An overview of biological basis of pathologic scarring

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Summary
Aims and objectives: To review the current mechanisms and biologic processes leading to the formation of pathologic scars.
Materials and Methods: A computerised literature search was carried out using MEDLINE for all published articles on "pathologic scarring". The medical subject headings "scarring" were combined with "mechanisms". A review of selected relevant literature was then undertaken.
Results: Scarless embryonal healing tends to be characterised by minimal inflammatory reaction mediated by reduced IL6, IL8 and hyaluronidase while there are elevated levels of hyaluronic acid MMP1 to 3, as well as IL 10. The multifunctional cytokine TGF-B, its several isoforms as well as its postreceptor signalling mechanisms appears to play the key role in the scarring process. There is also evidence to show that PDGF, IGF and other cytokines regulate scarring. While conventional antiscarring agents target the fibroplasia phase, others such as tamoxifen, calcium channel blockers, and imidazoquinolines targets various phases of the scarring process.
Conclusion: It appears that multiple mechanisms are involved in the phenotypical appearance of abnormal scarring. A deeper understanding of these mechanisms is pivotal to the development of better antiscarring therapies in the very near future.

Key words: Pathological scarring, healing mechanisms, transforming growth factor B.

Introduction
The healing of wounds appears to be basic to the survival of every living organism. In the developed nations over 100 million patients acquire scars each year following surgical procedures. A significant number of these scars ultimately become symptomatic. Abnormal scarring causes significant functional, aesthetic, psychological, and social problems costing an estimated 4 billion US dollars to treat in the US each year. Between 4% and 16% of post operative scars are thought to become pathological. ¹

The major brunt of problematic scarring appears to be in people of the darker races who are known to exhibit a more exuberant scar formation with a reported keloidal scarring prevalence of between 4% and 16% among African Blacks (figure). While scars are the end point of the normal processes of mammalian tissue repair, the final product should ideally be the complete total regeneration of a new tissue, an exact replica of the uninjured template. Man has apparently lost the potential for scarless healing as a price for evolutionary survival following mammalian tissue repair, the final product should ideally be the complete total regeneration of a new tissue, an exact replica of the uninjured template. Man has apparently lost the potential for scarless healing as a price for evolutionary survival following mammalian tissue repair, the final product should ideally be the complete total regeneration of a new tissue, an exact replica of the uninjured template. 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Both autosomal dominant and recessive types with variable penetrance have been described. There are also wide phenotypic heterogenicity described in literature. The altered expression of p53 and bcl family protein have been implicated in several familial keloids. Apart from these influences, evidence has shown that dermal fibroblasts respond to mechanical forces outside the granulation tissue by up-regulating fibroblast activity and extracellular matrix protein deposition. This may explain the predilection of cutaneous scarring in high tension areas such as the deltoid and sternal region. The natural biologic nature of native tissue also play a role in scarring as intraoral wounds rarely result in pathological scars. Hormonal influence has also been known to play a role in the aetiopathogenesis of abnormal scarring, as it has been shown that testosterone receptors have been identified in hypertrophic scars and keloids; and IGF has also been implicated in the invasive activity of fibroblasts. This may explain the common presentation of pathologic...
scarring in adolescents and young adults and the rarity in the very young and elderly.

**Inflammatory phase and foetal corollaries**

Focal wound healing processes have been studied as a comparative healing model to the fibrotic healing that occurs in adult skin. The inflammatory reaction to the injured skin appears to be key to the initiation of scarring as minimal inflammation defines scarless foetal healing. The fibrin clot fills the site of the injury maintains homeostasis and provides a matrix for repair. Characterised by the abundance of neutrophils and monocytes, and later by macrophages, this phase lasts between 1 and 3 days and is marked by the removal of the foreign bodies, bacteria and dead leukocytes from the injured area. The scarless embryonal healing is characterised by a reduced foetal platelet aggregation in response to collagen, lower levels of cytokine release, reduced leukocytes macrophages and activated macrophages as well as T and B lymphocytes. The pro inflammatory mediators (IL6, IL8) are reduced while the antiinflammatory IL10 is elevated in scarless foetal wound repair.

Other cytokines mediating this phase include interleukine 1 and 4, TGF-b, PDGF and IFG. The cellular expression of this is mediated by cell adhesion receptors called integrins. In scarless foetal healing, there is also an apparently reduced leukocyte endothelial cell interaction leading to reduced leukocyte emigration from the blood vessels into the wound bed as well as high levels of hyaluronic acid and low levels of hyaluronidase. Scarless foetal wounds demonstrate lower levels of COX-2 and contain less PE2. In adult wounds PE2 and analogues that induce collagen synthesis and fibrosis are typically elevated.

**Fibroplasia phase**

This phase is characterised by the predominance of fibroblast activity and the laying down of the precursor of the permanent scar - granulation tissue. In this phase, lasting between 2-6 days following injury, fibroblasts invade the provisional matrix and begin to lay down collagen within a matrix of numerous ground substances under the influence of IL-4 and TGF-b. Myo-fibroblasts, which are the predominant cell lines of this phase are specialised fibroblasts that express cytoskeletal protein similar to smooth muscle. They are produced in response to Granulocyte/monocyte colony stimulating factors on macrophages resulting in TFG-b and PDG-b production. They tend to persist long after wound has healed in pathological scarring and are responsible for the over 80% wound contraction occurring in cutaneous wounds.

