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Scarless wound healing: Transitioning from fetal research to regenerative healing

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Abstract

Since the discovery of scarless fetal skin wound healing, research in the field has expanded significantly with the hopes of advancing the finding to adult human patients. There are several differences between fetal and adult skin that have been exploited to facilitate scarless healing in adults including growth factors, cytokines, and extracellular matrix substitutes. However, no one therapy, pathway, or cell subtype is sufficient to support scarless wound healing in adult skin. More recently, products that contain or mimic fetal and adult uninjured dermis were introduced to the wound healing market with promising clinical outcomes. Through our review of the major experimental targets of fetal wound healing, we hope to encourage research in areas that may have a significant clinical impact. Additionally, we will investigate therapies currently in clinical use and evaluate whether they represent a legitimate advance in regenerative medicine or a vulnerary agent.

Keywords

fetal wound healing; fibrosis; regenerative medicine; scarless; scarring; wound healing

1 | INTRODUCTION

Though skin scarring is the normal and inevitable outcome of a full thickness dermal injury, many people fail to recognize the implications of fibrosis over true regenerative healing. In

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CONFLICT OF INTEREST

Michael T. Longaker is a founder and has an equity position in Neodyne Biosciences, Inc.

FURTHER READING

Atala, A., Lanza, R., Thomson, J., & Nerem, R. (2011). Scarless wound healing. In *Principles of regenerative medicine* (2nd ed.). London: Elsevier.

This article is categorized under:

Adult Stem Cells, Tissue Renewal, and Regeneration > Regeneration Plant Development > Cell Growth and Differentiation Adult Stem Cells, Tissue Renewal, and Regeneration > Environmental Control of Stem Cells

the skin and most organs, large enough insults in the form of traumatic wounds, surgical incisions, burns, major disease processes, or infections result in the deposition of collagen and recolonization with local cells that fail to recreate normal organ architecture. In the kidney, scarring of the nephrons leads to irreversible changes in glomerular filtration rate. In the liver, fibrosis leads to gradual accumulation of serum toxins as the organ loses its enzymatic ability. In the skin, scarring results in disfigurement, contractures, and at times severe psychological trauma. Skin healing can be "normal," resulting in a fibrotic and organized scar devoid of sensory, hair, and glandular appendages. Alternatively, the healing can be dysfunctional, leading to the more serious consequences of under and over healing, including ulceration and fibroproliferative scarring.

Skin scarring constitutes an enormous burden on individual patients and society. Every year, 100 million patients acquire scars from surgery in the developed world (Brown, McKenna, Siddhi, McGrouther, & Bayat, 2008). Patients with visible scars, particularly on the face, suffer from social stigma and psychological trauma (Brown et al., 2008; Hunt, Burden, Hepper, Stevenson, & Johnston, 2006). In the United States 500,000 patients per year are treated for burns, many of which leave scars and painful contractures that require major surgery (Asuku, Ibrahim, & Ijekeye, 2008; Egeland, More, Buchman, & Cederna, 2008). The total annual cost of medical care for burns in the United States is $7.5 billion (Finkelstein, Corso, & Miller, 2006), and much of this cost is related to the treatment of burn scars and contractures. Children are particularly affected by scarring, and can suffer from long-term physical dysfunction (Esselman, 2007; Sheridan et al., 2000) and psychological harm (Robert et al., 1999; Thomas et al., 2012; Van Loey & Van Son, 2003). Expectedly, there is an enormous market for consumer anti-scarring treatments, estimated at $12 billion annually in the United States (Sen et al., 2009). However, most commercial wound healing products fail to initiate skin regeneration.

In the 1970s, experiments in fetal lambs demonstrated the ability to heal skin without scarring in early gestation (Burrington, 1971). Since this discovery, several other animals including sheep, rats, mice, pigs, monkeys, and humans have demonstrated the same remarkable ability (Gurtner, Werner, Barrandon, & Longaker, 2008; Rowlatt, 1979). In each instance, cutaneous wounds made prior to the transition to the adult skin scarring phenotype resulted in complete organ regeneration, including the development of dermal appendages (Walmsley et al., 2015). In a subsequent series of reciprocal translocation experiments, tissue transplanted between postnatal and fetal animals implicated a cell intrinsic mechanism to the scarless wound healing phenomenon (Longaker et al., 1994; Lorenz et al., 1992). Since then, characterizing fetal wound healing has achieved deserved attention with the hopes of identifying the cells or cell signaling pathways that drive scarless wound healing in adult tissue (Buchanan, Longaker, & Lorenz, 2009; McPherson et al., 1988; Nwomeh, Liang, Diegelmann, Cohen, & Yager, 1998; Shah, Foreman, & Ferguson, 1995; Walmsley et al., 2015; Whyte et al., 2013; You & Han, 2014). For patients, scarless skin wound healing would allow complete functional recovery from burns, surgical incisions, and major tissue loss from trauma or other causes. In the pediatric population, this would mean contractures limiting normal growth and development could be avoided. As such, research into the mechanism behind fetal scarless wound healing has expanded significantly.

Below, we discuss the known mechanisms of adult fibrosis and fetal scarless wound healing. From there, we will investigate the growth factors, cytokines, enzymes, and cell-based strategies that attempt to achieve scarless wound healing in adults; whether through fetal mimicry or dampening the fibrotic response. Through our discussion, we hope to elucidate promising clinical targets and critically evaluate therapies currently in practice.

2 | MAMMALIAN SKIN ANATOMY AND EMBRYOLOGY

To understand skin wound healing, a basic understanding of skin anatomy and embryology is necessary. The skin, or integumentary system, is a complex organ that provides a moisture and microbial barrier to the outside world. It is divided into several basic layers that can then be subdivided further by cellular differentiation and cell types.

The most superficial layer of the skin is the epidermis. The epidermis is formed from a thin layer of embryonic ectoderm. After neuralization occurs in the fetus, the single cell layer of simple ectoderm expands to become a simple squamous epithelium known as the periderm. Eventually, the differentiating epithelium stratifies into distinct layers; the stratum germinativum/basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (also known as the horny layer, or cornified layer). The outermost layer (stratum corneum) sheds and sloughs off in adults. In the fetus, the development of the stratum corneum leads to the eventual shedding of the periderm.

The epidermis contains a deep layer of cells, known as basal cells, that serve as a stem cell reservoir for the development and differentiation of keratinocytes. Keratinocytes get their name from keratin, the characteristic protein of the epidermis. Keratinocytes begin their lifecycle at the basement membrane and gradually migrate up through the epidermis to the surface of the skin; where they expel their nuclei and flatten to form a stratified squamous epithelium.

Below the epidermis lies the dermis, a thick layer rich in extracellular matrix (ECM) proteins. The dermis houses the base of epidermal appendages, such as hair follicles, sweat glands, and also houses dermal fibroblasts. Fibroblasts deposit both normal and scar ECM proteins, which give the skin its tensile strength. Additionally, nerves and muscles are found in the dermis. The dorsal, ventral, and cranial dermis have different embryonic origins, though all mesodermal. The ventral dermis is derived from the somatic layer of lateral plate mesoderm while the cranial dermis is derived from the neural crest. In the dorsal dermis, cells are derived from the dermamyotome subdivision of somites. During fetal development, the dermis develops upward projections toward the epidermis known as dermal papillae, while the epidermis develops downward projections into the dermis, known as epidermal ridges (rete ridges).

From the epidermal ridges, several specialized epidermal structures develop, including hair, sebaceous glands, accrine sweat glands, apocrine sweat glands, mammary glands, teeth, and nails. Sebaceous glands secrete sebum, an oily substance that lubricates the skin and hair. Accrine sweat glands are widely distributed and function to secrete thermoregulatory sweat,

while apocrine sweat glands secrete odorous substances and are located mostly in the axilla and pubic regions in humans.

Hair is formed by an epidermal ridge that is indented at the bottom by a dermal papilla. Overlying the dermal papilla is the germinal matrix, a proliferating ectoderm that forms the base of the hair bulb. The germinal matrix undergoes a specialized keratinization process which leads to the development of a hair shaft that protrudes through the inner and outer epidermal root sheath. Hair color is produced by melanocytes secreting melanin at the base of the hair bulb. Progenitor cells for the hair follicle are located within the hair follicle bulge region. These cells differentiate and migrate from the bulge downward into the follicle to participate in hair follicle growth phases.

