A Paradigm of Fibroblast Activation and Dermal Wound Contraction to Guide the Development of Therapies for Chronic Wounds and Pathologic Scars

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Significance: Delayed wound healing and pathologic scarring are abnormal processes that can be thought of as occurring on a wound healing continuum, where insufficient wound contraction leads to nonhealing wounds, and overexuberant wound contraction leads to scarring. Chronic nonhealing wounds, including diabetic foot wounds, decubitus ulcers, and venous stasis ulcers, affect millions of people annually in the United States and costs billions of dollars. Similarly, pathologic scarring affects more than 40 million Americans annually and also costs billions of dollars.

Critical Issues: While there are multiple factors that contribute to chronic nonhealing wounds and pathologic scars, a derangement in wound contraction is common to both. In this article, we will present a paradigm of dermal wound contraction, derived from clinical observations and basic science evidence, which pertains to chronic nonhealing wounds and pathologic scars.

Recent Advances: We will review how select therapies currently under investigation and in development fit the paradigm.

Future Directions: The paradigm will facilitate translational research and enable the development of future innovative therapies.

SCOPE

WOUND HEALING PROCEEDS along a spectrum from nonhealing chronic wounds, to normal wound healing, to pathologic scarring (Table 1). Wound contraction is a fundamental physiologic mechanism that plays a central role across this spectrum. This article proposes that delayed wound contraction leads to chronic nonhealing wounds, normal wound contraction leads to healed wounds, and overexuberant wound contraction leads to pathologic scarring. This article will (1) provide a framework for understanding the clinical relevance of wound contraction, (2) briefly discuss what is known about the physiologic mechanisms/transla-

tional relevance of wound contraction as it relates to chronic wounds and scars, and (3) present case studies of devices and therapeutic agents under development that target specific aspects of wound contraction. The goal is to provide the reader with a paradigm that will guide research and development of therapies for chronic wounds and pathologic scars.

TRANSLATIONAL RELEVANCE

Chronic nonhealing wounds and pathologic scars are major medical problems that are estimated to cost billions of dollars annually.^{1,2} In the United States, chronic wounds affect 6.5 million patients. Chronic wounds

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Abbreviations and Acronyms

CTGF = connective tissue growth factor

NMMII = nonmuscle myosin II

ROCK = Rho kinase

TGFB1 = transforming growth factor beta-1

Table 1. Definitions of commonly used wound healing terminology

Term	Definition
Angiogenesis	Growth of new blood vessels from existing vessels.
Chronic wound	A wound that has failed to proceed through an orderly and timely series of events beyond 6 weeks to produce a durable structural, functional, and cosmetic closure.
Epithelialization	Growth of keratinocytes or epithelium over a granulation bed or the dermis.
Flap	A portion of the body, with intact blood supply, which is surgically moved to cover a wound in another region of the body. A flap may be composed of multiple tissue types such as skin, muscle, bone, tendon, nerve and vessels.
Granulation tissue	Tissue that is formed in a wound bed during the healing and repair process. It has a granular appearance due to sprouting of neo-capillaries in a collagenous matrix. It contains abundant fibroblasts/myofibroblasts, blood vessels, and inflammatory cells.
Healed wound	A wound that is epithelialized without breakdown for at least 3 months.
Myofibroblast	A specialized cell/differentiated fibroblast that has smooth muscle-like components. It has a role in the synthesis of ECM and is believed to predominate in force generation, which results in ECM reorganization and wound contraction.
Neovascularization	Growth of new blood vessels where there were no previous blood vessels.
Scar contracture	The long-term clinical outcome of excessive and continued wound contraction, which typically leads to soft tissue and joint deformation. They are disfiguring, prevent function, and may cause pain and itching.
Second intention healing	Healing of an open wound by granulation tissue formation and re-epithelialization without surgical intervention
Skin graft	A sheet of skin (epidermis and dermis) which has been harvested from one region of the body and applied to an open wound in another region. Split thickness skin grafts contain the epidermis and a portion of the dermis, typically the papillary dermis. Full thickness skin grafts include the epidermis and full-thickness dermis.
Contraction of wound, skin graft, flap, or scar	Progressive shrinking of a wound, skin graft, or scar by the centripetal displacement of surrounding skin edges. Contraction is caused by granulation bed fibroblasts or myofibroblasts which reside in open wounds or beneath skin grafts or scars. Recent studies suggest that it is the fibrocontractile apparatus of these cells that matters most for promoting contraction, rather than a single cell type, per say. These cells may reside either in the center of the wound or the periphery beneath the skin edges.
Pathologic scarring	Scarring that causes significant pain, pruritus and/or functional impairment of an organ
Primary intention healing	Healing of a wound by intentional closure and securing of the wound edges together

