ANNUAL REVIEWS

Annual Review of Biomedical Engineering

Regenerative Approaches for Chronic Wounds

François Berthiaume[1] and Henry C. Hsia[2]

[1]Department of Biomedical Engineering, Rutgers University, Piscataway, New Jersey, USA; email: fberthia@soe.rutgers.edu

[2]Department of Surgery, Yale University School of Medicine, and Department of Biomedical Engineering, Yale University, New Haven, Connecticut, USA

Annu. Rev. Biomed. Eng. 2022. 24:61–83

First published as a Review in Advance on February 28, 2022

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

https://doi.org/10.1146/annurev-bioeng-010220-113008

Keywords

skin, tissue engineering, angiogenesis, stem cells, microRNAs, wound healing

Abstract

Chronic skin wounds are commonly found in older individuals who have impaired circulation due to diabetes or are immobilized due to physical disability. Chronic wounds pose a severe burden to the health-care system and are likely to become increasingly prevalent in aging populations. Various treatment approaches exist to help the healing process, although the healed tissue does not generally recapitulate intact skin but rather forms a scar that has inferior mechanical properties and that lacks appendages such as hair or sweat glands. This article describes new experimental avenues for attempting to improve the regenerative response of skin using biophysical techniques as well as biochemical methods, in some cases by trying to harness the potential of stem cells, either endogenous to the host or provided exogenously, to regenerate the skin. These approaches primarily address the local wound environment and should likely be combined with other modalities to address regional and systemic disease, as well as social determinants of health.

Contents

1. INTRODUCTION

Chronic wounds afflict a broad spectrum of patients, with an estimated 2% of the overall US population suffering from an open wound (1). Nonhealing wounds can be the direct result of a complication of surgery or physical trauma, or they can be a consequence of an underlying medical condition such as diabetes or vascular insufficiency. Regardless of etiology, chronic wounds tend to have the greatest impact on those who are older, have impaired movement, and/or may be otherwise unable to engage in appropriate self-care due to dementia or other causes. The annual cost of medical expenditures related to wound care in the United States has been estimated to be in the range of $31.7 billion to $96.7 billion (2).

Normal wound healing starts with hemostasis and the activation of inflammatory cells, followed by proliferation and migration of fibroblasts and keratinocytes with matrix deposition and angiogenesis (3). Wound-healing processes continue even after formal wound closure with collagen matrix remodeling and scar maturation (**Figure 1**). Any disruption in these processes may lead to chronic wound development, but common clinical entities associated with the pathogenesis of chronic wounds include diabetes, peripheral atherosclerosis, venous stasis and insufficiency, and chronic pressure injuries (1, 3).

Appropriate diagnosis with correction of any underlying clinical condition is a mainstay of therapeutic approaches to chronic wound treatment (4). However, even successful treatment that results in complete closure of the chronic wound often leads to formation of a fibrotic scar that does not recapitulate the preinjury state, as it lacks hair follicles and sweat glands and also lacks

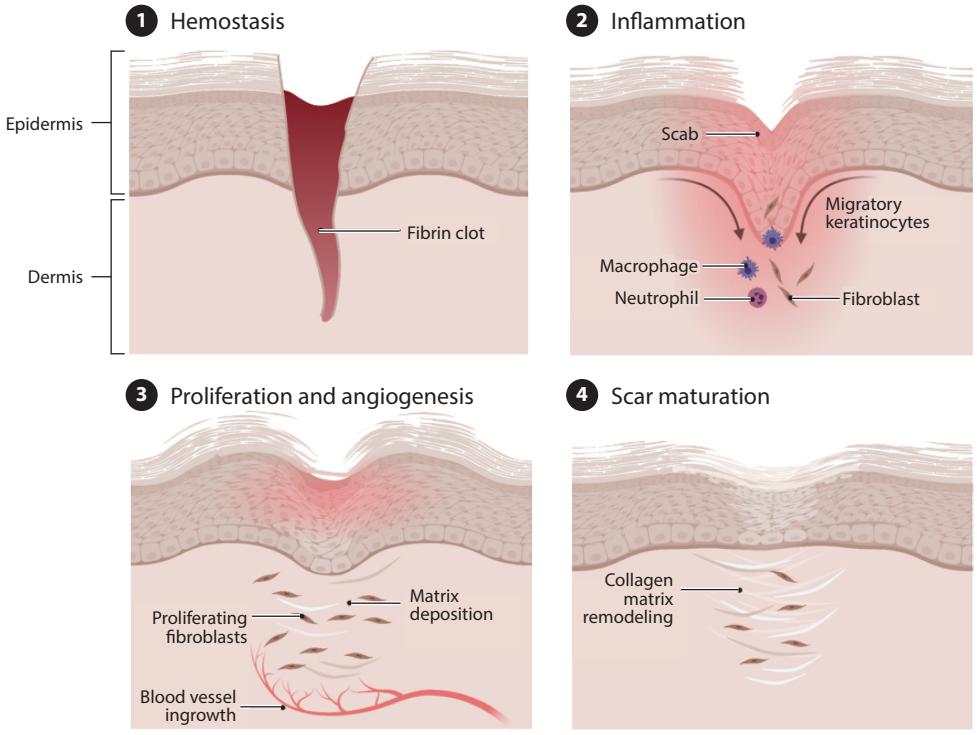

Figure 1

Phases of wound healing. (①) After injury, normal wound healing starts with the formation of a fibrin clot, followed by (②) activation of inflammatory cells including neutrophils and macrophages. (③) Migration and proliferation of other wound cells including fibroblasts and endothelial cells allow matrix deposition and angiogenesis to occur, resulting in the formation of an initial scar. (④) Although acute healing is typically completed by 3 weeks after initial injury, scar maturation with remodeling of the collagen matrix will continue for several months and years. Figure adapted from Wound Healing by **BioRender.com** (2021), retrieved from **https://app.biorender.com/biorender-templates**.

appropriate sensation and pigmentation (5). Fibrosis is characteristic of normal healing in human adults; however, observations of scarless healing in fetal human skin and complete fingertip regeneration in young children provide tantalizing evidence that regenerative healing in adult humans may be possible with complete restoration of the normal skin and subcutaneous tissue architecture (3). Therefore, current research efforts in developing novel treatments for chronic wounds emphasizes achieving wound closure that is less fibrotic and more regenerative and approximates as much as possible the preinjury state. The vast majority of recent and past studies on promoting healing of chronic wounds focus on methods that stimulate endogenous processes that may involve progenitor and stem cells as well as approaches that deliver stem cells to the wounds. The most relevant technologies are described in the remaining sections below and summarized in **Table 1** (see also **Supplemental Table 1**).

Supplemental Material >

2. PATHWAYS OF SKIN REGENERATION VERSUS REPAIR

Intrauterine fetal surgery led to the discovery that fetal skin could regenerate without a scar such that the repaired skin was nearly indistinguishable from intact uninjured skin (6). The ability to regenerate is, however, lost in later fetal developmental stages and after birth. This transition is

Table 1 Major technologies for promotion of regenerative healing of chronic skin wounds

Category	Modalities
Stem cells	Induced pluripotent stem cells Bone marrow–derived mesenchymal stem cells Adipocyte-derived stem cells
Biophysical approaches	Vacuum-assisted wound closure Electroceuticals Cold atmospheric plasma
Soluble factors	Defined growth factors and chemokines Conditioned media and secretomes Exosomes and microvesicles
Nucleic acids	Plasmids MicroRNAs Antisense DNA
Biomaterials	Porous materials via bulk processing Materials by additive manufacturing and 3D printing

somewhat correlated with maturation of the immune system, since regenerative wounds exhibit little inflammation. It is hypothesized that skin repair in the form of a scar (the process described in **Figure 1**) has evolved to involve a robust immune response to thwart pathogenic microorganisms and close the wound as rapidly as possible to ensure survival (7). This may no longer be optimal in the context of modern medical care, and efforts to steer the wound healing process toward the scarless regenerative pathways are thought to be one approach for improving chronic wound healing. A better understanding of the cues from embryonic wound healing is helping devise new promising approaches to promote chronic wound healing. For example, the subcutaneous fascia, which separates skin from other tissue layers underneath, plays a critical role in supplying fibroblasts and other accessory cells to the wound site during the proliferative phase of healing, for wounds that extend deep into the dermis (8). Furthermore, different fibroblast lineages exist in skin, and recent studies suggest that controlling the expression of specific genes, such as *Engrailed-1* (*EN1*), can be used as a switch between healing with a scar and scarless healing.

Mascharak et al. (9) have demonstrated that blocking mechanotransduction signaling using verteporfin, a drug approved by the US Food and Drug Administration to treat macular degeneration, allows a regenerative response in rodent wound healing, resulting in healed wounds that are virtually indistinguishable from unwounded skin, with hair growth and skin appendage–like structures present. The authors found that this regenerative response was mediated through a dermal subpopulation of fibroblasts lacking expression of *EN1*. Multiple subpopulations of myofibroblasts, which play a key role in wound contraction, have been identified by other investigators within both rodent and human wounds, and their relative numbers can be altered by their interactions with elements of the wound microenvironment such as wound macrophages (10). These myofibroblast subgroups demonstrate differential capabilities in key wound-healing processes such as collagen cross-linking that can have a significant impact on clinical wound-healing outcomes. Future research will focus on gaining a better understanding of these different wound cell subpopulations and how they can be manipulated to promote regenerative wound healing.

The absence of hair follicles in scars also provides other clues on the roles of specific cells during skin development and in particular on the contribution of a specific fibroblast lineage that is associated with the papillary dermis, which is progressively lost with age in rodents and also in humans, especially at ages of 50+ years (11, 12). The Wnt signaling pathways play an important role during skin development and wound healing and require coregulation by other transcription

factors, one of which is lymphoid enhancer-binding factor 1 (Lef1). A comprehensive analysis of gene expression in mouse regenerative and scarring wounds revealed that Lef1 is transiently expressed in neonatal papillary fibroblasts, at a stage during which skin can renew hair follicles in wounds (13). Overexpression of Lef1 in adult skin could restore the skin's ability to regenerate hair follicles, although the effect was dependent upon the stage in the hair follicle cycle, adding a layer of complexity to the approach (12). These hair follicles were thought to be more mature than those associated with wound-induced transient hair growth (14). Another observation from the same study was that there were many similarities in gene expression changes between regenerating and scarring wounds; thus, a few specific pathways tied to skin development may be critical to identify targets that promote regenerative skin responses. These cues from embryonic development provide avenues for future therapeutic endeavors.

