Dermatologic surgery

Strategies for prevention of scars: what can we learn from fetal skin?

Mohammad Reza Namazi[1], MD, Mohammad Kazem Fallahzadeh[2], MD, and Robert A. Schwartz[3], MD, MPH

[1]Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Dermatology Department, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, and [3]Department of Dermatology and Pathology, New Jersey Medical School, Newark, NJ, USA

Correspondence
Dr. Mohammad Kazem Fallahzadeh, MD
Dermatology Department
Faghihi Hospital
Shiraz
Iran
E-mail: kazem.fa@gmail.com

Funding sources: None.
Conflict of interest: None.

Abstract

Fetal wound healing occurs rapidly and without scar formation early in gestation. Studying the mechanisms of scarless repair can lead to novel scar-preventive approaches. In fetal wounds, collagen is deposited early and is fine and reticular with less cross-linking. Several important differences of fetal vs. postgestational wound-healing response have been determined, such as the presence of less inflammation, higher hyaluronic acid concentration and a greater ratio of collagen type III to type I. Compared with typical wounds, there are also altered ratios of signaling molecules, such as higher ratios of transforming growth factor (TGF)-β3 to TGF-β1 and -β2, and matrix metalloproteinases to tissue inhibitors of metalloproteinases. Furthermore, fetal fibroblasts do not exhibit TGF-β1-induced collagen production compared with their mature counterparts. Patterning genes (homeobox genes) involved in organogenesis are more active in the fetal period and are believed to be the "first domino" in the fetal cutaneous wound repair regulatory cascade. The recommended scar-preventive agents, such as Scarguard MD®, silicone gel and sheet, Sepraﬁlm® Bioresorbable Membrane, topical hyaluronan, onion extract, oral tamoxifen and 585-nm pulsed dye laser are reviewed in this study. Despite the lack of supporting evidence, there is a widespread false presumption that the acceleration of healing with the widely assumed scar-preventive commercial agents is associated with decreased scar formation. Humans are erroneously inclined to make a negative correlation between the healing rate and the degree of scar formation, while such a correlation does not exist in reality. Despite the importance of scar prevention, no FDA-approved therapy for this purpose is available in the 21st century, which reflects the important challenges, such as the presence of redundant pathways, that these approaches are facing.

Introduction

Scar formation is a major clinical problem resulting in adverse cosmesis, loss of function, especially if over joints, and hindrance of growth in children. Scars also have a dramatic impact on the patient's quality of life, and have been associated with anxiety, social avoidance, and depression.[1] Therefore, prevention of scar formation has long been important. Scar reduction is not only important to dermatologists, but is also salient in many other conditions, such as adhesions and strictures resulting from surgical procedures in abdominal and pelvic cavities, spinal cord ruptures, scarification of hand tendons after injury, corneal abrasions, glomerulonephritis, cirrhosis, human vascular restenosis lesions, myocardial infarction, systemic sclerosis, and diffuse fasciitis. The principles of anti-scarring therapy based on modulation of pro-scarring vs. anti-scarring factors appear to be promising for all the above-mentioned conditions, indicating that studies in the skin could have a broader clinical application.

Scarless healing

Scar and fibrosis are the end result of surgical and non-surgical skin injury. Aggressive wound healing may have once offered an evolutionary advantage for survival at the expense of scar formation. Amazingly, fetal cutaneous wounds, especially in the first 6 months of gestation, heal without scar formation.[2] Many clinicians hope that understanding the remarkable reparative capabilities of

the fetus may lead to the development of new wound-healing therapies that reduce or prevent scar formation and fibrosis.

Fetal monkey lip incisional wounds heal with restoration of normal appendage and dermal collagen architecture in midgestation. At the start of the third trimester, these wounds do not restore appendage (hair follicle and sebaceous gland) architecture, but still heal with a normal collagen pattern. Thus, a ''transition wound'' phenotype occurs. By the mid-third trimester, the wounds heal with a typical scar pattern, i.e. no appendages and collagen scar.[3] The transition point in a human fetal skin model also occurs after the second trimester of gestation.[4]

It is notable that not all fetal tissues share the anti-scarring properties of fetal skin; fetal wounds in the diaphragm and the gastrointestinal tract heal by fibrosis and contraction, as in the adults.[5,6] Fetal cutaneous wound healing is not only scarless but also rapid. The rapid epithelialization of fetal wounds may occur in part because of early deposition of tenascin and fibronectin, which are thought to be necessary for migration and cell anchoring, respectively.[7] As discussed below, overexpression of vascular endothelial growth factor (VEGF) may be another reason for rapid healing of fetal wounds. How is scarless wound healing different than the scarring wound healing? In scarless wounds, collagen is rapidly deposited in a fine reticular pattern indistinguishable from uninjured skin. In contrast, adult scarring wounds have disorganized and thick collagen bundles with more collagen cross-linking.[7]

Interestingly, amniotic fluid is neither essential nor sufficient for scarless repair. Fetal marsupials develop outside the uterus in a maternal pouch and heal cutaneous wounds without scar.[8] Adult sheep skin transplanted onto the backs of fetal sheep bathed in the amniotic fluid of the intrauterine environment heal with scarring of incisional wounds.[9]

Structure of fetal vs. postnatal wounds

Several structural and molecular differences between adult and fetal wounds exist.