ECM, extracellular matrix.

cost approximately \$25 billion annually to treat, and the expenses are rapidly growing. The annual wound care products market reached \$15.3 billion in 2010.³ There are ~34 million American patients who undergo surgical procedures. Two million people are injured in motor vehicle accidents and over 2.4 million patients are burned. In severe burns and blast injuries, >40% of patients develop large joint scar contractures.⁴ Between both chronic wounds and scars, there are more than 40 million Americans affected by these medical problems annually. It has been suggested that “the immense economic and social impact of wounds in our society calls for allocation of a higher level of research resources to understand biological mechanisms underlying the complexities noted in problem wounds.”³

CLINICAL RELEVANCE

Most chronic wound treatments and anti-scarring therapies in the clinic today have been empirically derived.⁵ They are re-application therapies that were often found to be effective in diseases and then applied to wounds or scars because of perceived pathogenic similarities between the intended disease and wound healing (e.g., steroids and antimetabolites) or they were approved by the Food and Drug Administration for use in humans, not intended for specific diseases (e.g.,

vitamin E, onion extract, and magnets), and then applied to wounds or scars. Most studies are observational case reports and retrospective case series. Studies, for the most part, have been poorly designed, underpowered, and anecdotal. Because of these reasons, there are many reported treatments in the literature, but therapies are truly only marginally effective.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Chronic wound therapies

Chronic wounds are classified by causality, including gradation therapy, venous stasis, diabetes, decubitus ulcers, chronic infection, and ischemia. While treatments for each type of chronic wound are based on the underlying mechanism of disease (e.g., diabetic foot wounds are managed by off-loading, maintenance of euglycemia, and optimized blood flow to the foot), there are several common therapies used to treat chronic wounds irrespective of causality. These therapies include debridement of necrotic/infected tissue, daily washing with a nontoxic cleanser, maintenance of a moist healing environment, avoidance of tobacco products, and increased protein intake.⁶ The most important endpoint in a clinical trial is time until full re-epithelialization.⁷ Additional considerations in clinical trials are time until 50% reduction in

wound size and stability of a healed wound for at least 3 months.

Scar reduction therapies

Scars are not classified by causality but by their physical characteristics, with persistence or absence of growth being an important component. There are (1) mature scars (light-colored, flat scars that have gradually regressed and are static), (2) immature scars (a red, sometimes itchy or painful, and slightly elevated scar in the process of regressing), (3) hypertrophic scars (a red, raised, sometimes itchy scar confined to the border of the original wound, which slowly regresses over time; 6 months to 2 years), (4) keloids (a raised scar that extends beyond the margins of the original injury and continues to grow over time), and (5) scar contractures (a shrunken scar that limits joint range of motion and persistence or absence of growth is not a consideration).⁸ Antiscarring therapies primarily consist of surgical scar revision with attention to minimizing tension, aligning scars along relaxed skin tension lines, minimizing tissue damage, and expediting closure of the open wound.^{9,10} There are no effective nonsurgical approaches to reduce scarring.¹¹ In clinical trials, relevant endpoints include scar growth, vascularity, pigmentation, thickness, pliability, or surface area.¹² Patient pain and itching is also a consideration.¹³ For contractures, elasticity and joint range of motion are primary outcomes.⁴

Investigating chronic wounds and phases of wound healing

What is needed in the wound healing field are rationally designed, basic and applied investigations to determine why chronic wounds and pathologic scars occur. The basis for rationally designed investigations comes from a combination of clinical observations, basic *in vitro* and *in vivo* research, and a fundamental understanding of granulation tissue and wound contraction. Dermal wound healing proceeds through an orderly sequence of partially overlapping phases: inflammation, proliferation, and remodeling.¹⁴ In the inflammatory phase, neutrophils and macrophages infiltrate the wound bed, remove pathogenic organisms, and secrete cytokines to recruit fibroblasts, endothelial cells, and keratinocytes. In the proliferative phase, quintessential granulation tissue forms. In wounds that are not primarily closed, granulation tissue forms. Granulation tissue is a friable tissue comprised of a newly synthesized extracellular cellular matrix and fibroblasts/myofibroblasts, blood vessels, and inflammatory cells, which provides volume to a wound, facilitates closure by driving

