly differentiating into skin cells. Bone marrow-derived MSCs have been found to improve cutaneous healing by accelerating re-epithelialization, increasing angiogenesis, and directly differentiating into epithelial cells expressing keratinocyte-specific marker [302,303]. MSCs have also been shown to contribute to ECM production and remodelling during the healing process [301]. The topical application or peripheral injection of bone marrow stem cells (BMSCs) into wounds has been explored in the management of various clinical conditions, including diabetic wounds, with encouraging results [300,304].

Adipose tissue has been identified as another source of multipotent stem cells, with characteristics resembling those of BMSCs. Although the healing mechanism is not completely understood, adipose tissue stem cells (ASCs) are speculated to contribute to wound repair and tissue regeneration by actively secreting growth factors, promoting angiogenesis and the proliferation of keratinocytes or dermal fibroblasts [305]. Furthermore, ASCs differentiate into adipocytes, providing a supportive architecture for dermal regeneration and re-epithelialization [300]. Applied directly to the wound or included in skin substitutes, ASCs are a promising strategy for wound repair and tissue engineering.

Stem cells isolated from umbilical cord blood, umbilical cord MSCs (UC-MSCs), have been shown to differentiate into epithelial cells *in vitro* [306,307]. A recent *in vivo* study confirmed that blood from the umbilical cord could improve skin wound healing by

accelerating wound closure [308], and that MSCs derived from umbilical cord blood are able to differentiate into keratinocytes in the wound bed. UC-MSCs are considered a promising alternative to BMSCs in tissue repair and regeneration [300,304]. Both *in vitro* and *in vivo* studies have demonstrated the potential of UC-MSCs to differentiate into epidermal tissue, suggesting the potential clinical application of UC-MSCs for epithelial reconstitution in wound healing [300].

The therapeutic potential of implanting human amniotic epithelial cells (AMEs) has been evaluated in a wound model in mice, resulting in significantly accelerated wound healing and increased cellularity and re-epithelialization. Transplanted AMEs exhibit high engraftment rates and express keratinocyte-specific proteins and cytokeratin in the wound area through the secretion of epithelialization growth factors [309].

Wound epithelialization has been shown to be influenced by a variety of microvesicles released from various cell types and delivered to recipient cells through a newly described cell-to-cell communication mechanism. These membrane-enclosed vesicles carry bioactive molecules, such as DNA, small RNAs, proteins, and membrane lipids, which are released into the extracellular environment, where they regulate a number of cellular events through enhanced gene expression, suppressed gene translation, and/or the activation of signalling pathways [310]. Microvesicles and exosomes derived from a number of different cell sources, such as MSCs, PRP, keratinocytes, and endothelial cells, have revealed promising outcomes in wound healing studies and are currently under intensive research [291,310,311]. For example, studies have shown that MSC-derived exosomes promote re-epithelialization of cutaneous wounds by inducing epithelial cell proliferation and angiogenesis, activate collagen and elastin secretion by fibroblasts, and prevent myofibroblast formation, thereby reducing scaring [310].

4.6 Extracellular matrix-related strategies

A recent review of the involvement of ECM proteins in epidermal regeneration highlighted its high level of complexity [32]. To date, the main targeted applications of ECM proteins, such as collagens, glycosaminoglycans, and polysaccharides, in wound repair concern tissue granulation formation and dermal repair, with various examples described throughout this review. Several ECM-based therapeutic systems for tissue repair and regeneration have reached the clinic or are in clinical trials [312]. Collagen- or fibrin-based products are the most established ECMs being used clinically to guide the regeneration of different tissues, including skin. These products are used as carriers for transplanted cells, acellular scaffolds, or immediate coverage for large trauma- or disease-associated skin defects.

Glycanase-resistant biopolymer engineered to mimic heparan sulphate has been shown to accelerate ulcer closure, re-epithelialization, and ECM remodelling by protecting growth factors and ECM proteins against degradation [313].

