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Scar management in burn injuries using drug delivery and molecular signaling: current treatments and future directions

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Abstract

In recent decades, there have been tremendous improvements in burn care that have allowed patients to survive severe burn injuries that were once fatal. However, a major limitation of burn care currently is the development of hypertrophic scars in approximately 70% of patients. This significantly decreases the quality of life for patients due to the physical and psychosocial symptoms associated with scarring. Current approaches to manage scarring include surgical techniques and non-surgical methods such as laser therapy, steroid injections, and compression therapy. These treatments are limited in their effectiveness and regularly fail to manage symptoms. As a result, the development of novel treatments that aim to improve outcomes and quality of life is imperative. Drug delivery that targets the molecular cascades of wound healing to attenuate or prevent hypertrophic scarring is a promising approach that has therapeutic potential. In this review, we discuss current treatments for scar management after burn injury, and how drug delivery targeting molecular signaling can lead to new therapeutic strategies.

Graphical abstract

Signaling pathways targeting wound management might provide a unique opportunity to prevent and/or manage scar post thermal injury.

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Introduction

A burn injury is one of the most severe forms of trauma to the skin and each year 300,000 people die due to burns. Improvement to acute burn care has reduced mortality in the past few decades. As a result, patients are surviving burn injuries that cover up to 100% of total body burn surface area (TBSA). Despite this improvement, the greatest unmet challenge in burn recovery is cutaneous scarring that results in reduced quality of life and reintegration into society. The prevalence of hypertrophic scarring after burn injury is approximately 70% [1], and scarring contributes to neuropathic pain, surface irregularities, stiffness, and disabling contractures. Therefore, therapies that reduce scarring postburn are crucial to improving patient rehabilitation. In this review paper, we discuss scar management in burn injuries and the use of drug delivery to target molecular signaling pathways involved in scar formation.

Hypertrophic Scarring

After cutaneous burn injury, there is linear collagen deposition that creates a scar that lacks the flexibility of uninjured skin. This collagen deposition occurs in excess resulting in the development of a pathological scar that is thick and non-pliable [2]. Moreover, the scar is itchy and painful which worsens the patient quality of life and outcome [3]. What is more desirable is scarless healing which leads to a flat, pliable scar with minimal skin discoloration. Unfortunately, this does not occur after burn injury leading to significant challenges. There are two types of scarring that arise from burn injury – hypertrophic scar or keloid. The pathophysiology underlying each type of scar is different. Hypertrophic scars occur within the confines of the wound, are elevated, erythematous, and appear within 4-8 weeks after initial injury [4]. The scar will mature and gradually regress to a flatter scar over a period of 2 years. Keloids, on the other hand, develop months to years after injury and spread beyond the confines of the wound [5]. Like hypertrophic scars, keloids are elevated and erythematous. In both hypertrophic scarring and keloids there is increased collagen deposition that is oriented in thick bundles, however, the arrangement of collagen fibers differs. In hypertrophic scarring, histology reveals an abundance of collagen type III fibers oriented parallel to the surface of the epithelium [6]. In contrast, keloids are composed of disorganized sheets of type I and type II collagen arranged randomly to the epithelium surface [6]. We will focus on the most common type of scar in severely burned patients – the hypertrophic scar. To understand the use of drug delivery for scar management after burn

injury, it is first important to understand the pathophysiology of wound healing and how this becomes disrupted in hypertrophic scarring.

Pathophysiology of Wound Healing and Hypertrophic Scarring

Skin is composed of two layers: the epidermis which forms a protective barrier to the environment and the dermis that is composed of connective tissue providing skin with its mechanical properties [7]. The epidermis contains a stratified epithelium with hair follicles and sebaceous glands [7]. The dermis can be divided into the superficial papillary region and the deeper reticular dermis. Both regions are made up of collagen, elastic fibers, and extracellular matrix [7]. Cutaneous injury begins a well-orchestrated cascade of cellular and biochemical events which leads to healing. The intensity of the response is dependent on the size of the wound, type of wound, and the severity of injury [8]. There are several distinct but overlapping phases of wound healing: hemostasis and inflammation, proliferation, and lastly maturation and remodeling (Figure 1). These phases correspond to activation of several key signaling pathways; these include Wnt/ β -catenin, growth factor/cytokine pathways such as TGF- β pathway, Notch, and Sonic hedgehog.

Stages of Wound Healing

The immediate response to injury is hemostasis and inflammation which prevents blood loss at the site of injury. A cascade of events such as coagulation, vascular constriction, platelet aggregation leads to the formation of a fibrin clot (Figure 2) [9-11]. This fibrin clot acts as a provisional matrix that supports the migration of neutrophils, macrophages, and lymphocytes to the wound site [9-11]. Foreign objects are removed by neutrophils whereas foreign particles and dead neutrophils are phagocytosed by macrophages. These inflammatory cells are attracted to the site of injury by cytokines such as transforming growth factor beta (TGF- β) and platelet-derived growth factor (PDGF), and tumor necrosis factor (TNF- α) [9, 10]. In addition to removing debris, macrophages also release TGF- β and other growth factors that stimulate the migration of fibroblasts and epithelial cells into the wound [11]. This is a crucial step in the initiation of the proliferation phase of wound healing.

The proliferative phase begins about three days after injury in humans and is characterized by re-epithelialization of the epidermis and repair of the underlying dermal layer with accompanying neovascularization (Figure 2) [11]. The dermis is restored by proliferating mesenchymal-like fibroblasts that produce ECM proteins such as fibronectin, secrete growth factors like TGF- β , and promote collagen deposition (Figure 2) [12]. These cells are derived from different sources. For instance, it has been shown that skeletal muscle progenitor cells (Pax7⁺) [13] and myeloid lineage cells contribute to granulation tissue formation [14]. The passage of fibroblasts and keratinocytes into the wound bed is possible due to the formation of a temporary matrix resulting from the cleavage of the ECM by matrix metalloproteinases (MMPs) [11, 15]. After migration, fibroblasts produce immature ECM components such as EDA fibronectin and type III collagen, and adult/mature collagen type I. To appreciate the complexity of this response, many growth factors facilitate the proliferative phase by regulating migration and the formation of new capillaries and blood vessels [7, 12, 16].

These include epidermal growth factor (EGF), keratinocyte growth factor (KGF), fibroblast growth factor (FGF), FGF-2 TGF- β , heparin-binding EGF, and vascular endothelial growth factor (VEGF). Closure of the epithelial gap and restoration of the epithelium is achieved by the coordination of the aforementioned events. Recently, it has been shown that hair follicle stem cells and interfollicular epidermal stem cells also contribute to re-epithelialization during wound healing wounds [17, 18].

Following adequate collagen deposition and closure of the epithelial gap, remodeling phase begins where the properties of the tissue are changed. Fibroblasts transform into a contractile myofibroblasts phenotype which initiates wound contraction [19]. The reorganization of the tissue begins as type III collagen and fibronectin (immature ECM) are degraded and replaced by organized type I collagen [7]. These changes lead to wound contraction and scar formation, and these changes can continue to unfold for weeks to years. Unlike highly regenerative organs like liver [20], scar formation is the minimal consequence of skin injury. Unfortunately, the new scar tissue has reduced tensile strength compared to uninjured skin [11]. Overall, efficient skin healing is dependent on the synchronized and timely response of these three phases, and any abnormalities or disruptions in these phases can lead to the formation of hypertrophic scars.

Molecular Pathways in Wound Healing and Scar Formation

Several key molecular pathways are activated during the coordinated phases of cutaneous wound healing. Here we will focus on five that have been shown to be of particular importance: Wnt/ β -catenin, TGF- β , Notch, Sonic Hedgehog, and ras/MEK/ERK signaling pathways. Interestingly, these molecular pathways are activated in both embryonic and adult wound healing. However, embryonic healing leads to a scarless wound. As such, there are critical differences in these molecular pathways that account for the inability of adult skin repair to recapitulate the structure and function of the uninjured skin. In recent years, by using several animal models of skin injury (thermally and non-thermally) [21, 22] involvement of several signaling pathways during skin healing has been documented. Targeting these differences and developing drugs that modulate these pathways may be of therapeutic benefit in scar management after burn injury. We will briefly discuss the role of these molecular pathways in wound repair and how alterations in these pathways lead to scar formation.

Wnt/ β -Catenin Signalling

Wnts are secreted glycoproteins that are fundamental to development, maintenance of homeostasis, and cutaneous wound healing [23]. Here we will focus on the canonical Wnt signaling pathway mediated through β -catenin, as this pathway plays a crucial role in wound healing. β -catenin is a transcriptional coactivator that is normally targeted for proteasomal degradation in the absence of Wnt signaling (Figure 3). A cytoplasmic destruction complex composed of axin, glycogen synthase kinase (GSK) 3 β , and adenomatous polyposis coli protein (APC) promotes the degradation of β -catenin [24, 25]. The binding of Wnt to the G-protein-coupled membrane receptor, Frizzled, stimulates the activation of Dishevelled in the cytoplasm [26]. Dishevelled is important as it inhibits the activity of the cytoplasmic

destruction complex resulting in the stabilization of β -catenin [26]. This allows for the nuclear translocation of β -catenin where it binds to transcriptional activators leading to transcription of target genes that influence wound healing [24, 27]. β -catenin has also been shown to be an important structural protein that forms part of the adherens junctions that mediate cell to cell contacts [24].