wound contraction, and enables healing by promoting re-epithelialization. In second intention wound healing or in wounds that have been treated with a flap or graft, granulation tissue persists after a wound has re-epithelialized. That persistence, which lasts throughout the remodeling phase of repair, is what causes continued scar contraction and increases scar tensile strength. The right balance between too little and too much wound contraction, in addition to other complex factors, is what determines second intention healing.

Granulation tissue formation, wound contraction, and epithelialization as it relates to nonhealing wounds

In nonhealing wounds, there is a failure of granulation tissue formation, wound contraction, and epithelialization. Interestingly, the degree by which wound contraction or epithelialization promotes healing in a chronic wound depends upon regional anatomy and is not related to causality. For example, wound contraction would be expected to play a large role in diabetic wounds on the nape of the neck but not in diabetic wounds on the foot. The reason why wound contraction is regionally dependent is the anchoring ligaments that attach skin to bone. In loose tissue, such as the back, abdomen, or face, anchoring ligaments are sparse and lax; as such, wound contraction plays a large role. In tightly adherent tissue such as the palm, foot, or anterior tibial region, skin ligaments are dense and taut; as such, wound contraction plays a small role and epithelialization is the predominant mediator of healing. In practical terms, this means that the wound fills with granulation tissue and that the epithelium has a larger surface to cover since there is no wound contraction. It also means that there is little granulation tissue underlying the re-epithelialized surface; therefore, the repaired tissue is relatively unstable. Tissue instability is undesirable and an indication for complex surgical soft tissue reconstruction.

Granulation tissue formation, and wound contraction as it relates to scarring

In scars with large surface areas, such as burn wounds, an important point to understand is that granulation tissue does not resolve immediately after a wound has re-epithelialized. Granulation tissue can persist beneath an epithelialized burn wound for 6 months to 2 years after healing.¹⁵ Activated granulation tissue persistently, incrementally contracts the subepithelial extracellular matrix (termed "scar contraction," which occurs in the remodeling phase of repair), leading to scarring. The mechanisms of granulation tissue

contraction are analogous whether occurring in open wounds or contracting scars.

Fibroblasts/myofibroblasts are the primary mediators of granulation tissue contraction

Granulation tissue is composed primarily of endothelial cells that form new blood vessels and fibroblasts/myofibroblasts, with fewer inflammatory cells such as mast cells, macrophages, and lymphoid cells. Fibroblasts/myofibroblasts are the workhorses of granulation tissue that promote healing. They proliferate, secrete cytokines, produce *de novo* extracellular matrix, align themselves parallel to each another, and actively shrink

the wound through coordination of individual cellular force generation. Cellular force generation is driven by stimulation of the cytoskeletal fibrocontractile apparatus, which mediates cell elongation, migration, and contractility.¹⁶ There are a number of stimulants that promote fibroblast activity (few suppressors are known) and a number of known fibroblast effector functions that promote wound contraction (Fig. 1). From a translational perspective, if a therapy were to benefit chronic nonhealing wounds, one would need to show that the therapy increases fibroblast activation, wound contraction, and epithelialization. If a therapy were to benefit scarring, one would need to show that the therapy

Figure 1. A paradigm of fibroblast/myofibroblast activation as it relates to wound contraction, wound healing, and scar contracture. Fibroblasts/myofibroblasts are the cells that cause wound granulation bed contraction. In the proposed paradigm, there are afferent (out-in) and efferent (in-out) mechanisms, with fibroblasts as the central mediators, which lead to wound contraction. The afferent loop consists of extracellular modulators that promote cell responsiveness by stimulating cell surface receptors and regulation of downstream intracellular cascades including changes in the cytoskeleton, molecular signaling pathways, and gene upregulation. The efferent loop begins with the cell response. The fibrocontractile apparatus is assembled, *de novo* smooth muscle α -actin transcribed, cytokines and chemokines synthesized, enzymes secreted, and extracellular matrix elements produced. The extracellular matrix elements are deposited and the tissue is remodeled by enzyme degradation, cell traction, elongation, and contractility. Approaches to modulating wound granulation bed contraction to either promote closure of nonhealing wounds or prevent scar contracture include targeting fibroblast stimulants, fibroblast inhibitors, or fibroblast effectors. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

dampens fibroblast activation, reduces cellularity, and inhibits wound contraction without being cytotoxic. What follows are several recent case studies that demonstrate how therapies under development fit the paradigm of dermal wound contraction.