Among other ECM proteins, FN is an adhesive molecule that plays a crucial role in all phases of wound healing, particularly ECM formation and re-epithelialization [32,314]. Several recent reports suggested a therapeutic role for the topical application of autologous FN to promote the healing of chronic skin and corneal ulcers. FN supports wound healing by contributing to haemostasis, assisting in the control of infection and debridement of wounds, and promoting re-epithelialization, granulation tissue, and the restoration of adequate tensile strength in connective tissue [315]. For example, topical treatment with plasma FN improves diabetic wound healing in rats by increasing fibroblast activity and the release of TGF-β1 [316]. Furthermore, FN and EPO exhibit additive activities when combined to treat wounds in diabetic mice [317]. The major cell-binding

site in FN, which contains the active cell-binding amino acid sequence (arginine-glycine-aspartic acid) known as the RGD, has also been considered for improvements in wound healing. The RGD sequence is not unique to FN and can be found in several other ECM molecules, such as laminin and vitronectin. Many different cell types respond to exposed RGD-moieties, and the peptide has been investigated for its use in tissue engineering as RGD-functionalized nanoparticles or biomaterials for many tissues, including skin [318,319]. Due to its limited effect on wound healing when used alone, the RGD peptide is preferentially combined with a number of bioactive scaffolds [320,321]. However, its direct use for keratinocyte adhesion and re-epithelialization has had controversial results. The combination of vitronectin and growth factors in an HA-based formulation was shown to promote re-epithelialization of a 3D de-epidermized dermis [322]. The topical application of recombinant collagen VII onto murine skin wounds accelerates wound closure by increasing re-epithelialization and is stably incorporated into the newly formed DEJ of healed wounds [323]. This is accompanied by the reduced expression of connective tissue growth factor, fewer α smooth muscle actin-positive myofibroblasts, and less deposition of collagen in the healed neodermis, which is consistent with less scar formation.

Laminins appear to have the potential to support wound closure [324]. They are members of a family of large glycoproteins located in the basement membrane, a distinctive ECM structure found in epithelial, endothelial, muscle, nerve, and fat tissues [325]. Five α-, three β-, and three γ-laminin chains assemble to form more than 16 different $\alpha\beta\gamma$ laminin trimers. Peptides from the N-terminal domains of the $\alpha 1$ and $\gamma 1$ laminin chains, originally identified for their angiogenic properties, have been shown to have a stimulating effect on granulation tissue formation and re-epithelialization when tested on full-thickness wounds in rats [326,327]. A laminin-$\alpha 1$ C-terminus-derived peptide conjugated to a

chitosan membrane has been proposed as scaffold material to deliver keratinocytes to a wound bed [328,329]. Interest in epithelial laminin isoforms for wound healing comes from data showing early increased expression of laminin 332 by migrating keratinocytes during re-epithelialization [31,74,330]. Several peptidic sequences were identified in the C-terminus of the laminin-$\alpha 3$ subunit, with integrin or syndecan-mediated cell adhesion properties and promotion of wound re-epithelialization in animal models [331-334]. In addition, a heparin-binding sequence has demonstrated antimicrobial properties and chemotactic activity for mononuclear cells [137]. Tethering an $\alpha 3\beta 1$ integrin binding peptide to microfibrous chitin matrices or collagen I scaffolds improves wound healing parameters, including re-epithelialization in rat and rabbit wounds [331,335,336]. A $\beta 4$ integrin subunit blocking antibodies that disrupt cell adhesion to laminin 332 was shown to significantly block wound re-epithelialization in a human skin equivalent model [337]. Although laminin 332 appears to be an important ECM component in epidermal regeneration, the topical application of purified native mature laminin 332 on wounds created in a diabetic mouse model does not trigger improved wound closure [338]. The ECM proteoglycan perlecan has been shown to preserve keratinocyte stemness in an HB-EGF-dependent mechanism [339].

Angiopoietin-like 4, which belongs to a group of secreted factors that play important roles in lipid and glucose metabolism, has been shown to influence wound re-epithelialization through its interaction with specific matrix proteins, delaying their degradation [340].

Bioactive peptides liberated from collagenase-digested vascular endothelial ECM and thrombin-derived peptides from PRP have been shown to enhance wound vascularization, granulation tissue formation, and re-epithelialization in both normal and diabetic mice [341,342].

