REVIEW ARTICLE

Scarless Wound Healing

Ian H. Bellayr, BS,^{a,b} Thomas J. Walters, PhD,^c Yong Li, MD, PhD^{a,b,d,*}

^aChildren's Hospital of the University of Pittsburgh Medical Center, Pittsburgh, PA, USA

^bUniversity of Pittsburgh, Pittsburgh, PA, USA

^cUnited States Army Institute of Surgical Research, Fort Sam Houston, TX, USA

^dUniversity of Pittsburgh, School of Medicine, Pittsburgh, PA, USA

KEYWORDS:

Fibrosis;
MMP;
Scarless;
Scarring;
TGF- β ;
Wound healing

Abstract Scarring results from injuries and disease in mammalian adults and can cause pain and loss of function in the afflicted tissues. This negative aspect of wound repair is not always true for certain amphibians and during fetal development of mammals. Based on this knowledge, scientists and clinicians are investigating the mechanisms and growth factors that contribute to or deter a suitable environment for wound healing. This review summarizes these aspects and challenges for scarless repair. © 2010 Elsevier Inc. All rights reserved.

One of the most intriguing perplexities of wound healing for mammals is understanding the elements that significantly impact scarring during injuries and repair. Scarring or fibrosis is the deposition of excess *extracellular matrix* (ECM), typically collagen, which impairs tissue or organ regeneration.^{1–3} Fibrosis can result from unexpected trauma or from genetic diseases such as Duchenne muscular dystrophy or cystic fibrosis.^{4,5} When this scarring occurs, mechanisms of wound repair, such as cell migration and proliferation, become impeded.

Not all organisms are subjected to the problematic conditions of scar formation. Certain lower-order species, such as amphibians (eg, newts), are specifically known for their ability to regenerate severed limbs, tails, and some damaged organs. This incredible capacity for regeneration stems from their specialized healing, in which an *apical epithelial cap* (AEC) is formed from epithelial cells local to the site of injury, and considerably minimizes further tissue damage or fluid loss by providing crucial stimulatory

signals.⁶ After formation of the AEC, both local cells and cells migrating to the injury site transform into an earlier unspecialized multipotent state that forms a *blastema* in a process known as *dedifferentiation*.⁷ This process occurs over approximately 70 days and fully restores the function, structure, and contour of the afflicted tissue.^{8,9} Although this phenomenon is prominently studied in newts, it has also been observed for other organisms, including *Drosophila*, zebrafish, and Murphy-Roth-Large mice, which have a notably higher regenerative capacity than normal mice do. Unfortunately, the exact mechanism by which blastema formation occurs to incite scarless wound healing is uncertain, and its variance across different species is unknown.

For mammals in fetal development, skin wound healing occurs without the scarring and inflammation that are typically associated with adult wounds. A number of circumstances have been found to contribute to the differences in healing, including faster speed to wound closure, faster cell proliferation, and a faster and better organized deposition of ECM proteins.¹⁰ Compared with adults, fetal skin wounds have a reduced inflammatory response because inflammatory proteins such as interleukin 6 and interleukin 8 are expressed at

Conflict of interest: The author reports no conflicts of interest.

* Corresponding author.

E-mail address: yongli@pitt.edu

a much lower level.¹¹ Furthermore, the immature nature of platelets at early stages of development prevents the secretion of essential growth factors that participate in the inflammatory response. Once platelets are thoroughly developed, they are more inclined to manufacture the growth factors such as platelet-derived growth factor, insulinlike growth factor, and *transforming growth factor- β 1* (TGF- β 1), the main contributor to scarring after injury.^{10–14} This transition from scarless healing to scarring usually occurs during the third trimester of pregnancy. The environment where fetal repair takes place has no impact on scarless healing. Instead, studies have demonstrated that adult skin grafts heal with scarring within a fetal environment; thus some intrinsic property of fetal fibroblasts, such as greater hyaluronic synthesis, allows for scarless repair.^{12,15,16}