3. STEM CELL–BASED THERAPIES

With the ability to renew themselves and differentiate into multiple different cell types, stem cells have generated considerable interest as potential therapeutic approaches for promoting regenerative closure of chronic wounds, especially since deficiencies in healthy and functional stem cells are considered a common feature of chronic wounds regardless of their original etiology (4). Stem cells can be classified on the basis of the range of their differentiation potential (15). Totipotent stem cells have the broadest range and can differentiate into any cell found within the embryo as well as extraembryonic structures such as the placenta and umbilical cord. Pluripotent stem cells are not able to form into extraembryonic structures but can differentiate into any cell found within embryonic structures including all three germ layers of the embryo, while stem cells that are not able to form all three germ layers and have a more restricted spectrum of differentiation are classified as multipotent stem cells.

Normal wound repair and regeneration relies, at least in part, on a contribution from tissue-specific multipotent stem cells already present in skin, as well as stem cells recruited from the circulation. Keratinocyte progenitors, a type of skin stem cell, are located in the basal layer of the epidermis and hair follicles (16); these cells continuously replicate both to self-renew and to generate more differentiated cells that migrate and further differentiate into a nonmitotic cornified layer toward the skin surface. They play a key role in the reepithelialization process of the wound, and the availability of local keratinocyte stem cells confers a high degree of regeneration potential to the epidermis.

Restoration of the epidermis is nevertheless dependent upon a suitable wound bed that can supply nutrients and oxygen. For example, angiogenesis and regeneration of the vasculature are critically important to support the metabolic demands of the healing wound, as cells actively proliferating and migrating consume significantly higher levels of metabolites than cells under basal nonstimulated conditions. A functional dermis may therefore be needed to enable the epidermis to close the wound. Cells of the dermis have, however, a more limited endogenous repair capacity. Mesenchymal stem cell (MSC) sources have been identified in the skin (17), and a contribution from circulating stem cells has also been documented, which may be enhanced by increasing their levels in the circulation, for example, through known methods used clinically for hematopoietic stem cell (HSC) mobilization before autologous bone marrow transplantation (18).

Because these endogenous regenerative and repair processes are impaired in chronic wounds, the use of exogenously applied stem cells (usually after inducing some level of differentiation) is a promising avenue to develop novel therapeutics for wound healing. Research on potential stem cell–based therapies for wound healing has largely focused on the use of pluripotent and multipotent stem cells.

3.1. Pluripotent Stem Cells

Pluripotent stem cells include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). For wound-healing applications, the potential of ESCs for use in developing epidermal sheets has been investigated (19). Ethical concerns, however, have greatly limited enthusiasm and research into clinical applications with these cells (20). Since they can be derived from adult cells, iPSCs have received more attention in recent years after Takahashi & Yamanaka (21) published their initial finding in 2006 that iPSCs can be derived from adult fibroblasts using the transcription factors Oct4, Sox2, Klf4, and c-Myc. In the years since, in vitro studies have demonstrated that these adult-derived pluripotent cells can be differentiated into a wide range of cell types for tissues and organs throughout the human body, with clinical application first reported in Japan in 2014 to correct age-related macular degeneration (22, 23).

Besides macular degeneration, human clinical trials using iPSC-based therapies have also been initiated in recent years to examine their potential to treat osteoarthritis and graft versus host disease (22). Multiple skin cell types required for regenerative wound healing have been successfully derived from iPSCs including fibroblasts, vascular cells, keratinocytes, melanocytes, and multipotent stem cells found in hair follicles and skin-associated fat tissue. There is also evidence that iPSC-derived cells may be less affected by their donors' comorbid conditions such as diabetes. However, concerns about tumorigenicity and immunogenicity by iPSCs in recipient patients remain a challenge for rapid clinical translation, especially with regard to chronic wound treatments (24). Therefore, in addition to direct derivation of cells, iPSC-derived secretomes, exosomes, and matrix scaffolds have also been investigated for potential application in chronic wound treatment, as discussed further below.

3.2. Multipotent Stem Cells

MSCs are multipotent cells that have been the most studied stem cells to date for human wound-healing applications as they can be readily isolated from adult bone marrow and adipose tissue as well as extrafetal tissue such as placenta and umbilical cord blood (25). MSCs are phenotypically difficult to distinguish from fibroblasts and are defined by agreed-upon criteria that include the presence of cell surface markers CD105, CD73, and CD90 and the absence of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR as well as functionally by their ability to differentiate into multiple tissue types including bone, cartilage, and fat (26). MSCs also do not appear to elicit an immune reaction in recipients, making these stem cells potentially useful for allogeneic as well as autologous applications (27). Bone marrow has been a common source for MSCs being studied for potential clinical applications; however, the need for bone marrow aspirate procedures, which are painful and can be highly morbid procedures carrying the risk of osteomyelitis for the patient, and the relatively low yield of cells from these procedures present a significant barrier for widespread clinical application of bone marrow–derived MSCs (BM-MSCs) for the treatment of chronic wounds (25). In contrast, adipose-derived stem cells (ADSCs) can be easily obtained in abundant numbers from aspirates obtained during liposuction, a much less morbid procedure from the patient perspective, greatly increasing the feasibility of autologous stem cell applications. The lack of donor morbidity is also considered an advantage for the use of MSCs isolated from umbilical cord blood, as this material along with other extrafetal tissue following a birth is usually discarded as medical waste (25).

3.3. Mechanism of Action of Stem Cells

The exact mechanism of action by which stem cells such as iPSCs and MSCs can promote regenerative healing remains to be elucidated; however, regardless of their source, stem cells are

postulated to have multifaceted positive effects on wound healing not only through their ability to differentiate directly into necessary wound cell types but also through their paracrine activity, including immunomodulatory effects that can decrease inflammation and promote cell migration and proliferation within the wound site (22, 28). The ability of stem cells to create a microenvironment that may be conducive for regenerative healing has led to interest in whether conditioned media from stem cells and/or the extracellular vesicles secreted by these cells can also have therapeutic applications for chronic wounds (22, 28). Combinatorial approaches where stem cells are seeded onto matrices to produce advanced scaffolds that can promote wound healing have also been proposed (22, 29, 30). Several preclinical studies supporting the potential therapeutic use of stem cells either directly or by leveraging their secretomes have been published in recent years, and some of the key findings are reported in the sections below.

To date, human clinical trials involving stem cells for wound-healing applications have been limited to MSCs and their secretomes, with more than 50 trials recently identified as being in progress or completed and the large majority of those being done in patients with diabetic foot ulcers, though other etiologies including burns and critical limb ischemia are also being investigated (28). These trials are mostly phase 1 and 2 investigations and have been limited by relatively small cohorts. While large-scale randomized double-blinded controlled trials remain to be done, at the very least MSCs appear to be safe and are well tolerated in human patients when injected around the wound or applied topically in some form (e.g., fibrin spray, hydrogel, or scaffold).

Variables such as source tissue, harvesting and isolation techniques, and vehicle delivery can impact the biological characteristics of stem cells, and such changes can in turn impact their therapeutic potential. Clinical comorbidities and genetic characteristics of the stem cell donors add further variability that can make it difficult to assess stem cell–based therapies in a rigorous fashion. A key challenge will be accounting for the heterogeneity among stem cells and developing standardized approaches in isolation, preparation, and delivery that will allow consistent and predictable therapeutic outcomes for chronic wound treatments derived from stem cells.

3.4. Molecular Approaches to Cellular Reprogramming

Cellular reprogramming is a novel approach to promote the proliferation and differentiation of skin cells. This approach was originally developed using gene transfection methods, and new ways to induce differentiation of stem cells into skin using small molecules and protein signals have been developed (31). Such molecules may be designed for optimal stability and efficient permeation into cells while avoiding genetic manipulations (32). These methods are used to try to emulate the temporal sequence of signals that is involved during embryonic development. For example, keratinocytes can be derived from iPSCs using a combination of bioactive molecules, and these stem cell–derived keratinocytes appear to function similarly to regular keratinocytes. While typical differentiation protocols use naturally occurring chemokines and growth factors, recent studies show the ability to derive synthetic compounds that bind cell receptors to activate fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), and sonic hedgehog pathways (33).

These methods assume that iPSCs and ESCs may be available to differentiate ex vivo before implantation; however, these cells must be derived from a source. Current protocols for the dedifferentiation of somatic cells (most often fibroblasts) to iPSCs generally use transfection methods to introduce genes encoding the Sox2, Oct4, c-Myc, and Klf4 proteins, followed by chemical-based reprogramming. Recently, some new chemical-based methods to perform the dedifferentiation step have been reported (34). Differentiation of iPSCs into vascular endothelial cells has been reported in multiple studies using activin A, BMP4, FGF2, and Wnt ligands or GSK3 inhibitors (35, 36).

Alternatively, strategies to promote the local recruitment of HSCs, MSCs, and ADSCs at the wound site followed by their differentiation in situ may be more easily implemented (17). Transdifferentiation of fibroblasts directly into endothelial cells has also been reported; this method obviates the need to go through an iPSC-like stage by reprogramming fibroblasts to endothelial progenitors, using either lentiviral vectors (37) or a sequence of chemicals that target chromatin condensation, such as 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor, and trichostatin A, a histone deacetylase inhibitor (38). Reported results show the transdifferentiation of fibroblasts into keratinocytes and vascular endothelial cells, and iPSC-derived endothelial cells were able to form capillary-like structures in culture and to integrate with the recipient's own blood vessels in vivo (39). Sweat gland–like cells have also been reported to form in response to various treatments applied onto MSCs, including conditioned medium from sweat gland cells and epimorphin (40) or keratinocyte growth factor (41).

A regenerative response in skin can be stimulated in situ using some of the same bioactive molecules that are sometimes used to promote dedifferentiation and transdifferentiation ex vivo. For example, thymosin 4 has been touted as a molecule that promotes regeneration of the dermis through pleiotropic effects (42). Other compounds that have been reported to improve skin wound healing through proangiogenic and anti-inflammatory effects include retinoids (43), deferoxamine, and plant extracts such as hydroxysafflor yellow A (44).