Studies in MMP-9-null mice have revealed that wounds exhibit compromised re-epithelialization and reduced clearance of fibrin clots. Mice lacking MMP-9 exhibit abnormal matrix deposition but normal tensile strength [343]. Thus, MMP-9 seems to be required for normal progression of wound closure under normal conditions. Conversely, topical treatment with a selective MMP-9 inhibitor accelerates wound healing and re-epithelialization in diabetic mice [344]. More recently, the use of an inhibitor of MMP-2, MMP-9, and MMP-14 was shown to accelerate wound healing in diabetic mice by reducing inflammation and enhancing angiogenesis and re-epithelialization. Furthermore, topical administration of active recombinant MMP-8 accelerates diabetic wound healing as a consequence of complete re-epithelialization, diminished inflammation, and enhanced angiogenesis. An additive effect of these two treatments has been observed [345]. These results show how the systemic condition of an individual impacts the wound healing process.

4.7 Innovative tissue-engineered scaffold supporting re-epithelialization

The ECM is at the heart of actual and future technological developments for innovative tissue engineering. Intact ECM derived from living tissues has emerged as a major biomaterial in regenerative medicine [346,347]. More recently, pH- or temperature-sensitive hydrogels based on ECM proteins extracted from various tissues, including adipose tissue and dermis, were developed as injectable scaffolds for cell delivery and skin regeneration [348,349].

In tissue engineering strategies using cellular or acellular scaffolds, complete re-epithelialization requires the migration of keratinocytes from either the graft or the wound edges to bridge the open wound surface. Creating a microenvironment in which keratinocytes are rapidly recruited and their migration promoted for faster re-

epithelialization is advantageous. Electrospun nanofibrous matrices, featuring a high surface-to-volume ratio, high porosity, and good interconnectivity, have great potential to mimic the skin ECM in regards to both morphology and composition [350,351]. Electrospinning technology has attracted great interest in recent decades thanks to its easy and effective processing of a broad range of polymeric materials in the form of nanofibres. Type I collagen-containing electrospun nanofibres have been reported to promote the adhesion and spreading of human epidermal keratinocytes and support the formation of epidermal layers [352]. Analysis of an electrospun polycaprolactone/collagen nanofibrous matrix coated with an ultrafine type I collagen network in an *in vitro* wound gap model revealed significant acceleration of keratinocyte migration over this matrix through the activation of β1 integrins, acquisition of a polarized phenotype, deposition of laminin-332, and expression of active MMPs [353]. The development of materials that mimic the natural tissue architecture is a promising strategy. Plating keratinocytes on a microstructured collagen membrane mimicking the natural 3D architecture of the papillary dermis of human skin has revealed that a microfabricated dermal papilla template can direct keratinocyte behaviour [354]. Keratinocytes cultured on microfabricated matrices with channels mimicking the native topographical microenvironment of the DEJ have demonstrated that keratinocyte differentiation increases as channel depth increases and channel width decreases. Furthermore, channels with the narrowest openings have enhanced epithelialization [355,356]. Adding fibroblasts to that model revealed that keratinocytes in narrower channels exhibit a more proliferative phenotype, whereas keratinocytes in wider channels exhibit enhanced synthesis of laminin-332 [357]. Nanofibres synthesized from natural biodegradable polymers, such as chitosan or gellan, have gained special attention for wound healing because they are biocompatible, non-antigenic, and can easily be washed from the wound surface. *In vitro* studies have

revealed that gellan containing nanofibres promotes enhanced keratinocyte adherence and proliferation [358]. The use of these scaffolds on full-thickness wounds in rats promotes rapid re-epithelialization in the early phases of wound healing [358].

Scaffolds made from synthetic materials have also been investigated, but they do not elicit biological cues similar to native ECM, have mechanical properties similar to that of skin, and in many case are not biodegradable [359]. Plant-derived nanofibrous 3D scaffolds have been developed, and the use of an electrospun soy protein-based tissue scaffold as a bioactive dressing in a pig model of full-thickness excisional wounds improved the quality of the wound healing process, particularly re-epithelialization, with the formation of sweat glands and hair follicles [360].

4. Conclusion

Understanding and addressing the challenges in the treatment of chronic wounds will lead to a better clinical outcome, resulting in improved patient quality of life and reduced healthcare costs.

Several strategies are currently available for the treatment of skin lesions including wound re-epithelialization, such as the application of autografts, allografts, wound dressings, and tissue-engineered substitutes. Although proven to be clinically effective, these strategies are still characterized by key limitations, such as patient morbidity, inadequate vascularization, low adherence to the wound bed, an inability to reproduce skin appendages, and high manufacturing costs. Advanced strategies offer an attractive alternative by combining biomaterials, cells, growth factors, and advanced biomanufacturing techniques. There are a number of advanced therapies for chronic wounds. However, most do not have a high level of evidence or even nonrandomized

prospective studies assessing their efficacy. More evidence for the efficacy of current and future advanced wound therapies is required for their appropriate use.