Sebaceous glands in most parts of the body are associated with and bud off of the hair follicle shaft as a diverticula. The gland then expands into the dermis where it branches to form secretory acini or alveoli. In sebaceous glands, the proliferating stem cell population resides in the basal layer. The basal layer stem cells then divide and differentiate to supply the gland with a constant source of new mature secretory epithelial cells.

Below the dermis is the hypodermis. The hypodermis is a layer of subcutaneous fat containing the skin's perforating blood vessels and adipose tissue. Blood vessels in the skin arise from deeper muscle tissue and then penetrate the dermis and epidermis. In the dermis and epidermis, blood vessels give off fine capillary tributaries to supply skin cells with necessary oxygen and nutrients.

In the skin, other specialized resident cells include Langerhans cells (which function as antigen-presenting cells) and melanocytes (which give the skin and hair color from the production of melanin). Melanin also serves as a natural sunscreen. Merkel cells also are present in the epidermis; where they act as pressure-sensitive mechanoreceptors. Lastly, scattered inflammatory cells like macrophages and mast cells scavenge debris and perform additional roles as resident antigen-presenting cells (Figure 1; Driskell et al., 2013; Forni, Trombetta-Lima, & Sogayar, 2012; Fuchs, 2007; Ge et al., 2017; Schoenwolf & Larsen, 2009; Zhu et al., 2014).

3 | ADULT SKIN HEALING

Our understanding of the formation of scar tissue is inseparably linked to the process of normal wound healing. In clinical and experimental practice, specific elements of the wound healing process are usually targeted to reduce scar formation. However, it is likely that every event in the wound healing cascade contributes in some way to the eventual formation of scar tissue.

Human skin wound healing is often described in three overlapping stages: inflammation, proliferation, and remodeling (Gurtner et al., 2008). The inflammatory phase involves the influx of a diverse array of cells, including neutrophils, macrophages, and lymphocytes, that cleanse the wound space of bacteria, debris, and dead cells (Gurtner et al., 2008). These cells also release a mixture of cytokines that regulate the behavior of other cells and allow the wound healing process to continue (Box 1).

The inflammatory phase is followed by the proliferative phase, during which fibroblasts create and organize collagen and other ECM molecules to form new tissue. In adult wounds, new collagen is laid down in parallel tightly spaced bundles. This is a hallmark of scar tissue, and is quite unlike uninjured tissue, where collagen exists in a looser, basket-weave configuration (van Zuijlen et al., 2003). Another characteristic of human scar tissue is that the dermal appendages in normal skin, including hair follicles and sweat glands, do not regenerate and are not present (Gurtner et al., 2008). The absence of these appendages may have implications for the regeneration of tissue during healing, since stem cells residing within these structures contribute to skin regeneration under homeostatic and injured conditions (Plikus et al., 2012; Figure 2).

The final remodeling phase of wound healing takes place over months to years and involves the gradual structural evolution of the scar tissue. Scar tissue initially contains high levels of type III collagen, but during the remodeling phase it is gradually replaced with type I collagen (Monaco & Lawrence, 2003). Scars that are initially raised, hyperpigmented, and pruritic may lose these characteristics over time (Baum & Arpey, 2005). Additionally, scars contract to reduce their overall size. Lastly, the tensile strength of scarred skin increases as remodelling occurs, but never reaches the full strength of uninjured skin (Levenson et al., 1965).

4 | FETAL SCARLESS HEALING

Surgical incisions in a mammalian fetus heal rapidly without scar formation (Burrington, 1971) and are nearly indistinguishable from uninjured tissue (Beanes et al., 2002). In 1979, the same observation was reported in human fetuses (Rowlatt, 1979). Fetal scarless healing was likely seen as a minor biological curiosity until the 1980s and 1990s, when it was recognized as a study vehicle through which scarless adult healing could be achieved (Adzick & Longaker, 1992). The most significant advances were made in the late 1980s through a series of experiments delineating the differences between adult and fetal wound healing in lambs (Longaker et al., 1990, 1991; Longaker, Chiu, et al., 1989; Longaker, Harrison, Crombleholme, et al., 1989; Longaker, Harrison, Langer, et al., 1989; Longaker, Whitby, et al., 1989). An initial hypothesis was that factors present in the fetal environment but extrinsic to the fetal tissue, such as complete sterility, growth factor-rich amniotic fluid, and low oxygen tension were responsible for scarless healing (Longaker & Adzick, 1991). However later experiments showed that these factors are not sufficient, and that scarless healing is intrinsic to fetal tissue (Longaker et al., 1994). Current efforts in the field of scarless healing are aimed at identifying factors intrinsic to fetal tissue that allow for scarless healing.

Inherently, fetal wound healing is difficult to study because the fetus develops in the protective and closed environment of the uterus. Fetal animal models are thus used extensively because of the difficulties in studying healing in human fetuses (Adzick & Longaker, 1991). Early studies in fetal wound healing noted several histological and chemical differences between healing fetal and adult wounds (Longaker & Adzick, 1991). Healing fetal wounds contain far fewer inflammatory cells compared with adult wounds (Longaker et al., 1990). The absence of these cells likely results in a very different

microenvironment with respect to cytokine and growth factor expression, and this may influence fibroblasts to lay down ECM molecules in a regenerative rather than scarring pattern. Hyaluronic acid is present in greater concentrations in fetal wounds (Longaker et al., 1989) and the ECM molecule fibronectin is deposited more rapidly (Longaker et al., 1989). These molecules likely interact with cells involved in healing and may encourage a regenerative phenotype. There is also a higher level of matrix metalloproteinase activity in fetal wounds, which may allow for early remodeling of skin wounds and scar collagen (Dang et al., 2003).

Fibroblasts deposit ECM molecules, whose configuration is an important element of regenerated versus scarred tissue. Therefore, it is possible that changes in fibroblasts populations or fibroblast phenotype in the wound play a role in the loss of scarless healing. *In vitro* studies show that fetal fibroblasts express more collagen III and IV compared with adult fibroblasts (Larson, Longaker, & Lorenz, 2010; Table 1). Additionally, fetal fibroblasts are capable of proliferating while simultaneously depositing collagen, whereas adult fibroblasts undergo proliferation in the wound and then later deposit collagen (Larson et al., 2010).

Rinkevich et al. recently reported a population of fibroblasts, defined by embryonic expression of the gene engrailed 1 *(Enl)* that are responsible for the production of normal and scar collagen in the dorsal skin of adult mice (Rinkevich et al., 2015). Initial comparisons of gene expression between *En1*-derived and *En1*-negative fibroblast populations revealed differences in a limited number of genes, including *Hoxc10* (homeobox C10), *Slit2* (slit guidance ligand 2), *Foxp1* (forkhead box P1), *Lepr* (leptin receptor R), *Mylk* (myosin light chain kinase), and *Acta1* (actin alpha 1). Of note, *En1* is only expressed early in development, and allows only for lineage tracing these cells. However, CD26 identifies the same population of *En1*-derived cells in adulthood, with 94% of *En1*-derived fibroblasts expressing CD26. Further studies will determine the extent to which these transcriptional differences contribute to regenerative versus scarring healing (Box 2).

5 | EVIDENCE OF ADULT SCARLESS WOUND HEALING

In adult mammals, the oral mucosa represents a model of regenerative healing, where injury results in minimal to no scar formation. While inflammation at the wound bed is known to contribute to the formation of a scar, the oral mucosa, and fetal skin are relatively immune privileged during wound healing (Walraven, Talhout, Beelen, van Egmond, & Ulrich, 2016). Additionally, in the oral cavity, salivary mucins act as barriers protecting the underlying mucosa from mechanical damage and direct interaction with pathogens and other harmful substances (Amerongen & Veerman, 2002; Wong et al., 2009).

Analysis of healthy human oral mucosa reveals significantly lower numbers of neutrophils and macrophages compared with healthy skin (Glim, Beelen, Niessen, Everts, & Ulrich, 2015). The attenuated inflammatory microenvironment of the oral mucosa may partially explain the reduction in scar formation. But also, ECM composition prior to wounding may be a major contributor to scarless healing. Relative to skin, the oral mucosa exhibits increased fibronectin and its splice variant ED-A (Glim, Everts, Niessen, Ulrich, & Beelen,

2014). Interestingly, the pro-fibrotic myofibroblast ACTA2 (smooth muscle alpha actin) expression relies on cell surface ED-A fibronectin interaction and TGFB1 to initiate scar contraction (Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002). Conversely, the presence of elastin is more pronounced in the skin than the oral mucosa.

