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Skin Wound Healing and Scarring: Fetal Wounds and Regenerative Restitution

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Abstract

The adverse physiological and psychological effects of scars formation after healing of wounds are broad and a major medical problem for patients. In utero, fetal wounds heal in a regenerative manner, though the mechanisms are unknown. Differences in fetal scarless regeneration and adult repair can provide key insight into reduction of scarring therapy. Understanding the cellular and extracellular matrix alterations in excessive adult scarring in comparison to fetal scarless healing may have important implications. Herein, we propose that matrix can be controlled via cellular therapy to resemble a fetal-like matrix that will result in reduced scarring.

Keywords

fetal wounds; fibrosis; ECM; CXCR3

INTRODUCTION

Skin has an almost unmatched capacity to heal wounds in a restorative mode. Still the end result falls short of the original skin, and with larger and full thickness wounds, dysfunctional and disfiguring scars can result. Scarring results from the failure of the wound to properly transition from the regenerative phase to the resolving phase extension of matrix deposition and chronic inflammation pathologies (Harty et al., 2003; Wynn, 2008). Thus, there is a need to develop strategies that better recreate the skin ontogeny when responding to a wound. Interestingly, in the fetus there is a unique environment that has the extraordinary ability to heal skin wounds without a scar in a truly regenerative manner (Rowlatt, 1979; Lane, 1986; Lorenz et al., 1992a; Armstrong and Ferguson, 1995). Given the major health problems that result from scarring and loss of function, there is great interest in recreating the fetal situation of scarless healing.

In the last several decades, researchers have sought to manipulate the regenerative phenotype of the fetus involved in wound healing to reduce or even prevent scarring. Initial

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studies focused on the intrauterine wound environment, which was postulated to play a pivotal role in the wound phenotype; however, late in utero, wounds do lead to scars. Thus, the current focus has shifted to the cellular and molecular aspects of fetal wound healing and the alterations in extracellular matrix, mechanical stress, and inflammation that result in postnatal wound healing pathologies such as excessive scarring. Prevention of scar formation and scar reduction has long been important, not just in skin pathologies but also other conditions such as systemic sclerosis, pulmonary and renal fibrosis, and others that arise from post-surgical procedures. The modulation of pro-scarring versus anti-scarring has been a key focus of anti-scarring therapy exploration To-date, clinical approaches range from molecular, mechanical, and drug-based therapies, along with engineered approaches such as scaffolds and skin substitutes. However, these therapies are suboptimal and met with varying degrees of clinical success.

The questions that must be asked are: what exactly would it take to achieve scarless healing and at what are the costs? Do we want scarless healing postnatal or just reduced scarring? In this review, we will discuss the unique and specific wound environment in both fetal and adult skin and provide a perspective of what controls each regenerative phenotype or lack thereof. We will focus on the role of the matrix and chemokine signals that drive the matrix environment in fetal and adult wound healing and alterations that result in excessive scarring (Table 1). Lastly, we will propose novel cell therapy strategies to reduce scarring in adult wounds that will attempt to direct cell behavior, such as migration, proliferation, differentiation, maintenance of phenotype, and apoptosis, to remodel a more function dermal matrix.

OVERVIEW OF ADULT WOUND HEALING

Adult integumentary wounds scar results from a dysfunction in remodeling of the two skin compartments, the ectodermally derived epithelial epidermis and the mesodermally derived mesenchymal dermis (Gurtner et al., 2008; Longaker MT et al., 1994). Immediately upon injury a series of events is initiated that triggers the start of the wound healing response. There is a delicate series of events that starts with the initiation of proliferation, migration, and phenotypic alteration of cells for several origins, working toward the ultimate goal of restoring tissue integrity and homeostasis (Singer and Clark, 1999). The normal adult response to injury is characterized as a series of four, not mutually exclusive, time-dependent phases: hemostasis, inflammation, tissue formation, and tissue remodeling/ resolution (Martin, 1997; Singer and Clark, 1999).