The early phases of cutaneous wound healing (24 hours to 7 days) are marked by the expression of various Wnts [28]. Migrating epithelial cells express Wnt10b, hair follicles express Wnt4, 5, and 10b, and the dermis expresses Wnt 4 [28]. Interestingly, Wnt signaling is essential during regeneration of hair follicles in large wounds in mice [29]. In a lineage tracing study, it is demonstrated that the source of new hair follicle cells is not the hair follicle stem cell niche, but other epidermal cells [29]. With regards to the role of Wnt/ β -catenin signaling in re-epithelization, there is much to be discovered. Nevertheless, mouse studies have unraveled key aspects of Wnt/ β -catenin's signaling in re-epithelization. In terms of migration capacity, keratinocytes isolated from mice that have β -catenin conditionally ablated have comparable migration capacity to normal keratinocytes [30]. Moreover, pharmacological stabilization of β -catenin inhibits keratinocyte migration in vitro [31], and the edges of chronic ulcers have increased accumulation of β -catenin in the epidermis [31]. These studies suggest that Wnt/ β -catenin signaling may have an inhibitory effect on re-epithelialization at a certain level, but more studies are required to elucidate the mechanisms involved.

An essential role has been documented for β -catenin in the regulation of fibroblasts during the proliferative phase of dermal repair [32]. The proliferative phase of healing in both humans and mice is characterized by increased β -catenin protein level and mRNA expression in dermal fibroblasts as well as target genes such as MMP7 and fibronectin [32, 33]. While Wnt proteins are required for the activation of β -catenin, they are not crucial to maintain persistent β -catenin levels during the proliferative phase [34]. When mice are treated with the Wnt inhibitor, Dickkopf (DKK1), during the proliferative phase of wound healing, there is not a decline in β -catenin [34, 35], suggesting that other factors likely play a role in maintaining β -catenin levels. Indeed, in dermal fibroblasts, TGF- β 1 increases β -catenin levels [30, 36, 37] and ECM components such as fibronectin have been shown to regulate β -catenin activity through GSK3 β -dependent pathways [34]. EDA-fibronectin deficient mice have fewer fibroblasts, decreased wound length, and lower β -catenin levels illustrating the regulation of β -catenin by fibronectin [34]. Wound healing can be rescued through activation of β -catenin in these mice by either pharmacological stabilization of β -catenin with lithium chloride or genetically by crossing these EDA-fibronectin deficient mice with mice that express conditionally stabilized β -catenin [34]. During development, Wnt/ β -catenin are involved in the formation of the epidermis and dermis, in part due to epithelial-mesenchymal transitions [38-41]. Hence, a unique role for Wnt/ β -catenin during wound healing may be facilitating epidermal-mesenchymal transition (EMT) interactions. An interesting study has shown that epidermal stabilizing β -catenin remodels the adult dermis to express immature collagens, proliferation of fibroblasts, and downregulation of ECM genes, thus gaining characteristics of the fetal dermis [42]. Another study found that hair follicle stem cells secrete Nephronectin, a Wnt/ β -catenin target gene that forms an attachment site for smooth muscle precursors of the erector pili muscle [43]. Whether these

epithelial-mesenchymal interactions can be manipulated through modulation of Wnt/ β -catenin signaling during wound healing remains to be seen.

Persistent β -catenin activity may contribute to hypertrophic scarring after burn injury. Mouse models where β -catenin is either conditionally stabilized or ablated have shown that high β -catenin levels are associated with an enlarged, hypercellular dermis, whereas low levels are associated with a smaller and less cellular dermis [30]. Preventing the proteasomal degradation of β -catenin in the dermis by fibroblast-specific deletion of GSK3 β leads to a fibrotic response in mice [44]. At the cellular level, β -catenin has an essential role in different cell types. In myeloid lineage cells and macrophages, it has been shown that downregulation or ablation of β -catenin alter macrophages migration and adhesion, the two essential cell behaviours that are important during wound healing [14]. As mentioned, one-fourth of fibroblast-like cells in the dermis of healing mice are the progeny of Pax-expression muscle progenitor cells [13]. These cells have activated β -catenin activity and knocking down β -catenin in Pax7 positive cells results in a smaller scar size and fewer fibroblast-like cells in the dermis [13]. This not only shows a role for β -catenin in scar formation but also highlights a cell source that might contribute to scar formation. Similarly, hypertrophic scars and keloids from human patients exhibit elevated β -catenin levels [33]. It appears that increased β -catenin activity during the proliferative phase is required for successful wound repair, but persistent β -catenin activity beyond the proliferative phase leads to scar formation. These β -catenin-dependent mechanisms of hypertrophic scarring need to be further elucidated so that drugs that augment this pathway and reduce scarring can be developed.

TGF- β 1 Pathway

TGF- β s are a group of major cytokine mediators comprised of TGF- β 1, TGF- β 2, TGF- β 3, isoforms; TGF- β 1 is particularly active during cutaneous wound healing [45, 46]. These isoforms are secreted by various cell types such as fibroblasts and macrophages as inactive precursors bound to latent TGF- β binding proteins [47, 48]. Conformational changes caused by integrins in response to wound contraction release TGF- β allowing it to exert cell-specific effects by binding to serine/threonine kinase TGF- β receptor I and TGF- β -receptor II heterodimers [45, 46]. This leads to phosphorylation of Smads 2 and 3. Smads 2 and 3 associate with Smad 4 and translocated to the nucleus where they modulate gene expression in conjunction with other transcription cofactors [49] (Figure 4).

TGF- β is well-known for its fibrotic mechanisms during wound healing. TGF- β 1 plays a role in the proliferation of fibroblasts, synthesis of ECM components like collagen I and fibronectin, the transformation of fibroblasts to a myofibroblastic phenotype, and lastly wound contracture [50, 51]. Wound healing remedies like the jelly part of human umbilical cord enhance wound healing mainly through activation of a myofibroblastic phenotype [52]. Genetic mouse models have provided insight into the fibrotic mechanisms of TGF- β 1. For instance, mice with TGF- β 1 and Scid knocked out have delays in all phases of wound healing and exhibit a disorganized and thin dermis [53]. Smad 3 knockout mice have smaller wounds than wild-type mice along with fewer fibroblasts and diminished ECM deposition [54]. Ablation of TGF- β 2 receptor also leads to defects in dermal repair like TGF- β 1 [55].

Fibroblasts isolated from TGF- β 2 receptor knockout mice show reduced contractile abilities [55, 56]. These defects are confirmed *in vivo* as fibroblast-specific deletion of TGF- β 2 receptor results in reduced granulation tissue formation, reduced collagen deposition, and decreased scar size [55]. Considering TGF- β 1's effects on fibroblast proliferation and migration, it would seem plausible that TGF- β 1 promotes re-epithelialization. However, the involvement of TGF- β pathway in re-epithelialization is inconsistent in literature. TGF- β 1 has an inhibitory effect on keratinocyte proliferation suggesting it may impair re-epithelialization [57]. For example, deletion of Smad3 in mice leads to increased proliferation and accelerated epidermal closure [54]. Moreover, transgenic mice that overexpress inhibitory Smad7 in the epidermis show increased keratinocyte proliferation, keratinocyte migration *in vitro*, most importantly faster wound closure [58]. Lastly, in porcine burn wounds, TGF- β 1 antagonist treatment leads to more wounds that heal with complete re-epithelialization [58]. In contrast, there are numerous studies showing that TGF- β pathway enhances re-epithelialization. TGF- β 1 and Scid knockout mice mentioned earlier have delayed re-epithelialization [53], and administration of topical TGF- β 1 leads to faster epithelial wound closure in rats [50]. Furthermore, α 3 β 1 integrin-deficient mice have delayed re-epithelialization, in part due to upregulation of Smad 7 and impaired TGF- β 1 in keratinocytes [59]. It sounds that the timing and the interaction of dermis with epidermis govern the outcome effect of TGF- β .

There is significant crosstalk between the dermis and epidermis during wound healing as re-epithelialization and dermal reconstitution are not completely independent processes [60]. TGF- β signaling is crucial to this crosstalk [60]. Secretion of TGF- β 1 by fibroblasts influences keratinocyte behavior [57], and in turn, keratinocytes can downregulate TGF- β 1 expression in fibroblasts [61]. Fibroblast-specific deletion of the TGF- β 2 receptor leads to several different changes in re-epithelialization [56]. There is increased keratinocyte proliferation and increased epidermis size which is associated with increased keratin 5 expression [56]. It should be noted that there have been inconsistencies in the reporting of the speed of re-epithelialization in mice with fibroblast-specific deletion of TGF- β 2 receptor. One study showed faster re-epithelialization [56], while another found no significant changes between wild-type and null mice [55]. One factor that may account for conflicting findings is the use of a metal stents to fix the wound periphery in the first study which allowed them to limit wound contracture and focus on re-epithelialization. The use of these stents may have changed the trajectory of healing as mice primarily rely on contraction to heal as opposed to re-epithelialization like humans. Like the aforementioned studies, epidermal TGF- β signaling has been shown to influence dermal wound healing. The temporal expression of dermal collagen is different during wound healing in mice that over-express Smad7 in keratinocytes [59]. Researchers also observed effects on inflammatory cells and angiogenesis as well. To summarize, these studies provide support for the notion that the epidermis and dermis communicate with each other during wound healing and TGF- β signaling is a mediator between the two compartments.

Like Wnt/ β -catenin signaling, excessive TGF- β 1 activity leads to the formation of hypertrophic scars. Interestingly, these fibrotic mechanisms of TGF- β 1 may occur through mechanisms mediated by β -catenin and/or connective tissue growth factor (CCN2) [30, 31]. In mesenchymal-like cells treated with TGF- β , β -catenin protein levels increase in a

dose-dependent manner [37]. Furthermore, TGF- β and Wnt regulate each others ligand production and the Smad/ β -catenin/Lef complex regulates a number of shared target genes between the two signaling pathways [62]. Despite this cross-talk, there are some differences in how each pathway regulates fibroblast behaviour in wound repair. TGF- β regulates the induction of the collagen lattice contraction, whereas β -catenin regulates cell motility [63]. Embryonic wounds from rats have lower TGF- β 1 levels and higher TGF- β 3 levels leading to scar-free wound healing [64-66]. In fact, application of TGF- β 1 to embryonic skin wounds produces scarring that is similar to adult wound healing [64]. TGF- β 3 potentially can serve as anti-fibrotic effects, however the mechanisms have yet to be elucidated. A rodent wound model which treated wounds with TGF- β 3 has shown reduced scarring [67], however another study showed that TGF- β 3 accelerates wound healing but does not alter scarring [68]. The molecular mechanisms involved are likely modulation of matrix production, cell movement, and reduction of inflammatory mediators [69]. The use of TGF- β 3 as an anti-scarring remedy remains controversial. A clinical trial reported that pharmaceutical administration of TGF- β 3 showed accelerated healing in phase I/II studies testing [70], however, phase III trials were unsuccessful later. Both Wnt/ β -catenin signaling and excessive TGF- β 1 activity can contribute to hypertrophic scarring through various processes and common target genes (Figure 5).