Limitations of these methods include the need to perform ex vivo cellular manipulations and the generally low transdifferentiation efficiency. Other challenges include the need for methods to screen for agents that promote the desired phenotypic changes toward dedifferentiation to the stem cell–like stage, followed by differentiation toward the desired cell type. Several agents typically need to be used at different times, complicating process optimization. Furthermore, in vitro cell culture systems are often used for such purposes and may not always predict in vivo behavior. Better in vitro systems that mimic chronic wounds while maintaining relatively simple yet physiologically relevant readouts would help advance this field (45).

4. BIOPHYSICAL APPROACHES: BIOMECHANICS AND BIOELECTRICITY

4.1. Vacuum-Assisted Wound Closure

Vacuum-assisted wound closure, also known as negative-pressure wound therapy (NPWT), is used extensively for wound care, either by itself or in combination with foam dressings (46). In this approach, the wound is covered with a specialized wound dressing consisting of a foam with open pores, and this dressing is then connected to a vacuum source; typically a negative pressure of 125 mm Hg is used, on the basis of early studies demonstrating that this pressure resulted in the most local blood flow increase in an animal model. Intermittent and pulsatile pressure application has also been suggested and shown to sometimes yield better angiogenesis (47).

The effects of NPWT are multifactorial. NPWT tends to bring wound edges closer to each other, thus helping to close the wound. Furthermore, fluid drainage is enhanced, thus decreasing tissue swelling and potentially increasing fluid percolation through the tissue surrounding the wound, thereby increasing nutrient delivery and waste removal. The use of NPWT in combination with foam dressings induces micromechanical deformation in the micrometer- to millimeter-sized scale at the tissue–wound dressing interface, and pore size has been correlated with the rate of granulation tissue formation (48). Instillation devices may be used together with the vacuum system to enable local administration of fluids containing bioactive compounds and antibiotics.

NPWT is thought to induce angiogenesis through the supply of angiogenic factors due to fluid drainage, via mechanical stimulation, and also by increasing blood flow through existing patent

vessels, which dilate when outside pressure is decreased. These mechanisms cannot easily be uncoupled; thus, it is not known to date which one(s) may be predominant. The proangiogenic effects have also been documented when NPWT is used in conjunction with engineered skin substitutes, as it accelerates vascularization of the dermal component of the skin substitute, and subsequently the survival and take of autologous skin grafts, which are often used to close the wound (49). This effect opens up the possibility of applying a dermal substitute and covering it with a skin graft in a single one-step procedure, as opposed to the traditional two-step procedure whereby the dermal substitute must revascularize (usually for ~3 weeks) before the skin graft is applied (49).

4.2. Electroceuticals

Electroceuticals are a class of therapies that involves delivering electric signals (as opposed to pharmacological or chemical signals) to tissue. Endogenous electrical fields that arise from injury that has caused a discontinuity in the skin tissue have been reported for a long time and are thought to play a key role in directing cell migration toward wound closure, a phenomenon called electrotaxis or galvanotaxis. The use of externally applied pulsed electrical fields has been reported in several clinical studies to improve blood flow perfusion and promote wound area reduction (50). Evidence for the benefits and relative safety of using electric fields for wound healing continues to accumulate; however, there is not yet a consensus on the optimal parameters of the electrical stimulation (voltage, frequency, wave shape, and so on) (51). Magnetic stimulation has also been reported with favorable results on skin wound healing, both in animal models and in humans (52). Magnetic and electrical stimulation are likely closely related, since variable magnetic fields cause inductive currents and appear to elicit many similar cellular responses.

Most of these treatments required patients to be treated in a hospital setting where the specialized equipment was located. In recent years, new self-powered wearable electronic devices that are able to harness and convert, using piezoelectric and triboelectric effects, mechanical energy from body motions into electricity have been developed and shown to be able to deliver electrical stimulation. For example, one system was described to be capable of promoting electrically stimulated wound healing in a rat model, and hair regeneration in rats and mice, and the effect correlated with increased production of vascular endothelial growth factor and keratinocyte growth factor (53, 54).

Typical electromagnetic stimulation studies use relatively low field powers; however, recent work suggests that high-intensity pulsed electric fields may be useful for treating skin wounds. For example, field strengths in excess of 500 V/cm cause irreversible electroporation and can serve as a modality to simultaneously debride and sanitize skin wounds (55, 56). The surroundings of the targeted tissue area are invariably exposed to sublethal electric field doses, and some regions are therefore exposed to dose ranges similar to those used in traditional electrical stimulation, on the order of 30 V/cm or less. In a recent cell culture study, it was reported that cell monolayers that were electroporated regained confluence much more quickly than cell monolayers that were cleared of cells over a similar area but using mechanical scraping (57). Furthermore, recent small-animal studies suggest that the skin repair process after electroporation is more akin to a regenerative response and that it restores skin without a scar (58). One potential explanation for this result is that electroporation leaves the extracellular matrix (ECM) largely in place, and the ECM may serve as a scaffold that inhibits wound contraction. This mechanism may be similar to that postulated for the efficacy of dermal matrices such as the dermal regeneration template (DRT) that was originally designed to mitigate scarring in skin burns (59).

4.3. Cold Atmospheric Plasma

Cold atmospheric plasma (CAP) consists of ionized gas generated by flowing gas through a dielectric barrier discharge or dielectric barrier electrode device (60). The device is usually held close

to—although without actually touching—the skin and is scanned across the treated area. Numerous studies have shown a beneficial effect of CAP on wound healing, and some systems have reached clinical use for treating recalcitrant wounds and other skin conditions (61). CAP may also be used to increase skin permeability to enhance transdermal drug delivery (62). Originally, the use of CAP on skin wounds was motivated by CAP's ability to decrease bacterial load, especially since it is very effective against a variety of bacterial strains, including antibiotic-resistant ones (63). However, experimental evidence accumulated over time has led to the realization that CAP may have more direct effects on the host's cells.

While the mechanism of CAP action is not fully elucidated, in vitro and in vivo data point to a role for reactive oxygen species (ROS) and reactive nitrogen species (RNS), which emanate from the ionized gas as mediators of cellular responses. In particular, proangiogenic effects have been extensively documented; however, because CAP-derived chemical species are generally short-lived and therefore may not penetrate deeply through tissue, it is not likely that much of this response is a direct effect of CAP on endothelial cells in the wound bed. Indeed, CAP treatment of cultured fibroblasts and keratinocytes causes the release of factors that trigger angiogenic responses (e.g., tube formation) in cultured endothelial cells, suggesting that CAP effects may also, if not mostly, be mediated via paracrine signaling mechanisms (64). CAP has also been shown to alter integrin-ECM interactions, at least transiently, in a way that promotes cellular migration in a variety of cell types (65), an effect that may also contribute to the healing benefit.

Although CAP can generate precise doses of ROS and RNS, the ionized gas contains a multitude of chemical species and as of yet it is unclear which one(s) may be the most effective in causing the wound-healing response. The relative amounts of these chemical species can be altered by changing the gas composition (with helium and argon currently being the most commonly used gas feeds) and electric field within the device (66); thus, future studies could focus on optimizing these parameters for treating wounds. Furthermore, the effective CAP dose is highly dependent upon the distance between the CAP device and skin, the duration of treatment, and host endogenous factors. For example, how many endogenous antioxidant species are present in different cell types will likely affect how much of the deposited ROS and RNS can actually participate in triggering cellular responses (67). Treatment efficacy and reproducibility may also be further improved using novel plasma-generating systems that conform to wound shape and can deliver plasma over a large area, such as recently described paper-based devices (68).

5. SOLUBLE FACTORS: SMALL MOLECULES, CHEMOKINES, GROWTH FACTORS, AND PROTEASE INHIBITORS

5.1. Approaches Using Individual Bioactive Factors

Various compounds have been tested as potential topical treatments to mitigate inflammation and promote the proliferative phase of healing. To address the former, anti-inflammatory cytokines and protease inhibitors (69), as well as proresolution molecules derived from unsaturated fatty acids (such as resolvins) (70), have shown beneficial effects on wound healing in animal models. Many of these approaches also aim to decrease proinflammatory macrophages (also known as M1) in favor of proresolution macrophages (also known as M2). The M1-to-M2 transition is hypothesized to be critical in moving from the inflammatory to the proliferative phases of wound healing (71); this transition fails to take place in chronic wounds. Proinflammatory diabetic pathways may be mitigated by inhibitors that target the receptor for advanced glycation end products (72).

Numerous growth factors and chemokines to stimulate proliferation have been shown to result in accelerated healing when used topically in laboratory animals (73); however, only one such compound—platelet-derived growth factor (PDGF)—has so far reached clinical use. Many of

these compounds are not stable in the wound environment and therefore repeated high-dose applications may be necessary, thus adding cost and inconvenience. It is noteworthy that a potential concern with the use of these factors is the potential side effects due to their role in cancer progression; this has severely impeded the use of PDGF (74). Protein engineering strategies that increase stability in the local wound environment, such as the use of nanoparticle-forming elastin fusion proteins, allow for longer lasting formulations with lower concentrations of growth factor (75). Another limitation of this approach is that single factors may not be optimal in driving a multifactorial healing process. Thus, combinations of factors may be more effective; furthermore, there is recent interest in exploring sequential treatment methods that attempt to match the use of different factors for different phases of healing (76).

5.2. Secretome-Based Approaches

Prior studies have shown that the secretome of MSCs and ADSCs can be used to promote the healing of chronic wounds (77). The use of conditioned media preparations alleviates several issues associated with transplantation of therapeutic living stem cells on skin wounds, including safety concerns due to risk of teratoma formation and batch-to-batch variability in the stem cell performance. Furthermore, the wound environment is poorly suited for the survival and function of the cells; for example, hyperglycemic conditions decrease the production of key growth factors such as VEGF, FGF-2, and stromal cell–derived factor 1 alpha, although this can be reversed by engineering the cells to overexpress hypoxia-inducible factor (HIF) (78).