Hemostasis initiates the healing process with a platelet plug in response to injury that severs blood vessels (Rivera et al., 2009). Starting moments after injury, the serum proteins, acted upon by the platelet lysate, provide a provisional matrix that both serves to re-establish the barrier function and as the foundational support of the migrating cells (Nieswandt et al., 2009). This process includes platelet aggregation, and platelet α-degranulation to release clotting factors that act on the serum proteins to form this proteinaceous covering (Arnout et al., 2006). Simultaneously, vasoconstriction occurs to limit blood loss under the effects of vasoactive mediators (epinephrine, prostaglandins, serotonin, and platelet factor 4 (PF4) released by the platelets), causing temporary blanching of the wound (Rendell et al., 2002; Velnar et al., 2009).

The platelets also release a number of factors that initiate the subsequent overlapping stages of wound healing. These factors include PF4 and platelet-derived growth factor (PDGF), proteases, and the vasoactive agents, serotonin, and histamine (Ross et al., 1986; Demidova-Rice et al., 2012). Chemokines released by platelet activation attract inflammatory cells to the area, heralding the next phase in the healing process. The inflammatory phase may even

be considered to coincide with hemostasis as elements of innate immunity are trapped in the platelet plug. The subsequent inflammatory infiltration that truly marks this phase is influenced by the deposition of fibrin in addition to the increase in the permeability of nearby vasculature as a result of activated thrombin (Nieswandt et al., 2009). This allows cells and factors access to the wound bed from the intravascular space. The persistence of the vasodilation is mediated by histamine and prostaglandins and allows the wound bed to have the continued blood flow necessary for the inflammatory cells that fight infection and debride the wound of devitalized tissue (Rendell et al., 2002; Velnar et al., 2009).

The chemotactic stimulus, mediated by resident macrophages and dendritic cells, recruits the neutrophils and later attracts the lymphocytes. Neutrophils are the most abundant cell type for the first 48 hr after injury and cleanse the wound site of bacteria and necrotic matter by phagocytosis. Also they release inflammatory mediators and bactericidal oxygen free radicals (Theilgaard-Monch et al., 2004). However, it has been shown that the absence of neutrophils does not prevent healing. In fact, mice studies have shown that neutrophils are not essential to the wound-healing process (Martin et al., 2003). Macrophages however are essential, and suggested to be the most important cells in the early phase. Not only does the macrophage phagocytose debris, they also secrete collagenases and other enzymes that break down necrotic tissue (Werner and Grose, 2003). Macrophages play a secondary role as a source of chemoattractants and growth factors, such as transforming growth factor alpha (TGF-α), heparin binding (HB)- epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGF-β). These factors act on the formed elements of the wound to promote immigration, proliferation, and survival. Macrophages provide the signaling cascade that attracts endothelial cells to the wound and promote angiogenesis and fibro-blasts to regenerate the dermis. Further, macrophages can and do produce many of the matrix elements and matrix-remodeling metalloproteinases (Lucas et al., 2010).

As the healing process continues, the regenerative phase dominates by hyperplasia of both the epidermal and dermal compartments. In this phase, epithelialization occurs to form a barrier epithelium over a denuded surface. This epithelial layer provides a seal between the underlying wound and the environment. The process begins within hours of tissue injury. Epidermal cells at the wound edges undergo a transient mesenchymal transition, having an undifferentiated and less mature phenotype (Yan et al., 2010). In a leapfrog fashion, the keratinocytes migrate across the wound separating the overlying eschar from the newly formed viable tissue (Kubo et al., 2001). When epithelialization is complete, the epidermal cells redifferentiate and assume their original epithelial phenotype. Subsequent to this, the basement membrane is restored with the hemidesmosomal linkages.