As discussed above, the quality and speed of wound epithelialization are often associated with the features of the underlying formation of ECM and vascularization. Ongoing developments concentrate on targeting epidermal regeneration through ECM adhesion supports and/or specific growth factors combined with an innovative tissue-engineered scaffold, providing a cue for prompt epithelialization.

The elucidation of molecular and cellular mechanisms underlying epidermal closure will undoubtedly pave the way for the discovery of new therapeutic strategies. Besides, a better understanding of the differences between various types of chronic wounds at the molecular and cellular levels should improve treatment approaches, leading to better healing rates, and facilitate the development of more effective therapies.

For instance, a better understanding of collective cell migration is clinically relevant. In wound healing, the primary goal for epithelial cells is to restore the epithelial barrier. Therefore, it is important that, while the epithelial cells migrate over the wound bed, proper cell-cell adhesion is maintained so that the epithelial barrier is not further compromised. Understanding the dynamics of cell-cell and cell-ECM interactions during collective cell migration, their cross-talk, and the mechano-transduction pathways involved in physiological and pathological conditions should help identify the most appropriate adhesion substrates or growth factors to include in modern dressings. This knowledge should help decipher anomalies in pathological situations such as chronic wounds and adapt therapeutic strategies accordingly.

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Table 1

Effect of selected growth factors, hormones and related products on wound epithelialization

Molecule tested	*In vivo* model used	Improved re-epithelialization	Impact on other wound healing parameters	Reference
Growth factor				
EGF	Venous ulcers, human	No	Greater reduction in ulcer size and more healed ulcers	[361]
EGF	Full-thickness wounds, rat	Yes	Collagen deposition, wound contraction	[362]
	Full-thickness wounds, pig	Yes	Wound closure	[363]
	Full-thickness wounds, db/db mice (*loaded in a collagen/HA dressing*)	Yes	Granulation tissue formation, increased angiogenesis	[364]
FGF-1	Splinted excisional wounds, NONcNZO10/LtJ type 2 diabetic mice	Yes	Wound closure	[365]
bFGF	Full-thickness wounds, induced diabetic mice *(loaded ultrafine fibers for sequential release)*	Yes	Regeneration of skin appendages, increased capillaries, and collagen deposition	[366]
	Full-thickness excisional wounds, db/db mice *(sequential release by acidic gelatin hydrogel microspheres)*	Yes	Granulation tissue formation and contraction	[367]
bFGF gene therapy	Full-thickness wounds, induced diabetic rats *(loaded electrospun fibers for sequential release)*	Yes	Formation of skin appendages, improved vascularization, and enhanced collagen deposition and maturation	[368]
FGF-7 gene transfer	Full-thickness scald burn, rat	Yes	Granulation tissue formation, neo-	[369]

			angiogenesis	
	Human skin equivalent model grafted on mice	Yes	Overall healing process	[370]
GH	Severe burn, human	Yes	Wound closure, thicker epidermis	[371]
GH + IGF-1	Full-thickness burns, rat	Yes	Wound closure, increased lean muscle and total body weight	[372]
HB-EGF	Excisional full-thickness wounds, mice (*coacervate delivery system*)	Yes	Granulation tissue, angiogenesis	[373]
HGF/SF	Full-thickness excisional wounds, db/db mice	Yes	Granulation tissue formation, neo-angiogenesis	[374]
IGF-1	Full-thickness wounds, estrogen-deficient mice	Yes	Decreased inflammation	[375]
IGF-I + IGFBP-1	Full-thickness wounds, db/db mice and rabbit ear model	Yes	Granulation tissue, increased capillaries	[376]
IGF-1 gene therapy	Full-thickness wounds, rat	Yes	Wound closure	[377]
	Partial-thickness wounds, diabetic Yorkshire pigs *(combined with autologous keratinocyte transplantation)*	Yes	Wound closure	[378]
KGF-2	Ischemia-impaired dermal ulcer, rabbit	Yes	Granulation tissue formation	[379]
PDGF	Punch wound behind each ear, human	Yes	Faster wound closure, granulation tissue formation, less contraction	[380]