Notch

Notch signaling pathway during skin homeostasis regulates epidermal differentiation and vascular maintenance [71]. Its role in wound healing is less characterized, but blocking Notch expression delays wound healing and administration of Notch ligand accelerate wound closure [72]. Notch signaling is necessary during the inflammatory response of wound healing. Mice lacking a Notch allele (Notch1^{+/-}) have reduced macrophage recruitment and TNF- α expression [73]. Notch1 regulates vascular endothelial growth factor 1 (VEGF1) expression and inflammatory cytokine expression in macrophages in vitro [73]. Notch signaling has also been shown to mediate adhesion of bone marrow-derived vascular cells to the ECM, thus facilitating matrix formation [74]. These results suggest that Notch expression during the inflammatory phase is crucial for wound healing or there will be delayed wound healing [72]. Lastly, Notch interacts with Wnt/ β -catenin and sonic hedgehog, two important signaling pathways in wound healing [75]. The exact mechanisms by which Notch promotes healing is not as well defined as Wnt/ β -catenin.

Sonic Hedgehog (Shh)

Like Notch, Sonic Hedgehog (Shh) signaling its role in wound healing is not fully understood, however, it does play an important role in skin development [76]. Inhibiting Shh signaling with cyclopamine results in delayed wound closure due to compromised wound vascularity and cell proliferation in mice [77]. In diabetic mice that experience deficient wound healing, Shh treatment is beneficial. Researchers have shown that Shh treatment increases wound vascularity and cellularity [78]. An increase in VEGF expression and recruitment of endothelial progenitors was also observed [78]. The mechanisms of Shh in wound healing need to be further elucidated. Since little is known about the role of Notch and Shh signaling in skin fibrosis, further studies are needed, particularly in transgenic

mouse models and patients, to determine whether inhibition of Notch or Shh and/or their target genes lead to curative strategies for hypertrophic scarring.

ras/MEK/ERK Signaling

TGF- β induces its fibrotic response in part due to the activation of the ras/MEK/ERK which leads to induction of CCN2, collagen expression, and matrix contraction [79-84]. Interestingly, ERK activation is essential for CCN2 induction [84, 85] and CCN2-dependent fibrosis [86]. Similar to TGF- β , transcription factors that act downstream of ERK are ets-1 and Smad 1 [87, 88], and blocking TGF- β induced ERK activation has anti-fibrotic effects [85, 89, 90]. The emerging importance of ras/MEK/ERK signaling in fibrosis is illustrated by evidence showing ERK activation is increased in bleomycin model of lung fibrosis. While the literature in this pathway is mainly focused on fibrotic responses, it is important to unravel the role of this pathway during scar formation in burn patients or burn injury animal models.

Scar Management in Burn Injury – Current Treatments

The time to healing is correlated with burn wound depth and many superficial (partial thickness) burns heal without hypertrophic scarring. These wounds are washed with water and chlorhexidine and covered with a wound dressing for a week. Deeper (full-thickness) burns are managed differently. Currently, the standard of care for patients with deep burn wounds is early excision and coverage with split-thickness skin autograft. Early excision also prevents infection which is correlated with survival. In many cases, debridement is necessary with an bromelain-containing agent that dissolves dead tissue [91]. Dermal substitutes are also used to replace the burned dermis [92]. These substitutes are composed collagen and elastin and are degraded later to allow for the infiltration of new cells [92]. Optimising the current skin substitutes or developing new skin substitutes may not only decrease mortality of burn patients but also may alleviate the morbidities such as scar formation [93]. Patients still experience significant pain which may be associated with compromised wound healing due to systemic pain signaling.

Deeper (full-thickness) burns take longer to heal so there is increased risk of hypertrophic scarring in these patients. This is because deep cutaneous injuries such as burn injury activate dermal fibroblasts that produce a large amount of collagen and inflammatory cytokines such as TGF- β 1, a known player in fibrosis [94]. As a result, there is increased synthesis of ECM components like elastin and collagen by fibroblasts which provide a scaffold for cell migration and vascularization [94]. Furthermore, fibrocytes that have migrated from other locations differentiate into fibroblasts and secrete more TGF- β 1 [46]. Transformation of fibroblasts to myofibroblasts causes the wound to contract, and the high tension and stretching caused by myofibroblasts increases the likelihood of scar formation [46]. In other models of wound healing, scar formation is correlated with the extent of damage and type of damage. For instance, wounds caused by Picosecond IR Laser (PIRL) causes less damage to tissue surrounding the wound and leads to less scarring [95].

Burn wounds that do not heal within 3 weeks have been found to be at high risk for scar formation [96]. A comprehensive study evaluation over 500 pediatric burn patients found that time to healing was positively correlated with hypertrophic scoring [97]. That is, the longer a wound takes to heal the greater the likelihood a hypertrophic scar will develop. Recent advances have increased the accuracy with which the potential for hypertrophic scarring can be predicted. Laser Doppler imaging is the most commonly used method that has improved diagnostic accuracy [98]. This technique can predict the time to healing, inform wound management, and predict if the surgical intervention will be needed [98]. While the usage of laser Doppler imaging is encouraging, it is not 100% accurate in determining which wounds should be excised and which will heal without scarring. In this section, we will discuss surgical methods and non-surgical interventions currently used to prevent scar formation.

Surgical

Full-thickness burns are usually excised 24-48 hours' post injury or debrided [5]. Burns covering greater than 30% TBSA are covered with cadaver or porcine skin to cover the allograft and excised wounds [99]. With larger skin grafts the time to complete re-epithelialization is increased, and the risk for hypertrophic scarring becomes higher. Smaller scars can be treated successfully with excision and closure that repositions the skin in a way that minimizes tension [100]. For larger wounds, closure may not be possible, and the use of skin grafts and dermal substitutes leads to challenging reconstructive problems [92]. To combat hypertrophic scarring after burn injury, Allotransplantation is commonly used. Allotransplantation is a reconstructive plastic surgery method where tissue (muscle, skin, blood vessels, nerves etc.) from a donor is used to restore the burned tissue of the patient [101, 102]. The major disadvantage to this technique is immune rejection, therefore deregulating the immune response of the recipient is crucial for successful transplantation [101, 102]. In pediatric patients, allotransplantation of solid organs has had great success with 80% of patients surviving into young adulthood [103]. In contrast, allotransplantation in burn patients is difficult due to anti-HLA sensitization [104]. The effect of allografts on scars is not entirely clear and not well defined. However, others and we hypothesize that an early excision and grafting is beneficial for scar development and that early autograft is even more beneficial. Surgical procedures are also used for reconstruction or removal of scars and keloids, which we would consider last resource as the outcome is not always guaranteed.

Non-Surgical Procedures

Various forms of non-surgical interventions are used to manage scarring post burn injury. These include laser therapy, compression garments, and injection of adipose-derived stem cells into the scar and drug delivery. Typically, a combination of these techniques is used to provide the best outcome possible for patients. If these non-surgical techniques are ineffective, then surgical interventions are usually performed to correct any defects or abnormalities.

Laser therapy has recently been used to manage scar formation and it has become the standard of care in many burn centers. The advantages to using laser therapy include:

increasing the pliability of the scar, decreasing scar height, reduced pain, and improvement of color and texture [105, 106]. Pulsed dye lasers reduce scar volume by targeting and destroying microvessels to reduce scar vascularity [105, 106]. This is an effective treatment option because it relieves symptoms like itching, redness, and pain [107, 108]. Interestingly, pulsed dye lasers reduce the expression of TGF- β 1, MMP13, and CCN2, key mediators of the fibrotic response [109, 110] and can be an effective treatment option relieving additionally associated symptoms like itching, redness, and pain. Recently, to alter collagen formation, laser tissue welding (LTW) has been utilized [111]. Laser energy is directed to wound margins causing them to fuse. As a result, collagen fibers are fused and intertwined leading to an immediate wound seal [112, 113]. This promotes faster re-epithelialization and smaller formation of granulation tissue [111, 114]. Carbon dioxide lasers are used to ablate collagen and cause fibroblasts apoptosis allowing for natural collagen formation, however, rates of reoccurrence are 90% [37, 38]. While lasers are a promising intervention to manage scar formation, additional research and clinical trials are needed to elucidate the mechanisms of laser therapy and confirm its effectiveness.

Compression therapy is used to reduce blood flow and manipulate collagen remodeling leading to a reduction of scar size [115, 116]. Some studies have shown decreased scar thickness and scar flexibility, whereas a meta-analysis of several studies suggested there are no significant differences in scar morphology with compression therapy [117]. In burn injuries, scar massage (compressed air) is commonly used to reduce pain and itching, but studies illustrating the efficacy of this technique are absent [118]. Moreover, it is recommended that pressure garments are worn for 23 hours a day until the hypertrophic scar matures which could take up to 2 years [5]. This is a laborious task; therefore, studies are needed to optimize treatment strategies to improve efficacy and practicality. Injection of fat into the wound area is another method of scar management [119]. This procedure enhances wound closure, reduces scar height, and scar flexibility [119, 120]. This is likely due to the presence of adipose-derived stem cells, which have been shown to improve wound healing in acute and complex wounds. To reduce scarring post burn, lipids and blood cells are removed from the fat [120]. This processed fat is then injected into the wound every 8-12 weeks [121]. Although not as common as the laser therapy or compression therapy, the autologous fat transfer is a promising technique to improve wound healing and prevent scar formation post burn. Secretome of mesenchymal stem cells has been used for wound healing study and scar management. In animals, while the secretome of umbilical cord mesenchymal stem cells enhance wound healing [122], it did not have beneficiary effect on scar fibroblasts like human keloid fibroblasts [123].