The fibrin crosslinked fibronectin and collagen provide a scaffold for the migrating cells. The adhesive matrix protein fibronectin provides the adhesion property of the cells to be guided across the wound base (Clark, 1990; Baneyx et al., 2002). Importantly, as the regenerative phase starts, the fibrin-rich provisional matrix is supplemented not only with collagens and fibro-nectins but also with complex macromolecules, including tenascin C, entactin, and immature laminins. These multidomain/multifunctional matrix components promote cell migration and proliferation (Chakravarti et al, 1990; Steffensen et al., 2001). This path of migration is allowed because plasmin has been active to dissolve the clot.

The timing of re-epithelializtion and the start of fibroplasia are critical to proper wound maturation. Fibroplasia begins 3–5 days after injury. The key component of the formation of granulation tissue is skin fibroblasts that are responsible for the production of most of the collagen, fibronectin, tenascin C, and other matrix proteins, including critical remodeling metalloproteases (Trebaul et al., 2007). Fibroblasts proliferate as the need for the

inflammatory response is reduced. This happened as the chemotactic factors that call inflammatory cells to the wound are reduced or become inactivated. During this phase, these stromal cells assume an activated phenotype of proliferation at the edges of the wound, migrate into the matrix, and synthesize matrix in the wound bed. These matrix components and factors, such as PDGF, FGF, and TGF-β, in turn drive other stromal cells (Roberts et al., 1990; Raab and Klagsbrun, 1997; Werner and Grose, 2003).

As the matrix evolves collagens becomes the most abundant component of the developing extracellular matrix. The synthesis and deposition of collagen is critical to wound healing. Collagen type I and type III are major constituents of scar tissue; however, collagen type I is dominant in adult wounds (Varga et al., 1987; Chesney et al., 1998; Schultz et al., 2011). Collagens control tissue architecture, tensile strength, cell–matrix, and matrix–matrix interactions. Collagen forms strong crosslinks because of hydroxylation of lysine residues. Adults that are deficient in oxygen and vitamin C wounds tends to be weaker because of the underhydroxylated collagen that is less capable of forming strong crosslinks and, therefore, is more vulnerable to breakdown (Clark et al., 2004). Collagen fibers are deposited in a framework of fibronectin. An essential interaction seems to exist between fibronectin and collagen; experimental wounds depleted of fibronectin demonstrate decreased collagen accumulation. Age, tension, pressure, and stress affect the rate of collagen synthesis.

Angiogenesis is another process that is initiated by macrophages. A rich blood supply is vital to sustain the highly metabolically active newly formed tissue, with macrophages producing macrophage-derived angiogenic factor in response to low tissue oxygenation (Tonnesen et al., 2000). This factor functions as a chemoattractant for endothelial cells along with other chemokines. The provisional matrix, rich in collagen III, tenascin C, and entactin, and deficient in small leucine-rich proteoglycans (SLRP), such as decorin and lumican, also promote endothelial cell migration and formation of tubes that become patent vessels (Merline et al., 2009; Iozzo and Schaefer, 2010).

This exuberant cell response of the regenerative phase, with thickened epidermis, hypercellular dermis, and hyperemic blood flow is reverted in the resolving phase. As collagen synthesis switches to collagen type I, the wound contracts. The seed of this switch is borne in the very nature of the regenerative phase. Key regulators of this maturation are the chemokines that bind to the CXCR3 receptor which is found ubiquitously (Fig. 1). CXCL11 (IP-9, I-TAC) is expressed by keratinocytes as they redifferentiate upon contact inhibition after re-epitehlializing the wound (Tensen et al., 1999; Satish et al., 2003, Yates et al., 2008). CXCL10 (IP-10) is produced by microvasculature as it matures (Bodnar et al., 2009). These factors channel the transcellular contractility of migrating fibro-blasts into matrix contraction (Allen et al., 2002), even while they predispose the fibroblasts to apoptosis (Shao et al., 2008). In addition, these chemokines drive anoikis, detachment-driven apoptosis of endothelial cells and immature vessels (Bodnar et al., 2009). The immature matrix is replaced by a collagen I-rich dermis, which also suppresses the synthetic properties of the stromal cells. Thus, the skin reverts to its unwounded state of relative avascularity, paucicellular dermis, and a locale- and use-defined keratinized epidermis. As the nature and timing of this phase impacts both inflammation and other external stressors, signaling during this phase dictates the outcome of the wound. Interestingly, the CXCR3 signaling axis actuates a comprehensive set of behaviors that would transition a wound from the regenerative phase to the final phase of wound resolution via epidermal-dermal crosstalk.

