Published in final edited form as:

Cell Biochem Funct. 2015 January ; 33(1): 1–13. doi:10.1002/cbf.3086.

The role of nuclear hormone receptors in cutaneous wound repair

Sandra Rieger^{1,*}, Hengguang Zhao², Paige Martin¹, Koichiro Abe³, and Thomas S. Lisse^{1,*}

¹Center for Regenerative Biology and Medicine, MDI Biological Laboratory, Salisbury Cove, ME, USA

²Department of Dermatology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

³Division of Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Kanagawa, Japan

Abstract

The cutaneous wound repair process involves balancing a dynamic series of events ranging from inflammation, oxidative stress, cell migration, proliferation, survival and differentiation. A complex series of secreted trophic factors, cytokines, surface and intracellular proteins are expressed in a temporospatial manner to restore skin integrity after wounding. Impaired initiation, maintenance or termination of the tissue repair processes can lead to perturbed healing, necrosis, fibrosis or even cancer. Nuclear hormone receptors (NHRs) in the cutaneous environment regulate tissue repair processes such as fibroplasia and angiogenesis. Defects in functional NHRs and their ligands are associated with the clinical phenotypes of chronic non-healing wounds and skin endocrine disorders. The functional relationship between NHRs and skin niche cells such as epidermal keratinocytes and dermal fibroblasts is pivotal for successful wound closure and permanent repair. The aim of this review is to delineate the cutaneous effects and cross-talk of various nuclear receptors upon injury towards functional tissue restoration. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords

wound healing; tissue repair; nuclear receptors; vitamin D; regeneration; hormones; keratinocytes; innervation

Introduction

The skin is a barrier tissue providing protection against the environment and prevention of water loss. Dysfunction of the skin either due to the many modes of physical injury or illness leads to bodily complications and even fatality. Each year, there are an estimated 265 000

Copyright © 2014 John Wiley & Sons, Ltd.

*Correspondence to: Thomas S. Lisse and Sandra Rieger, Center for Regenerative Biology and Medicine, Mount Desert Island Biological Laboratory, Salisbury Cove, ME, USA. tlisse@mdibl.org; srieger@mdibl.org.

Conflict of Interest: The authors have declared that there is no conflict of interest.

death-related burns and severe abrasions requiring medical attention worldwide.¹ Importantly, skin wounds must be immediately repaired to prevent fluid loss and infection of underlying tissues. Recent advances in our understanding of both the cellular and molecular biology involved in wound healing and tissue regeneration have provided improvements in therapeutic strategies. For example, recent work has detailed the importance of the vitamin D nuclear receptor and vitamin D status for activation of the transforming growth factor (TGF)- β signalling pathway critical for proper cutaneous wound healing.² This review will focus on the role of nuclear hormone receptors (NHRs) during cutaneous wound repair and their potential targeting to promote optimal tissue repair.

Basic Biology of Cutaneous Wound Healing

Cutaneous wound healing is a multistep process that involves many cell types including epidermal keratinocytes, fibroblasts, sebocytes, sweat gland myoepithelial cells, melanocytes, endothelial cells and peripheral nerve cells. In general, a wound consists of an incision, puncture or abrasion of the skin epidermis, dermis and/or underlying subdermal muscle and fat tissues. Injury repair integrates soluble mediators and tropic factors, extracellular matrix (ECM) and its components, and numerous parenchymal cells to restore tissue integrity. The wound repair process consists of three major phases—inflammation, tissue formation and remodelling—that overlap in time involving parallel and interrelated pathways that are either activated or suppressed to initiate and maintain proper repair (Figure 1). During the process, a number of signalling factors regulate the cistrome, miRNAome, transcriptome and proteome for proper wound repair.

Inflammation

The inflammatory phase marks the initial time of injury that persists for 24–48 h³ (Figure 2A, B). Initially, blood vessels are disrupted, leading to extravasation of blood constituents, and the coagulation cascade leads to haemostasis. Damaged keratinocytes and platelets release interleukin (IL)-1 and platelet-derived growth factor (PDGF) during primary hemostasis to attract neutrophils and trigger the release of growth factors from neighbouring cells.^{4,5} Infiltrated neutrophils remove foreign particles, debris, necrotic tissue and bacteria. In the skin, nerve endings are apposed to epidermal and dermal cells.^{6,7} Cutaneous sensory fibres contribute to inflammation through activation of keratinocyte-derived pro-inflammatory cytokines by the neurotransmitter substance P and calcitonin gene related peptide.⁸ Furthermore, phagocytic cellular uptake and clearance (e.g. of damaged/degenerating dendrites) by epidermal keratinocytes is a known protective mechanism against various damage-inducing stimuli.^{9,10} The blood clot re-establishes homeostasis and generates a provisional ECM consisting of fibronectin that is essential for cellular migration by acting as a reservoir for growth factors.³ Meanwhile, the heterogeneous populations of resident and recruited cells secrete mediators and trophic factors of wound healing. Numerous vasoactive mediators and chemotactic factors are derived from coagulation and activated complement pathways, and from injured or activated parenchymal cells in the vicinity function to recruit inflammatory leucocytes to the injury site. Platelet and keratinocyte-derived TGF- β and adherences to specialized ECM proteins initiate the conversion of monocytes to activated inflammatory or reparative macrophages at the injury

site, leading to the gradual formation of granulation tissue and secretion of pro-inflammatory cytokines and growth factors, which promote angiogenesis.^{4,5,11} Hence, macrophages play a pivotal role in the transition from inflammation to tissue formation.

Tissue formation

Re-epithelialization of wounds, occurring within hours after injury, is an essential step during tissue repair that involves migration and proliferation of keratinocytes from the surrounding epidermis and cutaneous appendages to restore an intact epidermal barrier^{12,13} (Figure 2B). Sensory nerves promote the initiation of the re-epithelialization process by expression of the vasoactive intestinal peptide, a neuropeptide that can activate keratinocyte migration.²⁰ During reepithelialization, epidermal cells undergo pronounced phenotypic changes that include withdrawal of intracellular tonofilaments (an intermediate filament), degradation of most intercellular desmosome junction complexes and formation of peripheral actin filaments that promote lateral cell movement. Newly expressed integrin receptors on epidermal cells allow them to bind to a variety of ECM components (e.g. fibronectin) that are interspersed in the wound margin and blood clot. Epidermal keratinocytes migrate underneath the fibrin clot spanning the wound, separating the desiccated eschar from existing tissue. Production of collagenases, proteinases and plasminogen activator by epidermal cells is necessary to degrade the ECM for active migration. One to two days after injury, epidermal cells at the wound margin begin to proliferate. Local release of growth factors, such as epidermal growth factor (EGF), TGF- α and keratinocyte growth factor, and increased expression of growth factors stimulate reepithelialization. As re-epithelialization continues, the expression of basement membrane proteins reappear in a zipper-like fashion adjacent to the wound margin. Epidermal cells revert back to their original phenotype, associating with the reestablished basement membrane separating the underlying dermis from the epidermis.

Approximately 4 days after wounding, new stromal tissue (granulation tissue; Figure 2B, C) consisting of capillaries starts to consume the wound space. During this period, macrophages, fibroblasts and blood vessels invade the wound.¹⁴ The macrophages generate growth factors (e.g. PDGF and TGF- β 1) necessary to stimulate fibroplasia and angiogenesis. Fibroblasts also secrete growth factors (e.g. fibroblast growth factors) that play key beneficial roles for wound closure and granulation tissue formation.^{15,16} Angiogenesis is critical to replace damaged capillaries and provide oxygen, blood constituents and nutrients to the damaged area. Fibroblasts proliferate and express integrin receptors to help migrate to the wound. Infiltrating fibroblasts synthesize, deposit and remodel the new ECM (called the provisional matrix) to provide a scaffold for new cell and blood vessel ingrowth in order to sustain cell metabolism during tissue restoration.¹⁷ The provisional matrix initially consists of fibrin, fibronectin and hyaluronic acid and then is eventually replaced with a collagenous matrix.¹⁸ Cells in the wound area eventually undergo apoptosis, and in response to growth factors, resident fibroblasts trans-differentiate into myofibroblasts, playing a pivotal role in wound contraction.¹⁹ Hyper-innervation of the epidermis/dermis has been described during wound repair occurring within days of injury lasting in duration, suggesting a functional role during early and later stages of wound healing.^{7,20} Substance P stimulates keratinocyte proliferation, perhaps playing a role in tissue formation during wound repair.²¹ Further co-

culture studies with skin explants and neurons or neuropeptides suggest positive effects on tissue formation during wound healing.²²

Remodelling

During the second week of healing (Figure 2C, D), TGF- β 1-dependent²³ fibroblast-to-myofibroblast differentiation continues where cells assume a myofibroblastic phenotype that can last up to 2 years.²⁴ Myofibroblast characteristics include bundles of actin-myosin filaments along the cytoplasmic face of the plasma membrane and cell–cell and cell–matrix linkages corresponding to connective tissue compaction and the contraction of the wound. Myofibroblasts accelerate wound repair by contracting the edges of wounds and in time are also eliminated through apoptosis.²⁵ Collagen reorganization during the replacement of granulation tissue to an acellular scar is dependent on continued synthesis and catabolism of collagen by proteolytic enzymes such as matrix metalloproteinases secreted by macrophages, epidermal cells, fibroblasts and endothelial cells. In time, the collagen gain improves tensile strength with formation of larger collagen bundles and intermolecular cross-links.