Ratio of collagen types

Type I collagen is the predominant collagen of both adult and fetal extracellular matrix. However, fetal skin has a higher ratio of type III to type I collagen; with maturation, the relative amount of type III collagen decreases.[10,11]

Hyaluronic acid (hyaluronan, HA)

A glycoprotein called HA-stimulating activity (HASA) is found in fetal skin and is absent in adult wounds. This glycoprotein is suggested to be responsible for an increase in HA and the resulting enhanced fluidity, which allows better influx of fibroblasts.[12] By the nature of its hygroscopic properties, HA can occupy 10,000 times its own volume. Thus, HA allows proliferating cells to avoid inhibitory contacts.[13] Hyaluronic acid synthesis precedes mitosis and dissociates the dividing cell from its substratum, permitting cell movement.[13]

Fetal fibroblasts have more surface receptors for HA than adult fibroblasts, enhancing fibroblast migration.[2]

Experimental tympanic membrane perforations in rats treated with HA not only close faster but also heal with much less scar tissue than the untreated controls.[12]

Scarless wound-healing physiology

Transforming growth factor-β (TGF-β)

Transforming growth factor-β is secreted by most cells involved in wound healing, including neutrophils, lymphocytes, macrophages, keratinocytes, and fibroblasts. Transforming growth factor-β is first released from degranulating platelets.[14] Interestingly, TGF-β upregulates its own production in an autocrine pattern, leading to TGF-β overproduction and scar formation.[14] Transforming growth factor-β is a potent chemoattractant of macrophages, neutrophils and fibroblasts, and stimulates extracellular matrix synthesis and prevents its degradation by upregulating the expression of tissue inhibitors of metalloproteinases (TIMPs) and downregulating the expression of proteases. Three highly homologous TGF-β genes in mammals, designated TGF-β1, -β2 and -β3, have been identified.[14] Shah *et al.*[15] treated adult rodent wounds with isoform-specific neutralizing antibodies either alone or in combination. Exogenous addition of neutralizing antibody to TGF-β1 alone resulted in some reduction in the inflammatory and angiogenic responses, as well as reduction of extracellular matrix deposition in the early stages with a marginal reduction in cutaneous scarring. By contrast, neutralizing antibody to TGF-β2 alone had little effect on the inflammatory or angiogenic responses, and no effect on the resultant scar. However, when neutralizing antibodies to TGF-β1 and TGF-β2 were administered together, the synergistic effect resulted in a dramatic amelioration of scar formation. Surprisingly, exogenous addition of TGF-β3 to cutaneous wounds in adult rodents also produced the same effects. Wounds treated with TGF-β3 showed a marked reduction in the immunoreactivity for TGF-β1 and TGF-β2 on Days 5 and 7 post-wounding compared with untreated control wounds. TGF-β3 is thus believed to downregulate TGF-β1 and TGF-β2.[14]

Fetal wounds have higher ratios of TGF-β3 to TGF-β1 and TGF-β2, favoring less scar formation.[16,17]

Additionally, in a recent study by Rolfe *et al.*[18] it has been shown that fetal fibroblasts, in comparison with

mature fibroblasts, respond differently to TGF-β1 stimulation and fail to demonstrate the TGF-β1-induced production of collagen (mRNA and protein). This different response is due to a comparatively short-lived or rapid phosphorylation of several components of the TGF-β1 signaling pathways.

Interestingly, healing of oral mucosal wounds produces less scarring compared with skin. Expression of Integrin αvβ6 is increased in oral mucosal epithelium during wound healing; this protein enhances the secretion of both TGF-β1 and TGF-β3. In a recent study by Eslami *et al.*, expression of TGF-β molecules and β6 integrin was assessed in experimental human gingival wounds, and the gingiva and skin of red Duroc pigs by means of real-time polymerase chain reaction, gene microarrays, and immunostaining.[17] As with human wounds, β6 integrin expression was upregulated in the pig wounds 7 days postwounding, and remained enhanced for more than 49 days. The expression levels of β6 integrin and TGF-β3 were markedly higher in the pig gingival wounds compared with cutaneous wounds. It was concluded that the prolonged expression of Integrin αvβ6 resulting in a higher concentration of activated TGF-β3 protected gingival wound epithelium from scar formation.

Decorin, fibromodulin, and lysyl oxidase

Decorin is a proteoglycan component of connective tissue. It binds to type I collagen fibrils and plays a role in matrix assembly. Decorin's name is a derivative of the fact that it "decorates" collagen. Fibromodulin is a small interstitial proteoglycan, which may participate in the assembly of the extracellular matrix as it interacts with type I and type II collagen fibrils and inhibits fibrillogenesis *in vitro*. It may also downregulate TGF-β activities by sequestering TGF-β in the extracellular matrix. Decorin is downregulated in fetal wounds, while fibromodulin is upregulated.[2]

Lysyl oxidase levels also increase during fetal skin development, perhaps resulting in higher collagen cross-linking.[2]

Matrix metalloproteinases (MMP) and TIMPs

Scarless wounds have a higher ratio of MMP to TIMP, favoring remodeling and less accumulation of collagen.[2,19] This may be due to decreased expression of TGF-β1, as it decreases MMP and increases TIMP expression, favoring collagen accumulation and scarring.[20]

Platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF)

Scarring wounds have higher levels of the profibrotic cytokines PDGF and FGF. Platelet-derived growth factor is a potent mitogen and chemoattractant for fibroblasts.[2]