PDGF gene therapy	Full-thickness burns, Yorkshire pigs (*combined with meshed skin autografts*)	Yes	Improved adhesion of the graft	[381]
PDGF-BB	Excisional wounds in the ischemic ear, rabbit	Yes	Wound closure	[382]
	Rabbit dermal ischemic ulcer	Yes	Collagen deposition, formation of capillaries	[383]
	Full-thickness splinted excisional wound model, db/db mice	No	No wound healing improvement	[384]
VEGF	Splinted full-thickness wounds, mice *(VEGF encapsulated in PLGA nanoparticles)*	Yes	Granulation tissue formation, angiogenesis	[385]
VEGF + PDGF-BB	Full-thickness wounds, rat *(PDGF-BB nanoparticles embedded within VEGF nanofibers)*	Yes	Granulation tissue formation, angiogenesis	[386]
VEGF + bFGF	Full-thickness excisional wounds, db/db mice *(fibrin-based PLGA nanoparticles)* Full-thickness wounds, rat	Yes	Granulation tissue formation	[387]
VEGF gene therapy	Punch biopsy, induced diabetic mice	?	Global wound closure	[388]
	Full-thickness wounds, pig (*transplantation of transfected autologous minced skin particles*)	Yes	More newly formed blood vessels	[389]
Lysophosphatidic acid	Excisional ear wounds, mouse	Yes	Increased re-epithelialization in the	[390]

			early phase of healing	
Smad3 gene transfer in fibroblasts	Ear dermal ulcer, rabbit	Yes	Increased granulation tissue formation, capillaries, and myofibroblast differentiation	[391]

Cytokines

Interleukin-8 + MIP-3α	Full-thickness wounds, induced diabetic mice *(sprayable gelatin hydrogels*)	Yes	Thicker granulation tissue, vascularization	[392]
Interleukin 22	Full-thickness wounds, induced diabetic mice	Yes	Granulation tissue formation, vascularization	[393]

Antagonists

TGF-ß$_{1-3}$ peptide antagonist	Full-thickness burn injury, pig	Yes	Reduced wound contraction and scarring	[394]
TNF-α blockade	Surgical wounds, mice	No	No difference in wound healing, inflammatory response and granulation tissue formation. Higher degree of collagen at day 20.	[395]
	Partial-thickness burns, rat *(anti-TNF-α mAbs conjugated to hyaluronic acid)*	Yes	Reduced inflammatory response	[396]
	Full-thickness burns, rat *(TNF-α inhibitor)*	Yes	Granulation tissue formation	[397]
Anti-IL5 mAb	Full-thickness wounds, hamster	Yes	Decreased eosinophil infiltration	[398]

Hormones

DHA-derived lipid mediator	Full-thickness wound, db/db mice (*combined with autologous MSC administration*)	Yes	Angiogenesis, vasculature formation	[399]
Erythropoietin	Full-thickness excisional wounds, induced diabetic rats	Yes	Increased rate of wound closure	[400]
	Deep second-degree burn, mice	Yes	Complete skin regeneration, conical and hair follicles	[239]
	Full-thickness burn, induced diabetic pigs	Yes	Granulation tissue formation, angiogenesis	[241]
	Deep second-degree scald injury, mice (*infusion pump*)	Yes	Neovascularization	[240]
	Deep second-degree scald injury, rat (*topical application*)	Yes	New hair follicle neovascularization	[240]
Leptin	Full-thickness wounds, leptin-deficient and WT mice	Yes	Well-organized hyperproliferative epithelium, accelerated global wound healing	[401]
Mineralocorticoid receptor antagonist	Full-thickness wounds, induced diabetic mice Punch biopsy in glucocorticoids-induced impaired wound closure in mouse and human skin	Yes	Delayed re-epithelialization is restored	[402]

Neuropeptide

Substance P	Full-thickness wounds, rat	Yes	Leucocyte infiltration, granulation tissue, angiogenesis	[403]
	Full-thickness wounds, denervated rat skin	Yes	Wound contraction	[404]

	Full-thickness wound, db/db mice *(combined with a collagen mimetic peptide)*	Yes	Down-regulated collagen deposition	[405]

Table 2

Advantages and disadvantages of therapeutic approaches used to support wound re-epithelialization