Nuclear Hormone Receptors and Wound Repair

Nuclear hormone receptors and their ligands regulate cutaneous homeostasis and injury by modulating cellular metabolism, migration, proliferation, differentiation, apoptosis and inflammation and have been targeted for dermatologic therapy (Table 1). NHR signalling regulates the renewal of keratinocyte layers, the epidermal permeability barrier and hair follicular cycling. In humans, there are 48 distinct nuclear receptor sequences that constitute the largest family of transcription factors that include classic, orphan and adopted orphan receptors.²⁶ NHRs control gene expression by binding to an array of multicomponent regulatory proteins that control their DNA binding properties for both transcriptional and epigenetic outcomes.²⁷ Generally speaking, NHRs interact with DNA response sequences of target genes located within the gene promoter, intronic regions or at more distal regions such as enhancers in an effort to regulate the transcriptional and epigenetic machinery. Importantly, NHRs are not restricted to nuclear transcription as some NHRs are known to modulate extra-nuclear signalling pathways.²⁸ Certain NHRs need to bind a ligand in order to become activated, whereby other ligands have the ability to bind to several co-receptors.

Sex hormone receptors: estrogen and androgen receptors

Estrogen actions are regulated by binding to estrogen receptor (ER)- α or ER- β receptors, which then subsequently interact with estrogen-responsive elements (EREs) on target gene sequences for transcriptional induction or repression. Estrogen deprivation is associated with attenuated wound healing, while hormone replacement therapy positively improves acute wound healing and precludes the development of chronic wounds in ageing women.²⁹ These positive effects of estrogen during wound repair occur by targeting a collection of epithelial cells, fibroblasts and immune cells in the skin (Figure 3A). Importantly, many genes that play a role in wound healing are induced after estrogen treatment such as cyclin D1 (cell cycle), EGF receptor (cell growth/movement) and insulin-like growth factor 1 (cell maintenance/tissue remodelling).^{30,31}

Estrogen treatment is known to increase collagen content/deposition, thickness of the dermis (presumably via direct actions on fibroblasts and/or anagen hair follicles), elasticity and water content of skin, and reduction of sebaceous secretion.³² Animal studies have also shown that estrogen treatment determines the prognosis of wound repair, in that exogenous estrogen treatment of ovariectomized rats restores healing rates to normal levels.^{33,34} In menopausal women, both structural and functional changes occur in the skin such as reduced dermal collagen deposition.^{35,36} In contrast, exogenous estrogen can reverse the impaired wound healing observed in menopausal women associated with a reduction in local inflammation by downregulating macrophage migration inhibitory factor³⁶ and improving matrix synthesis and deposition, leading to amelioration of scarring.²⁹ Increased estrogen levels during pregnancy positively influence anagen hair growth,³² whereby follicular bulge stem cells and their transiently amplifying progeny are known to play an active role during wound repair.³⁷ On the other hand, pronounced hair loss is observed postpartum when estrogen levels plummet.

Glimpses into the mechanisms of action were revealed in studies that depicted ER signalling having limited effects on the vascularization component of wound repair by applying estrogen injections in rabbit models.^{35,36} Other findings suggest that estrogen may attenuate the inflammatory phase of wound repair with adverse effects observed in hypoenestrogenemia.³⁸ Molecular studies have suggested positive influences of estrogen by inducing immunomodulatory (suppressor) TGF- β 1 production,³⁹ increasing collagen synthesis and decreasing collagenolysis by dermal fibroblasts.^{40–42} In terms of differential receptor actions during chronic wounding, a polymorphism of ER- β signalling was found to be involved in regulating inflammation during venous ulceration.⁴³ Furthermore, during wounding, ER- α , but not ER- β , expression is restricted to the epidermis at the wound edge, suggesting a protective role for the receptor in cells of this compartment.⁴⁴ Under homeostatic conditions, ER- α is expressed in dermal fibroblasts and sebocytes, suggesting the recruitment of these progenitors to the wound site after injury.⁴⁵ Recently, the ERE-luciferase transgenic reporter mouse line was used to monitor *in vivo* ER activation after skin injury.⁴⁶ In the study, ER was activated specifically within neo-epidermal keratinocytes and wound margin dermal cells, suggesting a functional role of ER in peri-wound activity. In the same study, differential receptor signalling was investigated using agonists that revealed ER- β signalling in both epidermal and dermal cells, but ER- α activation in dermal cells alone. Although these findings are intriguing, whether they are biologically relevant and accurate depends on whether the ERE transgenic line entirely recapitulates endogenous ERE relationships with distal (looping) enhancers, for example. In another study, it was recently determined that epidermal ER- β was responsible for the beneficial effects of ER, independent of its anti-inflammatory effects, towards cutaneous wound healing.⁴⁷ The authors utilized ovariectomized ER- β -null mice treated with exogenous estrogen and witnessed a delay in wound healing. The results collectively highlight cell type-specific differential estrogen signalling and offers insights into the development of targeted therapies for wound repair.

The major circulating androgen is testosterone, which is predominately produced by Leydig cells in the male testis and functions by binding to the androgen receptor (AR) to induce or

repress target gene expression by binding to DNA androgen response elements.⁴⁸ Gonadal disorders that result in elevated androgens, for example, highlight cutaneous phenotypes that cause hirsutism, acne and hyperpigmentation of the skin and androgenic alopecia in female and male subjects.⁴⁹ The precise function of AR during wound repair is not entirely clear (Figure 3B). Androgen signalling impairs wound healing,⁵⁰ and castration is known to enhance wound repair associated with a limited immune response and improved matrix deposition and metalloproteinase production.^{51,52} Systemic inhibition of testosterone metabolizing enzyme 5 α -reductase enhances cutaneous wound healing in rats with attenuated inflammatory cell infiltration, IL-6 and tumour necrosis factor α expression, while the TGF- β 1-expressing cell number is increased at wound sites.⁵³ AR is also expressed in fibroblasts and suprabasal keratinocytes in chronic wounds, possibly regulating collagen production and epidermal differentiation or proliferation.⁴⁴ *In vitro* studies suggest that treatment with the potent testosterone derivative dihydrotestosterone (DHT) inhibits keratinocyte migration.⁵⁴ Global AR deficiency in mice leads to faster re-epithelialization, reduced inflammation and increased collagen deposition during cutaneous wound repair after injury,⁵⁵ suggesting inhibitory effects of AR. However, re-epithelialization was delayed in keratinocyte-specific AR knockout mice without influencing keratinocyte proliferation, suggesting that AR might actually promote keratinocyte migration.⁵⁵ These conflicting results must be collectively rationalized in the context of cell-type AR ablation, whereby systemic AR ablation of niche cells may overshadow its keratinocyte-specific role.

Apart from the role of sex hormones in the respective gender physiology, the function of estrogen towards wound healing in men is unclear. In men, the primary biologically active estrogen is oestradiol that results from the aromatization of circulating testosterone.⁵⁶ Extraglandular formation of estrogen has been shown in human skin fibroblasts after DHT treatment, which is known to increase in men with age.⁵⁷ Despite having elevated estrogen with age, epidemiological studies have also shown that acute wounds heal more slowly in elderly men than in their female counterparts.^{38,58} Paradoxically, in men, the production of androgens diminishes with age, having effects on the local immune responses during cutaneous wound healing.⁵⁵ Yet the aromatization of testosterone to oestradiol remains persistently high because of a variety of factors such as increased age-associated abdominal fat emitting excessive levels of oestradiol into the blood stream, suggesting important cross-talking mechanisms and potential inhibitory effects of oestradiol towards wound repair in men.

Retinoid (retinoic acid and retinoid X receptors)

Retinoids are a family of vitamin A metabolites, whereby retinoic acids including all-*trans*-retinoic acid (ATRA) and 13-*cis*-retinoic acid can bind and activate retinoic acid receptors (RARs) α , β and γ and 9-*cis*-retinoic acid, which can bind to both RARs and retinoid X receptors (RXRs) α , β and γ . Related to skin disorders, retinoids are known to display anti-inflammatory, cytoprotective and antifibrotic features^{59,60} (Figure 3C). Although retinoic acids are widely used for treatment of skin diseases such as psoriasis and hyperkeratosis due to their inhibitory actions on keratinocyte terminal differentiation and proliferation,⁶¹ their effects on keratinocytes during skin wound repair are unclear. RXRs play a promiscuous role in NHR signalling in their ability to impact other receptor signal transduction pathways.

Mechanistically, it was proposed that retinoic acids suppress the nuclear factor FOXO1, thereby affecting the transcription of genes for the AR, proliferative factors, lipid biogenesis and certain inflammatory cytokines.^{62–64} The positive wound healing response due to retinoic acid-induced FOXO1 suppression might be restricted to immune and sebaceous cells, as induction of keratinocytespecific FOXO1 is known to promote cell migration during wound healing in mice.⁶⁵ Retinoids may amplify TGF- β signalling actions to promote wound healing. For example, in mesenchymal stem cells, ATRA was shown to elevate SMAD message levels and its nuclear localization.⁶⁶ ATRA was also shown to elevate TGF- β 2 in keratinocytes both *in vitro* and *in vivo*,⁶⁷ suggesting that retinoids may function to improve tissue repair by suppressing immune responses and/or promoting ECM formation.