Hypoxia and VEGF

The fetal skin is relatively hypoxic. Midgestational fetal lamb tissue pO_2 is about 16 mmHg, whereas adult tissue pO_2 ranges from 45 to 60 mmHg. TGF-β1 production by fetal fibroblasts may be blunted in hypoxemic conditions.[14] Physiologically low oxygen concentrations in fetal skin upregulate hypoxia-inducible factor 1, which is a potent transcriptional regulator of oxygen-dependent genes such as VEGF and TGF-β3.[21] Vascular endothelial growth factor, a mitogen for endothelial cells, increases twofold in scarless wounds, while its expression remains unchanged in scarring fetal wounds. Interestingly, one novel, non-protein angiogenic factor is nicotine, which acts through nicotinic acetylcholine receptors to stimulate angiogenesis. The use of nicotine accelerates angiogenesis and wound healing in the diabetic mouse model.[20] On the other hand, it is shown that endostatin, an angiogenesis inhibitor, minimizes scarring of mouse wounds.[20] It is difficult to interpret these results given that VEGF is expressed more in scarless healing. Therefore, the role of VEGF in scarless healing needs more exploration.

Fibroblasts and myofibroblasts

Fibroblasts are major players in wound healing after developmental maturity; by gaining contractile proteins such as α-smooth muscle actin they transform into myofibroblasts. These cells have certain clinical features of smooth muscle cell, and contribute to scar formation and contraction; however, their presence during fetal wound healing is controversial. Some studies have shown their absence[22] while others, such as Cass *et al.*,[23] have shown their presence. In the above-mentioned study by Rolfe *et al.*,[18] fetal fibroblasts exposed to TGF-β1 have been found to be capable of maturation into myofibroblasts; however, this differentiation was found to be quicker and more transitory compared with mature fibroblasts with less production of collagen.

The contractile forces generated by myofibroblasts may alter the orientation of collagen fibrils and contribute to scarring. Adult fibroblasts need to proliferate first before being able to lay down collagen, while fetal fibroblasts are capable of proliferating simultaneously while making collagen. As a result, collagen deposition is delayed in adult wound healing, contributing to scar formation.[7,12] Additionally, fetal fibroblasts at early gestation express a lower level of TGF-β receptor II than fetal cells later in gestation. It is also demonstrated that receptor tyrosine kinases express differentially between fetal and adult rat fibroblasts, suggesting that further elucidation of the TGF-β receptor signaling process in fetal fibroblasts may help to understand how fetal cells respond to TGF-β differently from adult cells.[24] Moreover, as mentioned

earlier, fetal fibroblasts have more HA receptors than adult fibroblasts.

Dot cells

Recently, Kong *et al.*[25] identified a novel, previously unidentified group of E-cadherin-positive cells in the blood of fetal and adult mice, and named them "Dot cells". Dot cells have a tiny dot shape with a diameter between 1 and 7 μm. It is suggested that Dot cells are relatively primitive cells or stem cells because of their unusually small size and expression of stem cell markers such as E-cadherin, integrin β1 and CD34 by these cells. The percentage of Dot cells in fetal mice blood is more than 20 times higher compared with adult blood. Dot cells migrate to wounds and differentiate into dermal cells, which release less interstitial collagen and reduce scarring. Transplantation of Dot cells to adult mice heals skin wounds with less scarring due to reduced smooth muscle actin and collagen expression in the repair tissue. These results infer that Dot cells are a previously unidentified component in scarless wound healing.

Inflammation and HA

Scarless fetal wounds have less inflammatory infiltrate, which may lead to improvement in the final wound-healing process. Decreased inflammation may be partly due to decreased fetal platelet degranulation and aggregation with the resultant lower levels of the chemoattractants TGF-β and PDGF.[2] Moreover, fetal neutrophils may not possess the chemotactic ability of adult neutrophils.[25] Both interleukin (IL)-6 and IL-8, which are important in chemotaxis and activation of inflammatory cells, are significantly lower in early fetal fibroblasts. Treating adult mouse wounds with IL-10, which has an anti-inflammatory effect through decreased production of IL-6 and IL-8, reduces inflammation and helps produce scarless healing. This may have therapeutic implications in human adult wounds.[2,26–28]

Insomnia, through increasing IL-6 levels,[29] might predispose patients to scar formation. If proven by controlled studies, this concept can have therapeutic implications, especially after myocardial infarction, severe burns and trauma.

High levels of HA may also contribute to decreased inflammation of fetal wounds. In *in vitro* systems, the chemotactic and random migration of white blood cells can be inhibited by HA. The phagocytic activity of mononuclear phagocytes is also inhibited by even a relatively low concentration of HA (≤0.05 mg/ml). Therefore, HA exerts biomechanical regulation over processes such as inflammation through its ability to modify the activity of cells involved in the inflammatory response.[13] The HA molecular network is able to exclude large molecules, such as fibrinogen and other proteins. Steric hindrance by the HA matrix may alter the chemotactic gradient, and thereby influence the magnitude and nature of the inflammatory response. Hyaluronic acid also serves as a high-capacity free radical scavenger. It is suggested that the presence of HA has a physical effect on the fibrin matrix that forms. Hyaluronic acid also limits fibrin formation through binding to the fibrinogen molecule, with the resultant prevention of excessive collagen deposition.[13]

The balance of cytokines in the fetus favors HA expression. Proinflammatory cytokines, such as IL-1 and tumor necrosis factor-alpha, which downregulate HA expression, are underexpressed in the fetal wounds, whereas in adults there is upregulation of these cytokines in response to wound healing. As mentioned previously, HASA also contributes to the higher production of HA in fetal skin.