SCARRING AS DEFICIENT WOUND RESOLUTION

Scar is a condition in which the dermis is dysfunctional, and this underlying pathology also affects the epidermis and its accessory organs, as well as the vasculature and nerves. Poor healing is characterized by an accumulation of excess collagen that is weaker than unwounded skin because it is aberrantly processed and crosslinked. The collagen fibers in hypertrophic scars are loosely arrayed in a wavy pattern (Hynes, 2009). Thus, the thickened skin is also more fragile. Hypertrophic scars stay within the limit of the original wound and do tend to regress over time due to reduction of the excessive collagen; however, they are not remodeled into more functional tissue (Singer and Clark, 1999). Hypertrophic scars are generally seen soon after tissue injury and develop over months. It has been hypothesized that hypertrophic scarring results from the fibroplasia and its overproduction of extracellular matrix that produces abundant immature matrix secondary to abnormalities in epidermal-dermal crosstalk.

During the healing process the rate-limiting step is the delicate balance of granulation tissue generation versus resolution. Too much granulation tissue production results in slowly healed resolved wound or deposition of scar tissue, which is the marker of fibrotic disease. Since fibroblasts/stromal cells are highly important in sensitizing and producing the dermal matrix, they are vital to the remodeling of the dermis. These cells orchestrate both synthesis and degradation of ECM components (Raghow, 1994). Under the control of chemokines and growth factors, fibroblasts mediate the rate of collagen synthesis and degradation. Scarring results when the balance of collagen synthesis and degradation is altered, creating prolonged dermal cellularity, including excessive vascularity. These cells also produce fibronectin to reinforce the granulation tissue scaffold for the deposition of collagen (Yates et al., 2011). In scar tissue, it seems that the delay in production of fibronectin and thus fibrin–fibronectin matrices delays the immigration of fibroblast into the wound bed (Wierzbicka-Patynowski and Schwarzbauer, 2003). Additionally, tenascin-C, a unique matrikine that has both adhesive and anti-adhesive interactions, acts as a chemokinetic agent that promotes survival, cell distribution, and is anti-apoptotic for all the adherent cells. In excessive scarring, there is a persistence of tenascin-C and it is speculated that this may prolong fibrosis. In contrast, there are suppressive molecules, such as SLRP decorin, that “decorate” collagen fibrils. This SLRP regulates the formation and degradation of collagen fibrils, and is involved in matrix organization via TGF-β signaling. Yet in scarring, decorin signaling is downregulated, altering the TGF-β signaling that contributes to the profibrotic actions of TGF-β (Reed and Iozzo, 2002; Zhang et al., 2007).

The extent of scarring is influenced by a variety of factors, including ongoing infection, allergic reactions, and a person’s genetic background. However, the specific molecular pathways and cell behaviors that lead to the excessive but dysfunctional collagen accumulation are ill-defined. Recent studies have shown that the signaling system involving CXCR3 stops the transition from the regenerative phase into the final phase, which is key in excessive scar prevention (Fig. 1). In wound healing in mice, the absence of the stop signal, the CXCR3, wound bed continues to undergo the regenerative phase processes and even experiences an inflammatory reactivation over an extended time period, leading to hypertrophic scarring (Yates et al., 2010). Ablating the CXCR3-mediated stop signals results in an immature matrix environment that was consistent with weak mechanical properties (Yates et al., 2007). Fibronectin and matrikine tenascin C, both integrin ligands, that alter the adhesiveness are both highly expressed at six months post-wounding (Tran et al., 2004; Tran et al., 2005). Under normal healing conditions these levels are seen only during the regenerative phase. The dermal profile of the cellular elements is proliferative and migratory. Furthermore, tenascin C presents cryptic ligands that activate EGF receptors to promote fibroblast and endothelial cell immigration (Iyer et al., 2007, 2008). This

activation contributes to matrix immaturity and the feed-forward loop in which the wound bed not only fails to heal but remains in an active, immature state contributing to the further scarring.