6 | TARGETS OF SCARLESS SKIN REGENERATION

6.1 | Growth factors

6.1.1 | Wingless-type signaling—Wingless-type (WNT) signaling involves many highly evolutionarily conserved cell signaling pathways that control various phases of embryonic development. Though WNT activates three separate and independent signaling cascades, canonical WNT signaling has been the major focus of wound healing research and scarless fetal healing. This class of secreted glycoproteins, of which humans have 19 individual subtypes, acts on cells locally to regulate proliferation and differentiation (Leavitt et al., 2016). In the most basic signaling cascade, WNT binds to the frizzled cell receptor on WNT-responsive cells to initiate the transcriptional activation of β-catenin, a protein that once translocated to the nucleus in high concentrations forms a protein complex with TCF/LEF (T-cell factor/lymphoid enhancer factor). TCF then activates transcription of several growth factors involved in embryonic development, organogenesis, and tissue repair and regeneration (Houschyar et al., 2015).

In adult dermal tissue, many WNT proteins (WNT 1, 3, 4, 5, and 10)—both canonical and otherwise—are activated during normal wound healing, similar to cell proliferation signals for dermal fibroblasts and keratinocytes (Whyte et al., 2013). WNT responsive cells in dermal tissue include hair follicle bulge cells, basal interfollicular epidermal cells, and dermal fibroblasts. In each of these locations, WNT responsive cells represent a population capable of recruitment for re-epithelialization and the development of granulation tissue (Reya & Clevers, 2005). In mouse fetal skin, WNT4, WNT5a, and WNT11 are expressed in the dermis and play important roles in hair follicle morphogenesis (Whyte et al., 2013). Additionally, WNTs interact with a number of other important embryonic proteins, including SHH (sonic hedgehog), BMP (bone morphogenic protein), and NOTCH (Reya & Clevers, 2005).

Recently, the study of wound-induced hair follicle neogenesis, a phenomenon restricted to murine skin wound healing, revealed the role of FGF9 (fibroblast growth factor 9) and WNT2A in murine dermal wound healing (Gay et al., 2013). Through a signaling cascade initiated by resident dermal γδ T cells, mice can develop new hair follicles in the base of a scar likely through initiating a communication between epidermal keratinocytes and dermal fibroblasts (Gay et al., 2013). Coming from these studies, recombinant WNT proteins have emerged as a potential therapeutic (Whyte et al., 2013). Specifically, WNT3A has shown promising results as a vulnerary agent, where several studies demonstrated its ability to enhance healing of full-thickness cutaneous wounds (Whyte et al., 2013). However, WNT's usual obligate palmitate appendage often renders the protein hydrophobic and therefore unreliable when delivered to living tissues (Clevers, Loh, & Nusse, 2014). While still an attractive study candidate for scarless wound healing, reliable recombinant WNTs are difficult to generate (Carthy, Engstrom, Heldin, & Moustakas, 2016).

6.1.2 | Transforming growth factor beta—The transforming growth factor beta (TGFB) superfamily receives attention for its ability as a ubiquitous growth factor in mammalian wound healing. The TGFB group has three major subtypes, 1, 2, and 3, each intimately involved in the process of cell recruitment, proliferation, differentiation, migration, and potentially immune modulation (Lichtman, Otero-Vinas, & Falanga, 2016). These proteins, while having striking structural homology, are thought to exhibit different and potentially competing actions in the wound environment. *In vivo,* the TGFB subtypes have specific temporal and spatial distributions—potentially indicating their individual roles in wound healing and regenerative repair. Also, an important part of TGFB function is that it can bind to and be stored on the ECM, where until cleaved by serum proteases, it remains quiescent and as an indolent reservoir for augmenting wound healing when provoked by injury (Lichtman et al., 2016).

The TGFB subtypes 1 and 3 are perhaps the most important in wound healing, with TGFB1 being involved in the scarring mechanism and implicated in the development of systemic sclerosis, while TGFB3 is thought to be anti-fibrotic and pro-regenerative. TGFB1 also plays an important role in the early phases of adult wound healing after being released from activated platelets. Once released, TGFB1 recruits macrophages and other inflammatory cells to the wound where it functions as a pro-inflammatory mediator, ultimately perpetuating the fibrotic healing mechanism. TGFB3, however, may play a role in fetal scarless wound healing (Lichtman et al., 2016). In the adult wound environment, TGFB3 appears in the early and later phases of wound healing where it aids in macrophage recruitment, ECM deposition, and may reduce cell proliferation. In the fetal environment, many studies revealed elevated levels of TGFB3 relative to the amount of TGFB1 and TGFB2 seen in adult wound environments, potentially pointing to TGFB3 as a pro-regenerative cellular signal (Occleston et al., 2011). However, recent studies highlight the importance of each of the TGFB subtypes in adult and fetal wound healing. While proportions of TGFB subtypes do differ in fetal and adult wounds, attempting to alter these ratios does not significantly impact adult wound healing. In part, this may be because the TGFB subtypes have different functions in adult and fetal healing that are not explained by concentration or ratio alone (Penn, Grobbelaar, & Rolfe, 2012). Yet, adult wounds treated with TGFB1 inhibitors tend to heal with an improved appearance and smaller resultant scars, while those treated with recombinant TGFB3 reveal the same outcome (Shah et al., 1995).

Attempts at enhancing expression or delivery of TGFB proteins are unsuccessful to date. Between 2008 and 2011, Avotermin received press as a recombinant TGFB3 for improved scar formation in acute wounds and scar revisions (So et al., 2011). Unfortunately, this product failed phase III clinical trials and has subsequently been abandoned. Ultimately, study of the TGFB pathway is necessary for achieving a complete understanding of the wound healing mechanism. But given the involvement of TGFB in a number of complex cellular pathways (Hameedaldeen, Liu, Batres, Graves, & Graves, 2014), targeting TGFB as a wound repair therapeutic is problematic.

6.1.3 | Fibroblast growth factor 9—Another important group of growth factors in wound healing are the fibroblast growth factors (FGFs). FGFs target fibroblasts and several other cell types involved in wound healing to induce proliferation, differentiation, and cell

migration. In humans, 20 fibroblast growth factor subtypes exist, each structurally similar to the next but with a distinct biological function (Ornitz & Itoh, 2015). As FGF receptors are tyrosine kinases, they can directly influence intracellular activity, making them important in wound healing research (Potthoff, Kliewer, & Mangelsdorf, 2012).

In the fetus, FGFs are highly involved in organogenesis and cell differentiation. FGFs also control cell signaling cascades that influence the development of many organs including the limbs, lungs, brain, inner ear, liver, and pancreas (Ornitz & Itoh, 2015; Potthoff et al., 2012). Locally in the adult, FGFs influence wound healing by creating feedback loops to initiate fibroblast growth and differentiation. As is commonly the case, FGFs are closely associated with the ECM and are usually bound to heparin sulfate proteoglycans, limiting the growth factor's systemic potential (Ornitz & Itoh, 2015).

Though many FGFs are involved in wound healing and embryonic development, FGF9 has emerged as an exciting new target due to its role in wound-induced hair follicle neogenesis (Gay et al., 2013). Activation of cells by FGF9 often leads to downstream upregulation of other FGFs, including FGF10, and also upregulation of the WNT2A/β-catenin pathway (Ornitz & Itoh, 2015). Through these signaling cascades, FGF9 may be able to re-establish a communication between dermal and epidermal tissues, resulting in invagination of the epidermis and the eventual development of new hair placodes (Gay et al., 2013). As a result of these findings, current experimental treatments with FGF9 focus on male patterned baldness (Fan et al., 2011). However, FGF9 may also be an important new target for wound healing since it is the only small molecule capable of regenerating skin appendages.

6.1.4 | Hypoxia inducible factor 1 alpha subunit—The transcription factor hypoxia-inducible factor-1 (HIF1), composed of a dimer of an alpha and a beta subunit, plays a major role in cellular adaptation and survival under hypoxic stress. The subunits of HIF1 bind together to acquire transcriptional properties, allowing it to regulate the transcriptional activity of hundreds of genes that promote cell survival in hypoxic conditions. A master regulator of oxygen homeostasis, HIF1 acts predominantly under hypoxic conditions, such as in the wound bed with its disrupted vascular supply. The HIF1B subunit is constitutively expressed whereas the HIF1A subunit is oxygen regulated (Semenza, 2011a). Regulation of HIF1 is thus determined by the rapid posttranslational degradation or stabilization of the HIF1A subunit (Semenza, 2010). In normal tissue oxygen conditions, HIF1A is rapidly and continuously degraded following translation (Tamama et al., 2011). Tissue hypoxia, however, induces a sustained increase in the expression of HIF1A. Levels of HIF1A protein increase exponentially as oxygen concentration declines (Semenza, 2011b).