Angiotensin-converting enzyme (ACE)

Recent studies indicate that upregulated ACE may participate in cutaneous pathological scar formation,[30] suggesting that ACE inhibitors may exert an anti-scar effect. Moreover, ACE inhibitors also retard Smad3 function, which plays an important role in the TGF-β-induced fibrosis.[31]

Homeobox genes

Homeobox genes are transcription factors that are implicated in the patterning and cell type specification events during development. Human homeobox genes *MSX-1*, *MSX-2*, and *MOX-1* are differentially expressed in skin development. Moreover, fetal scarless repair is associated with decreased expression of *HOXB13* and increased *PRX-2* expression.[2] This implies that *PRX-2* activation is an important stimulant to dermal generation. Conversely, *HOX-B13*, which is strongly expressed in normal second trimester fetal skin, is markedly downregulated in response to wounding. Thus, *HOX-B13* may be an inhibitor of dermal proliferation, and its constant expression may be involved in maintaining a static dermal architecture rather than promoting dermal growth.[32]

Hox genes, another subgroup of homeobox genes, encode for a family of transcription factors that are major regulators of tissue migration and cell differentiation during embryogenesis. In a recent study, Jain *et al.*[33] reported the increased expression of Hox gene Hoxd8 in excisional wounds of mid-gestational mice compared with the normal skin samples of mid-gestational mice and excisional wounds of late-gestational mice; therefore, it is proposed that this gene may have anti-scarring effects in fetal wound healing. Conversely, expression of another Hox gene Hoxd3 was found to be increased in both excisional wounds and normal skin samples of mid-gestational mice compared with late-gestational mice; hence,

this gene is considered to be expressed constitutively in the skin of mid-gestational mice.

It is likely that fetal mammals have the ability to heal large skin defects made early in gestation because transcription factor patterning genes, such as homeobox genes, are more active in the fetal environment. As a result, researchers have hypothesized that transcription factors, like homeobox genes, may be the ''first domino'' in the fetal cutaneous wound repair regulatory cascade.[32]

How these homeobox genes may coordinate scarless fetal wound repair is currently under investigation. Several possible targets have been identified, including the promoter regions of members of the TGF-β superfamily, various cellular adhesion molecules, and cell surface proteins such as integrins.[32]

Improvement of scar cosmesis with age

Wound healing is delayed in old age, but there is less scarring. Diminished inflammatory responses due to impaired macrophage and T-cell functions, loss of fibroblast responsiveness and motility, as well as reduced quantity and distribution pattern of various growth factors, including TGF-β, and their receptors may contribute to this effect. Estrogen upregulates TGF-β1 through binding to its receptors on fibroblasts. The decline of estrogen after menopause may explain decreased scarring in the postmenopausal period. Systemic hormone replacement therapy for 3 months accelerates re-epithelialization and collagen deposition of acute wounds in postmenopausal females.[34]

In normal aging skin, the levels of proteases such as MMPs increase, while the levels of proteolytic inhibitors (e.g. TIMPs) decrease, leading to less scar formation.[35,36]

TGF-β3 increases at Day 7 postwounding in old mice, while TGF-β1 and TGF-β2 increase at all time points in the wounds of younger mice. This may also explain the better cosmesis of wound healing in aging.[12]

The differences between fetal and adult wound healing discussed above are summarized in Table 1.

Table 1 Comparison of fetal vs. adult wound healing

	Fetus	Adult
Wound healing	Scarless, rapid	Scarring, slow
Collagen deposition	Rapid	Delayed
Collagen quality	Fine, reticular, less cross-linking	Thick, disorganized, more cross-linking
Collagen type III to type I ratio	Higher	Lower
Tenascin and fibronectin deposition	Early	Delayed
Lysyl oxidase levels	Lower	Higher
TGF-β1 and -β2 to TGF-β3 ratio	Lower	Higher
TGF-β1-induced collagen production	Absent	Present
Decorin and fibromodulin levels	Lower/higher	Higher/lower
MMP to TIMP ratio	Higher	Lower
PDGF and FGF levels	Lower/lower	Higher/higher
VEGF level	Higher	Lower
Myofibroblasts	Absent	Present
Fibroblast HA and TGF receptors	More/less	Less/more
Dot cells	Present	Absent
HASA	Present	Absent
HA (and tissue fluidity)	More	Less
Inflammation (and inflammatory cytokines)	Inconspicuous	Conspicuous
Homeobox genes	More active	Less active

HA, hyaluronan; HASA, HA-stimulating activity; FGF, fibroblast growth factor; MMP, matrix metalloproteinases; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteinases; VEGF, vascular endothelial-derived growth factor.