Such a situation leads to the persistence of an immature and inflammatory matrix, which in turn attracts macrophages. This persistent, though sterile inflammatory response, whether primary or secondary, serves to further accentuate an active turning over of the wound bed. After an extended period, a hypertrophic scar ensues. This suggests that the timely and fully penetrant transition to wound resolution is critical to limit or prevent scarring, and may be a key to regenerative healing.

OVERVIEW OF FETAL WOUND HEALING

In the fetus, at least through the second trimester, skin and bone wounds heal in a regenerative manner. The fetus has numerous unique situations and environments that may contribute. However, this is not simply due to the aseptic environment or the amniotic fluid, as this regenerative capacity wanes in the third trimester. Even more remarkable is that the scarless fetal wound repair is organ-specific, occurring mainly in skin and bone.

Cutaneous wound healing in the early gestation fetus is remarkably different from that in the adult. The most striking features of the fetal wound response are the speed and the absence of obvious scarring, an observation that was first reported more than 30 years ago (Rowlatt, 1979). However, after 24 weeks of gestation, neonatal skin fetal repair is histologically indistinguishable from adult skin. This fetal specific phenotype appears to be dictated by quantitative and qualitative alterations in both the inflammatory and regenerative phases, compared to adult normal wound healing (Lorenz et al., 1992a). The processes that are responsible for these occurrences are typically divided into two events: cellular and extracellular matrix (Table 2).

The most obvious difference is a cellular event—the absence of acute inflammation. The fetal wound environment is a warm, sterile, and growth factor-rich amniotic environment (Werner and Grose, 2003). This environment was thought to be essential for scarless fetal regeneration, yet evidence suggests that it is neither essential nor sufficient, as seen in late stage fetuses in which scarring occurs. Thus, in adult peritoneal wounds, which also consist of a sterile moist and growth factor-rich environment, scars ensue. The minimal acute inflammatory infiltrate is mainly macrophages and relatively devoid of leukocytes and lymphocytes (Hopkinson-Woolley et al., 1994). This may explain the decreased fetal platelet degranulation and aggregation. Fetal platelets produce less fibrogenic PDGF, TGF-β1 and TGF-β. Minimal inflammatory infiltrate leads to reduced phagocytic activity, and results in only a few neutrophils arriving to the wound site. This may be contributory, as the induction of inflammation in fetal wounds will lead to scar formation (Hopkinson-Woolley et al., 1994). In the absence of neutrophils, other cells must clear the debris of the devitalized tissue; this is accomplished by macrophages and wound fibroblasts (Lorenz et al., 1995; Coolen et al., 2010). While these differences might be contributory, they are not the whole or even major part of the answer. In normal wounds there is a precise balance between pro- to anti-inflammatory activity from the inflammatory cells that is coordinated via chemokines, cytokines, growth factors, and other soluble mediators (Wulff et al., 2012). However, in fetal wounds, pro-inflammatory chemokines, such as inter-leukin (IL)-8, are diminished (Liechty et al., 1998). In contrast, anti-inflammatory cytokines, such as IL-10, are highly expressed (Liechty et al., 2000). In mice lacking IL-10, fetal wounds display substantial inflammatory cell infiltrates and develop scars.

A second aspect of the cellular element is the presence of stem-like cells. While there is scant evidence that they are required for adult wound healing at its base level (they are required for the restoration of accessory elements such as hair and glands), there is increased content in fetal wounds and even in unwounded fetal skin (Oshima et al., 2001; Wagers et al., 2002; Rendl et al., 2005). Still, they are a minority of the cells. However, these may be educating the stromal cells and directing their behaviors toward regeneration rather than a simpler repair with scarring.