In terms of actual skin wounding experiments, topical tretinoin (also known as ATRA) and oral administration of the second-generation retinoid acitretin (used for psoriasis) were recently assessed during full-thickness skin lesion healing in a rat model.⁶⁸ Based on histological studies, the complete recovery time was longer in all the retinoid treatment groups compared with controls, suggesting a delay in secondary wound healing, epithelialization and angiogenesis. On the other hand, oral (systemic) tretinoin administration was shown to improve the rate and integrity of incisional wound healing in dorsal rat skin when compared with the vehicle group,⁶⁹ suggesting the drug delivery method and tissue metabolism as crucial determinants of tissue repair outcomes for certain retinoids. Mechanistically, it is known that retinoic acid upregulates the proteolytic plasminogen activator system in epidermal keratinocytes, which facilitates cell migration and re-epithelialization during cutaneous wound repair.⁷⁰ Lastly, topical application of MDI301, a non-irritating variant of 9-*cis*-retinoic acid, was shown to accelerate skin abrasion wound healing when compared with vehicle-treated controls in three rat models of damaged/atrophic skin.⁷¹ The effect of MDI301 was attributed to increases in soluble type I collagen and decreased matrix metalloproteinases 2 and 9 expression in wounded tissues during healing, without induction of proinflammatory cytokines.

Vitamin D receptor

Epidermal keratinocytes generate 7-dehydrocholesterol, which is necessary for the photochemical production of the parent vitamin D.^{72,73} Importantly, the skin is the only tissue capable of vitamin D production and activation, expressing all the required synthesizing and metabolizing enzymes.⁷⁴ The vitamin D receptor (VDR) heterodimerizes with the RXR to induce transcriptional changes by binding to specific DNA vitamin D response elements to regulate mRNA and miRNA expression.^{75–77} The capacity of the VDR to interact with multiple regulatory co-activators and co-repressors allows the fine-tuning of gene expression in response to ligand or unliganded conditions within keratinocytes.^{78–81} Alopecia is variably observed in patients with hereditary vitamin D-dependent rickets with abnormal (type IIA) or normal (type IIB) VDR and in VDR-null mice, suggesting a biological and physiological role of the VDR in the epidermis and hair follicle.^{82–88} The VDR is present in all epithelial cells of the skin,⁸⁹ whereby 1,25(OH)₂D₃ (1,25-dihydroxyvitamin D₃) is the primary active physiological metabolite for the VDR and promotes the differentiation and inhibition of proliferation of epidermal keratinocytes *in*

*vitro*⁹⁰ and *in vivo*.^{91–93} In addition, the VDR has a ligand-dependent immunomodulatory function enabling keratinocytes to ward off skin infections through activation of innate immunity and antimicrobial cathelicidin peptide production,^{94–98} which likely plays a role during cutaneous wound repair.

With regard to cutaneous tissue, under basal conditions, VDR-null mice exhibit defects that may affect subsequent wound healing outcomes that include increased and enlarged sebaceous glands, reduced number of post-morphogenic anagen hair follicles and bulge stem cells, development of epidermal cysts, lack of epidermal differentiation with increased hedgehog signalling, elevated number of dendritic epidermal T cells and hyperkeratosis.^{81,99–103} Given the impaired post-morphogenic hair cycle and follicles in VDR-null mice and the relationship of hair bulge stem cells and their transiently amplifying progenitor cells to wound repair,¹⁰⁴ the hair-specific defects in the VDR-null mice may also have direct effects on the onset of wound healing responses.^{105,106}

Given the basal cutaneous defects observed in adult VDRnull mice, it is not surprising that wound healing defects associated with injury are reported, suggesting a protective role of the VDR (Figure 4A). In recent studies, 8-week-old VDR-null or vitamin D-deficient mice exhibited impaired healing of cutaneous wounds consisting of a 3.5-mm punch biopsy, characterized by scarcity of infiltrating macrophages in the early inflammatory phase of wound repair and disrupted vascular invasion.² Specifically, early deficiencies include the reduction of F4/80-positive macrophages and MCP-1-labelled (monocytes/macrophages) cells in the granulation tissue of VDR-null and ligand-deficient mice 2 days post-injury. These results suggest that the VDR is required for normal recruitment of macrophages during the inflammatory phase of cutaneous injury repair in the presence of normal neutrophil recruitment. Other early defects include impaired granulation tissue in VDR-null mice characterized by avascularization and reduction in overall ECM content and organization. In the same study, the investigators appraised TGF- β /SMAD3 signalling in the context of granulation tissue (i.e. fibroblasts). The authors suggest that activation of the TGF- β signalling pathway in the granulation tissue was dependent on liganded VDR signalling as there was a decrease in the number of pSMAD3 nuclear staining and protein expression in VDR-null and vitamin D-deficient mice when compared with controls. Based on the previous results of defective TGF- β signalling in the dermal component of cutaneous wounds, the authors investigated activation of TGF- β signalling using (unstarved) primary dermal fibroblasts isolated from wild-type or VDR-null mice treated with TGF- β . The results suggest that the VDR is necessary for the activation of TGF- β signalling in dermal fibroblasts, as VDR-null cells had reduced TGF- β induction of immune (MCP-1) and motility/contraction (α -sma)-related target genes critical for granulation tissue contraction, for example. Although the investigators looked at immune-related and motility-related gene responses in fibroblasts, the functional outcome on provisional ECM (e.g. collagen or fibronectin synthesis and disposition) was not monitored after TGF- β treatment as this might be another mechanism to regulate cellular infiltration and function.

Based on these studies, it is still unclear if VDR-dependent cytokine expression is necessary for the inflammatory and tissue granulation phases, and from which cell types might these defects arise to regulate cellular infiltration and tissue formation. For example, it is known

that $1,25(\text{OH})_2\text{D}_3$ regulates the expression of PDGF-A/B¹⁰⁷ and IL-1¹⁰⁸ in normal keratinocytes; therefore, it is hypothesized that these cytokines may be dysregulated in VDR-null mice to affect the wound healing outcome. Also, we have a limited understanding of whether or not VDR signalling may suppress TGF- β profibrotic genes at later wound repair phases as seen in hepatic stellate cells in mice.¹⁰⁹ Furthermore, recent studies using the vitamin D analogue calcipotriol suggests an overall net antifibrotic response in fibroblasts over extrafibroblast cytokine effects in experimental scleroderma.¹¹⁰ These studies offer new insights on how the VDR and TGF signalling pathways co-regulate the wound healing process, requiring further studies.

Apart from its role during wound repair in cutaneous tissue, recent studies utilizing extracutaneous injury models have strengthened the notion that the VDR plays an overall positive role during tissue repair. For example, mice on a vitamin D-deficient diet are predisposed to colitis and result in reduced levels of aquaporin 4 (i.e. a membrane solute channel) transcripts in the colon intestinal epithelium.¹¹¹ It is known that aquaporins play important roles in maintaining proper cutaneous wound repair by directly regulating keratinocyte migration/proliferation.¹¹² In other recent studies, VDR-null corneal epithelium showed defects in tight junction integrity and number, suggesting that the VDR plays a positive role in corneal epithelium wound healing.¹¹³

Peroxisome proliferator-activated receptor

PPARs have been recently linked to protective healing processes of wound repair¹¹⁴ (Figure 4B). PPARs are a group of nuclear receptor proteins that function as transcription factors by heterodimerizing with RXRs. Endogenous ligands for the PPARs include free fatty acids and their derivatives, eicosanoids. PPARs are master regulators of lipid metabolism, and all three isoforms (α , β/δ and γ) are expressed in the pilosebaceous unit, playing a pivotal role in barrier function and sebocyte differentiation.¹¹⁵ Furthermore, there appears to be a certain degree of cross-talk between AR and PPAR- γ signal transduction pathways given that activation of both receptors has an additive effect on lipid droplet formation in sebocytes.¹¹⁶

PPARs play no role during epidermal maturation, as all PPAR isoform-null mice have no gross cutaneous defects.¹¹⁴ On the other hand, both PPAR- α and PPAR- β/δ play key roles during epidermal injury repair^{117–119} (Figure 4B). During the early wound repair phase, PPAR- α is only transiently expressed, while PPAR- β/δ levels persist for the duration of the healing process. Thus, a transient delay in wound re-epithelialization was present in PPAR- α -deficient mice¹¹⁷ versus a more long-term delay observed in PPAR- β/δ -deficient mice.^{118,119} When fitted along the normal wound healing timeline, this would suggest that the transient nature of PPAR- α expression might control early inflammation, as it is known to have inhibitory effects on nuclear factor κB actions.^{119,120} Furthermore, both PPAR- α and PPAR- γ are expressed after proliferative stimuli, for example after injury at early-phase wound edges.¹¹⁷ PPAR- β/δ is induced by pro-inflammatory cytokines after tissue injury, and as a consequence, Akt-1 becomes activated to suppress apoptosis, as well as integrin-linked kinase, to support subsequent keratinocyte movement.¹²¹ This series of events helps maintain viable wound keratinocytes to become activated during the overlapping phases of re-epithelialization and tissue formation. Thus, PPAR- β/δ -deficient keratinocytes are

movement deprived, and mice exhibit a delayed wound closure.¹¹⁷ As tissue repair continues to remodel, TGF- β 1 release by dermal fibroblasts increases macrophage and neutrophil recruitment to stimulate ECM deposition for wound remodelling. These events lead to sustained PPAR- β/δ expression in wound keratinocytes. TGF- β 1/SMAD3 signalling in later wound healing stages results in suppression of PPAR- β/δ by loss of AP-1 activity,¹¹⁷ forming a feedback loop. Thus, the cross-talk between PPAR and TGF- β 1 pathways after skin injury is required for the spatiotemporal regulation and completion of wound healing.