The TGF- β family consists of 3 structurally related isoforms, TGF- β 1, TGF- β 2, and TGF- β 3, which are generally secreted by macrophages and largely contribute to the scarring that occurs in the final stages of wound healing. TGF- β 1 has been indicated to stimulate the initial ECM proteins that provide the supporting architecture for repairing wounds. However, the ability of TGF- β 1 to increase the synthesis and deposition of ECM proteins at injury sites ultimately leads to fibrotic scarring.^{10,13} In addition, TGF- β 1 has been observed to transform a variety of cell types into myofibroblasts and promote their survival during apoptosis.^{14,17,18} These cells are typically identified for expression of α -smooth muscle actin (SMA) and vimentin and encourage fibrosis by secreting a number of inflammatory, fibrotic, and angiogenic factors. Although the period of inflammation appears as the sole instigator of fibrotic scar tissue formation, other mediators, such as vascular damage, oxidative stress, and mechanical tension, foster a profibrotic state for scar tissue.^{19–23} Another area in which fibrosis formation is concerned is the foreign body reactions to implanted tissue engineering biomaterials.²⁴ Tissue engineering products are constructed from a variety of materials (eg, polymers, ceramics, and metals) for the purpose of mimicking the ECM to improve wound healing, but these products can elicit unwanted inflammatory or immune responses that lead to fibrogenic development.^{25,26}

With the expected impairment that results from scarring, researchers have examined using anti-inflammatory therapies or anti-TGF- β medications as preventive measures to deter fibrosis formation. It has been found that scarring effects from TGF- β 1 secretion can be prevented in injured tissues through multiple methods, including blocking the actual TGF- β ligand; blocking the TGF- β receptors, which interferes with downstream signaling; or selectively inhibiting coactivators of TGF- β that lead to scar tissue formation.²⁷ Although other mediators, such as platelet-derived growth factor, *connective tissue growth factor* (CTGF), *monocyte chemoattractant protein-1* (MCT-1), and interleukin(IL)-13, are involved in fibrosis development, most of the clinical studies have focused on TGF- β in antiscarring medications. Several remedies that have been explored

for treatment are *decorin*, which inactivates the effect of TGF- β ; *interferon (INF)- γ* , which interferes with the downstream TGF- β signaling; and *suramin*, which blocks TGF- β by competitively binding to the TGF- β receptors.^{28–31} Another peptide examined for its effect on muscle tissue recovery is *relaxin*, a member of the insulin-like growth factor (IGF) family, which was observed to obstruct the potential of myofibroblasts from TGF- β 1 by reducing their proliferation and expression of α -SMA while having no adverse effects on collagen deposition.^{32,33}

Despite the advantages of these biological remedies, they prove beneficial only when they are used prior to fibrotic tissue formation. Individuals seeking medical attention for detrimental scarring often seek it only after fibrotic scar tissue has reached an irreversible stage. One group of enzymes under investigation for their extraordinary ability to remodel the ECM by promoting cell proliferation, migration, differentiation, and apoptosis is the matrix metalloproteinases (MMPs).^{34,35} MMPs play an integral role in the scarless limb regeneration of newts and the minimal scarring during regeneration of the *Drosophila*, zebrafish, and Murphy-Roth-Large mice. There are currently 25 known MMPs that are found in a plethora of different tissues, aid in minimizing damage, and encourage proper functional recovery. They are categorized into groups: collagenases, gelatinases, matrilysins, membrane-type MMPs, metalloelastases, stromelysins, and others, all of which have the capability to degrade some component of the ECM for tissue remodeling. In several studies, MMP1 has been examined for its ability to degrade collagen type I in existing fibrotic tissue and then promote cell migration and proliferation at the site of reconstruction.^{36–38} Other MMPs, including MMP2, 3, 9, 13, and 14, have been observed to aid in degrading certain elements of the ECM.^{39–42} The favorable aspects of MMPs are undermined when *tissue inhibitors of metalloproteinases* (TIMPs) are present. There are 4 known TIMPs (TIMP 1, 2, 3, and 4), which inhibit MMPs (as well as members of the *adamalysin* group, a disintegrin and a metalloproteinase) and control the extent of tissue remodeling that occurs during injury or disease. While expression of TIMPs during wound healing might be considered negative, they do prevent the overexpression of MMPs, which is known to contribute to various degenerative disorders such as corneal melting, arthritis, or even tumor metastasis.^{43–46}

Research on wound healing is helpful for doctors in determining how best to treat a patient injury or disease. This field of study is becoming ever larger and more detailed in cellular and molecular biology. The 2 areas of wound healing that must be distinguished in terms of health care are preventive methods in treating injuries before scarring occurs or treating fibrosis once the healing process has terminated. One approach to augment wound repair is to allow a greater expression of healing factors, such as MMPs, while minimizing the expression of negative factors such as TGF- β and platelet-derived growth factor (PDGF) or TIMPs (Figure 1). However, caution must be used to ensure that this unbalanced nature of protein expression

Figure 1 The key to achieving scarless healing for adults may lie with having a greater ratio of matrix metalloproteinases (MMPs) to factors that induce fibrosis. However, mediators or TIMPs must prevent the overexpression of MMPs, which can lead to erroneous healing or degenerative disorders. CTGF: connective tissue growth factor; PDGF: platelet-derived growth factor; TIMPs: tissue inhibitors of metalloproteinases.

does not result in an overexpression or underexpression of certain therapeutic components, a situation that can subsequently lead to improper healing and degenerative disorders.