The matrix and signaling events are better characterized. An important distinction between fetal and adult wound healing lies within fibroblast activity. Fetal fibroblasts proliferate at a faster rate and the fetal fibroblast migratory properties are equally enhanced in both intrinsic properties and in response to exogenous cues (Nodder et al., 1997). The growth and migration rates of dermal fibroblasts decrease with age (Shiraha et al., 2000). Unlike adult wound healing, fetal fibroblast ECM synthesis and deposition is highly coordinated as the fetal fibroblasts have a robust synthetic and secretory phenotype. With regard to myofibroblasts, little is known of their role in fetal wound healing. It has been shown that human fetal fibroblasts can differentiate into myofibroblasts when stimulated with TGF-β1 in vitro (Estes et al., 1994; Soo et al., 2003; Rolfe et al., 2007). Additionally, adult wound closure is different as wound contraction is driven by active movement of connective tissue and epidermis, whereas fetal wounds close in a purse string fashions via actin cables (Martin and Lewis, 1992; Brock et al., 1996).

This persistence of an immature wound matrix in adult wounds may be thought of as counter to the hypothesis above, but this is accompanied by a number of other differences. First, in fetal wounds there is an increase in proteoglycans, although this is significantly less than that of normal adult wounds, including the SLRP decorin. Decorin regulates collagen fibrillogenesis, growth factor activity, and cellular proliferation in fetal wound healing (Hildebrand et al., 1994; Beanes, et al., 2001). Second, the increase in levels of glycosaminoglycans such as hyaluronic acid (HA) changes the rheology of the matrix toward a more pliable one (Mast et al., 1993; Alaish et al., 1994). As stiffness of the extracellular matrix is known to drive a mesenchymal phenotype and affect cell survival, this may be critical for the scarless healing. HA is not only at higher levels but remains in the wound bed longer in fetal wounds compared to adult wounds. This promotes both the proliferation and migration of cells. HA-rich matrices can bind growth factors and cytokines, creating temporal and spatial differences of these factors (Longaker et al., 1989; Mast et al., 1993).

Fetal fibroblasts differ from adult fibroblasts in collagen synthesis in terms of speed of deposition, variation in collagen type ratios, and quantity of collagen. Most striking is the persistence of excess collagen type III over collagen type I, with the healed wounds in the fetus remaining at 3:1 instead of 1:3 as in the adult healed wounds (Merkel et al., 1988). Similar to uninjured skin, new collagen is deposited in a fine reticular weave pattern (Longaker et al., 1990; Lorenz et al., 1992b). Fibronectin in fetal wounds also shows an earlier expression, which may contribute to the migratory properties of fetal fibroblasts during healing (Schwarzbauer, 1991; Whitby et al., 1991; Carter et al., 2009). Tenascin C shows earlier deposition in fetal wounds as well, and this may further explain their ability to re-epithelize rapidly with a reduced presence of inflammatory cells. Overall, the intrinsic ability to synthesize a mature, well organized dermal matrix is superior in fetal fibroblast to adult fibroblast.

Importantly, growth factors are altered in fetal wounds. The levels of pro-angiogenic and scarring factors, TGF-β, PDGF, EGF, and basic FGF, are dramatically lower in fetal wounds (Haynes et al., 1994; Peled et al., 2001). In fact, in fetal wound healing there is a rapid

induction of TGF-β1, but at lower levels than adult. Still, fetal healing has a more rapid clearance from the wound site compared to adult wounds (Chen et al., 2005). If TGF-β1 is increased experimentally in fetal wounds, scarring will ensue. On the other hand, EGF mRNA, known to be mitogenic for a number of cell types, shows decreased levels with increasing gestational age (Peled et al., 2001).

The role that VEGF and angiogenesis play is not well defined in either scar formation or scarless healing. However, while forming scars appear to present excessive vascularity, it is also seen that scarless fetal repair has little angiogenesis, reflecting the reduced expression of growth factors associated with angiogenesis, such as VEGF (Colwell et al., 2005).