Nutrition

A key factor in scar management after burn injury that may be overlooked when trying to prevent scar formation is nutrition. Nutrition plays an important role in scar management post burn. Patients that are malnourished are at a greater risk for delayed wound healing, infections, and pressure ulcers [124]. Furthermore, several important processes in wound healing such as the proliferation of fibroblasts, collagen formation, immune cell function, and angiogenesis may be hindered [125, 126]. These notions are supported by the fact that patients with chronic wounds are commonly malnourished [124]. The Wound Healing

Society and the American Society for Parenteral and Enteral Nutrition recommended a caloric intake of 30-25 kcal/kg/day for optimal wound healing [124, 127]. This recommendation increases in underweight patients to 35-40 kcal/kg/day [124, 127, 128]. Older patients who often suffer from delayed and insufficient wound healing require an individualized nutrition plan. This is especially important in elderly burn patients that have higher morbidity and mortality compared to younger individuals. Indeed, optimizing the nutrition plan of at-risk patients using oral nutrition supplements (250-500 kcal per serving) greatly reduces the incidence of pressure ulcer development by 25% in at-risk patients such as the elderly [129].

Multivitamin supplementation is recommended for patients with large wounds [124, 130, 131]. Vitamin C is important for supporting the immune system, facilitating collagen deposition and promoting re-epithelialization during wound healing [125, 130, 132]. For severe wounds such as burn injury, 1000-2000 mg/day orally of vitamin C is recommended and vitamin C supplement is even recommended for patients with chronic wounds [130, 133]. The risk of kidney stone formation with high doses of Vitamin C should be considered, however [124]. Remarkably, Vitamin A supplementation (10,000-15,000 IU/day) has been shown to reverse delayed wound healing in patients taking steroids [20, 25]. Because elderly burn patients experience delayed inflammatory response and wound healing, vitamins may help slightly in augmenting this response.

Managing protein intake after burn is important for wound healing. Protein is essential for the formation of connective tissue, synthesis of enzymes for wound healing, the proliferation of cells, and lastly collagen formation [125, 128, 129, 131, 134-140]. The amino acid glutamine is crucial for the synthesis of amino acids and nucleotides which may have implications for the correct function of epithelial cells and fibroblasts [125, 130]. Protein intake is suggested at 0.8 g/kg/day in adults and 1 g/kg/day for elderly; this intake is increased for the healing of chronic wounds (1.25-1.5 g/kg/day) [126, 130, 132]. In situations of cachexia such as in burn injury, the recommendation may be higher [126, 130]. Protein intake should be monitored due to the risk of dehydration with higher intakes [125, 130].

Drug Delivery

Pathway Targeted Drugs

Wnt/ β -catenin Targeted Drugs—Pharmacological manipulation of the Wnt/ β -catenin signaling pathway, specifically β -catenin activity, could be a promising therapeutic approach in the treatment of hypertrophic scars. As mentioned previously, β -catenin is essential for appropriate wound repair, but excessive β -catenin activity is associated with hypertrophic scars and keloids in humans [33, 154]. Furthermore, the level of β -catenin activity is positively correlated with scar size in wound healing [30]. R-spondin2 is a Wnt/ β -catenin agonist that has been shown to thicken the epidermis *in vitro* which is an effect that is not ideal in scarring [155]. In contrast, Nefopam (5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine) is a centrally-acting, non-opioid analgesic that reduces β -catenin to baseline even when cells are stimulated with Wnt ligand [156]. Nefopam's effects are specific to hypertrophic wounds as it decreases cell proliferation and viability of hypertrophic scar

fibroblasts while leaving normal fibroblasts unaffected [156]. In a murine model of skin fibrosis, Nefopam treatment for 14 days reduced scar diameter by 50% compared to control group [156]. The exact mechanisms by which Nefopam decreases β -catenin levels and inhibits hypertrophic fibroblasts is unknown [156]. Targeting the downstream complex of TCF/ β -catenin may be useful in reducing hypertrophic scars. Antagonists that inhibit protein-protein interactions between TCF and β -catenin effectively reduce β -catenin transcriptional activity. The anti-fibrotic ability of two small molecules, PKF118-310 and ICG-001, have been evaluated in bleomycin-induced dermal fibrosis [157]. PKF118-310 inhibits TCF and β -catenin, whereas ICG-001 prevents the recruitment of β -catenin co-activators [157]. Treatment with these small molecules reduced Axin-2 mRNA expression which led to the inhibition of Wnt signaling, thereby reversing bleomycin induced fibrosis [157].

In hypertrophic scarring and other fibrotic diseases, Wnt is upregulated, whereas inhibitors of the pathway are downregulated [158]. While targeting β -catenin might be the most obvious method of reducing fibrosis, modulating Wnt activity is a delicate approach since β -catenin has different roles in different cell type that contribute to skin healing and scar formation. Recently, targeting Specific Wnt and/or Wnt receptors has received significant attention in several cancers [158]. In mouse models of prostate cancer, intraperitoneal injections of Wnt3A-neutralizing antibodies decreases proliferation and induces apoptosis [159]. Similarly, targeting Frizzled receptors with antibodies has shown effectiveness in targeting tumors in preclinical models of colon, breast and liver cancer, and leaves normal tissue unaffected [160, 161]. OMP-18R5 is a monoclonal antibody that specifically binds to Frizzled receptor 7 (FZD7) which blocks Wnt signaling induced by multiple different Wnt ligands [162]. OMP-18R5 inhibits the growth of tumors in patient-derived mouse xenograft models and reduced the potential for new tumor growth [162]. Overexpression of the Wnt inhibitor, Dkk-1, ameliorated fibrosis *in vitro* in various models that mimic different stages of human disease [163, 164]. Dkk-1 may be an attractive target for treating hypertrophic scarring [163, 164]. The lesson learned from these studies in cancer can be examined in the context of wound healing and scar formation as several cancers-particularly sarcomas- share similar pathway activation such as Wnt/ β -catenin activation [165-168].

Inhibition of various intracellular mediators of related to Wnt is another method of reducing Wnt signaling. Another mechanism of decreasing Wnt signaling is targeting the PDZ domain of Disheveled (DVL). Three different compounds (NSC 668036, FJ9 and 3289-8625) have been shown to inhibit Frizzled receptor-PDZ domain interactions *in vitro* [169]. Molecules that stabilize the level of Axin and promote phosphorylation-dependent degradation of β -catenin are called tankyrase inhibitors [170]. Tankyrase is an enzyme that regulates the level of Axin in the β -catenin destruction complex [170]. XAV939 is a tankyrase inhibitor that has been shown to reduce Wnt signaling and reduce bleomycin-induced lung fibrosis in mice [171]. There is a risk of intestinal toxicity when using tankyrase inhibitors, therefore the use of these drugs is limited [172]. Using GSK3 β inhibitors such as lithium chloride to pharmacologically manipulate Wnt signaling is a promising therapeutic approach in several pathologies including skin fibrosis [173-177]. Furthermore, GSK3 β is important in the inflammatory response, therefore inhibitors of GSK3 β also provide anti-inflammatory properties that may be beneficial in pathological

conditions associated with inflammation such as hypertrophic scarring [178]. Despite the wealth of knowledge known about this pathway, there has been little research that has attempted to modulate this pathway for managing scar formation after burn injury.

TGF- β Pathway Targeted Drugs

Excessive TGF- β 1 and growth factor activity leads to hypertrophic scarring in humans, thus pharmacological interventions that target TGF- β 1 may have therapeutic benefit. Targeting growth factors such as fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor (PDGF) may influence TGF- β 1 activity. This is because fibroblasts secrete FGF-2, PDGF, and TGF- β 1 during the healing process [179]. Moreover, the effectiveness of each growth factor may be dependent on its relative concentration to the other growth factors present [179]. FGF-2 is expressed at the wound site and is functionally important for wound healing. Mice lacking all three isoforms of FGF-2 have delayed wound healing [180], and transgenic mice lacking FGF-2 specifically in keratinocytes have delayed re-epithelialization [181]. In chronic wounds that show deficient healing human recombinant platelet-derived growth factor-BB (PDGF-BB; Becaplermin) is used to improve healing [182]. In three multicenter prospective randomized control trials, topical Becaplermin treatment (100 μ g/g) was found to be safe, well-tolerated, and easily used by diabetic ulcer patients [183-185]. Most importantly, Becaplermin treatment had higher incidence of complete wound healing illustrating its potential in improving wound healing [183-185]. Perhaps developing antagonists of growth factors like TGF- β 1 and PDGF-BB can help reduce scarring post burn injury.

Scleroderma is a systemic autoimmune disorder characterized by skin fibrosis [186]. The molecular mechanisms underlying this pathology are similar to hypertrophic scarring, therefore treatments aimed at scleroderma may be useful in scar management after burn injury. Similar to hypertrophic scarring, increased expression of TGF- β 1 and TGF- β 2 leads to excess ECM deposition and scarring in sclerosis [186]. Therapeutic approaches to scleroderma are focus on inhibiting TGF- β activity. CAT-192 is a TGF- β 1 neutralizing antibody that showed no evidence of efficacy in the treatment of patients with early-stage systemic scleroderma [187]. Another TGF- β 1 inhibitor, GC-1008 (fesolimumab), is improved symptoms and reduced expression of TGF- β 1 target genes in patients with systemic sclerosis [188]. Lastly, topical application of the P144 peptide inhibitor of TGF β 1 blocks bleomycin-induced skin fibrosis in mice [189] and improves scars in a human hypertrophic scar model in nude mice [190]. P144 is currently undergoing phase II clinical trials and a press release claims it has shown some efficacy in reducing skin fibrosis associated with systemic sclerosis [191]. Intradermal injection of TGF- β 3 improves scar appearance [192].