References

- Li ZB, Kollias HD, Wagner KR: Myostatin directly regulates skeletal muscle fibrosis. *J Biol Chem.* 2008;283(28):19371–19378.
- Wynn TA: Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008;214(2):199–210.
- Crosby LM, Waters CM: Epithelial repair mechanisms in the lung. *Am J Physiol Lung Cell Mol Physiol.* 2010;298(6):715–731.
- Bernasconi P, Torchiana E, Confalonieri P, et al: Expression of transforming growth factor-beta 1 in dystrophic patient muscles correlates with fibrosis: pathogenetic role of a fibrogenic cytokine. *J Clin Invest.* 1995;96(2):1137–1144.
- Collins FS: Cystic fibrosis: molecular biology and therapeutic implications. *Science.* 1992;256(5058):774–779.
- Christensen RN, Tassava RA: Apical epithelial cap morphology and fibronectin gene expression in regenerating axolotl limbs. *Dev Dyn.* 2000;217(2):216–224.
- Gurtner GC, Callaghan MJ, Longaker MT: Progress and potential for regenerative medicine. *Annu Rev Med.* 2007;58:299–312.
- Brookes JP: Amphibian limb regeneration: rebuilding a complex structure. *Science.* 1997;276(5309):81–87.
- Yokoyama H: Initiation of limb regeneration: the critical steps for regenerative capacity. *Dev Growth Differ.* 2008;50(1):13–22.
- Adzick NS, Lorenz HP: Cells, matrix, growth factors, and the surgeon: the biology of scarless fetal wound repair. *Ann Surg.* 1994;220(1):10–18.
- Liu W, Cao Y, Longaker MT: Gene therapy of scarring: a lesson learned from fetal scarless wound healing. *Yonsei Med J.* 2001;42(6):634–645.
- Bullard KM, Longaker MT, Lorenz HP: Fetal wound healing: current biology. *World J Surg.* 2003;27(1):54–61.
- Shen W, Li Y, Zhu J, Schwendener R, Huard J: Interaction between macrophages, TGF-beta1, and the COX-2 pathway during the inflammatory phase of skeletal muscle healing after injury. *J Cell Physiol.* 2008;214(2):405–412.
- Li Y, Foster W, Deasy BM, et al: Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. *Am J Pathol.* 2004;164(3):1007–1019.
- Longaker MT, Whitby DJ, Ferguson MW, Lorenz HP, Harrison MR, Adzick NS: Adult skin wounds in the fetal environment heal with scar formation. *Ann Surg.* 1994;219(1):65–72.
- Roh TS, Rah DK, Park BY: The fetal wound healing: a review. *Yonsei Med J.* 2001;42(6):630–633.
- Zhang HY, Phan SH: Inhibition of myofibroblast apoptosis by transforming growth factor beta(1). *Am J Respir Cell Mol Biol.* 1999;21(6):658–665.
- Li Y, Huard J: Differentiation of muscle-derived cells into myofibroblasts in injured skeletal muscle. *Am J Pathol.* 2002;161(3):895–907.
- Kisseleva T, Brenner DA: Mechanisms of fibrogenesis. *Exp Biol Med (Maywood).* 2008;233(2):109–122.
- Suematsu M, Suzuki H, Delano FA, Schmid-Schönbein GW: The inflammatory aspect of the microcirculation in hypertension: oxidative stress, leukocytes/endothelial interaction, apoptosis. *Microcirculation.* 2002;9(4):259–276.
- Piotrowski WJ, Marczak J: Cellular sources of oxidants in the lung. *Int J Occup Med Environ Health.* 2000;13(4):369–385.
- Hinz B, Mastrangelo D, Iselin CE, Chapponnier C, Gabbiani G: Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol.* 2001;159(3):1009–1020.
- Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chapponnier C: Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol Biol Cell.* 2001;12(9):2730–2741.
- Luttikhuisen DT, Harmsen MC, Van Luyn MJ: Cellular and molecular dynamics in the foreign body reaction. *Tissue Eng.* 2006;12(7):1955–1970.
- Tabata Y: Biomaterial technology for tissue engineering applications. *J R Soc Interface.* 2009;6(suppl 3):S311–S324.
- McCullen SD, Ramaswamy S, Clarke LI, Gorga RE: Nanofibrous composites for tissue engineering applications. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2009;1(4):369–390.
- Varga J, Pasche B: Antitransforming growth factor-beta therapy in fibrosis: recent progress and implications for systemic sclerosis. *Curr Opin Rheumatol.* 2008;20(6):720–728.
- Fukushima K, Badlani N, Usas A, Riano F, Fu F, Huard J: The use of an antifibrosis agent to improve muscle recovery after laceration. *Am J Sports Med.* 2001;29(4):394–402.
- Foster W, Li Y, Usas A, Somogyi G, Huard J: Gamma interferon as an antifibrosis agent in skeletal muscle. *J Orthop Res.* 2003;21(5):798–804.
- Chan YS, Li Y, Foster W, Fu FH, Huard J: The use of suramin, an antifibrotic agent, to improve muscle recovery after strain injury. *Am J Sports Med.* 2005;33(1):43–51.
- Chan YS, Li Y, Foster W, et al: Antifibrotic effects of suramin in injured skeletal muscle after laceration. *J Appl Physiol.* 2003;95(2):771–780.
- Negishi S, Li Y, Usas A, Fu FH, Huard J: The effect of relaxin treatment on skeletal muscle injuries. *Am J Sports Med.* 2005;33(12):1816–1824.
- Paz Z, Shoenfeld Y: Antifibrosis: to reverse the irreversible. *Clin Rev Allergy Immunol.* 2010;38(2–3):276–286.
- Bellayr I, Mu X, Li Y: Biochemical insights into the role of matrix metalloproteinases in regeneration: challenges and recent developments. *Future Med Chem.* 2009;1(6):1095–1111.
- Ra HJ, Parks WC: Control of matrix metalloproteinase catalytic activity. *Matrix Biol.* 2007;26(8):587–596.
- Bedair H, Liu TT, Kaar JL, et al: Matrix metalloproteinase-1 therapy improves muscle healing. *J Appl Physiol.* 2007;102(6):2338–2345.
- Kaar JL, Li Y, Blair HC, et al: Matrix metalloproteinase-1 treatment of muscle fibrosis. *Acta Biomater.* 2008;4(5):1411–1420.