Recently, investigators addressed the importance of PPAR- γ in dermal fibroblasts during cutaneous tissue repair.¹²² The premise was based on reduced PPAR- γ expression in dermal fibroblasts from scleroderma patients and the ability of PPAR- γ agonists to block fibrosis in skin fibroblasts. The authors showed that cutaneous deletion of PPAR- γ in fibroblasts accelerated dermal wound closure through increased phosphorylation of SMAD3, Akt and extracellular signal-regulated kinase and cell proliferation. These results suggest that fibroblast PPAR- γ functions to suppress cutaneous tissue repair via inhibition of proliferation and that pharmacological cell type-specific antagonists may benefit wound closure. In a different cell type, PPAR- γ activator ciglitazone was recently shown to induce aquaporin 3 (AQP3) mRNA and protein expression in human keratinocytes with implications towards permeability barrier function and wound healing.¹¹² Aquaporins are pore-forming integral membrane proteins that regulate the transport of ions and other solutes such as water, glycerol and H₂O₂ in and out of the cell.¹²³ Furthermore, AQP3-null mice have delayed cutaneous wound healing due to impaired keratinocyte migration and proliferation.¹²⁴ In the Jiang YJ *et al.*¹¹² study, it appears that both water and glycerol channelling by AQP3 plays an important role in hydration, migration and metabolism of lipids in the mammalian skin epidermis, which was also shown to regulate the proliferation and differentiation of keratinocytes *in vitro*. Importantly, when applied topically, the PPAR- γ activator induced AQP3 mRNA expression in the mouse epidermis. Interestingly, as hydrogen peroxide is known to promote the migration of keratinocytes,¹²⁵ it then begs the question of whether the increase in AQP3 promotes the migration of these cells through increased H₂O₂ diffusion and permeability.¹²³

Glucocorticoid receptor

Glucocorticoids, such as cortisol, are lipophilic antiinflammatory molecules that diffuse into the cell and bind to glucocorticoid receptors (GRs) with diverse developmental and metabolic functions. GRs are abundantly expressed in various skin niche compartments³¹ affecting all major steps of wound repair processes.¹²⁶ Elevated GR actions as a result of its overexpression (adrenal tumour) or by excessive administration of glucocorticoids result in epidermal and dermal atrophy, vascular instability and acne,¹²⁷ perhaps by way of counteracting the vitamin D system.¹²⁸ Thus, it is not surprising that topical application of glucocorticotropic and adrenocorticotropic hormones leads to impaired wound healing in rheumatoid arthritis patients.^{129,130} Furthermore, pharmacological inhibition of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1), the cortisone reductase that generates active hormone cortisol, was shown to accelerate wound repair based on findings that showed more *de novo* 11 β -HSD1-derived corticosterone in mouse skin compared with precursor

progesterone itself.¹³¹ These results implicate the potential for pharmacological inhibition of 11 β -HSD1 to improve tissue injury.

The preclinical and clinical data suggest that corticosteroids impair wound healing, although the mechanisms are unclear (Figure 4C). The mechanism of impaired early wound healing by endogenous glucocorticoids may lie in the exaggerated granulation tissue formation through increased TGF- β 2 expression and fibroblast proliferation that was observed in mice harbouring a DNA binding impaired mutant version of the GR (GR^{dim} mice)¹³². In other murine model studies, acute cortisone treatment results in a dose-dependent decrease in remodelling wound tensile strength.¹³⁰ Along this line, Cushing syndrome patients who are administered excess adrenocorticoids exhibited a 40% reduction in skin wound tensile strength.¹³³

Moving forward: other less-characterized nuclear receptors implicated in cutaneous wound repair

There are several understudied nuclear receptors that have been recently implicated in cutaneous wound repair. The stratum corneum, the outermost layer of the skin, is formed by corneocytes embedded in a lipid matrix consisting of cholesterol, ceramide and fatty acids. The nuclear liver X receptor (LXR) is a cholesterol sensor involved in biological processes such as lipid metabolism and cholesterol efflux.¹³⁴ The LXR may function to regulate the epidermal skin barrier, as LXR is expressed in human keratinocytes involved in cholesterol export.¹³⁵ Also, LXR and PPAR activators regulate the expression of SULT2B1b, the key enzyme in the synthesis of cholesterol sulfate, which is a potent regulator of epidermal differentiation and corneocyte desquamation.¹³⁶ Recently, LXR activation and binding were shown to directly induce the cytokine vascular endothelial growth factor expression in macrophages to possibly promote angiogenesis and neovascularization in the context of wound healing.¹³⁷

Innate lymphoid cells (ILCs) represent a novel subset of haematopoietic effector cells that have rapid and potent cytokine synthesizing capabilities and are enriched in mucosal tissues, including skin, implicated in tissue homeostasis and repair.¹³⁸ Recently, it was shown that the orphan nuclear receptor RAR-related orphan receptor gamma thymus (ROR γ t) was essential for the development and function of IL-22-generating group 3 ILC to maintain mucosal barrier function and tissue regeneration.^{139,140} IL-22 is a well-known effector cytokine and potent mediator of inflammation implicated in mucosal host defence responses against extracellular pathogens.^{141,142} In skin, it was recently found that IL-22 signalling occurs in dermal fibroblasts to direct ECM gene expression and myofibroblast differentiation to promote wound healing.¹⁴³ Collectively, these findings implicate the less obvious nuclear receptor, ROR γ t, through IL-22 as a potential regulator of protective immunity and fibroblast function within cutaneous tissues.

Another group of orphan receptors, the highly conserved NR4A subfamily including Nur77 (NR4A1), Nurr1 (NR4A2) and NOR1 (NR4A3), has emerged as pivotal transcriptional regulators of tissue wound repair mechanisms.¹⁴⁴ Although NR4A subfamily members have been extensively investigated in the context of inflammation and are known to be induced during stress conditions, recent developments in wound healing assays performed in mice

revealed that Nur77 improved wound healing by *cis*-targeting of the integrin $\beta 4$ promoter in endothelial cells.¹⁴⁵ This study revealed Nur77 as a potential candidate for proangiogenic therapy, and identifying natural ligands and novel agonists for NR4A members may be worthwhile to provide added benefits for wound repair.

Conclusion

Tissue repair is a dynamic and highly coordinated process. Injury-induced cell migration, organization and rates of cell death, division and differentiation are controlled by intrinsic and extrinsic signals. In this review, we focused on cutaneous tissue repair regulated by NHRs from the perspective of both clinical and animal model studies, whereby a recurrent observation was the protective effects of certain nuclear receptors and ligands after wounding. This is not to say that hidden within those studies that showed inhibitory effects of nuclear receptor signalling exist certain cell type-specific beneficial effects that have not been investigated to date. Future studies are necessary to identify other potential NHR candidates such as orphan receptors whose actions after injury regulate the wound repair outcome. Although this review highlighted several NHR pathways/activities that may represent potential therapeutic targets, they also represent promising areas needing more research. For example, anti-inflammatory steroids and retinoids have antagonistic effects on growth factors and tissue deposition of collagen in wounds; thus, understanding the spatiotemporal control may be relevant for treatment options in the clinical setting. This notion can also be extended towards vitamin D as a potential therapeutic agent in improving skin wounds through coupling anti-inflammatory, angiogenesis, fibrosis, keratinocyte migration and ECM remodelling interactions. In conclusion, understanding the NHR cross-talk during wound repair will better assist our ability to apply cellular therapy for local tissue repair, fibrosis, organ development and even cancer.

Acknowledgments

The authors would like to thank Elizabeth Brochu (MDI Biological Laboratory) for critically reviewing the manuscript. Research reported in this publication was supported by Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 1P20GM104318 and P20GM0103423, and an institutional award from the US Department of Army (USAMRMC) under grant number W81XWH-BAA. H.G.Z was funded by a research fellowship from the Department of Dermatology, the First Affiliated Hospital of Chongqing Medical University.

References

1. Peck MD. Epidemiology of burns throughout the world Part II: intentional burns in adults. *Burns*: ISBI. 2012; 38:630–637.10.1016/j.burns.2011.12.028
2. Luderer HF, Nazarian RM, Zhu ED, Demay MB. Ligand-dependent actions of the vitamin D receptor are required for activation of TGF-beta signaling during the inflammatory response to cutaneous injury. *Endocrinology*. 2013; 154:16–24.10.1210/en.2012-1579 [PubMed: 23132743]
3. Junker JP, Philip J, Kiwanuka E, Hackl F, Caterson EJ, Eriksson E. Assessing quality of healing in skin: review of available methods and devices. *Wound Repair Regen: Official Publication of the Wound Healing Soc [and] Euro Tissue Repair Soc*. 2014; 22(Suppl 1):2–10.10.1111/wrr.12162
4. Clark RA. Biology of dermal wound repair. *Dermatol Clin*. 1993; 11:647–666. [PubMed: 8222349]
5. Clark RA. Basics of cutaneous wound repair. *J Dermatol Surg Oncol*. 1993; 19:693–706. [PubMed: 8349909]