Tamoxifen citrate is a drug that has shown promising results in the treatment of hypertrophic scars and keloids [193, 194]. Tamoxifen is a selective estrogen receptor modulator (SERM) that is used to treat breast cancer, but in the context of scarring it is believed to down-regulate TGF- β [195, 196]. Several other drugs such as calcium channel blockers, calcineurin inhibitors, retinoids, and IL-10 modulators are thought to have potential in

treating hypertrophic scars and keloids. However, results are contradictory or inconclusive with low power making clinical use limited [197].

Notch Targeted Drugs

In murine models of systemic sclerosis (SSc), inhibiting Notch via the γ -secretase inhibitor DAPT and by overexpression of a Notch-1 antisense construct, prevents bleomycin-induced fibrosis [198]. Notch inhibition also led to regression of established fibrosis [198]. Moreover, several studies show that inhibiting the Notch pathway leads to attenuation of fibrosis in the liver, kidney, and heart [199-201]. Recent evidence indicates that Notch signalling regulates myofibroblast differentiation in fibrosis of the lung [202-204], liver [205-207], kidney [208-210], heart [211, 212], and skin [198, 213]. These studies suggest that Inhibiting Notch signaling may be a viable technique for reducing scarring. There are currently four different strategies used to control the Notch pathway in fibrosis, and some of these may be viable to use in hypertrophic scarring. These strategies include blocking activation of 1) Notch receptors γ -secretase inhibitors, 2) targeting the Notch ligand Jagged-1 directly, 3) inhibiting Notch signal transduction, and lastly 4) targeting the Notch intracellular domain (NICD). Several of them have been demonstrated to attenuate fibrosis. DAPT is a γ -secretase inhibitor that inhibits liver fibrosis [214]. Another γ -secretase inhibitor, RO4929097, reduces liver fibrosis by modulating hepatic cell differentiation [215]. The use of γ -secretase inhibitor systemically through oral administration is limited by side effects such as cytotoxicity in the gastrointestinal tract. However, they have low cost and the risk of gastrointestinal side effects can be avoided through the local topical application of γ -secretase inhibitor [216]. Targeting the Notch ligand Jagged1 with shRNA in pulmonary endothelial cells reduces lung fibrosis and repairs the lung [217]. The vitamin D analogue, paricalcitol, inhibits Notch signaling by attenuating TGF- β 1 induced Smad2 phosphorylation and reducing the upregulation of Jagged1 [218]. Targeting the NCID is the only approach that has been attempted in skin fibrosis [198]. Using a Notch1 antisense construct, researchers showed targeting the NCID prevents experimentally induced fibrosis in mice [198]. Notch3 shRNA [219] and microRNA's mir-148 and mir-34a [215, 219] have been utilized to specifically target NCID in fibrosis of other organs. Several plants produce the phytoalexin, resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), in response to injury [220]. Interestingly, this compound has been shown to protect against liver fibrosis by suppressing Notch1 and Notch3 gene expression [221]. Whether resveratrol has potential in treating hypertrophic scarring is unknown. Like resveratrol, several other drugs such as scutellarin [222], dibenzazepine [223], and olmesartan target Notch signal transduction [224]. A major disadvantage is the specificity as these drugs may indiscriminately inhibit many signaling pathways in different cell types, leading to unwanted side effects. Increasing the specificity and affinity for Notch is extremely important. Further studies are needed to elucidate the therapeutic potential of these drugs in scar management.

Shh Targeted Drugs

The role of Shh in wound repair and skin fibrosis is relatively unexplored when compared to its role in skin development. Currently, the most effective way to target Shh pathway is modulating Smoothed (SMO) via agonists or antagonists. Binding of Shh to the

transmembrane protein PTCH leads to the de-inhibition of SMO allowing SMO to regulate target genes [76]. Several drugs have been developed that target SMO. Itraconazole is a competitive antagonist that inhibits SMO [225, 226]. Targeting Gli1 through Gli1 siRNA or Trisenox (arsenic trioxide) inhibits cell growth and promotes apoptosis by inhibiting Shh signaling [227-229].

ras/MEK/ERK Targeted Drugs

Although more research is needed into unraveling the mechanisms of ras/MEK/ERK in hypertrophic scarring, some studies have illustrated ERK inhibition may have therapeutic benefit. Moreover, treatment with the highly selective inhibitor of MAP/ERK kinase1 (MEK1) activation, PD98059, blocks bleomycin-induced lung fibrosis [230]. In another model of lung fibrosis in which TGF- α is overexpressed, the MEK inhibitor ARRY-142886 (ARRY) could prevent TGF α induced cell proliferation and matrix production, thus mitigating fibrosis [230]. Recent studies suggest that ras/MEK/ERK signaling might be a viable therapeutic target in fibrotic diseases such as hypertrophic scarring.

NADPH oxidase enzymes

Recent studies have implicated reaction oxygen species (ROS) in the progression of fibrosis [231-236]. Normally, ROS production by phagocytic cells helps defend against pathogens. However, in the context of chronic pathology like fibrosis, ROS production is persistent and promotes the production of TGF- β 1, fibroblasts survival, and differentiation of fibroblasts to myofibroblasts [232, 233, 237]. ROS production is caused by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), and several of these Nox enzymes have been shown to promote fibrosis. In hypertrophic scars, the final step of cross-linking is mediated by hydroxyallysine which results in the formation of pyridinoline cross-links [238]. These crosslinks are resistant to degradation leading to excessive accumulation of collagen that is characteristic of hypertrophic scars. There is some evidence that Nox enzymes are involved in the formation of these pyridinoline cross-links [239]. After exposure to Nox-derived ROS, human normal skin ex vivo forms pyridinoline cross-links [239]. This is further supported by the finding that pyridinoline cross-links can be reduced *in vivo* after treatment with the antioxidant, catalase [240]. Perhaps inhibition of NOX-induced ROS may prevent the formation of these irreversible pyridinoline cross-links making the management of hypertrophic scars easier. Nox-enzymes can be modulated by inhibiting the migration of cytosolic subunits to the cell membrane or by competitively inhibiting Nox with molecules that mimic NADPH. Nox activation is dependent on the translocation of the cytosolic subunit, Rac1, to the cell membrane [241]. Statins are a group of lipid-lowering (primarily cholesterol) compounds which may prevent this cytosolic migration. The statin, pravastatin, has been shown to reduce dermal thickening, collagen deposition, and inflammation in mice with chronic graft-versus-host-disease (CGVHD) [242]. Another therapeutic strategy of targeting Nox enzymes is competitive inhibition. GKT137831 is a small molecule that is structurally similar to NADPH, and it is highly selective for Nox1 and Nox4 [243]. Furthermore, it has a low toxicity profile, lacks antioxidant activity, and is highly specific [244]. Thus far, GKT137831 is currently being considered for use in phase I clinical trials for the treatment of diabetic nephropathy [241, 245, 246]. In several preclinical

models, GKT137831 has been shown to be effective in treating idiopathic pulmonary fibrosis, liver fibrosis, and diabetic nephropathy [236, 247-249].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

To suppress inflammation, non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed as oral medication [250]. These drugs work by inhibiting the aromatase activity of Cox-1 and Cox-2 which are needed for the synthesis of pro-inflammatory cytokines like IL-1 β and prostaglandins [251, 252]. Because of their anti-inflammatory effects, NSAIDs can reduce pain and swelling during the acute phase of skin injury [253]. Additionally, NSAIDs like Diclofenac inhibit DNA synthesis of bacteria, thus they have mild antibacterial effects in addition to their anti-inflammatory properties [254]. In the context of wound healing, NSAIDs may be detrimental as may target the essential inflammatory phase for skin healing. Administration of aspirin during wound healing in mice is associated with impaired wound healing [255]. Moreover, Diclofenac treatment leads to inhibited collagen deposition in the granulation tissue of mice [251]. The mechanism by which NSAIDs inhibit collagen deposition is likely the inhibition of MMPs which are crucial for the early phases of wound healing [256-258]. Since inflammation is crucial in the early stages of wound healing, perhaps administration of NSAIDs during the remodeling phase of wound healing will attenuate hypertrophic scarring post burn. NSAIDs may also function by inhibiting Wnt/ β -catenin signaling, as such this might be another potential mechanism by which they exert their effects [259, 260]. In patients with familial adenomatous polyposis [261], a disease associated with dominant mutations in the APC gene and overactivation of Wnt/ β -catenin signaling, NSAIDs reduce polyp formation [262, 263]. Therefore, NSAIDs may be beneficial in reducing Wnt/ β -catenin signaling which may attenuate hypertrophic scarring in burn patients.

Corticosteroids

Intralesional corticosteroid injections have been commonly used to treat scars since the 1960's. The use of corticosteroids leads to regression of hypertrophic scarring and keloids, but this treatment is not 100% effective [142]. Studies have shown that steroid injections reduce inflammation in the wound which decreases collagen synthesis and glycosaminoglycan synthesis, and decreases fibroblast proliferation [264, 265]. An insoluble version of triamcinolone acetonide (TAC) at a concentration of 10 to 40 mg/ml is the most regularly used treatment. TAC can be combined with lidocaine to reduce pain that accompanies injection [266]. Randomized prospective studies have shown that injected TAC leads to a patient response rate of 50-100% and recurrence rates range from 9-50% [266, 267]. A study by Manuskiatti and Fitzpatrick found that TAC injections alone led to the clinical improvement of keloids and hypertrophic scars with no significant side effects. TAC treatment alone showed comparable results to TAC + 5-fluorouracil and pumped pulsed-dye laser (PDL) treatment [266]. Overall, steroid injection is an effective treatment for keloids and it remains a second-line therapy for hypertrophic scarring. The method of delivering steroids needs to be improved as injection leads to significant pain for patients [268]. Furthermore, 63% of patients experience other side effects such as hypopigmentation which

may develop into lymphogenous uptake of corticosteroids crystals [269, 270]. Subcutaneous fat loss and unresponsiveness are also common [271].