Clinical strategy	*Advantages*	*Disadvantages*	*Development stage*
Moist and interactive dressings	Management of most acute and chronic wounds Faster wound healing Faster epithelialization rate Improved dermal/wound bed healing responses (e.g., cell proliferation, extracellular matrix synthesis) Reduced scarring Retention of growth factors at wound site Lower wound infection rates Reduced pain perception Enhanced autolytic debridement	Bacterial proliferation under some occlusive dressings Some practical disadvantages linked to each type of dressing: - hydrocolloids may dislodge with shearing or friction - could be expensive if exudate requires daily dressing changes - lack of transparency - sometimes adhere to the wound	Numerous semipermeable films, hydrocolloids, alginates, foam dressings available on the market [229,406]
Scaffolds and bioactive dressings	Numerous applications, such as partial- and full-thickness wounds, second degree burns, chronic non-healing wounds Success in clinical results Biocompatibility, biodegradability, and non-toxic nature Form part of the natural tissue matrix and some of play an important role in new tissue formation Absorb exudates and provide a moist environment	Animal origin of some components with potential viral infections Insufficient mechanical properties High cost	Hyaluronic acid, collagen, chitosan, elastin scaffolds available on the market [229,406]
Growth factors	Targeted effect Can be delivered in biomaterial-based drug delivery systems Can be produced recombinantly	Limited effectiveness in clinical settings due to low *in vivo* stability in highly inflammatory context Restricted absorption through skin around wound lesions	Several approved medications containing the following growth factors are available as preparations for external use in the form of solutions, gels, creams, and

		Elimination by exudation prior to reaching the wound area Undesirable effects due to high local and/or systemic levels after topical administration Risk of antibody formation	ointments: rhPDGF, rhbFGF, rhEGF [228]
Platelet-rich plasma (PRP)	Autologous PRP therapy proposed in chronic and non-healing wounds Improves and shortens all wound healing parameters Cost-effective No risk of antibody formation or risk of donor disease	Depends on general status and haematological status of donor Large volume of blood is required Platelet count in the PRP varies among subjects	Several PRP preparation systems are available on the market [407]
Amniotic membrane (AM)	Deliver growth factors and used in all subtypes of chronic wounds with proven efficacy Avascular, low immunogenic, anti-inflammatory, anti-scarring Readily available in sufficient quantity No immunological problems Large size Simple to prepare and sterilize Histological structure similar to that of skin Rapid adherence to the wound bed - Increases angiogenesis. - Inhibits protease activity and inflammation - Induces rapid re-epithelialization	High cost Risk of viral infection transmission	Amniotic membranes are available in different forms [234-236]

Cytokines, hormones, and related products	Erythropoietin, insulin, substance P, opioid antagonists, and compounds presented in Table 1	Same drawbacks as growth factors	Not available on the market. *In vitro* and pre-clinical studies stages.
Gene therapy for growth factors	Promising technique for chronic wounds DNA more stable than protein Possibility of multiple DNA transfer	Repeated injections Inconsistent and short-term gene expression	Pre-clinical and clinical trials [249,257].
MicroRNAs	Powerful gene regulators that often target several genes within the same gene network	Same as gene therapy	Pre-clinical and clinical trials [408]
Full thickness skin grafts	Retain more of the characteristics of normal skin More resistant to mechanical wear Undergo less contraction while healing In children, are more likely to grow with the individual	Limited to relatively small, uncontaminated, well-vascularized wounds Do not have as wide a range of application as split-thickness grafts Donor sites must be closed primarily or, more rarely, resurfaced with a split-thickness graft from another site	"Golden standard" in skin regeneration, surgical procedure [268]
Split thickness skin graft	Better take under less optimal conditions Much broader range of application Donor sites heal spontaneously with cells supplied by the remaining epidermal appendages Donor sites may be re-harvested after healing is complete	Significant skill and/or expensive equipment needed More fragile Contract more during healing Do not grow with the individual Wound created at the donor site is often more painful than the recipient site Unpredictable change in colour	Surgical procedure [268]
Autologous	Include minimal scarring of the donor	Potential lack of donor skin availability	Surgical procedure