A proposed key mediator of scarring is COX-2 that functions by producing prostaglandins which in turn control many aspects of chronic inflammation. In scarless healing, low levels of COX-2 and prostaglandin-2 (PGE2) are present (Wilgus et al., 2004). Prostaglandin E2 is increased in fetal wounds. Prostaglandin E2 expression appears to be differentially regulated during fetal, neonatal, and adult dermal wound healing (Sandulache et al., 2006). However, both fetal and adult fibro-blasts show expression of the PGE2 receptors and PGE2 inhibits migration of both fibroblasts. The inhibition of adult fibroblast migration by PGE2 correlates with the disruption of the actin cytoskeleton, and PGE2 also inhibited the contraction of adult derived fibroblast populated collagen lattices (Sandulache et al., 2006; Parekh et al., 2007). PGE2, however, did not disrupt the actin cytoskeleton in fetal-derived fibroblasts and further did not prevent fetal fibroblast populated collagen lattices contraction.

NEW APPROACHES FOR REGENERATION

From the above, we have a situation wherein the education of the wound by the matrix, as generated by cells, appears to be key for scarring or scarless healing. Thus, changing the matrix may be a way to intervene in wounds to promote more regeneration and less scarring (Yates et al., 2011). One of the challenges in matrix alteration is the fact that the macromolecules are interactive. Attempts to simply add proteins have been disappointing due to poor incorporation and rapid degradation. These failures do not necessarily mean the component is non-contributory, but rather reflect on our inability to present components in the proper temporo-spatial manner to achieve the desired end (Rhett et al., 2008).

To address that, we propose that cell transplantation is an avenue to limit scarring. The goals of this cell transplantation are not for long-term incorporation and tissue formation, but for a sufficient time to educate the endogenous cells to generate a pro-regenerative matrix. If experiments support the concept of a transient transplantation as sufficient, then issues of autologous versus allogeneic are moot. Autologous transplants might suffer from having the same genetic predisposition to scarring or the patient may not have sufficient skin for harvesting after extensive wounds, thus negating this advantage. Allogeneic cells would be readily available for immediate application. An initial study suggests that this may be a viable approach in that the hypertrophic scarring in the CXCR3-deficient animals can be prevented in this manner (Yates et al., 2012). In fact, fetal, neonatal, and adult fibro-blasts can survive in an adult dermal wound bed through the inflammatory phase and late into the remodeling stages, and cell transplantation can be accomplished with autogenic, allogenic, and xenogenic derived cells. We propose that providing a pro-survival niche of matrix elements that support incorporation of stem cells with these stromal cells will provide accessory structures and a more physiological repair.

Most classical transplantation studies using stem cells such as mesenchymal stem cells (MSC) are allogeneic transplants of human MSC into immunodeficient mice. These studies

are not only clinically irrelevant but the survival of the transplant is low. Survival of these cells and methods of cell delivery are major limitations when it comes to MSC therapy for wound repair. In this regard, MSC-educated fibro-blast cell therapy using resorbable pro-survival matrices holds promise in that preliminary work suggests that the fibroblasts can educate the tissue to minimize scarring (Yates et al., 2012). Such a delivery system will allow MSC to survive longer, remain in a quiescent state and maintain stemness, and release trophic factors into the wound bed. Furthermore, the system will allow both the cell–cell interactions between the MSC and fibroblast to correct matrix production that results in excessive scarring. At the same time, cell–matrix interactions will be regulated by microenvironmental cues of the delivery system. This approach is a feasible one that is ultimately aimed at educating adult fibroblast to behave like fetal fibroblast to reduce scarring via cell therapy.