Antihistamines

Histamines released by basophils and mast cells of the immune system and are important mediators of the inflammatory response of wound healing [272-274]. Histamines play a role in restoring the mechanical integrity of the skin and clearing pathogens [275]. Histamine induces wound closure in vitro through the expression of histamines receptor 1-4 [275], and keratinocytes express several histamine receptors [276-278]. Keratinocytes exposed to histamines up-regulate genes involved in the migration of epithelial cells such as $\beta 5$ -integrin which leads to a dose-dependent acceleration in re-epithelialization of keratinocytes [275, 279]. Antagonists of histamine receptor 1 lead to less itching and are commonly used to treat pruritus [280]. Like NSAIDS, use of antihistamines reduces the inflammatory response which may impair wound healing. Administration of histamine receptor 1 blockers delay wound healing and impairs proliferation [281]. The anti-inflammatory and anti-proliferative may be useful in the treatment of hypertrophic scars after burn injury [282, 283]. The histamine antagonist, Tranilast, reduces the synthesis of collagen in keloid fibroblasts by suppressing the release of TGF- $\beta 1$ [284]. Diphenhydramine is another antihistamine that reduces the growth of fibroblasts in culture by 60%. Lastly, Pheniramine reduces DNA synthesis, collagen synthesis, and proliferation rate in fibroblasts derived by scars [284]. Overall, these studies suggest that the administration of antihistamines in the later stages of wound healing might prevent scarring by impairing the potential of fibroblasts to cause a fibrotic phenotype through altering proliferation [284].

Immunosuppressants and Immunomodulators

Intralesional steroid injections are commonly used to treat hypertrophic and keloid scars. Steroid injections are effective because they destroy collagen bundles and inhibit fibroblast growth [285]. A study evaluating the effectiveness of intralesional injection of triamcinolone acetonide in managing scar formation found that the treatment group had a 64% reduction in scar size and a 72% reduction in symptoms [286]. When steroid treatment was combined with beta-radiation and surgical scar removal reduction of the scar was achieved in 75% of patients [286]. Several more immunosuppressants and immunomodulators can be used to augment the inflammatory response and proliferation of cells involved in fibrosis.

Interferons (INFs) are immunomodulators that inhibit fibroblast collagen production [287]. One study reported that intralesional injection of INF- γ led to a significant reduction in keloid size in 6 out of 8 patients [288]. Another interferon, INF- α , reduces the recurrence rates of keloids from 58% to 19% when used in conjunction with scar excision [289]. While promising, interferons have high costs and adverse side effects like the flu are a risk [285]. A class of drugs termed Imidazoquinolines (e.g. imiquimod) are immunomodulators that activate toll-like receptors 7 and 8 leading to production of cytokines such as INF- α [287, 290]. Topical application of 5% imiquimod on keloids after scar operation led to no recurrence in all 8 patients after 12 months [291]. Another study found that post-surgical topical administration of 5% imiquimod led to a recurrence rate of 80% [292]. Altered

pigmentation is a common side effect of Imidazoquinolines [293]. Similarly to interferons, Bleomycin sulphate is a drug that works by inhibiting collagen synthesis by fibroblasts in the skin [294]. A study showed that injection of bleomycin sulphate into hypertrophic scars that were unresponsive to steroid treatment, 73% (11 out of 15) showed reduced scar size and patient symptoms lessened [295]. Unfortunately, 3 patients experienced hyperpigmentation and moderate skin atrophy [295] which is a common outcome in bleomycin treatment [296]. Several classes of immunomodulators exert their effects by impairing DNA synthesis in fibroblasts leading to reduced proliferation [297] [298] [299]. Fluorouracil is a cancer drug that competitively inhibits thymidylate synthesis thereby blocking DNA synthesis and fibroblast proliferation [297]. The active version of 5-Fluorouracil is suggested to increase fibroblast apoptosis by inhibiting DNA synthesis in rapidly proliferating cells [300]. A combination therapy of pulsed laser dye treatment and intralesional injection of 5-fluoruracil showed the highest success rate in patients compared to monotherapy [151]. Several studies highlight the effectiveness of intralesional injection of 5-fluoruracil in reducing scar size [301, 302]. In 1999, Fitzpatrick was the first to report that 5-Fluorouracil treatment reduces scar size [303]. Moreover, in a prospective randomized trial, 28 patients with keloids were given weekly injections of 5-Fluorouracil (50 mg/ml) for 12 weeks [304]. There was a reduction in scar size in the majority of patients and there was no reoccurrence of symptoms after a 24 week follow-up period [304]. Once again, hyperpigmentation is a side effect along with pain and tissue sloughing [285]. Methotrexate is a drug that inhibits dihydrofolate reductase, an essential enzyme for DNA synthesis [298]. Research on Methotrexate is limited, however, one study found that Methotrexate suppresses keloid reappearance after revision surgery, and no adverse effects were seen in a follow-up period of 10 years [305]. A third drug, Mitomycin C, cross links DNA strands impairing DNA replication [299]. Keloid fibroblasts treated with mitomycin C *in vitro* results in decreased DNA synthesis and cell density [306]. Topical application of mitomycin C after surgical excision of keloids led to 90% of patients avoiding keloid recurrence [299]. Another study in 8 patients showed that surgical therapy combined with topical mitomycin C treatment led to complete recurrence in 2 patients [307]. In contrast, a study by Sanders et al. showed that mitomycin C treatment after surgical incision had no beneficial effect in 15 patients [308]. The contradictory nature of these studies may be a result of small study power and short follow-up times. Moreover, there is tremendous variation in dosing and application intervals of mitomycin C which may also have accounted for the discrepancies observed [197].

Vitamins

Retinoids synthesized from Vitamin A are used in some cancers (e.g. acute promyelocytic leukemia) as a therapy and chemoprevention [158]. Whether Vitamin A can be utilized to treat hypertrophic scars is unknown. On the other hand, Vitamin D in its active form, 1 α ,25-dihydroxyvitamin D3, has chemopreventative effects through its modulation of Wnt/ β -catenin signaling [146]. The mechanism of inhibition has not fully elucidated, but it is thought that active vitamin D compete with transcriptional co-activators p300 and CBP which inhibits β -catenin [145-147]. Recently, it has been suggested that vitamin A and

Vitamin D induce the activity of Wnt/ β -catenin inhibitors such as Disable homolog 2 (DAB2) and DKK1 and DKK4, respectively [146].

One of the main mechanisms vitamins may attenuate skin fibrosis is through their antioxidants capabilities. While vitamins C and E are recognized as antioxidants, research has suggested they may not be effective in modulating skin fibrosis. A clinical investigation involving scleroderma patients found that oral vitamin E supplementation had no benefits [144]. Pentoxifylline, a theobromine methyxanthine analog, has been shown to inhibit the proliferation of human-derived fibroblasts in keloids and scleroderma [142]. Interestingly, a small clinical study involving 12 SSC patients found that treatment with Pentoxifylline and vitamin E significantly reduced scar thickness with minimal side effects [143]. The antioxidant nature of vitamins makes them a promising approach for managing hypertrophic scars. It may be possible that combined topical therapy comprised of vitamins and competitive Nox inhibitors discussed previously will lead to synergistic antioxidant effects that reduce scar formation.

Phytochemicals for wound healing

Curcumin

Various biologically active compounds in plants hold potential for supporting wound healing and prevent scarring. These biologically active compounds are termed phytochemicals. One of the most well studies phytochemicals for wound healing is curcumin, a chemical present in the spice turmeric. Curcumin's main mechanism of action is its anti-inflammatory properties. Curcumin suppresses TNF- α and IL-1 production in human macrophages in vitro [309], and curcumin is also a potent inhibitor of phosphorylase kinase (PhK) and NF- κ B activation [310, 311]. This makes curcumin a great phytochemical candidate for the treatment of hyper-inflammatory wounds such as chronic diabetic wounds and burns. Curcumin has been shown to be effective in treating hyper-inflammatory chronic wounds. In diabetic rats, topical curcumin leads to faster re-epithelization, increased migration of fibroblasts to the wound bed, higher collagen content, and improved vascularization [312]. Curcumin improves vascularization through upregulation of VEGF [313]. Curcumin's anti-oxidant properties may also be useful in managing scar formation. Transdermal application of curcumin in rat excisional wounds reduces reactive oxygen species (ROS) damage in fibroblasts and keratinocytes [314]. Another study showed the topical application of curcumin reduces lipid peroxidase levels and upregulates the production several anti-oxidant enzymes such as catalase, glutathione peroxidase, and superoxidase dismutase in rat excisional wounds [315].