Analysis of the conditioned medium shows that major components consist of ECM proteins, such as collagen fragments, fibronectin, and vimentin, as well as several key growth factors including transforming growth factor beta-1, VEGF, and connective tissue growth factor (79). Thus, the conditioned medium from these cells contains several different bioactive molecules that can potentially address multiple targets on several different wound cell types in a synergistic manner (80). The culture conditions, such as level of confluency and presence of serum (79), oxygen tension, and 2D versus 3D culture geometry (81), strongly affect the levels of some of these factors. For example, the addition of serum considerably increases the production of bioactive factors; however, serum-free preparations are still preferred to avoid the presence of animal proteins in the product. Concentrating serum-free culture supernatants through evaporation was reported to achieve acceptable bioactivity (79).

5.3. Application of Exosomes and Microvesicles

Exosomes, a type of extracellular vesicle (EV), are spherical liposomes bound by a single lipid bilayer and shed by living cells that contain various bioactive molecules, including lipids, proteins, and nucleic acids (82). They are 30–200 nm in size and originate from the cellular endosomal pathway. Microvesicles are larger in size (200–1,000 nm) and originate through the outward budding of the plasma membrane via a poorly understood pathway. EVs enable cell-to-cell communication wherein uptake by the target cells may be by direct membrane fusion or via endocytosis; in the latter case, the uptake is subject to selectivity depending upon the presence of cell-specific surface molecules (83). EVs may play a key role in mediating MSC-induced healing responses (84). Production of EVs by cultured cells is highly dependent upon a wide range of physicochemical stimuli, and optimization of the EV production process is an important subject in the literature (85). Several different systems have been developed for attempted mass production of EVs (86, 87). Besides large-capacity bioreactors, well-instrumented and continuous-flow systems, in particular, may be able to provide more precisely controllable environmental conditions and thus more reproducible product batches. Size-based separation methods are the most reliable in generating

high-purity EV fractions, albeit the yield remains low (88). EVs can be stored frozen for long periods, and lyophilization methods are being developed such that the shelf life can be similar to that of purified proteins (89, 90).

EVs from various cell types have been tested in wound-healing animal models; among these are EVs from MSCs and ADSCs, which have been shown to decrease proinflammatory macrophages (also known as M1) in favor of proresolution macrophages (also known as M2), along with improved angiogenesis and faster healing (91, 92). The M1-to-M2 transition is hypothesized to be critical in moving from the inflammatory to the proliferative phases of wound healing (71); this transition fails to take place in chronic wounds. Serum-derived EVs have also been shown to promote angiogenesis and healing in diabetic wound models (93). In a serendipitous discovery, when *Synechococcus elongatus* cyanobacteria were used on wounds in an attempt to locally deliver oxygen via photosynthesis, it was found that EVs secreted by the bacteria were proangiogenic and promoted healing (94).

EVs can be engineered for better therapeutic efficacy using various methods. EV-producing cells can be genetically modified to express or overexpress certain gene products that target specific wound-healing pathways, such as in the case of MSCs overexpressing long noncoding RNA (lncRNA) H19 (95), as well as ADSCs overexpressing nuclear factor erythroid 2–related factor 2 (Nrf2) (96). EVs can also be loaded with therapeutic agents and serve as carriers that essentially function as liposomes that enhance targeted delivery while protecting the bioactive compound from degradation in the wound environment. The loading may be done by introducing the desired compound into the living cells, allowing the compound to naturally become incorporated into the budding vesicles (97), or by ex vivo loading of vesicles using physicochemical methods (98). Examples include EVs from ADSCs loaded ex vivo with miR-21-5p mimics (99) and EVs from embryonic kidney cell lines overexpressing lncRNA H19 (95). Some of the challenges of using EVs currently include low loading efficiency for hydrophilic drugs and large proteins as well as decreased stability and altered surface properties of the EVs due to the presence of drugs and/or due to the loading method, which may alter interactions with target cells and cause aggregation of EVs.

6. NUCLEIC ACID–BASED APPROACHES

Therapeutic nucleic acids predominantly consist of oligonucleotides that aim to upregulate or inhibit specific pathways, with the most common ones being plasmid DNA, small interfering RNA (siRNA), microRNA (miRNA), and anti-miRNA, as well as antisense DNA (100). Nucleic acids that are destined for translation into protein may have more long-lasting effects than the delivery of the protein itself, and for intracellular targets such as transcription factors, using nucleic acids is likely more effective than attempting to deliver proteins across the cell membrane. Therapeutic nucleic acids are also capable of addressing noncoding RNAs involved in the wound-healing response (101). Furthermore, the physicochemical features of nucleic acids are largely independent of the specific nucleotide sequence; thus, multiple different targets may be addressed using the same delivery system. Rational design of the nucleotide sequence is also possible using widely available software, which reduces the optimization efforts by trial and error.

Nonviral delivery systems are currently preferred to viral delivery systems, as they have a lower perceived risk for possible insertional mutagenesis and carcinogenicity (102). Furthermore, viral delivery systems are more suitable for permanent gene modification, which is usually not a goal in wound-healing applications, given that wounds are transient in nature. The main functional targets of therapeutic nucleic acids are largely the same as those of small molecule and protein therapeutics, namely promotion of cellular migration and proliferation, enhancement of angiogenesis and tissue oxygenation, and mitigation of excessive inflammation and proteolytic activity (100).

6.1. Nucleic Acids Targeting Inflammation

A well-documented feature of chronic wounds is the elevated levels of matrix metalloproteinases (MMPs), in particular MMP-2 and MMP-9 (103). Thus, MMP-2 and MMP-9 have been popular targets for intervention using known extracellular inhibitors but also using a variety of siRNA-based approaches with several different delivery systems (104, 105). The inherent activity of the MMP can also be used as a regulator of its own release, as shown in a study where release of the bioactive siRNA from its depot was regulated by an MMP-2 susceptible linker (106).

A closely related chronic wound feature is the persistent inflammation that prevents progression of the wound-healing response to the proliferative phase. Thus, there have been many efforts to mitigate inflammation in chronic wounds, most specifically by targeting pathways that would favor a transition from the proinflammatory M1 to the proresolution M2 macrophage phenotypes. Some of these efforts focus on silencing well-known inflammatory mediators, such as tumor necrosis factor-alpha and monocyte chemoattractant protein-1 (107), or signaling pathways that are involved in the regulation of multiple inflammatory mediators, such as extracellular signal regulated kinase-1 (108). Plasmids that encode for anti-inflammatory miRNAs, such as miRNA-497, have also been described (109). A synthetic mimic of miRNA-146a, an inflammation regulator, has been shown to accelerate wound healing in mice (110). Similarly, a miR-223 mimic was used to promote M2 macrophages, resulting in increased vascularization (111).

6.2. Nucleic Acids Targeting Angiogenesis

Prior studies have determined the differentially expressed miRNAs in diabetic wounds compared with nondiabetic wounds (112), as well as other miRNAs of importance to healing (113), thus providing a roadmap to address delayed wound healing in diabetics through miRNAs. miRNAs are endogenous regulators of gene expression that bind complementary mRNA transcripts, thus preventing translation or causing their degradation. A single miRNA may control a large number of genes and thus is a potent target for therapeutic modulation. Inhibition of specific miRNAs, such as miR-615-5p, miR-135a-3p, and miR-26a, has been shown to promote angiogenesis and wound healing (114, 115). Another anti-miRNA, this one targeted to miR-92a, has also shown preclinical promise and is undergoing clinical trials (116). Recently, a photoactivatable version of this anti-miRNA has been developed so that it can be released in the local wound area by light exposure as a way to minimize off-target effects (117).

Promoting angiogenesis is a common aim for nucleic acid–based therapies. Angiogenic growth factors, such as epidermal growth factor (EGF), VEGF, platelet-derived growth factor (PDGF), and angiogenin, are not very stable in vivo; however, transient transfection of cells in the wound through DNA plasmids encoding for these peptides can increase local growth factor levels for days to weeks. Several different formulations for the delivery of the plasmids, including combination with antibacterials and incorporation into a bandage format, have been reported and shown to improve the healing response in animal models (118–120). Clinical trials using a DNA plasmid for hepatocyte growth factor and a chemically modified mRNA for VEGF are underway (100, 121).

HIF has also been a target in many studies, because a major endogenous pathway for promoting angiogenesis via VEGF and PDGF triggered by hypoxia is governed by HIF. For example, the use of a plasmid bearing a HIF-1α form that was truncated to increase its persistence in the cell enhanced angiogenesis in diabetic rat wounds (122). Biochemical features of hypoxic wound environments, such as low pH and increased levels of ROS, have been used as cues to control the degradation of polymer scaffolds releasing siRNA against prolyl hydroxylase domain protein 2, an intracellular protein that downregulates HIF and the angiogenic response (123).

Furthermore, restoration of the cellular redox regulator Nrf2 by introducing an siRNA against the Nrf2 repressor Keap1 improved healing of diabetic mouse wounds (124).

6.3. Nucleic Acids Targeting Reepithelialization

Keratinocytes, given their critical role in reforming the skin barrier function, are another common target for therapeutic nucleic acids. A plasmid encoding for keratinocyte growth factor was shown to enhance healing of experimental wounds in mice (125). An anti-miRNA (targeted to miR-210) was also used to hasten keratinocyte proliferation and migration, thus resulting in faster wound closure (126). Wound reepithelialization may also be accelerated using antisense DNA to decrease levels of connexin 43, which normally decreases after wounding but remains elevated in diabetes (127). The decrease in connexin 43 is typically associated with increased cell migration and proliferation, and other interventions that promote cell migration, such as decreasing miRNA-378a-5p, result in enhanced wound healing (128).

Therapeutic nucleic acids have typically shown low efficiency. Their efficiency may be improved using computationally designed double-stranded miRNAs, also known as miRNA mimics, which are more stable than their natural counterparts and have also been demonstrated to promote angiogenic responses, as is the case with a mimic of miR-148b (129). Another approach may be to codeliver other factors that are involved in siRNA-mediated silencing, such as argonaute proteins (130).