Accepted, potentially useful and questionable strategies for minimizing scars

• The use of proper surgical techniques, which ensure minimal tension and inflammation, and avoiding incisions over the midchest and joints, which are scar-prone sites, can lead to better cosmetic results.[34]

As mucosal wounds heal with reduced scar formation when compared with skin,[26] when possible there should be consideration of endoscopic surgical technique.[37]

Larger wounds may be more likely to heal with scar formation. It is not unreasonable to assume that larger wounds may extend the time of the healing response, thus exposing wound tissues to a different extracellular matrix and growth factor profile. In addition, the larger excisional wounds may stimulate the formation of myofibroblasts in the wound, resulting in scar formation.[32]

• Occlusive therapy can reduce scar formation. Occlusive therapy includes silicone gels and sheets, non-silicone occlusive sheets, cordran tape and Scarguard MD® (Redrock Laboratories, Great Neck, NY, USA; see below). Occlusion can reduce pro-inflammatory/pro-fibrotic cytokine levels. Increased temperature, even 1 °C, due to occlusion can upregulate collagenase expression.[34]

Topical silicone gel has been employed for scar reduction since the 1980s. It is probably of value by enhancing wound hydration as occlusive therapy with both silicone- and nonsilicone-based therapies appears to be of equal value.[38,39]

Scarguard MD® is a new topical over-the-counter medication containing silicone, vitamin E and hydrocortisone. In a pilot study on 12 patients, Scarguard MD® was applied twice daily after removal of a mole, and nothing

was applied after removal of a second mole. After 2 months, nine out of the 12 patients described a reduction in erythema and better cosmesis of the scar at the site treated with Scarguard MD® compared with the site that was not treated with Scarguard MD®. These results must be interpreted with caution, however, as the improved cosmesis may be the result of occlusion of a wound rather than the Scarguard MD product. An *in vitro* study showed that Scarguard MD® may stimulate the release of inactive collagenase precursors that may inhibit scar formation and reduce existing scars.[34]

• Seprafilm (Genzyme Corporation, Cambridge, MA, USA) is a type of absorbable barrier film composed of un-crosslinked, carboxymethylcellulose/sodium hyaluronate.[40] After application, by absorbing moisture from the application site, the dry film acquires a gel consistency over 24 hours.[40] This product decreases the incidence of adhesion formation, and enhances the repair of damaged tissues by acting as an occlusive barrier between damaged tissue sites.[40] Several studies have reported the effectiveness of Seprafilm in reducing postoperative intra-abdominal adhesion formation.[40–42] This product has also been shown to facilitate ileostomy closure.[43]

• Topical application of HA or saponins, which upregulate HA, could exert an anti-scarring effect.[44] The effects of topical exogenous HA on the healing of dermal wounds have been investigated by many independent investigators in recent years. Most researchers have found that HA provides a beneficial effect with regard to the quality of tissue repair scar formation. The results indicate that the greatest benefit is achieved using highly purified, high-molecular-weight HA at a concentration more than 1 mg/ml, and under conditions in which the HA is maintained at the wound site on a continuous basis for a prolonged period of time. In studies in which HA failed to promote or enhance the wound repair process, testing conditions were suboptimal. Low-molecular-weight and/or very dilute HA preparations rapidly diffuse from the injured site. The purity of exogenous HA is critical in order to limit contaminating substances, such as proteins, endotoxin, etc., which may be inflammatory in nature and may produce undesirable biological effects.[13]

The synthesis of HA appears to be limited by glucosamine availability.[45] Thus, administration of adequate amounts of glucosamine by mouth during the first few days after surgery or trauma has been hypothesized to enhance HA production in the wound, thereby promoting swifter healing and also diminishing complications related to scarring, such as adhesion bands.[45]

• For reduction of scar formation, clinical trials for the use of TGF-β3 and neutralizing antibodies to TGF-β1 and TGF-β2 are being conducted.[46] Neutralizing antibody-treated wounds contained fewer macrophages, less angiogenesis, and less collagen and fibronectin. Interestingly, the tensile strength of the scars of these wounds was comparable with controls.[47] Whether these antibodies are capable of reducing scar formation should be answered by the above-mentioned trials. Activation of latent TGF-β requires binding of the latent TGF-β to the mannose 6-phosphate/insulin-like growth factor-2 receptor in the presence of plasmin/urokinase. Activation could be blocked by mannose 6-phosphate or by antibodies to the mannose 6-phosphate receptor.[14]

Another potential target of therapy is connexin 43 (Cx43), which mediates TGF-β signaling. Studies on Cx43 antisense-based gene therapy to prevent scar formation are under way. As lithium increases Cx43 expression, patients who are taking it may exhibit increased susceptibility to scar formation. MicroRNAs, which are single-stranded RNA molecules partially complementary to one or more messenger RNA molecules, may be employed to downregulate gene expression.[46]

Smad3 plays an important role in the TGF-β-induced fibrosis. Downregulation of Smad3 expression in fibroblasts by small interfering RNA (siRNA) can significantly decrease procollagen gene expression and attenuate the process of fibrosis. RNA interference is a process in which brief RNA sequences, called siRNA, block signals from a particular gene. This process, called gene silencing, inhibits the gene from carrying out its function. However, this approach is facing some challenges, such as the need to develop improved siRNA delivery strategies that combine high specificity and efficiency with a low immunostimulatory and tumorigenic potential.[46,48]

• Tamoxifen is shown to downregulate the production of TGF-β1, basic FGF and VEGF.[49,50] Some *in vivo* reports indicate that it delays wound healing but ameliorates scar formation.[46]

• Starting at the day of suture removal, 585-nm pulsed dye laser treatment has improved the quality and cosmetic appearance of surgical scars in a controlled study on 11 patients.[51] Both 585-nm pulsed dye laser and the 1064-nm long-pulsed neodymium : yttrium-aluminum-garnet laser treatment of acne scars in the same patient showed both lasers to be effective modalities for the treatment of acne scars.[52]