Pathologic scars, including keloid and hypertrophic scars, are the result of an excessive fibrotic response that surpasses the rate of remodeling (Craig, 1975). Biopsies of these pathologic scars often demonstrate elevated levels of growth factors and upregulation of their receptors (Schmid, Itin, Cherry, Bi, & Cox, 1998). Consistent elevation of HIF1A protein levels resulting from increased transcription and translation, followed by stabilization of the HIF1A subunit, is observed in keloid and scleroderma tissues compared with normal skin (Distler et al., 2007), making this a potential target in wound healing and remodeling.

The complex nature of the HIF1 pathway and its critical role in cellular oxygen homeostasis limits the potential for molecular-targeted inhibition for applications in scarless wound healing. Many compounds described as HIF1A inhibitors are only effective at anti-proliferative and cytotoxic concentrations, limiting the clinical relevance of such studies (Giaccia, Siim, & Johnson, 2003; Semenza, 2003; Welsh & Powis, 2003). Translation of targeted HIF1A inhibition for scarless wound healing necessitates that future studies evaluate therapeutically useful HIF1A inhibitors and exclude cytotoxic compounds.

6.1.5 | Vascular endothelial growth factor—Vascular endothelial growth factor (VEGF) is a key factor in angiogenesis, another important component of wound healing. It is a homodimeric glycoprotein that regulates the permeability of blood vessels. In pathologic scars, expression of VEGF and the density of blood vessels tend to be higher than in normal skin (Bock, Schmid-Ott, Malewski, & Mrowietz, 2006; Ong et al., 2007). Several recent studies suggest a link between the surplus capillary growth seen in healing wounds and scar formation. In one such study, neutralization of VEGF via antibody treatment caused an approximately 50% reduction in peak wound vascularity in adult skin wounds. These treated wounds closed normally and showed a significant reduction in wound scar width (Wilgus, Ferreira, Oberyszyn, Bergdall, & Dipietro, 2008). More recent studies suggest an association of robust capillary growth with the development of keloids (Mogili et al., 2012). Moreover, a recent comparison of capillary content in human normotrophic and hypertrophic scars demonstrated that hypertrophic scar formation is associated with higher levels of angiogenesis (van der Veer et al., 2011). This evidence has led to the suggested use of anti-angiogenic therapy to reduce scar formation (Diao, Xia, & Guo, 2010; Wilgus et al., 2008).

Bevacizumab (Avastin, Roche Pharma, Basel, Switzerland) is an anti-VEGF-A monoclonal antibody. It is the first angiogenesis inhibitor approved by the U.S. Food and Drug Administration for the prevention of tumor growth and it is widely used in the management of diabetic retinopathy. Although bevacizumab reduces scar formation, it does have adverse effects including headache, high blood pressure, increased risk of bleeding, and intestinal perforation. Further research should be performed to reveal the mechanisms underlying its effect on cutaneous scar formation and to ensure that bevacizumab may be used as an anti-scarring therapy, without inducing these or other adverse effects.

6.2 | Cytokines

6.2.1 | Tumor necrosis factor-alpha—Tumor necrosis factor-alpha (TNFA) is a cytokine chiefly produced by macrophages that plays a critical role in the inflammatory response. In the context of wound healing, TNFA secreted by macrophages leads to the recruitment of inflammatory cells such as neutrophils (Olutoye, Zhu, Cass, & Smith, 2005). Interestingly, TNFA levels are elevated in chronic wounds (Mast & Schultz, 1996), suggesting that it may have a detrimental effect on healing and may perpetuate inflammation and inhibit tissue growth. However, the direct effects of elevated TNFA levels on healing are unclear, with some studies suggesting impairment of healing processes (Rawdanowicz, Hampton, Nagase, Woolley, & Salamonsen, 1994; So, Ito, Sato, Mori, & Hirakawa, 1992; Unemori, Hibbs, & Amento, 1991) and others suggesting pro-healing effects (Barrientos, Stojadinovic, Golinko, Brem, & Tomic-Canic, 2008; Brauchle, Angermeyer, Hubner, &

Werner, 1994; Kristensen et al., 1993). Inhibition of TNFA signaling using a neutralizing antibody improved the rate of re-epithelialization in a mouse wound model (Ashcroft et al., 2012). Unfortunately, the effects of TNFA modulation on human wound healing are less studied.

In a non-controlled human trial, patients with non-healing wounds that failed to respond to standard therapies were treated with TNFA inhibitor infliximab. The majority of test subjects experienced improved wound closure within 8 weeks (Streit, Beleznay, & Braathen, 2006). In a separate non-controlled study, patients with chronic venous leg ulcers were treated with the TNFA inhibitor adalimumab (Fox et al., 2016). Several of the wounds demonstrated healing during the study period, and the degree of healing correlated with a reduction of TNF staining seen on immunohistochemical examination of wound biopsies. From these studies, TNFA inhibition may be useful in the treatment of chronic wounds; however, high-quality randomized trials are needed before any conclusion can be made about its efficacy, particularly in acute wound healing.

6.2.2 | Interleukins—Interleukins are cytokines that regulate the function of immune cells. As such, they are ideal targets to mediate inflammation and ultimately wound healing. Interleukin-1 (IL1) is a key regulator of the acute phase of inflammation (McKay & Leigh, 1991). Studies have repeatedly demonstrated its ability to induce inflammatory cell and keratinocyte migration (Beck, Habicht, Benach, & Miller, 1986; Granstein, Margolis, Mizel, & Sauder, 1986; Sauder et al., 1989) and fibroblast proliferation (Schmidt, Mizel, Cohen, & Green, 1982). IL1 also promotes the production of pro-fibrotic cytokines like TGFB and IL6 (Aoki et al., 2006). As such, pharmacologic antagonism of the IL1 receptor (IL1R) could prove beneficial in the development of scarless wound healing products. To date, studies show that targeting the IL1R with antagonists or antibodies reduces fibrosis and accelerates reepithelization in mice *in vivo* (Mirza, Fang, Ennis, & Koh, 2013; Thomay et al., 2009; Yan et al., 2016) but these products have yet to be tested in human subjects.

Another interleukin of interest is the anti-inflammatory and anti-fibrotic cytokine IL10. Several studies demonstrate that fetal skin expresses increased IL10 relative to adult skin (Gordon et al., 2008; Liechty, Kim, Adzick, & Crombleholme, 2000; Yamamoto, Eckes, & Krieg, 2001) and specifically, that IL10 is necessary for scarless fetal skin repair (Liechty et al., 2000). Early studies demonstrated the ability of IL10 to downregulate pro-inflammatory cytokines like IL6 and IL8 (Gordon et al., 2008), while more recent evidence describes fibroblast-specific STAT3 (signal transducer and activator of transcription 3)-dependent regulation of hyaluronan metabolism (Balaji et al., 2016). Ultimately, most evidence points to IL10 as a mediator of ECM remodeling (Balaji et al., 2016; Gordon et al., 2008; Reitamo, Remitz, Tamai, & Uitto, 1994), an area with increasing research interest.

In clinical trials, IL10 has been shown to reduce scar formation in murine (Gordon et al., 2008; Kieran et al., 2013; Peranteau et al., 2008) and human research subjects (Kieran et al., 2013, 2014). Though the ideal timing and dosing of IL10 administration has not yet been determined in humans, studies suggest that scar appearance is optimized with low concentrations of IL10 administered multiple times over the course of scar maturation, likely

influencing scar remodeling over time (Kieran et al., 2013, 2014). Additional studies could further legitimatize IL10 as a wound healing therapy.

6.3 | Enzymes

6.3.1 | Lysyl oxidase—Lysyl oxidase (LO) is secreted as a 50 kDa proenzyme and then proteolytically cleaved to the 32 kDa active enzyme. It catalyses inter- and intra-molecular crosslinking in collagen and elastin by oxidatively deaminating lysyl residues in these proteins into peptidyl aldehydes (Kagan & Li, 2003). These aldehydes can then spontaneously condense with one another to yield a variety of covalent cross-linkages. Previous studies have reported increased lysyl oxidase expression associated with increased scar formation, congruent with the known role of lysyl oxidase in fibrotic cutaneous disorders such as scleroderma and hypertrophic scar formation (Fushida-Takemura et al., 1996). Lower lysyl oxidase expression in fetal scarless wounds likely results in less cross-linking and thus less accumulation of collagen.