38. Wang W, Pan H, Murray K, Jefferson BS, Li Y: Matrix metalloproteinase-1 promotes muscle cell migration and differentiation. *Am J Pathol.* 2009;174(2):541–549.
39. Zimowska M, Brzoska E, Swierczynska M, Streminska W, Moraczewski J: Distinct patterns of MMP-9 and MMP-2 activity in slow and fast twitch skeletal muscle regeneration in vivo. *Int J Dev Biol.* 2008;52(2–3):307–314.
40. Ohtake Y, Tojo H, Seiki M: Multifunctional roles of MT1-MMP in myofiber formation and morphostatic maintenance of skeletal muscle. *J Cell Sci.* 2006;119(Pt 18):3822–3832.
41. Furochi H, Tamura S, Takeshima K, et al: Overexpression of osteoactivin protects skeletal muscle from severe degeneration caused by long-term denervation in mice. *J Med Invest.* 2007;54(3–4):248–254.
42. Han YP, Yan C, Zhou L, Qin L, Tsukamoto H: A matrix metalloproteinase-9 activation cascade by hepatic stellate cells in transdifferentiation in the three-dimensional extracellular matrix. *J Biol Chem.* 2007;282(17):12928–12939.
43. Brejchova K, Liskova P, Cejkova J, Jirsova K: Role of matrix metalloproteinases in recurrent corneal melting. *Exp Eye Res.* 2010;90(5):583–590.
44. Rosler S, Haase T, Claassen H, et al: Trefoil factor 3 is induced during degenerative and inflammatory joint disease, activates matrix metalloproteinases, and enhances apoptosis of articular cartilage chondrocytes. *Arthritis Rheum.* 2010;62(3):815–825.
45. Piecha D, Weik J, Kheil H, et al: Novel selective MMP-13 inhibitors reduce collagen degradation in bovine articular and human osteoarthritis cartilage explants. *Inflamm Res.* 2009;59(5):379–389.
46. Overall CM, Kleinfeld O: Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and antitargets for cancer therapy. *Nat Rev Cancer.* 2006;6(3):227–239.