CASE STUDIES

Innovative research can be divided into that which arises from clinical observations and is reduced to study in the laboratory, or that which arises from basic research and is validated in animal models. In either scenario, the study of fibroblast activity is a necessity for both types of applied research, irrespective of the target of interest. Examples of clinical observations that have been reduced to study and are now being translated include observations that (1) infected wounds will not heal, (2) inflammation promotes scarring, and (3) increasing mechanical tension causes worsening of scars. Examples of basic science studies that have been validated in animal models include studies that observe (1) soluble factors promote fibroblast activity and thus cause scarring, (2) extracellular scaffolds facilitate fibroblast activity and thus promote chronic wounds to heal but also cause scarring, and (3) cytoskeletal activation upregulates fibroblast contractility and thus causes scarring.

Clinical observations leading to research and development studies

Wound infection

Clinical observations leading to hypothesis. While it is well-known that infected wounds will not heal and that removal of necrotic and infected tissue promotes healing, it is unknown why wounds that appear healthy and are colonized with bacteria will not heal. Scanning electron microscopy of chronic wounds reveals that more than 60% of chronic wounds have biofilms, as compared to 6% of acute wounds.¹⁷ These findings and the research of others have led to the hypothesis that bacteria form biofilms to escape the host response and evade antibiotic therapy; as such, biofilms cause non-healing wounds.¹⁸

Basic mechanistic studies. Biofilms are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix.¹⁹ In this environment, the pathogens have an increased growth rate and gene transcription. *Pseudomonas aeruginosa* displays multiple phenotypes when in a biofilm. The pro-pathogen biofilm environment severely impairs fibroblast function and inhibits healing.²⁰ Conditioned bio-

film methicillin-resistant *Staphylococcus aureus* media limits fibroblast migration, alters cytokine secretion, and causes cell death.²¹ Biofilms also inhibit granulation tissue formation and delay wound contraction in diabetic mice.^{20,22}

Intervention developed based on hypothesis. There are several interventions under development to eradicate biofilms.²³ These include, but are not limited to, topical antibiotics, silver-impregnated gauze, combination therapies, lasers, dressings, and application of serine proteases.^{24–26}

Preclinical/clinical trial research studies. One study that is currently seeking enrollment involves the use of *Staphylococcus epidermidis* JK16 cell supernatants or a serine protease to destroy pre-existing *S. aureus* biofilms.²⁷ The investigators hypothesize that the use of *S. epidermidis* JK16, culture supernatants or purified serine protease protein will disrupt *S. aureus* biofilms, decrease bacterial colonization of chronic wounds, and improve healing times. The primary outcome measure will be healing rate as calculated for each 6-week standard or experimental treatment periods.²⁸

Inflammation

Clinical observations leading to hypothesis. It has long been known that human burn wounds that are inflamed (red, hot, swollen, with large numbers of inflammatory cells) form worse scars than noninflamed wounds. Likewise, with the advent of fetal surgery, scientists discovered that human fetal wounds heal without scar and are associated with reduced inflammation. These two observations have led investigators to study the role of inflammation in scarring.

Basic mechanistic studies. There are multiple inflammatory cells that putatively contribute to scarring, including the macrophage, mast cell, and T cell.^{29–31} Castagnoli found abundant numbers of Langerhans cells, T cells, and macrophages in hypertrophic scar biopsies.³² Studies by Wong *et al.* demonstrated that scarring is reduced almost ninefold in T-cell-deficient mice.³³ Goren *et al.* and Mirza *et al.* used transgenic technology to selectively ablate macrophages and found that mice lacking macrophages had reduced collagen deposition, decreased cell proliferation, lower levels of transforming growth factor- β 1 (TGF β 1), loss of myofibroblast differentiation, and impaired wound contraction.^{34,35} Lucas *et al.* demonstrated

that depletion of macrophages in the early stage of repair significantly reduced granulation tissue formation, and minimized scar formation, whereas depletion in the mid-stage of repair prevented tissue maturation and wound closure and depletion in the late stage of repair did not significantly impact outcomes.³⁶ Foley *et al.* demonstrated that mast cells enhance fibroblast proliferation and fibroblast-populated collagen lattice contraction and Wulff *et al.* utilized the mast cell-deficient mouse to show that dermal mast cells in scarless wounds are fewer in number, less mature, and do not degranulate in response to wounding as effectively as mast cells of fibrotic wounds.^{37,38} Wulff's group also found that wounds that normally heal with a scar, healed with significantly smaller scars in mast cell-deficient mice as compared to control littermates.³⁸