7. BIOMATERIALS AND SKIN SUBSTITUTES

Humans have treated and covered skin wounds using various materials for thousands of years (131). The first human-made biologically inspired scaffold developed to promote skin tissue regeneration was developed in the 1980s and consisted of a cross-linked porous matrix of collagen and glycosaminoglycan dubbed the DRT (59). Since then, various biomaterials have been developed that are more specifically tailored for chronic wounds and that functionally address different aspects of the wound-healing response (132). Biomaterials that are specifically designed to promote skin regeneration usually combine properties of biocompatibility and in vivo biodegradability and exert bioactivity that emanates from the scaffold material itself or subsequently incorporated bioactive compounds (133). Bioactivity is dependent upon biophysical factors, such as pore size, orientation, and shape, as well as biochemical factors, either immobilized or in solution, that interact with cellular receptors. Methods for biomaterial synthesis have evolved from bulk processing approaches, such as lyophilization of suspensions and solutions of matrix materials, to layer-by-layer deposition and 3D printing methods that allow precise manufacturing of more in vivo–like heterogeneous structures (134). In parallel, injectable hydrogels have also been developed to allow use in hard-to-reach cavities or to enable better conformation and provide a seamless interface with the wound edges (135).

Collagen (and its close relative gelatin) is one of the most preferred components in biomaterials for skin regeneration because of its similarity with native skin ECM; however, collagen gels are weak mechanically and undergo biodegradation too rapidly. These issues were addressed early on with the DRT by cross-linking collagen with chondroitin sulfate (59). Other strategies have been pursued since then to develop mechanically competent collagen-based systems that allow for incorporation of a variety of bioactive compounds, such as incorporation of polycaprolactone (PCL), poly(glycolic acid), poly(DL-lactic-co-glycolic acid), and poly(L-lactic acid), with a resulting material supportive of the growth of ADSCs (136). Natural polysaccharides have also been incorporated with the collagen matrix to stabilize the hydrogel structure and have been shown to

result in a material that is permissive to various skin-relevant cell types, including MSCs, and that promotes angiogenesis and wound healing in mice (137). An interesting approach to slowing biomaterial degradation was attempted by switching the chirality of peptides used for cross-linking injectable microporous annealed particle scaffolds; this approach had an unexpected effect of triggering type 2 innate and adaptive immune responses against the D-amino acids and resulted in improved skin healing with features of regeneration based on hair follicle formation and restoration of preinjury collagen structure (138).

Incorporation of laminin, a natural component of basal membranes, has been shown to enhance the proangiogenic and healing effects of MSCs in collagen gels implanted in diabetic mice (139). The addition of sericin, a component of silk produced by the worm *Bombyx mori*, has been shown to promote epithelial differentiation of MSCs (140). Broadly speaking, silk-based materials have been extensively studied for skin wound-healing applications (141). Gelatin-based matrices containing the photosensitive polymer poly(3-hexylthiophene) and EGF showed that ADSCs could differentiate into epidermal cells upon light stimulation (142).

Another natural polymer, fibrin, which is already present in early stages of wound healing due to the activation of the clotting cascade, has been used in several experimental models and is available for clinical use as fibrin glue; however, it is mechanically weak and not easy to apply on a surface due to its rapid and difficult-to-control gelation process. Nevertheless, fibrin scaffolds loaded with ADSCs have shown an acceleration of healing in animal models (143). Other natural ECM proteins, such as laminin, have been used to functionalize PCL scaffolds and shown to support cultured stem cells and then promote wound healing in vivo with evidence for recovery of skin appendages (144). A thrombin-activated microporous annealed particle scaffold strongly promoted angiogenesis and wound healing in mice, even in the absence of exogenous growth factors; it also had the ability to support complex cellular network formation of a variety of cell types in vitro including fibroblasts, ADSCs, and BM-MSCs (145).

Additive manufacturing is a new trend in biomaterial design for skin tissue engineering, with the potential to provide more immediate treatment to the patient than traditional biomaterial manufacturing methods. For example, collagen-based beads on a scale of ~2 mm in size can reproducibly be generated on superhydrophobic surfaces created by treating polystyrene with perfluorodecyltriethoxysilane. Such beads can be loaded with ADSCs as well as other bioactives (in this case platelet lysate), stored frozen so they are available off the shelf, and then assembled into tissue layers that are stabilized by the formation of cell bridges across the beads (146). Porcine skin that is decellularized, thus leaving only the ECM, can be formulated as a printable bioink that can incorporate a variety of living cell types. The bioink is then either extruded or sprayed with an inkjet nozzle onto a surface. Prevascularized skin patches containing human endothelial progenitor cells and ADSCs were found to promote angiogenesis, wound blood flow, and healing in experimental wounds in mice (147). Direct printing of BM-MSCs and amniotic fluid–derived MSCs suspended in fibrin-collagen gels on wounds in nude mice showed increased vascularization and faster wound closure; interestingly, cell labeling showed that the therapeutic cells were not integrated into the host tissue, suggesting that the majority of the observed effects were due to paracrine interactions (148). Cell printing technology and bioink formulation are still relatively new endeavors, and further development of the methodology is needed (149).

Stem cells have also been used in conjunction with skin grafting to improve graft take. For example, a small clinical study combined autologous BM-MSCs and skin fibroblasts with the patient's skin graft to treat diabetic ulcers, resulting in ulcer improvement (150). Larger human studies comparing this approach with the standard of care (autologous graft with no added stem cells) would be needed to establish the true benefit of using MSCs.

8. FUTURE PERSPECTIVES

Currently, successful closure of a chronic wound more often than not leads to the formation of a fibrotic scar lacking skin appendages such as hair follicles and sweat glands and also lacking appropriate sensation and pigmentation (5). Achieving regenerative healing with full restoration of preinjury tissue architecture is the holy grail of the ongoing efforts to improve treatments for chronic wounds. Recent investigations have provided some tantalizing evidence that the normal fibrotic response wounds can be altered to produce a regenerative response. The above-reviewed literature provides a wide range of approaches that can address regenerative healing, to various degrees, in chronic skin wounds.

Most of the studies reviewed herein have been performed in animal models and focus on modulation of the biochemical and biophysical signals in the local wound environment. Individuals with chronic wounds most often have comorbidities, especially impaired circulation in the extremities, that are not addressed through local wound therapies and also are not included in the majority of animal models used to test potential regenerative therapies. Clearly, the hypoxic and nutrient-poor environments of the wound impede not only energy-requiring fundamental cellular processes of migration, proliferation, and differentiation in endogenous wound cells but also the function of exogenous therapeutic cells (151, 152). Thus, adjunct modalities, such as microvascular surgery, hyperbaric oxygen, and other modes to supply nutrients to the wounds, may still be necessary. Other aspects of the host environment, such as the disruption of normal growth factor and chemokine signaling due to the proinflammatory environment of diabetes, may also need to be addressed (72, 75).

Social determinants of health (SDOH), which are defined as environmental conditions including socioeconomic status, nutritional insecurity, and home living conditions, are increasingly recognized as key factors in determining therapeutic outcomes for patients suffering from a broad range of illnesses (153, 154). Although the relative impact of SDOH on patients with chronic wounds remains poorly studied (155), a detailed understanding of how these issues impact clinical healing will be critical to optimizing the success of new therapies for chronic wounds that are developed in the future, by allowing appropriate patient selection and defining the patient environment that will be most conducive to helping these treatments produce a regenerative outcome.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors would like to acknowledge financial support from the United States Department of Defense (grant W81XWH-17-1-0194) and the Plastic Surgery Foundation.

LITERATURE CITED

1. Sen CK. 2019. Human wounds and its burden: an updated compendium of estimates. *Adv. Wound Care* 8:39–48
2. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, et al. 2018. An economic evaluation of the impact, cost, and Medicare policy implications of chronic nonhealing wounds. *Value Health* 21:27–32
3. Eming SA, Martin P, Tomic-Canic M. 2014. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci. Transl. Med.* 6:265sr6
4. Frykberg RG, Banks J. 2015. Challenges in the treatment of chronic wounds. *Adv. Wound Care* 4:560–82