6. Winkelman RK. Cutaneous sensory nerves. *Semin Dermatol.* 1988; 7:236–268. [PubMed: 3153451]
7. Mihara M. Regenerated cutaneous nerves in human epidermal and subepidermal regions. An electron microscopy study. *Arch Dermatol Res.* 1984; 276:115–122. [PubMed: 6721571]
8. Paus R, Heinzelmann T, Robicsek S, Czarnetzki BM, Maurer M. Substance P stimulates murine epidermal keratinocyte proliferation and dermal mast cell degranulation in situ. *Arch Dermatol Res.* 1995; 287:500–502. [PubMed: 7542862]
9. Sayedyahosseini S, Nini L, Irvine TS, Dagnino L. Essential role of integrin-linked kinase in regulation of phagocytosis in keratinocytes. *FASEB.* 2012; 26:4218–4229.10.1096/fj.12-207852
10. Han C, Song Y, Xiao H, et al. Epidermal cells are the primary phagocytes in the fragmentation and clearance of degenerating dendrites in *Drosophila*. *Neuron.* 2014; 81:544–560.10.1016/j.neuron.2013.11.021 [PubMed: 24412417]
11. Wang XJ, Han G, Owens P, Siddiqui Y, Li AG. Role of TGF betamediated inflammation in cutaneous wound healing. *J Invest Dermatol Symp Proc/Soc Invest Dermatol Inc [and] Euro Soc Dermatol Res.* 2006; 11:112–117.
12. Arwert EN, Hoste E, Watt FM. Epithelial stem cells, wound healing and cancer. *Nat Rev Cancer.* 2012; 12:170–180.10.1038/nrc3217 [PubMed: 22362215]
13. Lu CP, Polak L, Rocha AS, et al. Identification of stem cell populations in sweat glands and ducts reveals roles in homeostasis and wound repair. *Cell.* 2012; 150:136–150.10.1016/j.cell.2012.04.045 [PubMed: 22770217]
14. Murphy PS, Evans GR. Advances in wound healing: a review of current wound healing products. *Plast Surg Int J.* 2012; 2012:190–436.10.1155/2012/190436
15. Broadley KN, Aquino AM, Woodward SC, et al. Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. *Lab Invest; J Tech Meth Pathol.* 1989; 61:571–575.
16. Ortega S, Ittmann M, Tsang SH, Ehrlich M, Basilico C. Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. *Proc Natl Acad Sci U S A.* 1998; 95:5672–5677. [PubMed: 9576942]
17. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature.* 2008; 453:314–321.10.1038/nature07039 [PubMed: 18480812]
18. O'Toole EA. Extracellular matrix and keratinocyte migration. *Clin Exp Dermatol.* 2001; 26:525–530. [PubMed: 11678882]
19. Mann J, Oakley F, Akiboye F, Elsharkawy A, Thorne AW, Mann DA. Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. *Cell Death Differ.* 2007; 14:275–285.10.1038/sj.cdd.4401979 [PubMed: 16763620]
20. Engin C. Effects of calcitonin gene-related peptide on wound contraction in denervated and normal rat skin: a preliminary report. *Plast Reconstr Surg.* 1998; 101:1887–1890. [PubMed: 9623832]
21. Tanaka T, Danno K, Ikai K, Imamura S. Effects of substance P and substance K on the growth of cultured keratinocytes. *J Invest Dermatol.* 1988; 90:399–401. [PubMed: 2450147]
22. Cheret J, Lebonvallet N, Buhe V, Carre JL, Misery L, Le Gall-Ianotto C. Influence of sensory neuropeptides on human cutaneous wound healing process. *J Dermatol Sci.* 2014; 74:193–203.10.1016/j.jdermsci.2014.02.001 [PubMed: 24630238]
23. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol.* 2007; 179:1311–1323.10.1083/jcb.200704042 [PubMed: 18086923]
24. Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol.* 2007; 127:526–537.10.1038/sj.jid.5700613 [PubMed: 17299435]
25. Hinz B, Gabbiani G. Fibrosis: recent advances in myofibroblast biology and new therapeutic perspectives. *F1000 Biol Rports.* 2010; 2:78.10.3410/B2-78
26. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. *Cell.* 1995; 83:835–839. [PubMed: 8521507]
27. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. *Science.* 2001; 294:1866–1870.10.1126/science.294.5548.1866 [PubMed: 11729302]

28. Kobayashi K, Sueyoshi T, Inoue K, Moore R, Negishi M. Cytoplasmic accumulation of the nuclear receptor CAR by a tetratricopeptide repeat protein in HepG2 cells. *Mol Pharmacol*. 2003; 64:1069–1075.10.1124/mol.64.5.1069 [PubMed: 14573755]
29. Margolis DJ, Knauss J, Bilker W. Hormone replacement therapy and prevention of pressure ulcers and venous leg ulcers. *Lancet*. 2002; 359:675–677.10.1016/S0140-6736(02)07806-6 [PubMed: 11879867]
30. Beer HD, Gassmann MG, Munz B, et al. Expression and function of keratinocyte growth factor and activin in skin morphogenesis and cutaneous wound repair. *J Investig Dermatol Symp Proc/Soc Investig Dermatol Inc [and] Euro Soc Dermatol Res*. 2000; 5:34–39.10.1046/j.1087-0024.2000.00009.x
31. Beer HD, Fassler R, Werner S. Glucocorticoid-regulated gene expression during cutaneous wound repair. *Vitam Horm*. 2000; 59:217–239. [PubMed: 10714241]
32. Brincaat MP, Baron YM, Galea R. Estrogens and the skin. *Climacteric: J Int Menop Soc*. 2005; 8:110–123.10.1080/13697130500118100
33. Roth GS, Harman SM, Lamberg SI. Altered ovarian regulation of wound healing during aging. *Proc Soc Exp Biol Med Soc Exp Biol Med*. 1981; 166:17–23.
34. Calvin M, Dyson M, Rymer J, Young SR. The effects of ovarian hormone deficiency on wound contraction in a rat model. *Br J Obstet Gynaecol*. 1998; 105:223–227. [PubMed: 9501791]
35. Ashcroft GS, Ashworth JJ. Potential role of estrogens in wound healing. *Am J Clin Dermatol*. 2003; 4:737–743. [PubMed: 14572296]
36. Ashcroft GS, Mills SJ, Lei K, et al. Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. *J Clin Invest*. 2003; 111:1309–1318.10.1172/JCI16288 [PubMed: 12727922]
37. Cotsarelis G. Epithelial stem cells: a folliculocentric view. *J Invest Dermatol*. 2006; 126:1459–1468.10.1038/sj.jid.5700376 [PubMed: 16778814]
38. Ashcroft GS, Greenwell-Wild T, Horan MA, Wahl SM, Ferguson MW. Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. *Am J Pathol*. 1999; 155:1137–1146.10.1016/S0002-9440(10)65217-0 [PubMed: 10514397]
39. Ashcroft GS, Dodsworth J, van Boxtel E, et al. Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta1 levels. *Nat Med*. 1997; 3:1209–1215. [PubMed: 9359694]
40. Ashcroft GS, Horan MA, Ferguson MW. Aging is associated with reduced deposition of specific extracellular matrix components, an upregulation of angiogenesis, and an altered inflammatory response in a murine incisional wound healing model. *J Invest Dermatol*. 1997; 108:430–437. [PubMed: 9077470]
41. Ashcroft GS, Horan MA, Herrick SE, Tarnuzzer RW, Schultz GS, Ferguson MW. Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. *Cell Tissue Res*. 1997; 290:581–591. [PubMed: 9369533]
42. Ashcroft GS, Kiely CM, Horan MA, Ferguson MW. Age-related changes in the temporal and spatial distributions of fibrillin and elastin mRNAs and proteins in acute cutaneous wounds of healthy humans. *J Pathol*. 1997; 183:80–89.10.1002/(SICI)1096-9896(199709)183:1<80::AID-PATH1104>3.0.CO;2-N [PubMed: 9370952]
43. Ashworth JJ, Smyth JV, Pendleton N, et al. The dinucleotide (CA) repeat polymorphism of estrogen receptor beta but not the dinucleotide (TA) repeat polymorphism of estrogen receptor alpha is associated with venous ulceration. *J Steroid Biochem Mol Biol*. 2005; 97:266–270.10.1016/j.jsbmb.2005.05.012 [PubMed: 16153823]
44. Strudwick X, Powell BC, Cowin AJ. Role of sex hormones in acute and chronic wound healing. *Prim Intent*. 2006; 14:35–38.
45. Kariya Y, Moriya T, Suzuki T, et al. Sex steroid hormone receptors in human skin appendage and its neoplasms. *Endocr J*. 2005; 52:317–325. [PubMed: 16006726]