epidermal skin grafts	site No risk of graft rejection. Automated and minimally invasive tools Sensory organs and hair follicles undisturbed		
Hair follicle grafting	Source of epidermal stem cells Rapid epithelialization of donor and recipient areas Absence of visible scarring	Potential lack of donor skin availability Graft fragile and susceptible to cell death	Preclinical and clinical studies
Epidermal cell spray grafting	Extremely rapid to prepare (30 min) Potentially decreased hospital lengths of stay Reduced costs Fast re-epithelialization, reducing the healing time and minimizing complications Viable and proliferating keratinocytes, melanocytes, fibroblasts, and Langerhans cells	Require specialized material and laboratory for amplification Difficulty handling, unpredictable uptake, and high cost, requiring a delivery system or support dressing Very expensive	Autologous cell harvesting devices available on the market [13]
Cultured autologous epithelial cell sheets	Used in burn care since 1981 Can be generated from a small biopsy of healthy skin Expanded into sheets for few weeks	Autologous: Time-consuming process, lack the ability to make differentiated structures (e.g., hair and sweat glands) Allogenic: cost of handling and storage, short shelf life Very expensive	Commercially available autologous epidermal/keratinocyte preparations [13,294]
Tissue engineered dermo-epidermal	Autologous permanent graft provides both epidermal and dermal components	Poor cell survival and functionality Critical safety issues	Promising clinical trials for autologous skin substitutes (not

skin	required to achieve functional wound closure Allogenic skin substitutes only provide temporary coverage and must be replaced by a split skin graft	High manufacturing costs Mechanical constraints of cell-scaffold systems.	available for commercial use), but dermal/epidermal allograft commercially available (Apligraft) [294].
Mesenchymal stem cells (MSC) derived from bone marrow, adipose tissue, umbilical cord	Use of adult MSCs provides an easily accessible source of multipotent precursor cells that could ease ethical concerns Autologous cell-based approaches are ideal to avoid immune rejection Multi-lineage differentiation potential Enhanced cutaneous regeneration Accelerated wound closure Enhanced histological parameters irrespective of cell isolation or delivery method	High susceptibility to protease activity Low stability in highly inflammatory context	Preclinical studies and clinical trials [300,409-412]
Extracellular Matrix (ECM) related products	ECM components are key regulators of cell adhesion, migration, proliferation, and differentiation during skin tissue repair Scaffolds from animal skin or made recombinantly Recombinant human proteins or fragments within scaffolds, combined to scaffolds, or delivered soluble	Ability of cell to be functional Difficulty including several cell types Difficulty industrializing highly complex technologies High costs	Commercially available ECM scaffolds are described as “bioactive scaffolds” and “tissue engineered skin” sections [312]; other ECM proteins and fragments tested in preclinical and clinical studies.
Innovative tissue-	Tremendous technological	High costs	Technological developments, pre-