LO is regulated by a variety of factors including serum conditions, TGFB1, and shear stress (Ando et al., 1996; Boak et al., 1994; Gacheru et al., 1997). A commonly used inhibitor of LO is BAPN (β-aminopropionitrile) (Tang, Trackman, & Kagan, 1983). Other inhibitors include diamines, heparin, amino nitrites, semicarbazides, and hydrazines (Gavriel & Kagan, 1988; Udupa, 1995). Molecular inhibition of LO during wound healing reportedly results in diminished tensile strength in addition to collagen content. However, further studies are necessary to evaluate the efficacy of these compounds, as they have yet to be tested using appropriate *in vivo* models of excisional wound healing or hypertrophic scar formation.

6.3.2 | Matrix metalloproteinases—Studies to date have thoroughly evaluated the role of matrix metalloproteinases (MMP) in scarless fetal wound healing. MMPs are proteases that play a significant role in ECM remodeling (Lee & Murphy, 2004). Since the fetal ECM is distinct from the adult ECM (Chin et al., 2001; Lovvorn et al., 1999), it follows that fetal MMP expression differs from that of the adult. The expression of MMPs also differs between uninjured and wounded skin (Chen, Fu, Ge, Sun, & Sheng, 2007; Dang et al., 2003). For example, MMP2 (gelatinase A), MMP9 (gelatinase B), known as the type IV collagenases, degrade collagen in the basement membrane in addition to other minor cleavage roles in the ECM. MMP2 and MMP9 expression is low in the uninjured skin of the early fetus, and high in the adult (Chen et al., 2007), but the opposite is true in wounded skin (Dang et al., 2003). In addition, wound healing studies in adult *Mmp9* knockout mice did not show any impairment in cutaneous wound healing (Liu et al., 1998), highlighting the complexity of scar formation in adult skin.

Research efforts demonstrate that MMP regulation can improve hypertrophic scarring models in rabbits (Li, Kilani, Rahmani-Neishaboor, Jalili, & Ghahary, 2014; Wang et al., 2014). Though there are many studies that characterize differences in MMP composition in both animals and humans (Gawronska-Kozak, 2011; Manuel & Gawronska-Kozak, 2006; Tanriverdi-Akhisaroglu, Menderes, & Oktay, 2009; Witte et al., 1998), clinical trials in humans have yet to be performed. Overall, while MMPs appear to be a promising target, the

complexity of their interactions with each other and at different times in the wound healing process may prove challenging when developing a therapy (Figure 3).

6.4 | ECM Components

6.4.1 | Hyaluronic acid—Hyaluronic acid (HA) is a glycosaminoglycan present in normal and wounded ECM and is distinguishable by its large molecular size (Vigetti et al., 2014). Since HA is highly expressed in fetal wounds compared to adult wounds, this molecule may be useful as a therapeutic agent to reduce scarring. Almost all human cells express the surface receptor CD44 that binds to HA as well as to other ECM molecules (Litwiniuk, Krejner, Speyrer, Gauto, & Grzela, 2016). In their interaction, HA and CD44 may modulate the inflammatory response of immune cells as well as promote fibroblast migration into wounded tissue (Litwiniuk et al., 2016). *In vitro* studies also indicate that HA directly modulates the expression of genes related to ECM production in fibroblasts (Mast, Diegelmann, Krummel, & Cohen, 1993).

Because of the central role played by hyaluronic acid in wound healing, there is widespread interest in its use as a therapeutic agent. Several commercial products utilize HA and its derivatives, particularly in the setting of chronic non-healing wounds. In one randomized trial, 34 patients with diabetic foot ulcers were treated with either an HA-based dressing or a conventional gauze dressing (Lee, Han, Choi, Chung, & Lee, 2016). Subjects treated with HA demonstrated greater rates of complete healing within the study period, as well as greater healing velocity. In a separate study, patients with second-degree burns were randomized to receive a topical cream containing either HA plus silver sulfadiazine (SSD) versus SSD alone (Costagliola & Agrosi, 2005). Those treated with the HA-SSD cream experienced shorter times to re-epithelialization. Several observational studies *without* control arms have shown satisfactory healing rates in wounds treated with HA products (Caravaggi, Grigoletto, & Scuderi, 2011; Gravante et al., 2007; Voinchet, Vasseur, & Kern, 2006), but it is impossible to determine from these studies whether the HA product truly improves healing.

Based on the important role that HA plays in wound healing, and evidence of a beneficial effect in chronic wounds, it is likely that HA-based products will serve as important tools for clinicians caring for patients with chronic wounds. To date, however, there is far less evidence that HA may be used in normally healing wounds to reduce scarring, and it has been incapable of promoting regeneration in adult skin.

6.4.2 | Type III Collagen—Histologic examinations of fetal wounds and skin consistently demonstrate a higher ratio of Type III to Type I collagen (Beanes et al., 2002). Type III collagen (COL3) is homotrimeric fibril-forming collagen believed to regulate Type I collagen (COL1) fibril diameter, altering the physical properties of tissue (Liu, Wu, Byrne, Krane, & Jaenisch, 1997). Recent studies in mice show that inhibiting COL3 leads to increased scarring potentially through excess COL1 deposition or organization (Volk, Wang, Mauldin, Liechty, & Adams, 2011). Additionally, hypertrophic scarring is associated with excess COL1 deposition (Colwell, Phan, Kong, Longaker, & Lorenz, 2005). In practice, collagen can be used to augment the wounded ECM through the use of tissue engineered

scaffolds that allow cells to attach, grow, and differentiate (Hsu, Hung, Liou, & Shen, 2010). COL1 may also be used as a therapy itself to coat nanofiber matrices providing accelerated wound healing (Rho et al., 2006). However, COL3 might be a more appropriate target for reduced or scarless wound healing. To consider COL3 or COL1 alone as a clinical target may not be relevant due to the density of cell signaling molecules, cells, and other matrix proteins that augment and regulate cellular signaling, cytokine release, cell migration, and scar maturation within the ECM (Vorotnikova et al., 2010). However, the histological assessment of COL1 and COL3 remains the standard of measuring outcomes in scarring research today (Cuttle et al., 2005; Gawronska-Kozak & Kirk-Ballard, 2013).

6.5 | Cell surface receptors

6.5.1 | Integrins—ECM adhesion proteins are particularly important for facilitating responses to the wound environment by embedding cells in the neo-ECM following cutaneous injury. Of the many adhesion proteins on the cell surface, the integrin family of receptors is the most abundant facilitator of cell-ECM interactions. Integrins are heterodimeric glycoproteins with two subunits, one comprised of 18 α-subunits and one of eight β-subunits (Eckes, Nischt, & Krieg, 2010). Interestingly, integrin protein expression differs in fetal fibroblasts and adult fibroblasts (Cass et al., 1998). In the fetus, there is increased α2 subunit expression coupled with decreased α1 and 3 expression when compared to the adult (Moulin & Plamondon, 2002).

In response to injury in adult skin, integrin expression is modified and upregulated to mediate fibroblast and keratinocyte adhesion and migration in addition to epidermal proliferation (DiPersio, Zheng, Kenney, & Van De Water, 2016). Notably, the β1 integrin is necessary for keratinocyte migration and wound closure. However, redundancy in integrin receptors makes it difficult to determine the specific roles of individual integrins *in vivo* (Kenny & Connelly, 2015).

Though it is difficult to target integrins themselves, studies demonstrate successful suppression of downstream signaling. The addition of lumican, a small leucine-rich proteoglycan (SLRP) detected in the ECM, was shown to suppress hypertrophic scarring in rabbits by inhibiting collagen-integrin α2β1-FAK signaling (Zhao et al., 2016). Though current knowledge is limited, future investigations into modulation of integrin adhesion protein activity could be used to improve cutaneous wound repair.