46. Emmerson E, Rando G, Meda C, Campbell L, Maggi A, Hardman MJ. Estrogen receptor-mediated signalling in female mice is locally activated in response to wounding. *Mol Cell Endocrinol.* 2013; 375:149–156.10.1016/j.mce.2013.05.015 [PubMed: 23727624]
47. Campbell L, Emmerson E, Davies F, et al. Estrogen promotes cutaneous wound healing via estrogen receptor beta independent of its antiinflammatory activities. *J Exp Med.* 2010; 207:1825–1833.10.1084/jem.20100500 [PubMed: 20733032]
48. Murashima A, Kishigami S, Thomson A, Yamada G. Androgens and mammalian male reproductive tract development. *Biochim Biophys Acta.* 2014.10.1016/j.bbagr.2014.05.020
49. Basaria S. Male hypogonadism. *Lancet.* 2014; 383:1250–1263.10.1016/S0140-6736(13)61126-5 [PubMed: 24119423]
50. Fimmel S, Zouboulis CC. Influence of physiological androgen levels on wound healing and immune status in men. *Aging Male: ISSAM.* 2005; 8:166–174.10.1080/13685530500233847
51. Ashcroft GS, Mills SJ, Ashworth JJ. Ageing and wound healing. *Biogerontology.* 2002; 3:337–345. [PubMed: 12510172]
52. Ashcroft GS, Mills SJ. Androgen receptor-mediated inhibition of cutaneous wound healing. *J Clin Invest.* 2002; 110:615–624.10.1172/JCI15704 [PubMed: 12208862]
53. Gilliver SC, Ashworth JJ, Mills SJ, Hardman MJ, Ashcroft GS. Androgens modulate the inflammatory response during acute wound healing. *J Cell Sci.* 2006; 119:722–732.10.1242/jcs.02786 [PubMed: 16449322]
54. Gilliver SC, Ruckshanthi JP, Hardman MJ, Zeef LA, Ashcroft GS. 5 α -Dihydrotestosterone (DHT) retards wound closure by inhibiting re-epithelialization. *J Pathol.* 2009; 217:73–82.10.1002/path.2444 [PubMed: 18855875]
55. Lai JJ, Lai KP, Chuang KH, et al. Monocyte/macrophage androgen receptor suppresses cutaneous wound healing in mice by enhancing local TNF- α expression. *J Clin Invest.* 2009; 119:3739–3751.10.1172/JCI39335 [PubMed: 19907077]
56. Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I. Estradiol in elderly men. *Aging Male: ISSAM.* 2002; 5:98–102.
57. Chabab A, Sultan C, Fenart O, Descomps B. Stimulation of aromatase activity by dihydrotestosterone in human skin fibroblasts. *J Steroid Biochem.* 1986; 25:165–169. [PubMed: 2943941]
58. Ashcroft GS, Horan MA, Ferguson MW. Aging alters the inflammatory and endothelial cell adhesion molecule profiles during human cutaneous wound healing. *Lab Invest; J Tech Meth Pathol.* 1998; 78:47–58.
59. Daly TJ, Weston WL. Retinoid effects on fibroblast proliferation and collagen synthesis in vitro and on fibrotic disease in vivo. *J Am Acad Dermatol.* 1986; 15:900–902. [PubMed: 3771855]
60. Sundstrom A, Alfredsson L, Sjolín-Forsberg G, Gerden B, Bergman U, Jokinen J. Association of suicide attempts with acne and treatment with isotretinoin: retrospective Swedish cohort study. *BMJ.* 2010; 341:c5812.10.1136/bmj.c5812 [PubMed: 21071484]
61. Lee DD, Stojadinovic O, Krzyzanowska A, Vouthounis C, Blumenberg M, Tomic-Canic M. Retinoid-responsive transcriptional changes in epidermal keratinocytes. *J Cell Physiol.* 2009; 220:427–439.10.1002/jcp.21784 [PubMed: 19388012]
62. Melnik BC. The role of transcription factor FoxO1 in the pathogenesis of acne vulgaris and the mode of isotretinoin action. *Giornale italiano di dermatologia e venereologia: organo ufficiale, Societa italiana di dermatologia e sifilografia.* 2010; 145:559–571.
63. Melnik BC. Is nuclear deficiency of FoxO1 due to increased growth factor/PI3K/Akt-signalling in acne vulgaris reversed by isotretinoin treatment? *Br J Dermatol.* 2010; 162:1398–1400.10.1111/j.1365-2133.2010.09754.x [PubMed: 20394627]
64. Melnik BC. FoxO1—the key for the pathogenesis and therapy of acne? *JDDG.* 2010; 8:105–114.10.1111/j.1610-0387.2010.07344.x [PubMed: 20151947]
65. Ponugoti B, Xu F, Zhang C, Tian C, Pacios S, Graves DT. FOXO1 promotes wound healing through the up-regulation of TGF- β 1 and prevention of oxidative stress. *J Cell Biol.* 2013; 203:327–343.10.1083/jcb.201305074 [PubMed: 24145170]

66. Dingwall M, Marchildon F, Gunanayagam A, Louis CS, Wiper-Bergeron N. Retinoic acid-induced Smad3 expression is required for the induction of osteoblastogenesis of mesenchymal stem cells. *Differ; Res Biol Divers.* 2011; 82:57–65.10.1016/j.diff.2011.05.003
67. Glick AB, Flanders KC, Danielpour D, Yuspa SH, Sporn MB. Retinoic acid induces transforming growth factor-beta 2 in cultured keratinocytes and mouse epidermis. *Cell Regul.* 1989; 1:87–97. [PubMed: 2519621]
68. Gunes Bilgili S, Calka O, Akdeniz N, Bayram I, Metin A. The effects of retinoids on secondary wound healing: biometrical and histopathological study in rats. *J Dermatolog Treat.* 2013; 24:283–289.10.3109/09546634.2012.697985 [PubMed: 22646471]
69. Gencoglan G, Tosun M, Gencoglan O. Isotretinoin-induced effects of mast cells on wound healing. *JDD.* 2010; 9:1207–1210. [PubMed: 20941944]
70. Braungart E, Magdolen V, Degitz K. Retinoic acid upregulates the plasminogen activator system in human epidermal keratinocytes. *J Invest Dermatol.* 2001; 116:778–784.10.1046/j.1523-1747.2001.01310.x [PubMed: 11348470]
71. Warner RL, Bhagavathula N, Nerusu K, et al. MDI 301, a nonirritating retinoid, improves abrasion wound healing in damaged/atrophic skin. *Wound Repair Regen: Wound Heal Soc [and] Euro Tissue Repair Soc.* 2008; 16:117–124.10.1111/j.1524-475X.2007.00338.x
72. Bikle DD, Nemanic MK, Gee E, Elias P. 1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation. *J Clin Invest.* 1986; 78:557–566.10.1172/JCI112609 [PubMed: 2426308]
73. Bikle DD, Nemanic MK, Whitney JO, Elias PW. Neonatal human foreskin keratinocytes produce 1,25-dihydroxyvitamin D3. *Biochemistry.* 1986; 25:1545–1548. [PubMed: 2423114]
74. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev.* 2008; 29:726–776.10.1210/er.2008-0004 [PubMed: 18694980]
75. Christakos S, Hewison M, Gardner DG, et al. Vitamin D: beyond bone. *Ann N Y Acad Sci.* 2013; 1287:45–58.10.1111/nyas.12129 [PubMed: 23682710]
76. Lisse TS, Chun RF, Rieger S, Adams JS, Hewison M. Vitamin D activation of functionally distinct regulatory miRNAs in primary human osteoblasts. *J Bone Miner Res: J Amer Soc Bone Miner Res.* 2013; 28:1478–1488.10.1002/jbmr.1882
77. Lisse TS, Adams JS, Hewison M. Vitamin D and microRNAs in bone. *Crit Rev Eukaryot Gene Expr.* 2013; 23:195–214. [PubMed: 23879537]
78. Demay MB, MacDonald PN, Skoriya K, Dowd DR, Cianferotti L, Cox M. Role of the vitamin D receptor in hair follicle biology. *J Steroid Biochem Mol Biol.* 2007; 103:344–346.10.1016/j.jsbmb.2006.12.036 [PubMed: 17223342]
79. Oda Y, Sihlbom C, Chalkley RJ, et al. Two distinct coactivators, DRIP/mediator and SRC/p160, are differentially involved in vitamin D receptor transactivation during keratinocyte differentiation. *Mol Endocrinol.* 2003; 17:2329–2339.10.1210/me.2003-0063 [PubMed: 12893881]
80. Oda Y, Uchida Y, Moradian S, Crumrine D, Elias PM, Bikle DD. Vitamin D receptor and coactivators SRC2 and 3 regulate epidermisspecific sphingolipid production and permeability barrier formation. *J Invest Dermatol.* 2009; 129:1367–1378.10.1038/jid.2008.380 [PubMed: 19052561]
81. Lisse TS, Saini V, Zhao H, Luderer HF, Gori F, Demay MB. The vitamin D receptor is required for activation of cWnt and Hedgehog signaling in keratinocytes. *Mol Endocrinol.* 2014; 28(10): 1698–1706. me20141043. 10.1210/me.2014-1043 [PubMed: 25180455]
82. Li YC, Pirro AE, Amling M, et al. Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. *Proc Natl Acad Sci U S A.* 1997; 94:9831–9835. [PubMed: 9275211]
83. Feldman D, Chen T, Cone C, et al. Vitamin D resistant rickets with alopecia: cultured skin fibroblasts exhibit defective cytoplasmic receptors and unresponsiveness to 1,25(OH)2D3. *J Clin Endocrinol Metab.* 1982; 55:1020–1022.10.1210/jcem-55-5-1020 [PubMed: 6288751]
84. Hewison M, Rut AR, Kristjansson K, et al. Tissue resistance to 1,25-dihydroxyvitamin D without a mutation of the vitamin D receptor gene. *Clin Endocrinol (Oxf).* 1993; 39:663–670. [PubMed: 8287584]