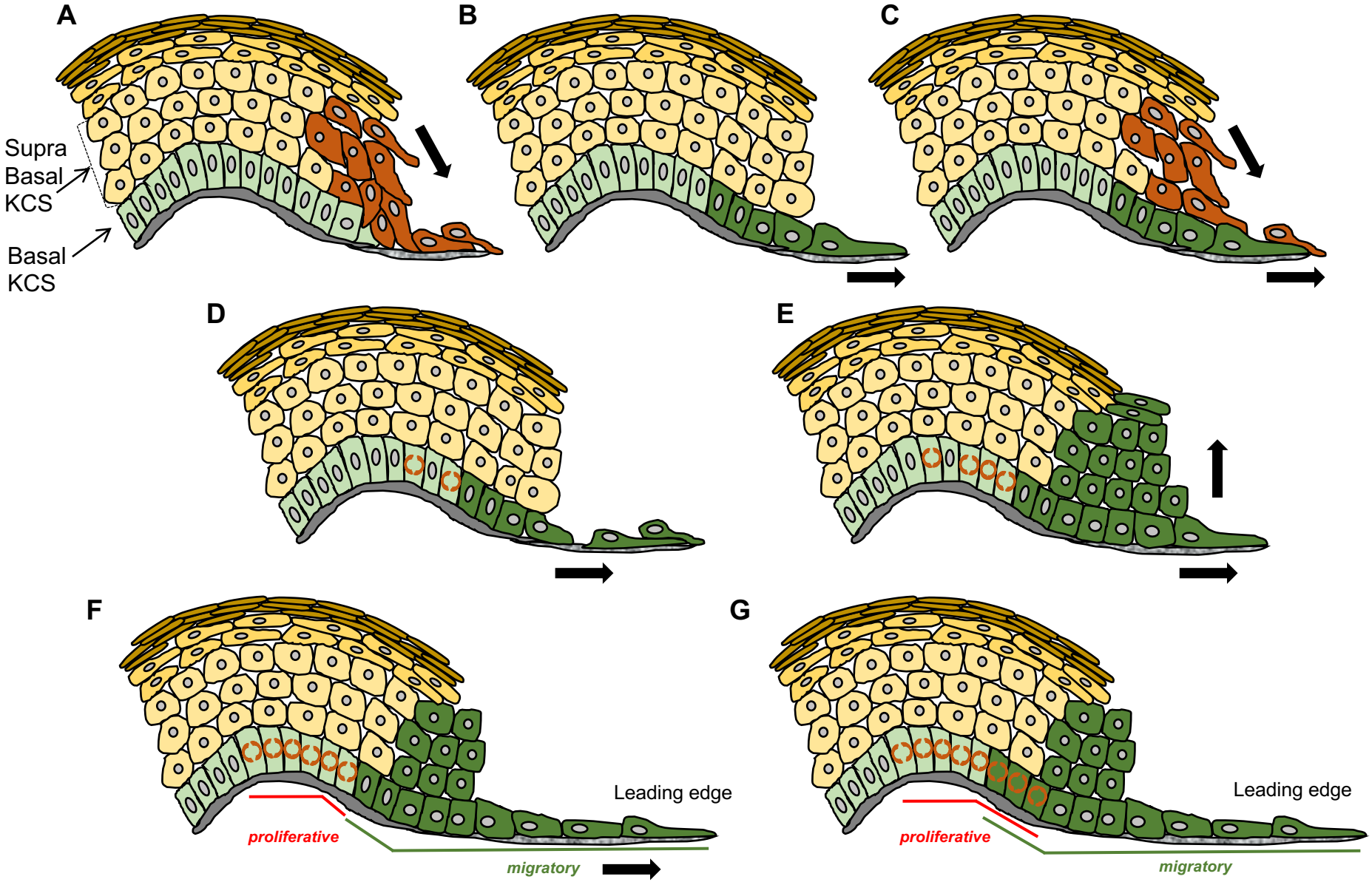
engineered scaffolds	developments in the design of tissue-engineered cellular or acellular scaffolds made of ECM, polysaccharide, or biomimetic synthetic materials Computer-controlled micro-fabrication of cell aggregates, scaffolds, fibres, hydrogels, microspheres, modular tissues combined with ECM, growth factor, or chemicals	clinical and clinical studies [406,413].

Figure legends

Fig. 1.

Diagram showing re-epithelialization models. (A) According to the rolling mechanism, the migrating suprabasal cells roll over leading basal cells and dedifferentiate to form new leaders at the epidermal tongue that migrate as a cohesive sheet [24,50-53]. (B) According to the sliding mechanism, keratinocytes from the basal layer move forward in a cohesive block at the leading edge, whereas the above cluster of superficial cells is passively dragged along [17,55-57]. (C) The model of Usui et al. [61] is an alternative to both previous models suggesting that suprabasal cells de-differentiate and participate, together with the basal cells, in reconstituting the new wound epithelium. (D) The model of Laplante et al. [62] involves the passive displacement of the superficial layers over the basal layers of keratinocytes which migrate individually over each other in agreement with the rolling model of migration. Pushing force are provided by dividing keratinocytes from the adjacent unwounded epidermis. (E) The model of Safferling et al. [72] suggested that collectively migrating basal keratinocytes of the epidermal tongue continuously build a multilayered epithelium in which suprabasal cells never contact the ECM. Keratinocyte proliferation occurs in a concentric pattern around the wound, producing new cells that migrate into the direction of the wound. (F) The molecular profiling of the migrating leading edge of a re-epithelializing wound in mice revealed that this zone is distinctive from a proliferative zone located behind [74]. (G) The proliferative and migratory zones overlap, and this area is the major source of surface expansion. Arrow indicates movement of basal and/or suprabasal keratinocytes. Dark green or orange colours means that basal (dark green) or suprabasal (orange) keratinocytes are activated.

Figure 1


A
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Leading edge
proliferative
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Leading edge
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