• Botulinum toxin has shown improvement in several patients when injected into the muscle underlying a wound immediately after surgery.[53] A recent evaluation of 19 patients found botulinum toxin type A injections of value in the treatment of hypertrophic scars.[54]

• An investigator-blinded, controlled study showed that the onion extract gel significantly improved scar softness, redness, texture and global appearance at the excision site.[55]

• A double-blind, randomized, placebo-controlled study did not demonstrate efficacy of imiquimod, an anti-fibrosis cytokine inducer, on scar cosmesis.[56]

• Vitamin E, a TGF-β inhibitor, was shown to be ineffective topically.[57]

• In one report, cyclooxygenase (Cox)-2 inhibition by celecoxib, which can decrease wound inflammation, was shown to decrease scar deposition,[58] but work by others suggested that antagonism of Cox-2 had no effect on the morphology of healed wounds.[59]

• Commercial products made by snail's secretion, such as BIO SKIN CARE™ Cream (Andes Natural Skin Care, Carson City, NV, USA) and Eilcina are claimed to have anti-scarring properties. In support of this claim, an early study reported that a secretion from the mollusk *Cryptomphalus aspersa* (SCA) stimulated skin regeneration after wound-healing impairment due to acute radiodermatitis.[60] Moreover, in a recent study, Brieva *et al.* reported that therapeutic properties of SCA in skin regeneration were due to the presence of antioxidant substances in this compound; SCA was also reported to have extracellular matrix remodeling capabilities.[61]

• Importantly, despite marketing claims, there is no evidence that acceleration of healing with some agents, such as Cicamosa emulsion (Lutsia, Paris, France), Cicalfate cream (Avene, Paris, France), trolamine (Biafine Genmedix, France) topical emulsion, or other commercial repair creams is associated with decreased scar formation.[62,63] Even if these agents do speed healing time, there is no known correlation between healing rate and the degree of scar formation. For example, postmenopausal wounds heal slower but with less scar formation in comparison to premenopausal wounds.[34,64] It should be noted that promotion of fibroblast activation as some of these products claim may theoretically induce more scarring.

Conclusion

Prevention of scar formation has long been important, and is salient in a variety of medical disorders and settings. With its tremendous widespread clinical value and commercially lucrative potential, one hopes for breakthroughs of clinical value. To achieve this, scientific advances and high-quality clinical trials are required. Given the data presented in this work, one can look to the future with optimism.

Acknowledgment

The principal author (MRN) wishes to dedicate this article to Prof. Ramin Miri, the former Vice-Chancellor for Research of Shiraz University of Medical Sciences, for his outstanding support of young scientists.

References

1 Rumsey N, Clarke A, White P. Exploring the psychosocial concerns of outpatients with disfiguring conditions. *J Wound Care* 2003; **12**: 247–252.
2 Colwell A, Longaker M, Lorenz PH. Fetal wound healing. In: Falabella AF, Kirsner RS, eds. *Wound Healing*. Boca Raton: Taylor & Francis, 2005: 9–16.
3 Lorenz HP, Whitby DJ, Longaker MT, *et al.* Fetal wound healing: the ontogeny of scar formation in the non-human primate. *Ann Surg* 1993; **217**: 391–396.
4 Lorenz HP, Lin RY, Longaker MT, *et al.* The fetal fibroblast: the effector cell of scarless wounds repair. *Plast Reconst Surg* 1995; **96**: 1251–1259.
5 Longaker MT, Whitby DJ, Jennings RW, *et al.* Fetal diaphragmatic wounds heal with scar formation. *J Surg Res* 1991; **50**: 375–385.
6 Mast BA, Albanese CT, Kapadia S. Tissue repair in the fetal intestinal tract occurs with adhesions, fibrosis, and neovascularization. *Ann Plast Surg* 1998; **41**: 140–144; discussion 144–147.
7 Longaker MT, Whitby DJ, Adzick NS, *et al.* Studies in fetal wound healing VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. *J Pediatr Surg* 1990; **25**: 63–68; discussion 68–69.
8 Armstrong JR, Ferguson MWJ. Ontogeny of the skin and transition from scar free to scarring phenotype during wound healing in the pouch young of *Monodelphis domestica*. *Dev Biol* 1995; **169**: 242–260.
9 Longaker MT, Whitby DJ, Ferguson MWJ, *et al.* Adult skin wounds in the fetal environment heal with scar formation. *Ann Surg* 1994; **219**: 65–72.
10 Whitby DJ, Ferguson MW. The extracellular matrix of lip wounds in fetal, neonatal and adult mice. *Development* 1991; **112**: 651–668.
11 Hallock GG, Merkel JR, Rice DC, DiPaolo BR. The ontogenetic transition of collagen deposition in rat skin. *Ann Plastic Surg* 1993; **30**: 239–243.
12 Dasgeb B, Phillips TJ. What are scars? In: Arndt KA, ed. *Scar Revision*. Philadelphia: Elsevier, Saunders, 2006: 1–16.
13 Balazs EA, Larsen NE. Hyaluronan: aiming for perfect skin regeneration. In: Garg HG, Longaker MT, eds. *Scarless Wound Healing*. New York: Marcel Dekker, 2000: 143–161.
14 Shah M, Rorison P, Ferguson MWJ. The role of transforming growth factors-beta in cutaneous scarring. In: Garg HG, Longaker MT, eds. *Scarless Wound Healing*. New York: Marcel Dekker, 2000: 213–227.
15 Shah M, Foreman DM, Ferguson MWJ. Neutralisation of TGF-β1 and TGF-β2 or exogenous addition of TGF-β3 to cutaneous rat wounds reduces scarring. *J Cell Science* 1995; **108**: 985–1002.
16 Hsu M, Peled ZM, Chin GS, *et al.* Ontogeny of expression of transforming growth factor-beta 1 (TGF-beta 1), TGF-beta 3, and TGF-beta receptors I and II in