6.5.2 | CD26—CD26, also known as DPP4 (dipeptidyl peptidase 4) is a cell surface marker that is implicated in fibrotic conditions in various organs including liver (Kaji et al., 2014) and kidney (Min et al., 2014). Rinkevich et al. (2015) showed that a lineage of fibroblasts that form scar collagen in healing wounds highly express CD26, while the fibroblasts that form normal collagen in uninjured skin do not. Inhibition of CD26 with a small molecule inhibitor reduced scar formation after excisional wounding without slowing the rate of healing. Interestingly, elevated CD26 levels are also seen in burn exudate in human patients (Prager, Sabeh, Baxter, Atiles, & Hartline, 1994), but there is little human evidence of CD26 expression in scar forming fibroblasts. From these studies, CD26 is emerging as a new clinical target in wound healing. Though its role is not well understood, it

may be an attractive target for reducing scar formation. To date, no studies on CD26 inhibition in the setting of human wound healing have been performed.

6.6 | Tissue and cell therapy

In the clinic, scaffolds and cell therapies are often used in the setting of refractory chronic wounds and acute burn injuries (burns less than 1 week after injury, status post- or pre-debridement). For acute wounds (clean noncontaminated wounds, less than 48 hr after injury), reliance on engineered products is less prevalent. In general, engineered products fall into any one of several categories: they may be synthetic or natural, two or three dimensional (3D), and may or may not involve the use of autologous or transplanted cells.

6.6.1 | Two- and three-dimensional scaffolds—Scaffolds mimicking the fetal or adult ECM can be used to recapitulate the fetal wound healing environment. Often, it is extrapolated that the loss of normal three-dimensional architecture may play a significant role in signaling scar tissue formation. In contrast, in the fetus where organizational cues abound, tissue can be regenerated without apparent healing defects. As mentioned in prior sections, the ideal scaffold should provide adequate structural and mechanical support while also allowing for cellular adhesion, proliferation, and differentiation (Zhong, Zhang, & Lim, 2010). In general, scaffolds can be divided by material composition into natural polymers, synthetic polymers, and composites.

Two-dimensional scaffolds are often used to aid in debridement, and/or to deliver therapeutic compounds. Most often, two-dimensional scaffolds are used in the setting of chronic wounds, where chronic skin barrier breakdown makes healing difficult. Three-dimensional scaffolds are used in slightly more physiologically challenging scenarios, such as burn injuries, where there may not be enough soft tissue available to cover an injury with an autologous tissue transplant. In these settings, scaffolds that mimic the three-dimensional architecture of skin are instrumental, and can serve as a medium to accept and protect skin cell allografts. Finally, three-dimensional scaffolds such as acellular dermal matrices are valuable in situations of extreme soft tissue damage and loss, and also in situations where tensile strength preservation is paramount, for instance, in hernia repair.

As mentioned before, collagen, a natural polymer, may be used to create ECM scaffolds. Other advancements in the fields of tissue engineering and biochemistry led to the creation of synthetic matrices as well (Benedetti et al., 1993). One such material used for matrices and scaffolds is semisynthetic HYAFF, which is biocompatible and biodegradable (Milella et al., 2002). With the ability to be fashioned into different structures depending on the therapeutic need, synthetic matrices can be used for sustained drug release, delivery of small molecules such as growth factors, and application of cells to any injured area (Zavan et al., 2009). This has proved invaluable in burn victims, allowing for cultured epithelial autografts to be applied (Atiyeh & Costagliola, 2007). One of the latest developments has been the use of siRNA within a silicon bilayer to block the TGFB1-mediated pathway, resulting in downregulation of COL1, COL3, and ACTA2—ultimately leading to inhibited scar formation during skin regeneration (Liu et al., 2013). Also, since the development of 3D printing technology, the ability of 3D scaffolds to accurately mimic skin has improved

significantly (Michael et al., 2013). Advancements in 3D printing has led to more complex natural and synthetic matrix production, but most excitingly, 3D printing has allowed for the development of 3D scaffolds comprised of printed cells. Several studies used 3D printers to develop scaffolds entirely comprised of adult keratinocytes and fibroblasts. Potentially, this technology could also harness and incorporate stem cell populations with the aim of ongoing tissue renewal and scarless healing (Abaci, Guo, Doucet, Jackow, & Christiano, 2017; Kim, Lee, Gao, & Cho, 2017; Lee et al., 2014; Michael et al., 2013). With their promising clinical results, two dimensional and 3D scaffolds, whether synthetic or otherwise, continue to find new applications in wound healing (Table 2).

6.6.2 | Neonatal stem cell therapy—Of the possible neonatal cell sources, umbilical cord epithelium (UCE), human amniotic epithelial cells, and human foreskin fibroblasts (HFF) have emerged as the most promising cell-based therapies for scarless wound healing. The UCE is structurally similar to the epidermis of the early fetus (Mizoguchi, Ikeda, Suga, & Ogawa, 2000). UCE can grow into a multilayered stratified epithelium (similar to cutaneous skin) when cultured or upon transplantation *in vivo* (Mizoguchi, Suga, Sanmano, Ikeda, & Ogawa, 2004). Surrounding the UCE is a gelatinous ECM known as Warton's Jelly (WJ). Mesenchymal stem cells (MSC) isolated from WJ have been shown to promote wound healing through paracrine signaling (Arno et al., 2014).

More recently, human amniotic epithelial cells were identified as another source of stem cells capable of forming tissue-engineered skin with similar morphology to normal skin (Jiang, Chen, & Lu, 2016). Also, HFF are used in allogeneic grafting. They can be seeded onto scaffolds and applied directly to wounds to impact chronic or acute wound healing. Transfected HFF from newborn males may have a potential role in scarless healing, as they can be grafted into wounds to increase expression of TGFB3 (Mahmoudi Rad, Mahmoudi Rad, & Mirdamadi, 2015). HFF have been successfully used to form induced pluripotent stem cells, also increasing their potential therapeutic range (Skrzypczyk, Giri, & Bader, 2016). Shown to have more superior scarless healing capacity than HFF, fetal cells used to construct fetal dermal matrices have marked upregulation in genes responsible for scarless healing (Pouyani, Papp, & Schaffer, 2012). As the technology advances, the use of allogenic cells from fetal tissue may have increasing significance, particularly in diseased individuals in whom the burden of scarless wound healing by another mechanism may be too prolonged or taxing (Figure 4).

6.6.3 | Adult stem cell therapy—With the ability of self-renewal and multilineage differentiation, MSCs are an attractive therapeutic consideration for many chronic diseases (Ding, Shyu, & Lin, 2011). MSCs have been shown to accelerate wound closure, reduce inflammation, improve angiogenesis, and positively augment the deposition of the ECM (Lee, Ayoub, & Agrawal, 2016). Some sources of stem cells for wound healing include: WJ, amnion, adipose tissue, and bone marrow. Directly compared, human adipose-derived stem cells (ADSC) have shown comparative results to human amniotic MSCs in inducing dermal fibroblast response to cutaneous wounds (Liu et al., 2013). Clinically more accessible and as efficacious, ADSC are being used in human clinical trials to assess their effect in wound healing (National Clinical Trial, NCT02590042). The future use of MSCs in scarless healing

will depend largely on our understanding of how MSCs function in their niche environment (Wong, Gurtner, & Longaker, 2013). Additionally, the use of biological scaffolds in combination with the application of MSCs may allow for improved cellular migration, proliferation, and differentiation. However, the mechanical and structural components of these scaffolds will affect how the impregnated MSCs function, therefore making careful development paramount (Wang et al., 2010).

6.7 | Targeting Mechanical Forces

Tension, compression, shear, and osmotic forces play an important role in cutaneous wound healing outcomes (Agha, Ogawa, Pietramaggiori, & Orgill, 2011). For example, incisions made parallel to Langer lines (naturally occurring bands of tension in human skin) experience reduced tension and typically heal with thinner scars containing less scar collagen than those placed perpendicular (Langer, 1978; Meyer & McGrouther, 1991; Silver, Siperko, & Seehra, 2003). In addition, areas of the body subjected to increased mechanical force, such as the back, sternum, and joints exhibit increased scar formation when compared to areas that experience reduced mechanical force, such as skin on the eyelid (Meyer & McGrouther, 1991; Wray, 1983).