85. Chen H, Hewison M, Adams JS. Functional characterization of heterogeneous nuclear ribonuclear protein C1/C2 in vitamin D resistance: a novel response element-binding protein. *J Biol Chem*. 2006; 281:39114–39120.10.1074/jbc.M608006200 [PubMed: 17071612]
86. Lisse TS, Liu T, Irmeler M, et al. Gene targeting by the vitamin D response element binding protein reveals a role for vitamin D in osteoblast mTOR signaling. *FASEB*. 2011; 25:937–947.10.1096/fj.10-172577
87. Lisse TS, Hewison M, Adams JS. Hormone response element binding proteins: novel regulators of vitamin D and estrogen signaling. *Steroids*. 2011; 76:331–339.10.1016/j.steroids.2011.01.002 [PubMed: 21236284]
88. Lisse, TS.; Chen, H.; Nanes, MS.; Hewison, M.; Adams, JS. Vitamin D response element binding protein In *Vitamin D*. Elsevier Academic Press; San Diego, USA: 2011. p. 251-271.
89. Reichrath J, Classen UG, Meineke V, et al. Immunoreactivity of six monoclonal antibodies directed against 1,25-dihydroxyvitamin-D3 receptors in human skin. *Histochem J*. 2000; 32:625–629. [PubMed: 11202159]
90. Pillai S, Bikle DD. Epidermal vitamin D metabolism, function, and regulation. *Adv Lipid Res*. 1991; 24:321–341. [PubMed: 1763717]
91. Bikle DD. Vitamin D regulated keratinocyte differentiation. *J Cell Biochem*. 2004; 92:436–444.10.1002/jcb.20095 [PubMed: 15156556]
92. Bikle DD, Chang S, Crumrine D, et al. Mice lacking 25OHD 1 α hydroxylase demonstrate decreased epidermal differentiation and barrier function. *J Steroid Biochem Mol Biol*. 2004; 89–90:347–353.10.1016/j.jsmb.2004.03.113
93. Bollag WB. Differentiation of human keratinocytes requires the vitamin d receptor and its coactivators. *J Invest Dermatol*. 2007; 127:748–750.10.1038/sj.jid.5700692 [PubMed: 17363957]
94. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006; 311:1770–1773.10.1126/science.1123933 [PubMed: 16497887]
95. Schaubert J, Gallo RL. Expanding the roles of antimicrobial peptides in skin: alarming and arming keratinocytes. *J Invest Dermatol*. 2007; 127:510–512.10.1038/sj.jid.5700761 [PubMed: 17299432]
96. Schaubert J, Dorschner RA, Coda AB, et al. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest*. 2007; 117:803–811.10.1172/JCI30142 [PubMed: 17290304]
97. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front Physiol*. 2014; 5:151.10.3389/fphys.2014.00151 [PubMed: 24795646]
98. Hewison M. Antibacterial effects of vitamin D. *Nat Rev Endocrinol*. 2011; 7:337–345.10.1038/nrendo.2010.226 [PubMed: 21263449]
99. Xie Z, Komuves L, Yu QC, et al. Lack of the vitamin D receptor is associated with reduced epidermal differentiation and hair follicle growth. *J Invest Dermatol*. 2002; 118:11–16.10.1046/j.1523-1747.2002.01644.x [PubMed: 11851870]
100. Teichert A, Elalieh H, Bikle D. Disruption of the hedgehog signaling pathway contributes to the hair follicle cycling deficiency in Vdr knockout mice. *J Cell Physiol*. 2010; 225:482–489.10.1002/jcp.22227 [PubMed: 20458748]
101. Teichert AE, Elalieh H, Elias PM, Welsh J, Bikle DD. Overexpression of hedgehog signaling is associated with epidermal tumor formation in vitamin D receptor-null mice. *J Invest Dermatol*. 2011; 131:2289–2297.10.1038/jid.2011.196 [PubMed: 21814234]
102. Meindl S, Rot A, Hoetzenecker W, Kato S, Cross HS, Elbe-Burger A. Vitamin D receptor ablation alters skin architecture and homeostasis of dendritic epidermal T cells. *Br J Dermatol*. 2005; 152:231–241.10.1111/j.1365-2133.2005.06392.x [PubMed: 15727633]
103. Cianferotti L, Cox M, Skoriya K, Demay MB. Vitamin D receptor is essential for normal keratinocyte stem cell function. *Proc Natl Acad Sci U S A*. 2007; 104:9428–9433.10.1073/pnas.0702884104 [PubMed: 17517646]
104. Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med*. 2005; 11:1351–1354.10.1038/nm1328 [PubMed: 16288281]

105. Ito M, Cotsarelis G. Is the hair follicle necessary for normal wound healing? *J Invest Dermatol.* 2008; 128:1059–1061.10.1038/jid.2008.86 [PubMed: 18408743]
106. Langton AK, Herrick SE, Headon DJ. An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. *J Invest Dermatol.* 2008; 128:1311–1318.10.1038/sj.jid.5701178 [PubMed: 18037901]
107. Zhang JZ, Maruyama K, Ono I, Kaneko F. Production and secretion of platelet-derived growth factor AB by cultured human keratinocytes: regulatory effects of phorbol 12-myristate 13-acetate, tretinoin, 1,25-dihydroxyvitamin D3, and several cytokines. *J Dermatol.* 1995; 22:305–309. [PubMed: 7673548]
108. Kong J, Grando SA, Li YC. Regulation of IL-1 family cytokines IL-1 α , IL-1 receptor antagonist, and IL-18 by 1,25-dihydroxyvitamin D3 in primary keratinocytes. *J Immunol.* 2006; 176:3780–3787. [PubMed: 16517748]
109. Ding N, Yu RT, Subramaniam N, et al. A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell.* 2013; 153:601–613.10.1016/j.cell.2013.03.028 [PubMed: 23622244]
110. Usategui A, Criado G, Del Rey MJ, Fare R, Pablos JL. Topical vitamin D analogue calcipotriol reduces skin fibrosis in experimental scleroderma. *Arch Dermatol Res.* 2014;10.1007/s00403-014-1466-6
111. Lagishetty V, Misharin AV, Liu NQ, et al. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. *Endocrinology.* 2010; 151:2423–2432.10.1210/en.2010-0089 [PubMed: 20392825]
112. Jiang YJ, Kim P, Lu YF, Feingold KR. PPAR γ activators stimulate aquaporin 3 expression in keratinocytes/epidermis. *Exp Dermatol.* 2011; 20:595–599.10.1111/j.1600-0625.2011.01269.x [PubMed: 21457357]
113. Elizondo RA, Yin Z, Lu X, Watsky MA. Effect of vitamin D receptor knockout on cornea epithelium wound healing and tight junctions. *Invest Ophthalmol Vis Sci.* 2014;10.1167/iov.13-13553
114. Michalik L, Wahli W. Involvement of PPAR nuclear receptors in tissue injury and wound repair. *J Clin Invest.* 2006; 116:598–606.10.1172/JCI27958 [PubMed: 16511592]
115. Rosenfield RL, Kentsis A, Deplewski D, Ciletti N. Rat preputial sebocyte differentiation involves peroxisome proliferator-activated receptors. *J Invest Dermatol.* 1999; 112:226–232.10.1046/j.1523-1747.1999.00487.x [PubMed: 9989800]
116. Makrantonaki E, Zouboulis CC. Testosterone metabolism to 5 α -dihydrotestosterone and synthesis of sebaceous lipids is regulated by the peroxisome proliferator-activated receptor ligand linoleic acid in human sebocytes. *Br J Dermatol.* 2007; 156:428–432.10.1111/j.1365-2133.2006.07671.x [PubMed: 17300229]
117. Michalik L, Desvergne B, Tan NS, et al. Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR) α and PPAR β mutant mice. *J Cell Biol.* 2001; 154:799–814.10.1083/jcb.200011148 [PubMed: 11514592]
118. Tan NS, Michalik L, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor-beta as a target for wound healing drugs. *Expert Opin Ther Targets.* 2004; 8:39–48.10.1517/14728222.8.1.39 [PubMed: 14996617]
119. Tan NS, Michalik L, Di-Poi N, Desvergne B, Wahli W. Critical roles of the nuclear receptor PPAR β (peroxisome-proliferator-activated receptor beta) in skin wound healing. *Biochem Soc Trans.* 2004; 32:97–102. doi:10.1042/. [PubMed: 14748722]
120. Tan NS, Michalik L, Di-Poi N, et al. Essential role of Smad3 in the inhibition of inflammation-induced PPAR β /delta expression. *EMBO J.* 2004; 23:4211–4221.10.1038/sj.emboj.7600437 [PubMed: 15470497]
121. Di-Poi N, Tan NS, Michalik L, Wahli W, Desvergne B. Antiapoptotic role of PPAR β in keratinocytes via transcriptional control of the Akt1 signaling pathway. *Mol Cell.* 2002; 10:721–733. [PubMed: 12419217]
122. Sha W, Thompson K, South J, Baron M, Leask A. Loss of PPAR γ expression by fibroblasts enhances dermal wound closure. *Fibrogenesis Tissue Repair.* 2012; 5:5.10.1186/1755-1536-5-5 [PubMed: 22502865]