5. Hetzler PT 3rd, Dash BC, Guo S, Hsia HC. 2019. Targeting fibrotic signaling: a review of current literature and identification of future therapeutic targets to improve wound healing. *Ann. Plast. Surg.* 83:e92–95
6. Moore AL, Marshall CD, Barnes LA, Murphy MP, Ransom RC, Longaker MT. 2018. Scarless wound healing: transitioning from fetal research to regenerative healing. *Wiley Interdiscip. Rev. Dev. Biol.* 7:e309
7. Marshall CD, Hu MS, Leavitt T, Barnes LA, Lorenz HP, Longaker MT. 2018. Cutaneous scarring: basic science, current treatments, and future directions. *Adv. Wound Care* 7:29–45
8. Correa-Gallegos D, Jiang D, Christ S, Ramesh P, Ye H, et al. 2019. Patch repair of deep wounds by mobilized fascia. *Nature* 576:287–92
9. Mascharak S, desJardins-Park HE, Davitt MF, Griffin M, Borrelli MR, et al. 2021. Preventing *Engrailed-1* activation in fibroblasts yields wound regeneration without scarring. *Science* 372:eaba2374
10. Shook BA, Wasko RR, Rivera-Gonzalez GC, Salazar-Gatzimas E, Lopez-Giraldez F, et al. 2018. Myofibroblast proliferation and heterogeneity are supported by macrophages during skin repair. *Science* 362:eaar2971
11. Haydont V, Bernard BA, Fortunel NO. 2019. Age-related evolutions of the dermis: clinical signs, fibroblast and extracellular matrix dynamics. *Mech. Ageing Dev.* 177:150–56
12. Phan QM, Fine GM, Salz L, Herrera GG, Wildman B, et al. 2020. Lef1 expression in fibroblasts maintains developmental potential in adult skin to regenerate wounds. *Elife* 9:e60066
13. Rognoni E, Gomez C, Pisco AO, Rawlins EL, Simons BD, et al. 2016. Inhibition of β-catenin signalling in dermal fibroblasts enhances hair follicle regeneration during wound healing. *Development* 143:2522–35
14. Wier EM, Garza LA. 2020. Through the lens of hair follicle neogenesis, a new focus on mechanisms of skin regeneration after wounding. *Semin. Cell Dev. Biol.* 100:122–29
15. Zakrzewski W, Dobrzynski M, Szymonowicz M, Rybak Z. 2019. Stem cells: past, present, and future. *Stem Cell Res. Ther.* 10:68
16. Jimenez F, Poblet E, Izeta A. 2015. Reflections on how wound healing-promoting effects of the hair follicle can be translated into clinical practice. *Exp. Dermatol.* 24:91–94
17. Motegi SI, Ishikawa O. 2017. Mesenchymal stem cells: the roles and functions in cutaneous wound healing and tumor growth. *J. Dermatol. Sci.* 86:83–89
18. Zhu J, Huang H, Chen H, Zhang X, Li Z, et al. 2018. Plerixafor and granulocyte-colony-stimulating factor for mobilization of hematopoietic stem cells for autologous transplantation in Chinese patients with non-Hodgkin's lymphoma: a randomized Phase 3 study. *Transfusion* 58:81–87
19. Guenou H, Nissan X, Larcher F, Feteira J, Lemaitre G, et al. 2009. Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. *Lancet* 374:1745–53
20. de Miguel-Beriain I. 2015. The ethics of stem cells revisited. *Adv. Drug Deliv. Rev.* 82–83:176–80
21. Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–76
22. Dash BC, Korutla L, Vallabhajosyula P, Hsia HC. 2021. Unlocking the potential of induced pluripotent stem cells for wound healing: the next frontier of regenerative medicine. *Adv. Wound Care*. In press. **https://doi.org/10.1089/wound.2021.0049**
23. Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, et al. 2017. Autologous induced stem-cell-derived retinal cells for macular degeneration. *N. Engl. J. Med.* 376:1038–46
24. Gorecka J, Kostiuk V, Fereydooni A, Gonzalez L, Luo J, et al. 2019. The potential and limitations of induced pluripotent stem cells to achieve wound healing. *Stem Cell Res. Ther.* 10:87
25. Kosaric N, Kiwanuka H, Gurtner GC. 2019. Stem cell therapies for wound healing. *Expert Opin. Biol. Ther.* 19:575–85
26. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, et al. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315–17
27. Kim I, Bang SI, Lee SK, Park SY, Kim M, Ha H. 2014. Clinical implication of allogenic implantation of adipogenic differentiated adipose-derived stem cells. *Stem Cells Transl. Med.* 3:1312–21
28. Malhotra P, Shukla M, Meena P, Kakkar A, Khatri N, et al. 2022. Mesenchymal stem cells are prospective novel off-the-shelf wound management tools. *Drug Deliv. Transl. Res.* 12(1):79–104

29. Dash BC, Xu Z, Lin L, Koo A, Ndon S, et al. 2018. Stem cells and engineered scaffolds for regenerative wound healing. *Bioengineering* 5:23
30. Gorecka J, Gao X, Fereydooni A, Dash BC, Luo J, et al. 2020. Induced pluripotent stem cell-derived smooth muscle cells increase angiogenesis and accelerate diabetic wound healing. *Regen. Med.* 15:1277–93
31. Chen D, Hou Q, Zhong L, Zhao Y, Li M, Fu X. 2019. Bioactive molecules for skin repair and regeneration: progress and perspectives. *Stem Cells Int.* 2019:6789823
32. Zhou J, Sun J. 2019. A revolution in reprogramming: small molecules. *Curr. Mol. Med.* 19:77–90
33. Horinouchi CDS, Oostendorp C, Schade D, van Kuppevelt TH, Daamen WF. 2021. Growth factor mimetics for skin regeneration: in vitro profiling of primary human fibroblasts and keratinocytes. *Arch. Pharm.* 354:e2100082
34. Yuan ZD, Zhu WN, Liu KZ, Huang ZP, Han YC. 2020. Small molecule epigenetic modulators in pure chemical cell fate conversion. *Stem Cells Int.* 2020:8890917
35. Williams IM, Wu JC. 2019. Generation of endothelial cells from human pluripotent stem cells. *Arterioscler. Thromb. Vasc. Biol.* 39:1317–29
36. Xu M, He J, Zhang C, Xu J, Wang Y. 2019. Strategies for derivation of endothelial lineages from human stem cells. *Stem Cell Res. Ther.* 10:200
37. Lee S, Park C, Han JW, Kim JY, Cho K, et al. 2017. Direct reprogramming of human dermal fibroblasts into endothelial cells using ER71/ETV2. *Circ. Res.* 120:848–61
38. Wary A, Wary N, Baruah J, Mastej V, Wary KK. 2017. Chromatin-modifying agents convert fibroblasts to OCT4+ and VEGFR-2+ capillary tube-forming cells. *PLOS ONE* 12:e0176496
39. Song W, Kaufman DS, Shen W. 2016. Efficient generation of endothelial cells from human pluripotent stem cells and characterization of their functional properties. *J. Biomed. Mater. Res. A* 104:678–87
40. Tao R, Sun TJ, Han YQ, Xu G, Liu J, Han YF. 2014. Epimorphin-induced differentiation of human umbilical cord mesenchymal stem cells into sweat gland cells. *Eur. Rev. Med. Pharmacol. Sci.* 18:1404–10
41. Xu Y, Hong Y, Xu M, Ma K, Fu X, et al. 2016. Role of keratinocyte growth factor in the differentiation of sweat gland-like cells from human umbilical cord-derived mesenchymal stem cells. *Stem Cells Transl. Med.* 5:106–16
42. Yang WS, Kang S, Sung J, Kleinman HK. 2019. Thymosin β4: potential to treat epidermolysis bullosa and other severe dermal injuries. *Eur. J. Dermatol.* 29:459–67
43. Al Haj Zen A, Nawrot DA, Howarth A, Caporali A, Ebner D, et al. 2016. The retinoid agonist tazarotene promotes angiogenesis and wound healing. *Mol. Ther.* 24:1745–59
44. Gao SQ, Chang C, Li JJ, Li Y, Niu XQ, et al. 2018. Co-delivery of deferoxamine and hydroxysafflor yellow A to accelerate diabetic wound healing via enhanced angiogenesis. *Drug Deliv.* 25:1779–89
45. Guenat OT, Geiser T, Berthiaume F. 2020. Clinically relevant tissue scale responses as new readouts from organs-on-a-chip for precision medicine. *Annu. Rev. Anal. Chem.* 13:111–33
46. Huang C, Leavitt T, Bayer LR, Orgill DP. 2014. Effect of negative pressure wound therapy on wound healing. *Curr. Probl. Surg.* 51:301–31
47. Baldwin C, Potter M, Clayton E, Irvine L, Dye J. 2009. Topical negative pressure stimulates endothelial migration and proliferation: a suggested mechanism for improved integration of Integra. *Ann. Plast. Surg.* 62:92–96
48. Argenta LC, Morykwas MJ. 1997. Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. *Ann. Plast. Surg.* 38:563–77
49. Zhang L, Weng T, Wu P, Li Q, Han C, Wang X. 2020. The combined use of negative-pressure wound therapy and dermal substitutes for tissue repair and regeneration. *Biomed. Res. Int.* 2020:8824737
50. Thakral G, Lafontaine J, Najafi B, Talal TK, Kim P, Lavery LA. 2013. Electrical stimulation to accelerate wound healing. *Diabet Foot Ankle* 4:10.3402/dfa.v4i0.22081
51. Rajendran SB, Challen K, Wright KL, Hardy JG. 2021. Electrical stimulation to enhance wound healing. *J. Funct. Biomater.* 12:40
52. Lv H, Liu J, Zhen C, Wang Y, Wei Y, et al. 2021. Magnetic fields as a potential therapy for diabetic wounds based on animal experiments and clinical trials. *Cell Prolif.* 54:e12982
53. Long Y, Wei H, Li J, Yao G, Yu B, et al. 2018. Effective wound healing enabled by discrete alternative electric fields from wearable nanogenerators. *ACS Nano* 12:12533–40