Specifically, mechanical forces influence cell gene expression, proliferation, and migration (Chiquet, Tunc-Civelek, & Sarasa-Renedo, 2007; Eckes et al., 2006; Kadi, Fawzi-Grancher, Lakisic, Stoltz, & Muller, 2008; Khetan et al., 2013; Yang, Tibbitt, Basta, & Anseth, 2014). These extracellular cues induce changes in mechanosensors (including FAK-SRC [the dual focal adhesion-tyrosine kinase complex], ROCK [Rho associated coiled-coil containing protein kinase], MAPK1 [mitogen-activated protein kinase 1], and PI3K [phosphoinositide 3-kinase] calcium/calcineurin) and ultimately influence gene expression (Das et al., 2015; Januszyk et al., 2014; Kenny & Connelly, 2015; Takada, Furuya, & Sokabe, 2014; Wang et al., 2015; Wong, Akaishi, Longaker, & Gurtner, 2011; Wong, Longaker, & Gurtner, 2012). Fibroblasts subjected to mechanical cues (e.g., suction, cyclical stretching) *in vitro* have been shown to exhibit increased proliferation, upregulation of genes typically expressed by fibroblasts (e.g., collagen 1alpha 1, FGF2, TGFB), increased migration, and reduced apoptosis via mechanisms related to the ITG and WNT mechanotransduction pathways (Huang et al., 2013; Lu et al., 2011; Webb et al., 2006). *In vivo,* mechanical stress in the early phases of cutaneous wound healing reduces AKT-dependent apoptosis, resulting in hypertrophic scaring (Aarabi et al., 2007).

The mechanotransduction signaling pathway is an ideal target for researchers attempting to achieve the scarless ideal partly because these same forces are not in play in the relatively immobile fetal environment. Techniques to offload mechanical stresses in the wound environment, such as Embrace Advanced Scar Therapy®, have been shown to reduce scar formation in pigs, and humans (Aarabi et al., 2007; Agha et al., 2011; Huang et al., 2013; Huang, Holfeld, Schaden, Orgill, & Ogawa, 2013; Lim et al., 2014; Longaker et al., 2014; Lu et al., 2011; Wang et al., 2015; Webb et al., 2006; Wong et al., 2011; Wong, Beasley, et al., 2013; Wong, Gurtner, et al., 2013). Further research into the field of mechanotransduction in wound healing could offer exciting new insights into the mechanism of scarless healing.

7 | CONCLUSION

By discussing the major experimental and clinical targets aimed at achieving scarless wound healing, it is evident that (1) none have been successful in regenerating all dermal appendages and (2) most can reduce scar thickness and appearance, but will not produce histological or functional similarity to uninjured skin. The study of fetal wound healing offers promising insights, with many obvious dissimilarities between fetal and adult wounds. However, few clinical tools mimic the fetal wound healing environment to reduce the adult fibrosis mechanism or cell signaling response. For instance, dermal substitutes often mimic adult uninjured skin but do not often contain the growth factors, cytokines, or other components of developing skin. Additionally, targeting components of adult healing, like inflammation, focuses our attention on adult cells instead of the few fetal inflammatory cells involved in *in utero* wound healing. Novel technology, such as tension offloading, and new molecular targets, such as CD26, offer promising advancements in our understanding of the adult skin healing mechanism. Ultimately, it is unlikely that dermal regeneration in adults will be accomplished without a complete understanding of dermal development in the fetus and of fetal scarless wound healing.

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BOX 1

IMPORTANCE OF MACROPHAGES IN WOUND HEALING

Not mentioned in the introduction of adult skin healing is the importance of macrophages in wound healing. Eliminating inflammatory cells has minimal effect on wound healing, except in the case of macrophages. Macrophages are thought to have a regulatory role in the process of wound healing. Macrophages appear to be responsible for recruiting inflammatory cells into the wound, for initiating dermal proliferation, and for determining collagen subtype deposition.

BOX 2

TARGETING CD26 IN *EN1* DERIVED FIBROBLASTS

CD26 is a major identifying cell surface marker in the scar fibroblasts derived from *En1* embryonic skin cells. When CD26 is targeted with a specific inhibitor, diprotin A, wounds will heal with significantly diminished scar collagen production. CD26 may also identify human scar fibroblasts and could be used as a therapeutic target.

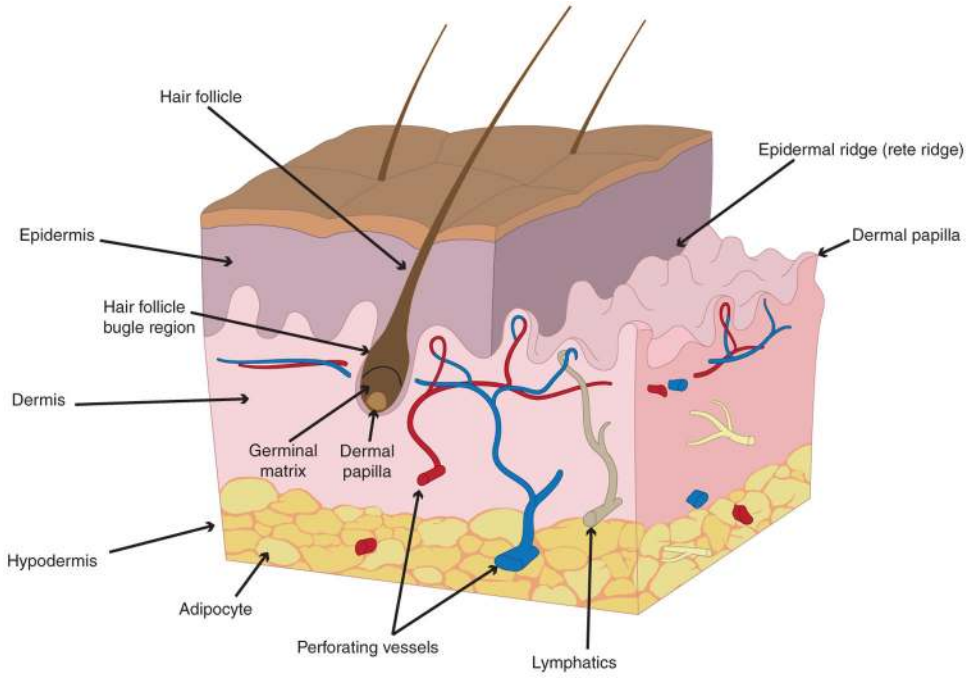

FIGURE 1.

Anatomy of human skin. The most superficial layer of the skin is the epidermis, followed by the dermis, and then hypodermis. Also depicted in this figure is a specialized skin structure: the hair follicle. Note the dermal papilla, germinal matrix, and bulge regions

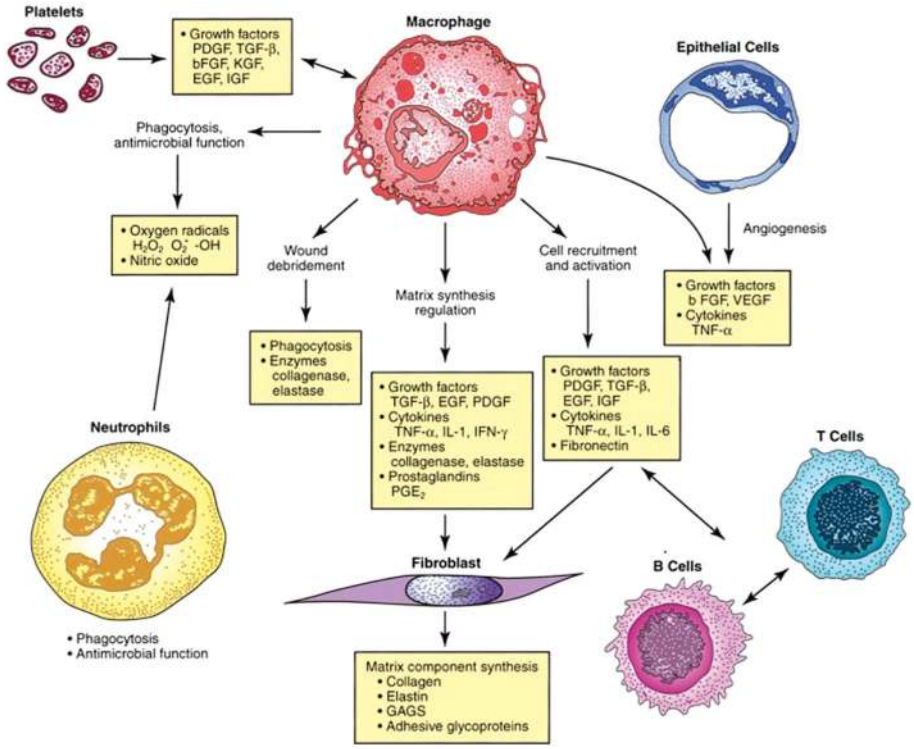

FIGURE 2.