Intervention developed based on hypothesis. Macrophages are the primary source of cytokines released during wound healing, and TGFB is the most potent class of cytokines that modulate scarring.^{39–41} TGFB1 increases scarring and TGFB3 reduces scarring. In 1992, Shah injected TGB-neutralizing antibody into rodent wounds and found reduced scarring.⁴² Khaw and colleagues later applied a novel recombinant monoclonal neutralizing antibody to human TGFB2 to the conjunctiva to reduce glaucoma filtration surgery scarring and found it significantly improved glaucoma filtration surgery outcome in an animal model of aggressive conjunctival scarring compared with control and was clinically safe, non-toxic, and well tolerated after subconjunctival administration.⁴³ These and related research studies led to the formation of a biotechnology company, Renovo, who then tested their TGFB modulators in humans.^{39,44–46}

Preclinical/clinical trial research studies. Renovo discovered and commercially developed avotermin, the first human recombinant TGFB3, a key protein involved in scar-free healing observed in embryos. They found in double-blind, randomized clinical studies that avotermin, administered as an intradermal injection at the time of surgery, led to short-term and longer-term improvements in scars.^{5,47,48} Later studies revealed that a second TGFB3 agonist, Juvista, when applied to scars led to an improvement in structural organization of the healing wound and reduction of subsequent scarring.⁴⁹ Unfortunately, in February 2011, it was reported that Juvista did not meet its primary or secondary endpoints in phase III trials.

Mechanical strain

Clinical observations leading to hypothesis. Perhaps as long as humans have lived, it has been known that mechanical strain leads to scarring, which is why a basic tenet in reconstructive surgery is to design the approach to reduce mechanical tension.⁵⁰ Scars along regions of the body where skin is under more tension, such as the chest and shoulders, are more prominent than scars along areas with less tension, such as the abdomen. Moreover, pressure garment therapy is standard of care for prevention and treatment of hypertrophic scarring after burn injury.⁵¹

Basic mechanistic studies. Biomechanical and histologic analysis of normal skin reveals that in unwounded skin fibroblasts are quiescent, native collagen is arranged randomly, elastin fibers are abundant, and matrix stiffness is low (0.75 N/mm).^{52,53} Conversely, in scar contractures fibroblasts are active, collagen is arranged in linear arrays, scars are thick, elastic fibers are few, and matrix stiffness is high (3 N/mm).⁵⁴ Matrix stiffness correlates with mechanical strain transmission to the fibroblast/myofibroblast as mechanical tension is transmitted from the extracellular matrix to the fibroblast's cytoskeleton (out-in) and from the fibroblast's cytoskeleton back to the extracellular matrix (in-out) to cause shortening of the extracellular matrix and contraction of the wound granulation bed along lines of stress.^{16,55} On a cellular level, increases in matrix rigidity are a potent activator of fibroblast contractility.

Intervention developed based on hypothesis. There have been multiple attempts at shielding scars from mechanical tension.^{56–58} A recent example is the creation of the Neodyne™ technology platform, invented by a team of bioengineers and surgeon scientists from Stanford. Neodyne is a mechanomodulating polymer device that, when applied across a scar, reduces mechanical tension on the scar. Utilizing the red Duroc swine, stress shielding of incisions reduced histologic scar area by 6- and 9-fold compared to control and elevated stress states, respectively ($p < 0.01$ for both) and dramatically decreased the histologic expression of profibrotic markers.⁵⁹

Preclinical/clinical trial research studies: The Neodyne technology is currently being tested in clinical trials. In a phase I clinical study, the stress-shielding polymer was used to treat abdominal wounds. Photographs taken between 8 and 12 months postsurgery revealed that stress shielding

significantly improved scar appearance ($p=0.004$) compared with within-patient controls.⁵⁹ In 2012, the Neodyne company reported that in a prospective, open label, randomized, multi-center study, their stress shielding device was effective at reducing scarring after abdominoplasties.⁶⁰ The device will be commercially available within the United States in the fourth quarter of 2012.