123. Bienert GP, Chaumont F. Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochimica et Biophysica Acta*. 2014; 1840(5):1596–1604.10.1016/j.bbagen.2013.09.017 [PubMed: 24060746]
124. Hara M, Ma T, Verkman AS. Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery. *J Biol Chem*. 2002; 277:46616–46621.10.1074/jbc.M209003200 [PubMed: 12270942]
125. Loo AE, Ho R, Halliwell B. Mechanism of hydrogen peroxide-induced keratinocyte migration in a scratch-wound model. *Free Radic Biol Med*. 2011; 51:884–892.10.1016/j.freeradbiomed.2011.06.001 [PubMed: 21699973]
126. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*. 2002; 96:23–43. [PubMed: 12441176]
127. Quax RA, Manenschijn L, Koper JW, et al. Glucocorticoid sensitivity in health and disease. *Nat Rev Endocrinol*. 2013; 9:670–686.10.1038/nrendo.2013.183 [PubMed: 24080732]
128. Issa LL, Leong GM, Barry JB, Sutherland RL, Eisman JA. Glucocorticoid receptor-interacting protein-1 and receptor-associated coactivator-3 differentially interact with the vitamin D receptor (VDR) and regulate VDR-retinoid X receptor transcriptional cross-talk. *Endocrinology*. 2001; 142:1606–1615.10.1210/endo.142.4.8068 [PubMed: 11250942]
129. Ragan C, Grokoest AW, Boots RH. Effect of adrenocorticotrophic hormone on rheumatoid arthritis. *Am J Med*. 1949; 7:741–750. [PubMed: 15394604]
130. Wang AS, Armstrong EJ, Armstrong AW. Corticosteroids and wound healing: clinical considerations in the perioperative period. *Am J Surg*. 2013; 206:410–417.10.1016/j.amjsurg.2012.11.018 [PubMed: 23759697]
131. Tiganescu A, Hupe M, Uchida Y, Mauro T, Elias PM, Holleran WM. Increased glucocorticoid activation during mouse skin wound healing. *J Endocrinol*. 2014; 221:51–61.10.1530/JOE-13-0420 [PubMed: 24464022]
132. Grose R, Werner S, Kessler D, et al. A role for endogenous glucocorticoids in wound repair. *EMBO Rep*. 2002; 3:575–582.10.1093/embo-reports/kvf119 [PubMed: 12034758]
133. Lindstedt E, Sandblom P. Wound healing in man: tensile strength of healing wounds in some patient groups. *Ann Surg*. 1975; 181:842–846. [PubMed: 1138634]
134. El Roz A, Bard JM, Huvelin JM, Nazih H. LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: relation to proliferation and apoptosis. *Anticancer Res*. 2012; 32:3007–3013. [PubMed: 22753765]
135. Sticozzi C, Pecorelli A, Belmonte G, Valacchi G. Cigarette smoke affects ABCA1 expression via liver X receptor nuclear translocation in human keratinocytes. *Int J Mol Sci*. 2010; 11:3375–3386.10.3390/ijms11093375 [PubMed: 20957101]
136. Jiang YJ, Kim P, Elias PM, Feingold KR. LXR and PPAR activators stimulate cholesterol sulfotransferase type 2 isoform 1b in human keratinocytes. *J Lipid Res*. 2005; 46:2657–2666.10.1194/jlr.M500235-JLR200 [PubMed: 16150827]
137. Walczak R, Joseph SB, Laffitte BA, Castrillo A, Pei L, Tontonoz P. Transcription of the vascular endothelial growth factor gene in macrophages is regulated by liver X receptors. *J Biol Chem*. 2004; 279:9905–9911.10.1074/jbc.M310587200 [PubMed: 14699103]
138. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol*. 2013; 13:145–149.10.1038/nri3365 [PubMed: 23348417]
139. Serafini N, Klein Wolterink RG, Satoh-Takayama N, et al. Gata3 drives development of RORgammat + group 3 innate lymphoid cells. *J Exp Med*. 2014; 211:199–208.10.1084/jem.20131038 [PubMed: 24419270]
140. Romera-Hernandez M, Aparicio-Domingo P, Cupedo T. Damage control: RORgammat + innate lymphoid cells in tissue regeneration. *Curr Opin Immunol*. 2013; 25:156–160.10.1016/j.coi.2013.01.007 [PubMed: 23433918]
141. Aujla SJ, Chan YR, Zheng M, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med*. 2008; 14:275–281.10.1038/nm1710 [PubMed: 18264110]
142. Ouyang W. Distinct roles of IL-22 in human psoriasis and inflammatory bowel disease. *Cytokine Growth Factor Rev*. 2010; 21:435–441.10.1016/j.cytogfr.2010.10.007 [PubMed: 21106435]

143. McGee HM, Schmidt BA, Booth CJ, et al. IL-22 promotes fibroblast-mediated wound repair in the skin. *J Invest Dermatol.* 2013; 133:1321–1329.10.1038/jid.2012.463 [PubMed: 23223145]
144. Zhao Y, Bruemmer D. NR4A orphan nuclear receptors: transcriptional regulators of gene expression in metabolism and vascular biology. *Arterioscler Thromb Vasc Biol.* 2010; 30:1535–1541.10.1161/ATVBAHA.109.191163 [PubMed: 20631354]
145. Niu G, Ye T, Qin L, et al. Orphan nuclear receptor TR3/Nur77 improves wound healing by upregulating the expression of integrin beta4. *FASEB.* 201410.1096/fj.14-257550
146. Grose R, Werner S. An aPPARently protective mechanism for keratinocytes in wounded skin. *Trends Mol Med.* 2002; 8:149–151. [PubMed: 11927266]
147. Hubner G, Brauchle M, Smola H, Madlener M, Fassler R, Werner S. Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine.* 1996; 8:548–556.10.1006/cyto.1996.0074 [PubMed: 8891436]

Figure 1.

Overlapping phases of skin wound healing in humans. The major phases of wound healing are marked by inflammation, tissue formation/proliferation and tissue remodelling. Image inserts, normal skin healing after an abrasion in a 3-year-old patient. Left insert, approximately 5 days after abrasion. Right insert, approximately 8 weeks after abrasion. Abbreviation: ECM, extracellular matrix

Figure 2.

Schematic presentation of the stages of cutaneous wound repair in humans. (A) Immediately after skin injury, the wounded area is filled with a blood clot consisting of blood elements/proteins and vasoactive amines from locally impaired blood vessels within the dermis. Coagulation occurs as platelets aggregate with fibrin. Platelets secrete factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)- β 1 that increase vascular permeability and attract neutrophils to the wound site, signalling the early phase of inflammation. Nerve endings are damaged and partially removed by phagocytic keratinocytes. (B) At 24–48 h after injury, neutrophils have undergone apoptosis, while macrophages become the principal inflammatory cell in the wounded area. Endothelial cells infiltrate into the transitioning clot and proliferate to form new blood vessels. Hyper-innervation occurs at the wound. Fibroblasts also migrate into the wounded area and proliferate to deposit a provisional extracellular matrix consisting of new tissue termed granulation tissue. Keratinocytes migrate down the injured dermis above the provisional matrix and begin to proliferate. (C) By 3–10 days after injury, the wound is filled with granulation tissue, and fibroblasts are recruited to the wound by growth factors from macrophages. Fibroblasts then trans-differentiate into myofibroblasts, leading to wound contraction and immature collagen deposition. At this phase, the apical wound portion is overlaid with a neo-epidermis. (D) During remodelling, the rate of collagen synthesis is reduced approximately 2 weeks post-injury. Collagen cross-linking and reorganization can occur for months/years after injury to restore tissue architecture and function. In scar tissue,

collagen alignment occurs in a single direction. Tensile strength of (scar) tissue increases over time, but never to that of the pre-injury level

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

A. Estrogen receptor signaling **B. Androgen receptor signaling** **C. Retinoid signaling** **Figure 3.**

Overview of estrogen, androgen and retinoid nuclear hormone receptor signalling during the phases of cutaneous wound repair. (A) Estrogen sex hormone nuclear receptor (ER) signalling. (B) Androgen nuclear receptor (AR) signalling. For clarification, AR activates immune responses that impair cutaneous wound repair processes.³³ (C) Retinoid acid (RAR) and X receptor (RXR) signalling. See text for references. (+) represents activation, while (–) represents inhibition. Precise outcomes are explained in the text. Abbreviations: del, deletion; KC, keratinocytes; MMIF, macrophage migration inhibitory factor; MMPs, metalloproteinases; PAS, plasminogen activator system; TGF, transforming growth factor; sol. (soluble)

A. Vitamin D receptor signaling **B. Peroxisome proliferator-activated receptor signaling** **C. Glucocorticoid receptor signaling** **Figure 4.**

Overview of vitamin D, peroxisome proliferator-activated and glucocorticoid nuclear hormone receptor signalling during the phases of cutaneous wound repair. (A) Vitamin D receptor (VDR) signalling. (B) Peroxisome proliferator-activated receptor (PPAR) signalling. (C) Glucocorticoid receptor (GR) signalling. See text for references. (+) represents activation, while (–) represents inhibition. Precise outcomes are explained in the text. Abbreviations: 11 β -HSD1, 11 β -hydroxysteroid dehydrogenase 1; AQP3, aquaporin 3; ECM, extracellular matrix; H₂O₂, hydrogen peroxide; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor

Table 1
Nuclear hormone receptors and cutaneous wound healing

Receptor	Heterodimers	Function during wound repair	Reference
ER	Liver X receptor (α , β)	Estrogen deprivation is associated with attenuated wound healing, HRT positively improves acute wound healing; ER- β signalling promotes wound healing in KCs; ER- α signalling in fibroblasts promotes wound healing; immune effects	29–31,47
AR	RAR (α , β , γ)	AR signalling inhibits KC migration; increases inflammation, a likely pathophysiological mechanism for decreased wound closure	38,39,54,55
RAR and RXR	AR, VDR, PPAR, GR	Retinoid signalling is cytoprotective, anti-inflammatory and profibrotic (fibroblasts); improves wound healing through suppression of MMPs (fibroblasts); KC-TGF- β role unknown; inhibition of KC terminal differentiation and proliferation?; $\uparrow$ PAS in KCs for re-epithelialization	61–64,70,71
VDR	RXR (α , β)	VDR promotes proper wound healing through recruitment of macrophages to the wound site and activating immunomodulatory TGF- β signalling in fibroblasts (ECM? Contraction?); may promote growth factor signalling; immunomodulatory role in KCs	2,94–98,107
PPAR (α , β , δ , γ)	RXR	Systemic: anti-inflammatory, anti-oxidant, metabolic (normalization of lipids) Cellular: re-epithelialization of wound; KC protection/migration; induction of AQP3; negative role in fibroblasts	114,117–119,122,146
GR	RXR (α , β , γ)	GR overexpression leads to dermal atrophy, vascular instability and acne; topical application of GCs impairs wound healing targeting KCs, fibroblasts and endothelial cells; endogenous GCs target fibroblasts (granulation tissue); leukocytes/macrophages ($\downarrow$ IL-1- α/β and TNF- α)	105,106, 133,147

Abbreviations: AQP3, aquaporin 3; AR, androgen receptor; ECM, extracellular matrix; ER, estrogen receptor; GCs, glucocorticoids; GR, glucocorticoid receptor; HRT, hormone replacement therapy; IL, interleukin; KC, keratinocyte; MMPs, metalloproteinases; PAS, plasminogen activator system; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; TGF, transforming growth factor; TNF, tumour necrosis factor; VDR, vitamin D receptor.