Interaction of cellular and humoral factors in wound healing. Note the key role of the macrophage. FGF2 = basic fibroblast growth factor; EGF = epidermal growth factor; GAGs = glycosaminoglycans; H_2O_2 = hydrogen peroxide; IFNG = interferon-gamma; IGF = insulin-like growth factor; IL1 = interleukin-1; IL6 = interleukin-6; KGF = keratinocyte growth factor; O_2^- = superoxide; -OH = hydroxyl radical; PDGF = platelet-derived growth factor; PGE2 = prostaglandin E2; TGFB = transforming growth factor-beta; TNFA = tumor necrosis factor-alpha; VEGF = vascular endothelial growth factor (Reprinted with permission from Townsend Beauchamp, Evers, and Mattox (2017). Copyright 2017 Elsevier)

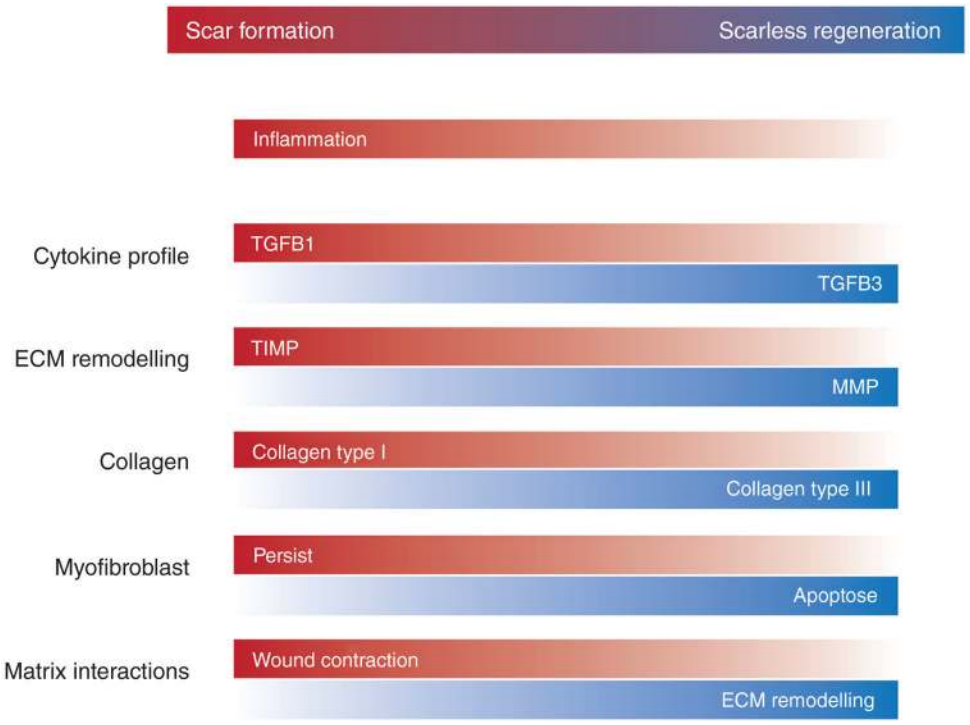

FIGURE 3.

Features of reparative scar formation and scarless regeneration in wound healing. ECM = extracellular matrix, MMP = matrix metalloproteinase; TGFB = transforming growth factor beta; TIMP = tissue inhibitor of metalloproteinase (Reprinted with permission from Leavitt et al., 2016. Copyright 2016 Springer)

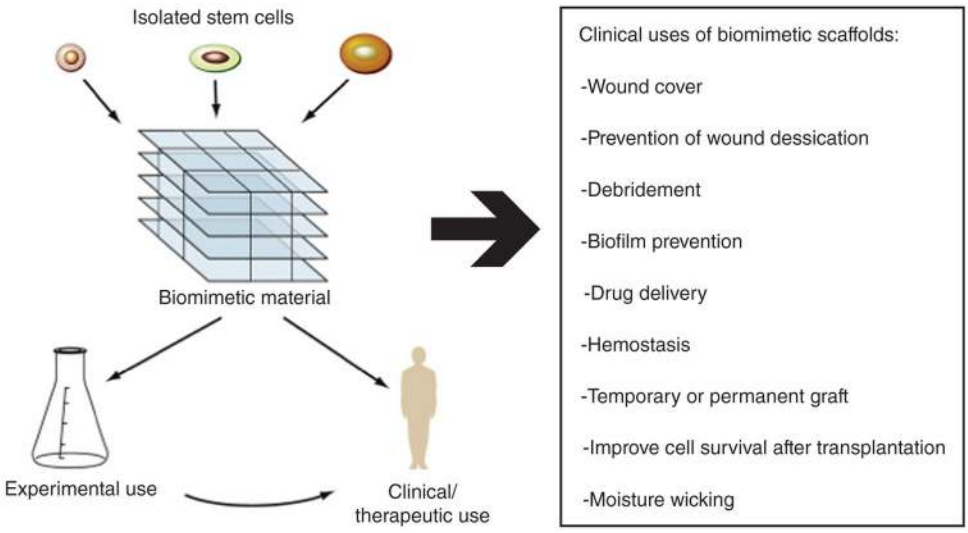

FIGURE 4.

Biomimetic materials are engineered to create favorable stem cell niches for in vitro experimental stem cell biology studies and for clinical use in regenerative medicine applications. Because all stem cells are exquisitely sensitive to environmental cues, the bioengineering component of regenerative medicine will be crucial to modulate and control stem cell behavior to allow effective cell-based therapies to be used clinically (Reprinted with permission from Townsend et al., 2017. Copyright 2017 Elsevier)

TABLE 1

Key differences between adult and fetal wound healing

	Fetal wounds	**Adult wounds**
Inflammation (Longaker et al., 1994; Lorenz et al., 1992)	Few or no inflammatory cells	Many inflammatory cells
Hyaluronic acid (Nwomeh et al., 1998; Shah et al., 1995)	High, prolonged levels, promotes cellular movement	Lower levels, inhibits cellular movement
Collagen (Gurtner et al., 2008; McPherson et al., 1988)	Elevated ratio of type III compared to that of type I	Elevated ratio of type I compared to that of type III
Keratin (Lorenz et al., 1992; Rowlatt, 1979)	K8 and K19 are present during fetal development	Absence of K8 and K19
Mast cells (Gurtner et al., 2008; Walmsley et al., 2015)	Low numbers and less mature	High numbers, more mature
TGF-β (Burrington, 1971; Rowlatt, 1979; Sen et al., 2009)	Low expression of TGF-β1 and TGF-β2	High expression of TGF-β1 and TGF-β2
Stem cells (Sen et al., 2009)	Higher levels of MSCs at injury sites with accompanying E-cadherin-positive cells	Lower levels of MSCs at injury sites without accompanying E-cadherin-positive cells

MSCs = mesenchymal stem cells (Reprinted with permission from Walmsley et al., 2015. Copyright 2015 LWW).

TABLE 2

Clinically relevant biomaterials for wound healing

Substrate	Origin	Composition	Clinical examples
ECM substitutes	Animal or plant tissue	Single to multilayer films with variable 3D architecture	Beriplast, Biocol, Hyaff, Hycoat, Hyalomatrix, Promogran, Puraply
Acellular dermal matrices	Porcine or human	Decellularized skin, chemically treated to achieve sterility and improve tensile strength	AlloDerm, DermaSpan, FlexHD, Surgimend
Synthetic compounds	Electrospinning, salt lithography, soft lithography stamping, solid free-form fabrication	Single and multi-compound materials can be impregnated with pharmaceuticals	Biobrane, Dermagraft, Integra
Cultured stem cells	Human amniotic membrane, dermal fibroblasts, BM-MSCs, ASCs, neonatal prepuce	Most often cells are embedded or seeded onto cultured scaffolds	Apligraf, denovoSkin, denovoDerm,Epicel, EpiFix, OrCel, StrataGraft, Transcyte, Triscover

Table includes data from Hu et al. (2015) and Varkey, Ding, and Tredget (2015). ASC = adipose-derived stromal cell; BM = bone marrow; ECM = extracellular matrix; MSC = mesenchymal stem cell (Reprinted with permission from Moore, Marshall, & Longaker, 2017. Copyright 2017 MDPI). Link to license https://creativecommons.org/licenses/by/4.0/.