54. Yao G, Jiang D, Li J, Kang L, Chen S, et al. 2019. Self-activated electrical stimulation for effective hair regeneration via a wearable omnidirectional pulse generator. *ACS Nano* 13:12345–56
55. Rubin AE, Usta OB, Schloss R, Yarmush M, Golberg A. 2019. Selective inactivation of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* with pulsed electric fields and antibiotics. *Adv. Wound Care* 8:136–48
56. Das B, Berthiaume F. 2021. Irreversible electroporation as an alternative to wound debridement surgery. *Surg. Technol. Int.* 39:sti39/1452
57. Das B, Shrirao A, Golberg A, Berthiaume F, Schloss R, Yarmush ML. 2020. Differential cell death and regrowth of dermal fibroblasts and keratinocytes after application of pulsed electric fields. *Bioelectricity* 2:175–85
58. Li X, Saeidi N, Villiger M, Albadawi H, Jones JD, et al. 2018. Rejuvenation of aged rat skin with pulsed electric fields. *J. Tissue Eng. Regen. Med.* 12:2309–18
59. Yannas IV, Tzeranis DS. 2021. Mammals fail to regenerate organs when wound contraction drives scar formation. *NPJ Regen. Med.* 6:39
60. Tabares FL, Junkar I. 2021. Cold plasma systems and their application in surface treatments for medicine. *Molecules* 26:1903
61. Gan L, Jiang J, Duan JW, Wu XJZ, Zhang S, et al. 2021. Cold atmospheric plasma applications in dermatology: a systematic review. *J. Biophotonics* 14:e202000415
62. Wen X, Xin Y, Hamblin MR, Jiang X. 2021. Applications of cold atmospheric plasma for transdermal drug delivery: a review. *Drug Deliv. Transl. Res.* 11:741–47
63. Bourke P, Ziuzina D, Han L, Cullen PJ, Gilmore BF. 2017. Microbiological interactions with cold plasma. *J. Appl. Microbiol.* 123:308–24
64. Arndt S, Unger P, Berneburg M, Bosserhoff AK, Karrer S. 2018. Cold atmospheric plasma (CAP) activates angiogenesis-related molecules in skin keratinocytes, fibroblasts and endothelial cells and improves wound angiogenesis in an autocrine and paracrine mode. *J. Dermatol. Sci.* 89:181–90
65. Schmidt A, Liebelt G, Niessner F, von Woedtke T, Bekeschus S. 2021. Gas plasma-spurred wound healing is accompanied by regulation of focal adhesion, matrix remodeling, and tissue oxygenation. *Redox Biol.* 38:101809
66. Gorbanev Y, Soriano R, O'Connell D, Chechik V. 2016. An atmospheric pressure plasma setup to investigate the reactive species formation. *J. Vis. Exp.* 117:54765
67. Mohamed H, Gebski E, Reyes R, Beane S, Wigdahl B, et al. 2021. Differential effect of non-thermal plasma RONS on two human leukemic cell populations. *Cancers* 13:2437
68. Xie J, Chen Q, Suresh P, Roy S, White JF, Mazzeo AD. 2017. Paper-based plasma sanitizers. *PNAS* 114:5119–24
69. Larouche J, Sheoran S, Maruyama K, Martino MM. 2018. Immune regulation of skin wound healing: mechanisms and novel therapeutic targets. *Adv. Wound Care* 7(7):209–31
70. Menon R, Krzyszczyk P, Berthiaume F. 2017. Pro-resolution potency of resolvins D1, D2 and E1 on neutrophil migration and in dermal wound healing. *Nano Life* 7(1):1750002
71. Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. 2018. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. *Front. Physiol.* 9:419
72. Olekson MP, Faulknor RA, Hsia HC, Schmidt AM, Berthiaume F. 2016. Soluble receptor for advanced glycation end products improves stromal cell-derived factor-1 activity in model diabetic environments. *Adv. Wound Care* 5:527–38
73. Nurkesh A, Jaguparov A, Jimi S, Saparov A. 2020. Recent advances in the controlled release of growth factors and cytokines for improving cutaneous wound healing. *Front. Cell Dev. Biol.* 8:638
74. Blume P, Bowlby M, Schmidt B, Donegan R. 2014. Safety and efficacy of Becaplermin gel in the treatment of diabetic foot ulcers. *Chronic Wound Care Manag. Res.* 1:11–14
75. Kang HJ, Kumar S, D'Elia A, Dash B, Nanda V, et al. 2021. Self-assembled elastin-like polypeptide fusion protein coacervates as competitive inhibitors of advanced glycation end-products enhance diabetic wound healing. *J. Control Release* 333:176–87
76. Jimi S, Jaguparov A, Nurkesh A, Sultankulov B, Saparov A. 2020. Sequential delivery of cryogel released growth factors and cytokines accelerates wound healing and improves tissue regeneration. *Front. Bioeng. Biotechnol.* 8:345

77. Lombardi F, Palumbo P, Augello FR, Cifone MG, Cinque B, Giuliani M. 2019. Secretome of adipose tissue-derived stem cells (ASCs) as a novel trend in chronic non-healing wounds: an overview of experimental in vitro and in vivo studies and methodological variables. *Int. J. Mol. Sci.* 20:3721
78. Xu J, Liu X, Zhao F, Zhang Y, Wang Z. 2020. HIF1α overexpression enhances diabetic wound closure in high glucose and low oxygen conditions by promoting adipose-derived stem cell paracrine function and survival. *Stem Cell Res. Ther.* 11:148
79. An YH, Kim DH, Lee EJ, Lee D, Park MJ, et al. 2021. High-efficient production of adipose-derived stem cell (ADSC) secretome through maturation process and its non-scarring wound healing applications. *Front. Bioeng. Biotechnol.* 9:681501
80. Cai Y, Li J, Jia C, He Y, Deng C. 2020. Therapeutic applications of adipose cell-free derivatives: a review. *Stem Cell Res. Ther.* 11:312
81. Kim MH, Wu WH, Choi JH, Kim J, Jun JH, et al. 2018. Galectin-1 from conditioned medium of three-dimensional culture of adipose-derived stem cells accelerates migration and proliferation of human keratinocytes and fibroblasts. *Wound Repair Regen.* 26(Suppl. 1):S9–18
82. Lou P, Liu S, Xu X, Pan C, Lu Y, Liu J. 2021. Extracellular vesicle-based therapeutics for the regeneration of chronic wounds: current knowledge and future perspectives. *Acta Biomater.* 119:42–56
83. French KC, Antonyak MA, Cerione RA. 2017. Extracellular vesicle docking at the cellular port: extracellular vesicle binding and uptake. *Semin. Cell Dev. Biol.* 67:48–55
84. Zhang B, Wang M, Gong A, Zhang X, Wu X, et al. 2015. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. *Stem Cells* 33:2158–68
85. Hahm J, Kim J, Park J. 2021. Strategies to enhance extracellular vesicle production. *Tissue Eng. Regen. Med.* 18:513–24
86. Patel DB, Santoro M, Born LJ, Fisher JP, Jay SM. 2018. Towards rationally designed biomanufacturing of therapeutic extracellular vesicles: impact of the bioproduction microenvironment. *Biotechnol. Adv.* 36:2051–59
87. Grangier A, Branchu J, Volatron J, Piffoux M, Gazeau F, et al. 2021. Technological advances towards extracellular vesicles mass production. *Adv. Drug Deliv. Rev.* 176:113843
88. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. 2019. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. *Cells* 8:307
89. Yuan F, Li YM, Wang Z. 2021. Preserving extracellular vesicles for biomedical applications: consideration of storage stability before and after isolation. *Drug Deliv.* 28:1501–9
90. Trenkenschuh E, Richter M, Heinrich E, Koch M, Fuhrmann G, Friess W. 2022. Enhancing the stabilization potential of lyophilization for extracellular vesicles. *Adv. Healthc. Mater.* 11:e2100538
91. An Y, Lin S, Tan X, Zhu S, Nie F, et al. 2021. Exosomes from adipose-derived stem cells and application to skin wound healing. *Cell Prolif.* 54:e12993
92. Hu Y, Tao R, Chen L, Xiong Y, Xue H, et al. 2021. Exosomes derived from pioglitazone-pretreated MSCs accelerate diabetic wound healing through enhancing angiogenesis. *J. Nanobiotechnol.* 19:150
93. Chen L, Qin L, Chen C, Hu Q, Wang J, Shen J. 2021. Serum exosomes accelerate diabetic wound healing by promoting angiogenesis and ECM formation. *Cell Biol. Int.* 45:1976–85
94. Yin H, Chen CY, Liu YW, Tan YJ, Deng ZL, et al. 2019. *Synechococcus elongatus* PCC7942 secretes extracellular vesicles to accelerate cutaneous wound healing by promoting angiogenesis. *Theranostics* 9:2678–93
95. Li B, Luan S, Chen J, Zhou Y, Wang T, et al. 2020. The MSC-derived exosomal lncRNA H19 promotes wound healing in diabetic foot ulcers by upregulating PTEN via microRNA-152–3p. *Mol. Ther. Nucleic Acids* 19:814–26
96. Li X, Xie X, Lian W, Shi R, Han S, et al. 2018. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp. Mol. Med.* 50:1–14
97. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, et al. 2020. Extracellular vesicles as drug delivery systems: why and how? *Adv. Drug Deliv. Rev.* 159:332–43
98. Sutaria DS, Badawi M, Phelps MA, Schmittgen TD. 2017. Achieving the promise of therapeutic extracellular vesicles: The devil is in details of therapeutic loading. *Pharm. Res.* 34:1053–66

99. Lv Q, Deng J, Chen Y, Wang Y, Liu B, Liu J. 2020. Engineered human adipose stem-cell-derived exosomes loaded with miR-21-5p to promote diabetic cutaneous wound healing. *Mol. Pharm.* 17:1723–33
100. Berger AG, Chou JJ, Hammond PT. 2021. Approaches to modulate the chronic wound environment using localized nucleic acid delivery. *Adv. Wound Care* 10:503–28
101. Herter EK, Xu Landén N. 2017. Non-coding RNAs: new players in skin wound healing. *Adv. Wound Care* 6:93–107
102. Picanco-Castro V, Pereira CG, Covas DT, Porto GS, Athanassiadou A, Figueiredo ML. 2020. Emerging patent landscape for non-viral vectors used for gene therapy. *Nat. Biotechnol.* 38:151–57
103. Caley MP, Martins VL, O'Toole EA. 2015. Metalloproteinases and wound healing. *Adv. Wound Care* 4:225–34
104. Castleberry SA, Almquist BD, Li W, Reis T, Chow J, et al. 2016. Self-assembled wound dressings silence MMP-9 and improve diabetic wound healing in vivo. *Adv. Mater.* 28:1809–17
105. Li N, Yang L, Pan C, Saw PE, Ren M, et al. 2020. Naturally-occurring bacterial cellulose-hyperbranched cationic polysaccharide derivative/MMP-9 siRNA composite dressing for wound healing enhancement in diabetic rats. *Acta Biomater.* 102:298–314
106. Kim HS, Yoo HS. 2013. Matrix metalloproteinase-inspired suicidal treatments of diabetic ulcers with siRNA-decorated nanofibrous meshes. *Gene Ther.* 20:378–85
107. Kasiewicz LN, Whitehead KA. 2019. Lipid nanoparticles silence tumor necrosis factor α to improve wound healing in diabetic mice. *Bioeng. Transl. Med.* 4:75–82
108. Tezgel Ö, DiStasio N, Laghezza-Masci V, Taddei A-R, Szarpak-Jankowska A, et al. 2020. Collagen scaffold-mediated delivery of NLC/siRNA as wound healing materials. *J. Drug Deliv. Sci. Technol.* 55:101421
109. Ban E, Jeong S, Park M, Kwon H, Park J, et al. 2020. Accelerated wound healing in diabetic mice by miRNA-497 and its anti-inflammatory activity. *Biomed. Pharmacother.* 121:109613
110. Sener G, Hilton SA, Osmond MJ, Zgheib C, Newsom JP, et al. 2020. Injectable, self-healable zwitterionic cryogels with sustained microRNA–cerium oxide nanoparticle release promote accelerated wound healing. *Acta Biomater.* 101:262–72
111. Saleh B, Dhaliwal HK, Portillo-Lara R, Shirzaei Sani E, Abdi R, et al. 2019. Local immunomodulation using an adhesive hydrogel loaded with miRNA-laden nanoparticles promotes wound healing. *Small* 15:e1902232
112. Ozdemir D, Feinberg MW. 2019. MicroRNAs in diabetic wound healing: pathophysiology and therapeutic opportunities. *Trends Cardiovasc. Med.* 29:131–37
113. Mulholland EJ, Dunne N, McCarthy HO. 2017. MicroRNA as therapeutic targets for chronic wound healing. *Mol. Ther. Nucleic Acids* 8:46–55
114. Icli B, Wu W, Ozdemir D, Li H, Cheng HS, et al. 2019. MicroRNA-615-5p regulates angiogenesis and tissue repair by targeting AKT/eNOS (protein kinase B/endothelial nitric oxide synthase) signaling in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 39:1458–74
115. Icli B, Wu W, Ozdemir D, Li H, Haemmig S, et al. 2019. MicroRNA-135a-3p regulates angiogenesis and tissue repair by targeting p38 signaling in endothelial cells. *FASEB J.* 33:5599–614
116. Gallant-Behm CL, Piper J, Dickinson BA, Dalby CM, Pestano LA, Jackson AL. 2018. A synthetic microRNA-92a inhibitor (MRG-110) accelerates angiogenesis and wound healing in diabetic and non-diabetic wounds. *Wound Repair Regen.* 26:311–23
117. Lucas T, Schafer F, Muller P, Eming SA, Heckel A, Dimmeler S. 2017. Light-inducible antimiR-92a as a therapeutic strategy to promote skin repair in healing-impaired diabetic mice. *Nat. Commun.* 8:15162
118. Shi R, Lian W, Han S, Cao C, Jin Y, et al. 2018. Nanosphere-mediated co-delivery of VEGF-A and PDGF-B genes for accelerating diabetic foot ulcers healing in rats. *Gene Ther.* 25:425–38
119. Wang S, Yan C, Zhang X, Shi D, Chi L, et al. 2018. Antimicrobial peptide modification enhances the gene delivery and bactericidal efficiency of gold nanoparticles for accelerating diabetic wound healing. *Biomater. Sci.* 6:2757–72
120. Mo Y, Guo R, Zhang Y, Xue W, Cheng B, Zhang Y. 2017. Controlled dual delivery of angiogenin and curcumin by electrospun nanofibers for skin regeneration. *Tissue Eng. Part A* 23:597–608
121. Sun N, Ning B, Hansson KM, Bruce AC, Seaman SA, et al. 2018. Modified VEGF-A mRNA induces sustained multifaceted microvascular response and accelerates diabetic wound healing. *Sci. Rep.* 8:17509