Basic science observations which have led to therapy development

Soluble factors

Background. As discussed above, TGF β is a well-known mediator of fibrosis, which has been targeted to prevent scarring. However, because of its pleiotropic effects, efforts to modulate TGF β have been limited by concerns about treatment specificity.⁶¹ TGF β induces connective tissue growth factor (CTGF) through several pathways. CTGF is thought to contribute to scarring because it mediates many of TGF β 's pro-fibrotic effects, and it induces scarring independent of TGF β .^{62–65}

Basic mechanistic studies. CTGF is a 38-kDa extracellular matrix protein. It stimulates a diverse array of cellular functions such as cell proliferation, migration, differentiation, angiogenesis, extracellular matrix production, and adhesion.^{66,67} CTGF is overexpressed in keloids, hypertrophic scars, and localized sclerosis.^{65,68} Using a rabbit ear model of hypertrophic scarring, Sisco *et al.* demonstrated that antisense oligonucleotides to CTGF mRNA markedly reduced hypertrophic scarring, myofibroblast formation, and genes associated with extracellular matrix turnover.⁶²

Intervention developed based on hypothesis. CTGF has been widely studied for its effects on scarring.^{65,69,70} Schultz and colleagues led a series of investigations to understand how antisense technology could be used to inhibit CTGF production and reduce scarring. He first assessed the role of CTGF antisense in the development of breast implant elastomer capsule formation over time. While implanting breast implants in the dorsum of rats, it was found that antisense technology reduced CTGF and that the technology reduced capsular contractures (scar contraction) based on histology.⁷¹ Subsequent studies were performed on corneal fibroblasts, since excessive fibroblast-mediated tissue contraction lead to contractile scarring in the eye. CTGF antisense reduced fibroblast populated collagen lattice contraction and inhibited TGF β 1-stimulated myofibroblast differen-

tiation and fibroblast populated collagen lattice contraction. Based on these studies, as well as the work of others, inhibition of CTGF activity or gene transcription could be a suitable target for anti-scarring therapies.^{72,73} Using his novel rabbit ear hypertrophic scar model, Sisco *et al.* found that CTGF increased as scarring progressed, and showed that CTGF antisense reduced hypertrophic scarring.⁶²

Preclinical/clinical trial research studies. Excaliard Pharmaceuticals, Inc., was later formed to commercialize the technology. In January 2011, they reported three studies that demonstrated EXC 001's efficacy in elective revision surgery of scars resulting from prior breast surgery (Study 203), efficacy in fine line scars in elective abdominoplasty surgery (Study 202) and activity in a biomarker study of abdominal scars (Study 201). The results showed a significant reduction in scar severity for EXC 001-treated scars versus placebo-treated scars in all three trials. EXC 001 was well tolerated with no important drug-related adverse effects observed. They are currently enrolling patients for a safety and efficacy study to demonstrate their technology improve the appearance of scars from prior breast surgery.⁷⁴

Extracellular matrix scaffolds

Background. In third-degree burns with >40% total body surface area skin loss there is a need for skin substitutes to replace the lost tissue. In addition to providing coverage when autografts are not available, skin substitutes eliminate donor-site morbidity, and can expedite healing, reducing length of stay and improving survival. Dermal templates should be designed to protect the wound from infection and fluid loss, provide a stable template that allows host incorporation, expedite healing, be easy to handle and be durable.⁷⁵ There is a long history of material development, and many groups have created different types of skin equivalents that can be categorized by anatomy, composition, or cellular makeup.⁷⁶ One approach that is instructive for case study is the use of fabricated, collagen scaffolds.