fetal rat fibroblasts and skin. *Plast Reconstr Surg* 2001; **107**: 1787–1794.
17 Eslami A, Gallant-Behm CL, Hart DA, *et al.* Expression of integrin αvβ6 and TGF-β in scarless vs scar-forming wound healing. *J Histochem Cytochem* 2009; **57**: 543–557.
18 Rolfe KJ, Richardson J, Vigor C, *et al.* A role for TGF-beta1-induced cellular responses during wound healing of the non-scarring early human fetus? *J Invest Dermatol* 2007; **127**: 2656–2667.
19 Lorenz V, Soo C, Beanes SR, *et al.* Differential expression of matrix metalloproteinases and their tissue derived inhibitors in scarless fetal wound healing. *Surg Forum* 2001; **52**: 397–401.
20 Yang GP, Lim IJ, Phan T-T, *et al.* From scarless fetal wounds to keloids: molecular studies in wound healing. *Wound Rep Reg* 2003; **11**: 411–418.
21 Scheid A, Wenger RH, Schäffer L, *et al.* Physiologically low oxygen concentrations in fetal skin regulate hypoxia-inducible factor 1 and transforming growth factor-beta3. *FASEB J* 2002; **16**: 411–413.
22 Estes JM, Vande Berg JS, Adzick NS, *et al.* Phenotypic and functional features of myofibroblasts in sheep fetal wounds. *Differentiation* 1994; **56**: 173–181.
23 Cass DL, Sylvester KG, Yang EY, *et al.* Myofibroblast persistence in fetal sheep wounds is associated with scar formation. *J Pediatr Surg* 1997; **32**: 1017–1021.
24 Liu W, Cao Y, Longaker MT. Gene therapy of scarring: a lesson learned from fetal scarless wound healing. *Yonsei Med J* 2001; **42**: 634–645.
25 Kong W, Li S, Longaker MT, Lorenz HP. Blood-derived small Dot cells reduce scar in wound healing. *Exp Cell Res* 2008; **314**: 1529–1539.
26 Liechty KW, Adzick NS, Crombleholme TM. Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair. *Cytokine* 2000; **12**: 671–676.
27 Liechty KW, Crombleholme TM, Cass DL, *et al.* Diminished interleukin-8 (IL-8) production in the fetal wound healing response. *J Surg Res* 1998; **77**: 80–84.
28 Gordon AD, Karmacharya J, Herlyn M. Scarless wound healing induced by adenoviral-mediated overexpression of interleukin-10. *Surg Forum* 2001; **52**: 568–569.
29 Bender BG, Ballard R, Canono B, *et al.* Disease severity, scratching, and sleep quality in patients with atopic dermatitis. *J Am Acad Dermatol* 2008; **58**: 415–420.
30 Morihara K, Takai S, Takenaka H, *et al.* Cutaneous tissue angiotensin-converting enzyme may participate in pathologic scar formation in human skin. *J Am Acad Dermatol* 2006; **54**: 251–257.
31 Namazi H. ACE inhibitors: a novel treatment for neurofibroma. *Ann Surg Oncol* 2008; **15**: 1538–1539.
32 Chin GS, Stelnicki EJ, Gittes GK, Longaker MT. Characteristics of fetal wound repair. In: Garg HG, Longaker MT, eds. *Scarless Wound Healing*. New York: Marcel Dekker, 2000: 239–263.
33 Jain K, Sykes V, Kordula T, *et al.* Homeobox genes Hoxd3 and Hoxd8 are differentially expressed in fetal mouse excisional wounds. *J Surg Res* 2008; **148**: 45–48.
34 Berman B, Zell D. Medical treatment of scarring. In: Arndt KA, ed. *Scar Revision*, 1st edn. China: Elsevier, Saunders, 2006: 1–16.
35 Ashcroft GS, Horan MA, Herrick SE, *et al.* Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. *Cell Tissue Res* 1997; **290**: 581–591.
36 Ashcroft GS, Herrick SE, Tarnuzzer RW, *et al.* Human ageing impairs injury-induced *in vivo* expression of tissue inhibitor of matrix metalloproteinases (TIMP)-1 and -2 proteins and mRNA. *J Pathol* 1997; **183**: 169–176.
37 Bender O, Balci FL, Yüney E, *et al.* Scarless endoscopic papillomectomy of the breast. *Onkologie* 2009; **32**: 94–98.
38 Wolfram D, Tzankov A, Pülzl P, *et al.* Hypertrophic scars and keloids – a review of their pathophysiology, risk factors, and therapeutic management. *Dermatol Surg* 2009; **35**: 171–181.
39 Chang CC, Kuo YF, Chiu HC, *et al.* Hydration, not silicone, modulates the effects of keratinocytes on fibroblasts. *J Surg Res* 1995; **59**: 705–711.
40 Falabella CA, Melendez MM, Weng L, *et al.* Novel macromolecular crosslinking hydrogel to reduce intra-abdominal adhesions. *J Surg Res* 2010; **159**: 772–778.
41 Fujii S, Shimada H, Ike H, *et al.* Reduction of postoperative abdominal adhesion and ileus by a bioresorbable membrane. *Hepatogastroenterology* 2009; **56**: 725–728.
42 Emre A, Akin M, Isikgonul I, *et al.* Comparison of intraperitoneal honey and sodium hyaluronate-carboxymethylcellulose (Seprafilm) for the prevention of postoperative intra-abdominal adhesions. *Clinics (Sao Paulo)* 2009; **64**: 363–368.
43 Kawamura YJ, Kakizawa N, Tan KY, *et al.* Sushi-roll wrap of Seprafilm for ileostomy limbs facilitates ileostomy closure. *Tech Coloproctol* 2009; **13**: 211–214.
44 Mast BA, Flood LC, Haynes JH, *et al.* Hyaluronic acid is a major component of the matrix of fetal rabbit skin and wounds: implications for healing by regeneration. *Matrix* 1991; **11**: 63–68.
45 McCarty MF. Glucosamine for wound healing. *Med Hypotheses* 1996; **47**: 273–275.
46 Rhett JM, Ghatnekar GS, Palatinus JA, *et al.* Novel therapies for scar reduction and regenerative healing of skin wounds. *Trends Biotechnol* 2008; **26**: 173–180.
47 Chang J, Siebert JW, Schendel DA, *et al.* Scarless wound healing: implications for the aesthetic surgeon. *Aesthetic Plast Surg* 1995; **19**: 237–241.
48 Wang Z, Gao Z, Shi Y, *et al.* Inhibition of Smad3 expression decreases collagen synthesis in keloid disease fibroblasts. *J Plast Reconstr Aesthet Surg* 2007; **60**: 1193–1199.
49 Ruffy MB, Kunnavatana SS, Koch RJ. Effects of tamoxifen on normal human dermal fibroblasts. *Arch Facial Plast Surg* 2006; **8**: 329–332.