Curcumin may hold potential in treating hypertrophic scarring post burn. In hypertrophic scarring, there is excessive TGF- β expression, fibroblast proliferation, and excess collagen and ECM synthesis [123]. Interestingly, curcumin inhibits TGF- β in keloid fibroblasts and reduces ECM production [316]. Moreover, fibroblasts isolated from keloid and hypertrophic scar patients have reduced proliferation when treated with curcumin [317]. Lastly, curcumin has been shown to inhibit TGF- β signaling in scleroderma, another fibrotic skin disease similar to hypertrophic scarring [318]. These studies indicate that curcumin may be a viable treatment for hypertrophic scarring post burn. Indeed, there have been 6 case reports of

curcumin gel used to improve wound healing and prevent scarring in patients post surgery [8], but randomized controlled trials are needed to determine the efficacy of this treatment. One standard first-line therapy for hypertrophic scarring is intralesional steroid injections which have potent immunosuppressive effects. Because curcumin's mechanism of action is similar to steroids, perhaps curcumin may be effective in attenuating scar formation in human trials. The main challenge is the use of curcumin is how the drug is delivered. Curcumin is a hydrophobic compound; hence dermal delivery is negligible [319]. As a result, different formulations have been created to enhance its topical effectiveness [8]. These formulations include polymetric bandages [320], collagen films [321], and alginate foams [322]. A delivery method that holds tremendous promise is lipid-core nanocapsule (LCN) which has been shown to significantly enhance dermal delivery of curcumin [323]. Recently, the use of curcumin-loaded chitosan nanoparticles [324], hyalosomes [319] and hydrogels [325] have been developed that show promise. Interestingly polyphenols found in plants such as quercetin, epigallocatechin-3-gallate (EGCG), resveratrol, and curcumin might be inhibitors of the Wnt/ β -catenin signaling pathway [326-330]. The exact mechanisms are unknown and this inhibitory effect might lack specificity as it acts on multiple pathways [326-330]. These polyphenols can inhibit Wnt signaling cascade through redox regulation of transcription [331].

Honey

Honey is a traditional medicine that is used as a topical treatment for chronic wounds and burns in many countries [332]. Honey has anti-bacterial effects making it useful in preventing microbial penetration in burns [333]. This is because the avascular wound bed is an ideal environment for microbial growth making burn injury particularly vulnerable to infection [334]. Aside from its anti-microbial effects, honey has immunomodulatory effects and promotes angiogenesis and fibroblast proliferation in human clinical trials [335]. Chronic inflammation is an issue in hypertrophic scarring. Honey exerts its anti-inflammatory effects through reducing TNF- α and IL-6 expression, inhibiting COX-2 and inducible nitric oxide synthase (iNOS) [336]. The polyphenol Caffeic acid phenol ester (CAPE) found in honey suppresses MMP2 and MMP2 in fibrosarcoma cells which may reduce degradation of ECM [337]. Honey also suppresses MMP2 and MMP9 in other cell types such as human glioblastoma multiform cell lines [338]. These antiinflammatory properties may be useful in the later stages of wound healing to reduce persist inflammation associated with hypertrophic scarring. Additionally, honey possesses anti-oxidant properties due to the prescience of various compounds such as flavonoids, phenolic acids, catalase, peroxidase, carotenoids ascorbic acid that counteract the abundance of free-radicals in fibrotic wounds [339, 340]. Most clinical trials utilizing honey focus on the treatment of chronic wounds that do not heal as opposed to hypertrophic scars. A study involving 59 patients with chronic ulcer wounds showed increased epithelialization in response to honey treatment [341]. Another study examining 30 patients with diabetic foot ulcers found complete healing in 43.3% of patients and only 6.7% did not respond to honey treatment [342]. In the context of burns, rat models of partial-thickness burn injuries reduced re-epithelialization time and increased wound contraction [343, 344]. In humans, a meta-analysis of 8 randomized-controlled trials found that honey was superior to silver

sulphadiazine-impregnated gauze in superficial and partial-thickness burn wounds [345]. Interestingly, honey could possibly be used as a preventative measure against hypertrophic scarring. A randomized-controlled trial in 104 patients with superficial burns found there was a significantly lower incidence of hypertrophic scarring in parities treated with honey in comparison to silver sulphadiazine [346].

Terminalia genus

The genus *Terminalia* is comprised of over 200 species of trees, and throughout history has been used as a medicine in Africa, Asia, and Australia [347]. Some *Terminalia* species have antioxidant properties and antimicrobial ability. Some studies have even shown the species *T. arjuna* and *T. chebula* to improve wound healing in rat excisional wounds [348, 349]. *T. arjuna* may hold potential in improving wound healing because it contains tannic acid (TA), a plant polyphenol that stabilizes collagen and elastin in the ECM. TA does this by inhibiting MMP collagenases while improving collagen cross-linking [350, 351]. Another species, *T. chebula*, enhances ECM deposition in granulation tissue in rat excisional wounds [352], and extracts from this species enhances keratinocyte and fibroblast growth *in vitro* [353]. Moreover, rat excisional wounds show reduced lipid peroxide levels which was confirmed by electronic spin resonance (ESR)-2,2, -diphenyl-1-picrylhydrazyl (DPPH) assays, illustrating the antioxidant role of *T. chebula* [352], which was confirmed by electronic spin resonance (ESR)-2,2, -diphenyl-1-picrylhydrazyl (DPPH) assays [352, 353]. *T. chebula* promotes angiogenesis through upregulation of VEGF expression leading to new vessel formation in rat models of wound healing [348]. While these effects of *T. chebula* are promising in improving wound healing, its anti-inflammatory properties may be useful in treating hypertrophic scars. Chebulagic acid (CA), an anti-oxidant compound extracted from *T. chebula*, significantly suppresses NF- κ B activation, and TNF- α and cyclooxygenase-2 (COX-2) expression when cultured with macrophages [354]. Topical application of *T. chebula* may be beneficial in hyper-inflammatory wounds such as burns. This is supported by the fact that *T. chebula* extract accelerates wound healing when compared to 1% silver sulfadiazine in burned rats [355].

Other plant-based sources of wound care

Phytochemicals seem to be used for wound healing in traditional medicine globally due to their anti-inflammatory and antioxidant activity. *Chromolaena odorata* is found in southern Asia and is commonly used to treat wounds in Vietnam. This plant promotes wound contraction [356] and promotes proliferation of fibroblasts *in vitro* [357]. *Chromolaena odorata* also protects fibroblasts and keratinocytes from oxidative damage caused by hydrogen peroxide [358]. Like *Chromolaena odorata*, *Bridelia ferruginea*, is widely used in Nigeria as a wound healing remedy due to its antioxidant properties and its ability to stimulate fibroblast growth [359]. Various other African plants such as *Ficus asperifolia* and *Gossypium arboreum*, possess similar properties and are traditionally used in Ghana [360].

Several phytochemicals have shown efficacy in animal models of wound healing after burn injury, however, only a handful have been used in human trials [361]. Aloe vera has been studied in animals and humans. Topical application of A. vera has been shown to reduce

inflammatory cytokines such as TNF- α and IL-6 in rat burn models [362]. In addition to its antiinflammatory properties, A. vera increased re-epithelialization in rat [363, 364] and guinea pig burn models [365]. Two randomized controlled trials involving partial-thickness or second-degree burn patients has shown that topical application of A. vera was superior to 1% silver sulphadiazine cream in promoting wound contraction and re-epithelialization [366, 367]. The use of phytochemicals to treat burn wounds and prevent scar formation is promising. Additional studies with more patients should investigate the efficacy of phytochemicals in treating burn wounds and whether they are useful in preventing hypertrophic scars compared to more traditional methods.

Gene Therapy

Gene therapy is the delivery of genes into a patients' cells to treat disease. In theory, it might be a promising method to cure numerous diseases including hypertrophic scarring. DNA genotyping of burn patients suffering from hypertrophic scars has provided some information on the genetic component of scarring. A study identified a variant of CUB and Sushi multiple domains 1 (CSMD1) gene as an indicator of less severe scarring in a group of burn patients [368]. Little is known about how this gene variant affects hypertrophic scarring, but it possibly is associated with inflammation and/or TGF- β 1 signaling [369]. The protein produced by CSMD1 is elevated in head and neck cancers [370] and is thought to be a tumor suppressor [371]. Further studies are needed in burn patients to identify novel gene variants that can be targeted to attenuate post-burn hypertrophic scarring. Finding the essential genes- gatekeepers- that contribute to the initiation and/or progression scar formation may pave the road toward the gene therapy of this disease.

Methods of Improving Drug Delivery (Transdermal Drug Delivery)

Transdermal Drug Delivery (TDD) is an alternative approach to oral delivery of drugs and hypodermic injection. The main goal of TDD is to ablate the skin stratum corneum layer to increase the permeability of drugs. The clinical use of TDD has increased over the past few years because of its ability to deliver small, lipophilic, low-dose drugs. While several delivery systems have been developed, here we will focus on thermal ablation, electroporation, and microneedles which have shown the most potential.

Thermal Approaches (Laser and Radio-Frequency Heating)

Thermal approaches heat the surface of the skin which removes the stratum corneum without damaging deeper tissues, thus allowing for the delivery of drugs. Because high temperatures are needed to achieve removal of the stratum corneum, the thermal exposure should be short. The most commonly used methods are laser and radiofrequency mediated thermal ablation of the skin. Thermal ablation greatly enhances the delivery of lipophilic and hydrophilic drugs into the skin lasers. The use of laser therapy alone in the management of hypertrophic scarring provides variable results. Laser therapy can be combined with different topical treatments to improve drug delivery and the penetration topical formulations. This is called transdermal drug delivery (TED), and it is a promising method of drug delivery. One example is the use of YAG or CO₂ laser to create microchannels in the

epidermis which increases drug permeability in the skin [372]. A study utilized ablative fractional radiotherapy (RF) to open microchannels in the epidermis before application of triamcinolone [372]. Triamcinolone is a synthetic glucocorticoid steroid that is used to treat keloid and hypertrophic scars via intralesional injection [373]. Complete resolution of scarring in the head and neck was observed after four sessions of triamcinolone treatment combined with RF [372]. A similar study treated hypertrophic scar patients with ablative fractionated CO₂ combined with a topical poly-L-lactic acid (PLLA) [374]. PLLA is an injectable filler commonly used to correct facial volume through subcutaneous injection. PLLA works by stimulating fibroblast proliferation and collagen synthesis [374]. It is reported that after 3 months of treatment, four blinded investigators evaluated the scars based on the Modified Manchester Scar Scale and found that 95% of the scars had improved significantly [374]. Overall, transdermal drug delivery is a promising technique to increase the efficacy of steroids and other treatments while minimizing the risk of localized atrophy and irregular appearances commonly observed in steroids such as triamcinolone [372, 373].