122. Thiersch M, Rimann M, Panagiotopoulou V, Ozturk E, Biedermann T, et al. 2013. The angiogenic response to PLL-g-PEG-mediated HIF-1α plasmid DNA delivery in healthy and diabetic rats. *Biomaterials* 34:4173–82
123. Martin JR, Nelson CE, Gupta MK, Yu F, Sarett SM, et al. 2016. Local delivery of PHD2 siRNA from ROS-degradable scaffolds to promote diabetic wound healing. *Adv. Healthc. Mater.* 5:2751–57
124. Rabbani PS, Zhou A, Borab ZM, Frezzo JA, Srivastava N, et al. 2017. Novel lipoproteoplex delivers *Keap1* siRNA based gene therapy to accelerate diabetic wound healing. *Biomaterials* 132:1–15
125. Kobsa S, Kristofik NJ, Sawyer AJ, Bothwell AL, Kyriakides TR, Saltzman WM. 2013. An electrospun scaffold integrating nucleic acid delivery for treatment of full-thickness wounds. *Biomaterials* 34:3891–901
126. Ghatak S, Li J, Chan YC, Gnyawali SC, Steen E, et al. 2016. AntihypoxamiR functionalized gramicidin lipid nanoparticles rescue against ischemic memory improving cutaneous wound healing. *Nanomedicine* 12:1827–31
127. Gilmartin DJ, Soon A, Thrasivoulou C, Phillips AR, Jayasinghe SN, Becker DL. 2016. Sustained release of Cx43 antisense oligodeoxynucleotides from coated collagen scaffolds promotes wound healing. *Adv. Healthc. Mater.* 5:1786–99
128. Li H, Chang L, Du WW, Gupta S, Khorshidi A, et al. 2014. Anti-microRNA-378a enhances wound healing process by upregulating integrin beta-3 and vimentin. *Mol. Ther.* 22:1839–50
129. Miscianinov V, Martello A, Rose L, Parish E, Cathcart B, et al. 2018. MicroRNA-148b targets the TGF-β pathway to regulate angiogenesis and endothelial-to-mesenchymal transition during skin wound healing. *Mol. Ther.* 26:1996–2007
130. Li J, Wu C, Wang W, He Y, Elkayam E, et al. 2018. Structurally modulated codelivery of siRNA and Argonaute 2 for enhanced RNA interference. *PNAS* 115:E2696–705
131. Shah JB. 2011. The history of wound care. *J. Am. Col. Certif. Wound Spec.* 3:65–66
132. Zhang X, Shu W, Yu Q, Qu W, Wang Y, Li R. 2020. Functional biomaterials for treatment of chronic wound. *Front. Bioeng. Biotechnol.* 8:516
133. Hosseini M, Shafiee A. 2021. Engineering bioactive scaffolds for skin regeneration. *Small* 17:e2101384
134. Nikolova MP, Chavali MS. 2019. Recent advances in biomaterials for 3D scaffolds: a review. *Bioact. Mater.* 4:271–92
135. Liang Y, Zhao X, Hu T, Chen B, Yin Z, et al. 2019. Adhesive hemostatic conducting injectable composite hydrogels with sustained drug release and photothermal antibacterial activity to promote full-thickness skin regeneration during wound healing. *Small* 15:e1900046
136. Rahmani Del Bakhshayesh A, Mostafavi E, Alizadeh E, Asadi N, Akbarzadeh A, Davaran S. 2018. Fabrication of three-dimensional scaffolds based on nano-biomimetic collagen hybrid constructs for skin tissue engineering. *ACS Omega* 3:8605–11
137. Wong VW, Rustad KC, Galvez MG, Neofytou E, Glotzbach JP, et al. 2011. Engineered pullulan-collagen composite dermal hydrogels improve early cutaneous wound healing. *Tissue Eng. Part A* 17:631–44
138. Griffin DR, Archang MM, Kuan CH, Weaver WM, Weinstein JS, et al. 2021. Activating an adaptive immune response from a hydrogel scaffold imparts regenerative wound healing. *Nat. Mater.* 20:560–69
139. Assi R, Foster TR, He H, Stamati K, Bai H, et al. 2016. Delivery of mesenchymal stem cells in biomimetic engineered scaffolds promotes healing of diabetic ulcers. *Regen. Med.* 11:245–60
140. Bhowmick S, Thanusha AV, Kumar A, Scharnweber D, Rother S, Koul V. 2018. Nanofibrous artificial skin substitute composed of mPEG–PCL grafted gelatin/hyaluronan/chondroitin sulfate/sericin for 2nd degree burn care: in vitro and in vivo study. *RSC Adv.* 8:16420–32
141. Farokhi M, Mottaghitalab F, Fatahi Y, Khademhosseini A, Kaplan DL. 2018. Overview of silk fibroin use in wound dressings. *Trends Biotechnol.* 36:907–22
142. Jin G, Prabhakaran MP, Ramakrishna S. 2014. Photosensitive and biomimetic core-shell nanofibrous scaffolds as wound dressing. *Photochem. Photobiol.* 90:673–81
143. Ozpur MA, Guneren E, Canter HI, Karaaltin MV, Ovali E, et al. 2016. Generation of skin tissue using adipose tissue-derived stem cells. *Plast. Reconstr. Surg.* 137:134–43

144. Bahrami H, Keshel SH, Chari AJ, Biazar E. 2016. Human unrestricted somatic stem cells loaded in nanofibrous PCL scaffold and their healing effect on skin defects. *Artif. Cells Nanomed. Biotechnol.* 44:1556–60
145. Griffin DR, Weaver WM, Scumpia PO, Di Carlo D, Segura T. 2015. Accelerated wound healing by injectable microporous gel scaffolds assembled from annealed building blocks. *Nat. Mater.* 14:737–44
146. Lima AC, Mano JF, Concheiro A, Alvarez-Lorenzo C. 2015. Fast and mild strategy, using superhydrophobic surfaces, to produce collagen/platelet lysate gel beads for skin regeneration. *Stem Cell Rev. Rep.* 11:161–79
147. Kim BS, Kwon YW, Kong JS, Park GT, Gao G, et al. 2018. 3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: a step towards advanced skin tissue engineering. *Biomaterials* 168:38–53
148. Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, et al. 2012. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl. Med.* 1:792–802
149. Masri S, Fauzi MB. 2021. Current insight of printability quality improvement strategies in natural-based bioinks for skin regeneration and wound healing. *Polymers* 13:1011
150. Vojtassak J, Danisovic L, Kubes M, Bakos D, Jarabek L, et al. 2006. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. *Neuro Endocrinol. Lett.* 27(Suppl. 2):134–37
151. Kosol W, Kumar S, Marrero-Berríos I, Berthiaume F. 2020. Medium conditioned by human mesenchymal stromal cells reverses low serum and hypoxia-induced inhibition of wound closure. *Biochem. Biophys. Res. Commun.* 522:335–41
152. Faulknor RA, Olekson MA, Ekwueme EC, Krzyszczyk P, Freeman JW, Berthiaume F. 2017. Hypoxia impairs mesenchymal stromal cell-induced macrophage M1 to M2 transition. *Technology* 5:81–86
153. Kleinman DV, Pronk N, Gomez CA, Wrenn Gordon GL, Ochiai E, et al. 2021. Addressing health equity and social determinants of health through Healthy People 2030. *J. Public Health Manag. Pract.* 27(Suppl. 6):S249–57
154. Lucyk K, McLaren L. 2017. Taking stock of the social determinants of health: a scoping review. *PLOS ONE* 12:e0177306
155. Sasson DC, Duan K, Patel SM, Junn A, Hsia HC. 2021. *The impact of social determinants of health on pressure ulcer prognosis: a retrospective chart and systematic review*. Poster presented at the 38th Annual Meeting of the Northeastern Society of Plastic Surgeons, Philadelphia