Basic mechanistic studies. Basic mechanistic studies and intervention developed based on hypothesis Yannas and Middelkoop have been lead investigators in this field.^{77–94} In 1989, Yannas *et al.* reported that the wound contraction inhibiting activity of collagen-based scaffolds depends upon pore size, and the scaffolds persistence as an

insoluble network over a critical period of time.⁹⁵ He concluded that the collagen-based scaffolds inhibit wound contraction because they interrupt the transference of mechanical stress over the scale of the wound, and prevent myofibroblast formation. He later argued, in 2005, that dermal templates promote regeneration by inhibiting wound contraction.⁸³ Middelkoop's group designed and developed an acellular double-layered skin construct, using matrix molecules and growth factors to promote wound closure with minimal contraction, early vascularization, and elastin formation.⁹⁴ Using a rat full-thickness wound model, they created a template where the "epidermal layer" was prepared using type I collagen, heparin, and fibroblast growth factor 7, while the porous dermal layer was prepared using type I collagen, solubilized elastin, dermatan sulfate, heparin, fibroblast growth factor 2, and vascular endothelial growth factor. They found that the double-layered skin construct showed more cell influx, less contraction, and increased angiogenesis at early time points in comparison to controls.

Preclinical/clinical trial research studies. One of the best examples of an artificial collagen-based product that has been created to improve healing is Integra™, pioneered by Burke and Yannas. Integra was applied to patients with life-threatening burns who underwent primary excision and grafting. Controls received the investigator's usual skin grafting material. At 1-year postgrafting, there was less hypertrophic scarring of the artificial dermis, and more patients preferred the artificial dermis to the control graft.⁹⁶ In 2011, Middelkoop's group reported the first subjective and objective results of a 12-year follow-up of dermal substitution in acute and reconstructive burn wounds, examining their acellular double-layered skin construct.⁹¹ They concluded that, after this long period, there was an improvement in scar parameters in both acute and reconstructive substituted wounds. Treated areas were significantly smoother than controls and there was increased elasticity in treated scars. Lastly, they believe that randomized controlled trials are needed to obtain more data on the effectiveness of dermal substitutes.

Cell cytoskeleton

Background. In the paradigm of fibroblast activation and dermal wound contraction, the fibrocontractile apparatus of the fibroblast/myofibroblast is centrally causative of granulation tissue contraction. The fibrocontractile apparatus consists

of a complex conglomerate of proteins that link cell surface receptors to the cytoskeleton. The fibrocontractile apparatus controls mechanotransduction. It enables cells to sense extracellular strain (afferent stimuli) and to transmit forces to the extracellular matrix (efferent stimuli).

Basic mechanistic studies. There are thousands of studies on different constituent proteins of the fibrocontractile apparatus, but perhaps one of the most interesting proteins to discuss from a translational perspective is Rho kinase (ROCK). ROCK is the predominant enzyme in the calcium-independent signaling pathway that promotes cell contractility, elongation, and migration. ROCK has at least three mechanisms of action: (1) ROCK phosphorylates myosin regulatory light chain and myosin phosphatase that promotes the nonmuscle myosin II (NMMII)-actin kinetic interaction, (2) ROCK activates transcriptional factors, myocardin-related transcriptional factor and serum response factor to promote transcription of cytoskeletal elements such as actin and NMMII, and (3) ROCK promotes (focal adhesion) FA formation.⁹⁷

Intervention developed based on hypothesis. Over the last 10–15 years, ROCK has become a common target of many pharmaceutical/biotechnology companies because of its role in different diseases.⁹⁸ It has been shown to reduce scarring in a variety of preclinical studies, where the skin is one example.^{99,100}

Preclinical/clinical trial research studies. Interestingly, there is a paucity, if not absence, of clinical trials investigating the role of ROCK inhibitors or other cytoskeletal antagonists in reducing scarring. It is unclear why this is the case. We speculate that perhaps development of a novel drug for reduction of scarring may be prohibitively expensive considering the market size and potential return on investment or that biotechnology/pharmaceutical companies have not considered the indication of scarring when developing compounds. If a company were to have a compound that modulates the fibrocontractile apparatus, it is clear from the literature that such a compound could be used to promote closure of nonhealing wounds or to prevent scarring.

CONCLUSIONS

Chronic nonhealing wounds and scars are epidemic problems. They are caused by either insufficient or overexuberant granulation tissue function. Granulation tissue function is regulated

by many putative stimulants, suppressants, and cell effectors that affect dermal fibroblast/myofibroblast activation. Fibroblast/myofibroblast activation leads to wound contraction, which causes wound healing and scar contracture. Translational therapies that are being studied and developed to treat nonhealing wounds and scars need to be tested for their effects on fibroblast activation, epithelialization, and wound contraction.

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