Microneedles

Another method of increasing transdermal drug delivery in scar management is the use of microneedles [375]. Microneedles are not invasive and can selectively permeabilize the stratum corneum [375]. Microneedles have been shown to increase skin permeability to a wide variety of molecules and protein without causing pain [376]. This increase in skin permeability by microneedles allow for drugs to not only travel across the epidermis but also the superficial dermis. Furthermore, water-soluble microneedles can deliver coated or encapsulated cargo during the insertion process [377]. Various compounds can be stored within the needle and these microneedles dissolve in the skin after several minutes [377]. The advantages of microneedles are plenty when compared with other transdermal delivery methods. Microneedles are minimally invasive, there is little chance for contact with skin nerves limiting pain, and microneedles can be manufactured out of biocompatible and biodegradable polymers [378, 379]. Moreover, with microneedle use the size of the drug is not an issue as microneedles can deliver small or large molecular weight compounds [380]. One study showed that microneedle treatment increases TGF- β 3 collagen fiber integration in scars from burn patients [381]. Another study reported that keloids from burn patients respond to microneedle treatment, however, these were inactive keloids [382]. More studies are needed to test the effectiveness of microneedles in treating hypertrophic scarring in burn injury in well planned randomized clinical trials.

Electroporation

Historically, electroporation has been used to reversibly disrupt the cell membrane for gene transfection. To electroporate the skin, electric currents are applied to the skin for milliseconds allowing for the passage of materials through the small electropores created [383]. This allows for the transdermal transport of small molecule drugs, peptides, and DNA [383]. It is shown that electroporation can control the density of hypertrophic scar fibroblasts *in vitro* [384]. Researchers used intermittently delivered pulsed electric fields (IDPEF) every 72 hours for 12 days and found the optimal stimulation that controls fibroblast density without causing excess cell death [384]. This finding is important because deregulation of

fibroblasts during scarring leads to uncontrolled growth and delayed apoptosis. Perhaps electroporation can be used as a method of controlling excess cell growth during scarring after burn. Recently, irreversible electroporation has been shown to prevent scar formation in burned rats, providing some *in vivo* evidence that electroporation might be an interesting route to explore [385]. Although electroporation has been used extensively in animals, the use of electroporation in humans is mainly limited by the complexity of designing a safe device. Moreover, it is possible that deeper tissues that contain sensory and motor neurons can be hit by the current leading to pain and muscle stimulation [383].

Conclusion

In this review, we covered a myriad of studies that highlight the importance of targeting the molecular pathways of wound healing that become dysfunctional during hypertrophic scar formation. Current approaches such as early surgical intervention and various non-surgical techniques have shown some efficacy and improvement in patient satisfaction. However, these approaches are inadequate in reducing or preventing scar formation. Ideally, developing treatments that improve wound healing and prevent scarring are preferred as opposed to simply managing symptoms. Emerging evidence points to drug delivery targeting molecular signaling as a promising avenue for treating hypertrophic scarring that holds therapeutic potential. Targeting molecular pathways such as Wnt/ β -catenin, TGF- β 1, Notch, Shh, and ras-MEK-ERK have been shown to attenuate fibrosis in various animal models (Table 3). Continued research is needed to further elucidate the molecular mechanisms of hypertrophic scarring so that novel preventative strategies can be developed through drug delivery. Once these drug delivery methods have passed animal and preclinical studies, well-designed randomized controlled trials are needed to confirm the efficacy of these treatments in improving scarring post-burn.

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Figure 1.

A) Phases of wound healing during normal physiologic skin healing. B) Hypertrophic scarring is characterized by a prolonged proliferation phase during wound healing which leads to excess ECM deposition.

Figure 2. Schematic illustrating each phase of wound healing with important cell types and genes involved in regulating this well-orchestrated process.

Figure 3.

In the absence of Wnt ligand, the transcriptional co-activator β -catenin is degraded by the cytoplasmic destruction complex composed of axin, glycogen synthase kinase (GSK) 3 β , and adenomatous polyposis coli protein (APC). The binding of Wnts to frizzled (FZD) receptor transduces a signal across the plasma membrane activating Dishevelled (DVL) protein. DVL inhibits the cytoplasmic destruction complex resulting accumulation of β -catenin in the cytoplasm. β -catenin then enters the nucleus and modulates gene transcription.

Figure 4.

Transforming growth factor (TGF)- β is normally bound to latent TGF- β binding proteins. During wound healing, conformational changes caused by α_v integrins and mechanical strain release TGF- β allowing it to bind to serine/threonine kinase TGF- β receptor I and TGF- β -receptor II heterodimers. This causes the phosphorylation of Smads 2 and 3 and their translocation to the nucleus where they modulate gene expression along with other transcription cofactors.

Figure 5.

Hypertrophic scarring is mainly caused by alterations in collagen synthesis, ECM degradation/remodeling, and fibroblast behavior during wound healing. Several target genes of the Wnt/ β -catenin and TGF- β 1 signaling pathways have been identified as directly involved in these processes.

Table 1

Molecular Pathways Involved in Wound Healing and Scarring.

Molecular Pathway	Role in Wound Healing	Role in Scarring
Wnt/ β -catenin Signalling	- Increases epidermal stem cell numbers and promotes hair follicle differentiation and regeneration in large wounds. - Increases fibroblast numbers, fibroblast migration, matrix production in the reconstitution of the dermis.	- Overexpression of stabilized β-catenin in mouse fibroblasts leads to enlarged and hypercellular dermis - Conversely, loss of β-catenin accelerated wound healing resulting in reduced numbers of fibroblasts and hypocellularity.
TGF- β Pathway	- Fibroblast proliferation, myofibroblast transformation, wound contraction, matrix production during proliferation and remodelling phase. - Inhibits re-epithelization.	- Excessive TGF-β1 activity leads to excessive myofibroblast activation and is associated with hypertrophic scar formation. - TGF-β3 is highly expressed in fetal wound healing and might have anti-scarring properties.
Notch	Not as well documented as Wnt/β-catenin and TGF-β, but role in macrophage behavior and angiogenesis.	N/A
Sonic Hedgehog (Shh)	- Regeneration of hair follicles. - Vascularity and cellularity of the wound.	N/A
ras/MEK/ERK Signaling	Interacts with TGFβ to induce CCN2 expression, collagen and matrix deposition.	Blocking ERK activation prevents lung fibrosis.

Table 2

Modifying nutrition to improve wound healing and attenuate scarring.

	Wound Healing	Scarring
Vitamin A	Improve delayed wound healing [20, 25]	N/A
Vitamin E	Anti-oxidant [141]	Anti-oxidant, reduce scar thickness [142], [143], [144]
Vitamin D	Vitamin D deficiency associated with impaired healing chronic leg ulcers	Inhibit Wnt/ β -catenin signaling [145-147]
Vitamin C	Promote re-epithelialization, anti-oxidant [125, 130, 132]	N/A
Zinc and Copper	Delayed wound healing when deficient [141, 148]	Reduced zinc levels in patients with scars [149]
Omega-6 and Omega-3	Alter pro-inflammatory cytokine production [150, 151]	Nutritional deficiency of Omega-3 correlated with keloids [152, 153]

Table 3

Summary of potential drugs targeting different molecular pathways involved in hypertrophic scarring.

Molecular Pathway	Drug	Effect	Species
Wnt/β-catenin	R-spondin2	Thicken epidermis in vitro [386].	Human [386].
	Nefopam	- Decreases cell proliferation and viability of hypertrophic scar fibroblasts [156]. - Nefopam treatment for 14 days reduced scar diameter by 50% in murine model of fibrosis[156]. - Decreases β-catenin levels [156].	Mouse [156].
	PKF118-310	Inhibits TCF and β-catenin [157].	Mice [157].
	Dkk-1	Wnt inhibitor shown to attenuate fibrosis <i>in vitro</i> in various animal models of fibrosis in different organs [163, 164].	Mice [163, 164].
	ICG-001	Prevents the recruitment of β-catenin co-activators [157].	Mice [157].
	OMP-18R5	Monoclonal antibody that specifically binds to Frizzled receptor 7 thus inhibiting Wnt signaling [162].	Human tumor xenografts in mice [162].
TGF-β1	Becaplermin	Randomized control trials utilizing topical Becaplermin improves wound healing and increases the incidence of complete healing [183-185].	Human [183-185].
	Fesolimumab	Reduces expression of TGF-β1 target genes in patients with systemic sclerosis [188].	Human [188].
	P144	- Blocks bleomycin-induced skin fibrosis in mice [189]. - Improves scars in a hypertrophic scar model in nude mice [190].	Mice [189, 190].
	Tamoxifen citrate	Down-regulates TGF-β [195, 196].	Human [195,196].
Notch	γ -secretase	Inhibiting Notch via the γ-secretase inhibitor, DAPT, prevents bleomycin-induced skin fibrosis [198].	Mice [198].
	Jagged1 shRNA	Reduces lung fibrosis and repairs the lung. Not used in skin [217].	Mice [217].
	Resveratrol	Protect against liver fibrosis by suppressing Notch1 and Notch3 gene expression. Not used in skin [221].	Rat [221].
Sonic Hedgehog	Trisenox	Inhibits cell growth and promotes apoptosis by inhibiting Shh signaling [227-229].	Mice [227-229].
	Itraconazole	Competitive antagonist that inhibits Smoothed (SMO) [225, 226].	Mice [225, 226].

Molecular Pathway	Drug	Effect	Species
	Cyclopamine	Competitive antagonist of SMO [76].	Mice [76].
ras-MEK-ERK	PD98059	Blocks bleomycin-induced lung fibrosis. Not used in skin fibrosis [230].	Mice [230]
	ARRY-142886γ	Prevents TGF-α induced cell proliferation and matrix production in lung fibrosis. Not used in skin [230].	Mice [230]