50 Morena AM, Oshima CT, Gebrim LH, *et al.* Early nuclear alterations and immunohistochemical expression of Ki-67, Erb-B2, vascular endothelial growth factor (VEGF), transforming growth factor (TGF-beta1) and integrine-linked kinase (ILK) two days after tamoxifen in breast carcinoma. *Neoplasma* 2004; **51**: 481–486.
51 Nouri K, Jimenez GP, Harrison-Balestra C, *et al.* 585-nm pulsed dye laser in the treatment of surgical scars starting on the suture removal day. *Dermatol Surg* 2003; **29**: 65–73; discussion 73.
52 Lee DH, Choi YS, Min SU, *et al.* Comparison of a 585-nm pulsed dye laser and a 1064-nm Nd:YAG laser for the treatment of acne scars: a randomized split-face clinical study. *J Am Acad Dermatol* 2009; **60**: 801–807.
53 Sherris DA, Gassner HG. Botulinum toxin to minimize facial scarring. *Facial Plast Surg* 2002; **18**: 35–39.
54 Xiao Z, Zhang F, Cui Z. Treatment of hypertrophic scars with intralesional botulinum toxin type A injections: a preliminary report. *Aesthetic Plast Surg* 2009; **33**: 409–412.
55 Draelos ZD. The ability of onion extract gel to improve the cosmetic appearance of postsurgical scars. *J Cosmet Dermatol* 2008; **7**: 101–104.
56 Berman B, Frankel S, Villa AM, *et al.* Double-blind, randomized, placebo-controlled, prospective study evaluating the tolerability and effectiveness of imiquimod applied to postsurgical excisions on scar cosmesis. *Dermatol Surg* 2005; **31**: 1399–1403.
57 Baumann LS, Spencer J. The effects of topical vitamin E on the cosmetic appearance of scars. *Dermatol Surg* 1999; **25**: 311–315.
58 Wilgus TA, Vodovotz Y, Vittadini E, *et al.* Reduction of scar formation in full-thickness wounds with topical celecoxib treatment. *Wound Repair Regen* 2003; **11**: 25–34.
59 Blomme EA, Chinn KS, Hardy MM, *et al.* Selective cyclooxygenase-2 inhibition does not affect the healing of cutaneous full-thickness incisional wounds in SKH-1 mice. *Br J Dermatol* 2003; **148**: 211–223.
60 Ledo E, de las Heras ME, Ledo A. Treatment for acute radiodermatitis with *Cryptomphalus aspersa* secretion. *Radioprotección* 1999; 23.
61 Brieva A, Philips N, Tejedor R, *et al.* Molecular basis for the regenerative properties of a secretion of the mollusk *Cryptomphalus aspersa*. *Skin Pharmacol Physiol* 2008; **21**: 15–22.
62 Cohen JL, Jorizzo JL, Kircik LH. Use of a topical emulsion for wound healing. *J Support Oncol* 2007; **5**: 1–9.
63 De Rauglaudre G, Courdi A, Delaby-Chagrin F, *et al.* Tolerance of the association sucralfate/Cu-Zn salts in radiation dermatitis. *Ann Dermatol Venereol* 2008; **Spec No 1**: 11–15.
64 Ashcroft GS, Dodsworth J, van Boxtel E, *et al.* Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta1 levels. *Nat Med* 1997; **3**: 1209–1215.