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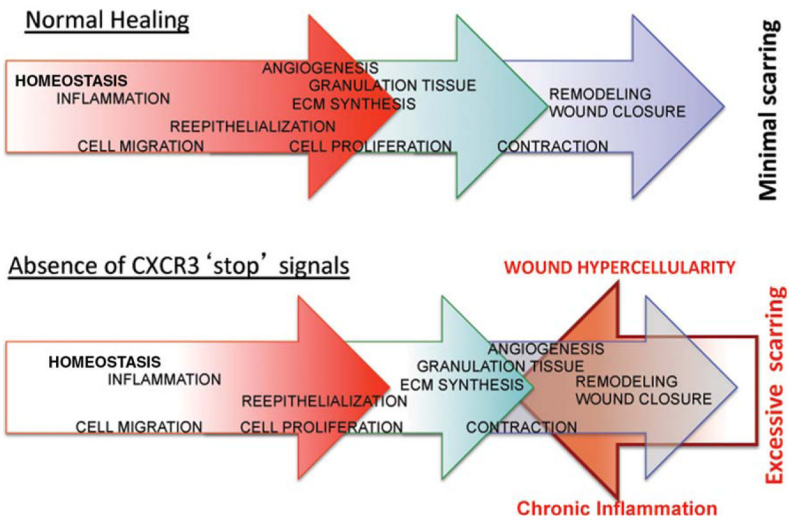

Figure 1.

CXCR3 "stop" signals in scar prevention. CXCR3 receptor is a seven transmembrane G protein coupled receptor. In human and rodent, the receptor is ubiquitous, but the ligands are regulated temporally and spatially. CXCR3 receptor is expressed on keratinocytes, fibroblast, and endothelial cells. Its ligands are CXCL10/IP-10 that appears in the dermis and is produced by endothelial cells of the neovasculature and CXCL11/IP-9 is expressed in redifferentiating keratinocytes behind the leading edge of the wound. Signaling through CXCR3 blocks growth factor-induced motility of fibro-blasts and endothelial cells by suppressing *m*-calpain activation. In contrast, this signaling promotes keratinocytes motility and more rapid re-epithelialization. In the absence of 'stop' signals of CXCR3, fibroplasia results in an immature matrix which in turn recruits more inflammatory cells that stimulate the stromal cells to produce more regenerative matrix. This drives scarring.

TABLE 1

Major Differences Between Fetal Scarless, Adult Normal

Select Component	Fetal	Adult	
Inflammation	Marginal	Robust	
Pro- inflammatory Cytokines *IL-6* & *IL-8*	Low levels	High levels	
Anti inflammatory Cytokines *IL-10*	High levels	Low levels	
Wound Closure	Actin cable	Myofibroblasts and Contraction	
Growth Factors			**Function**
EGF	High level	Decrease with Age	Mitogenic
PDGF	Low levels	High levels	Pro-fibrotic
FGF	Low levels	High levels	Matrix deposition, re-epithelialization
TGF-β1	Low levels	High levels	Matrix deposition, scarring angiogenesis
TGF-β2	Low levels	High levels	Matrix deposition, scarring angiogenesis
TGF-β3	High levels	Low levels	Anti-scarring
VEGF	Low levels	High levels	Angiogenesis Role in scar formation unknown

TABLE 2

Matrix Control in Fetal and Adult Healing

	Fetal Healing	Adult Healing	Function
Collagen 1	Low Fine, reticular, less cross-linking	High Thick, disorganized, more cross-linking	Tissue architecture, Tensile strength Cell-Matrix and Matrix-Matrix interaction, ECM remodeling
Collagen III	High	Low	Tissue architecture Matrix-Matrix interaction, maturation and ECM remodeling
Hyaluronic Acid	High Facilitates cellular movement High molecular weight	Low Inhibits cellular movement Low molecular weight	Cell-matrix interactions Matrix-matrix interactions Cell migration
Fibronectin	Early Deposition	Late Deposition	Tissue architecture Cell-matrix and Matrix-matrix interactions Cell proliferation and migration
Tenascin C	Early Appearance	Late Appearance	Modulates cell-matrix Anti-adhesive Anti-proliferative
Decorin	Low	High	Bind and storage of growth factors