IL-6 expression has been known to be increased in keloid fibroblast cultures, IL-6 induces further collagen synthesis by fibroblasts. There also is an upregulation of the IL-6 receptors (IL-6-R and IL6-Rβ). Analyses of these receptors have been noted to lead to a reduction in type I collagen synthesis by keloid fibroblast cultures. Though fibroblast from hypertrophic scars demonstrate elevated collagen production, their response to metabolic stimulation is similar to that of normal fibroblasts.

Though the mechanisms are still incompletely understood, it appears that basal ECM tension also causes fibroblast proliferation and biosynthetic activity to be up-regulated and this explains the development of hypertrophic scarring in areas of high skin tension and its resolution when the tension is relieved or redirected.

**Remodelling phase**

Collagen maturation from type 3 to the type 1 variety along with reduced angiogenesis in the granulation tissue are key features of this phase. There is re-arrangement of collagen fibres, reduction of wound size and apoptosis of the myofibroblasts. There also is marked cross linkage between the collagen fibres and reduction of scar cellularity and increased breaking strength. This phase takes several weeks and with a progressive increase in wound tensile strength with final scar maturation.

The interaction of monocyte derived macrophages with the Extracellular matrix protein appears to be mediated via cellular adhesion molecules - integrins.

There appears to be a highly regulated cell-cytokine ECM interaction keeping scar production within physiological limits. Certain factors however alter this fine balance in favour of excessive dermal scarring in some situations. Transforming growth factor B seem to be the key mediator in this event, as it is released by adjacent cells and through limited ECM breakdown. Intricate processes of ECM remodelling give rise to the finely balanced matured scar of the adult human. This balance is highly regulated by the Matrix enzymes, metalloproteinases, procollagenases serine proteinases which include plasminogen activator (PA) all of which effectively result in ECM breakdown, with an overall anti-fibrotic effect. On the other hand plasmin activator inhibitor (PAI) a tissue inhibitor of metalloproteinases promote ECM build up under the regulation of TGF-b. The complexity of this regulated system is further evidenced by the fact that plasmin can release active TGF-B from its latency-associated protein which further regulates PAI-1, several MMPs, TIMP-1 and several genes encoding ECM components. In scarless embryonal healing there are higher levels of MMP1, MMP2, MMP3 compared to adult wounds; in addition, these wounds express MMP1, MMP9, MMP14 more quickly than fibrotic wounds. Though MMP2, TIMP1, TIMP3 expression appear unaltered in scarless healings, MMP2 is reduced and TIMP1 and TIMP3 increased in fibrotic healings. There is some evidence to suggest that in keloids the finely regulated balance is tilted towards matrix synthesis as it has been shown that keloidal fibroblast exhibit a decreased capacity for fibrinolysis and fibrin clot degradation. There is also an increased PAI-1 expression found at mRNA and protein synthesis levels leading to reduction in plasmin mediated collagenase activation with a resultant reduced collagen degradation during the remodelling phase.

**Fibrogenic cytokines**

Probably the most intensely studied factor in the development of scar tissue is the role of TGF-B in fibrotic healing. TGF-B is a multifunctional cytokine which regulates cellular growth and differentiation in fibroblasts. It belongs to a family of structurally related protein including TGF-B1 to 5 and bone morphogenic proteins BMPs. This family has been implicated in a wide range of fibrotic disorders such as cutaneous fibrosis, scleroderma, visceral conditions such as liver cirrhosis, pulmonary and renal fibrosis. Several isoforms (TGF-b1 and TGF-b2) are well known fibrogenic cytokines which have been noted to be of lower concentration and more rapidly cleared in scarless healing. The TGF-b1 and TGF-b2 (receptors) have also been noted to be of lower concentration in scarless wounds. The introduction of TGF-b converts scarless healing into a fibrotic one. The isomer TGF-b3 has been shown to be anti-fibrotic.

TGF-B stimulates fibroblast proliferation and migration. It also increases extracellular matrix production as well as inhibiting its degradation. There are three human isoforms of TGF-B, namely: TGF-b, b1, b2 and b3. TGF-b1 and TGF-b2 have been found to be highly expressed in keloids and hypertrophic scars while TGF-b3 has been found to reduce cutaneous scarring in rats and pigs. The precursor to TGF-b1 is a C terminal mature TGF-b non-covalently bounded to a N-terminal latency associated peptide of 390 amino acids. The initial step in pathological scarring appears to be increased TGF-b1 gene expression by neo-vascular endothelial cells, thus stimulating fibroblasts production by TGF-b1. This results in an
Increased fibroblast activity with a resultant increased collagen deposition and reduced breakdown. TGF-b and its analogues like activin and Bone morphogenetic protein (BMP) have been shown to act via the heterodimeric transmembrane serine / thrombin kinase receptor. TGF-bR2 receptor is involved in the initial ligand binding, subsequently recruits TGF-bR1. It acts via a series of post receptor signalling involving receptor associated TAK-1 binding proteins and SMADs to activate transcription and ultimately collagen synthesis and ECM build up. Recent studies have suggested a role for the TGF-b receptors in pathological scarring as evidenced by increased TGF-bR1/ TGF-bR2 ratio found in keloids as opposed to normal scars. It has also been shown that keloidal fibroblast secrete normal levels of TGF-b1. The implication of which may mean the excessive scarring may be a receptor/post receptor phenomenon. Keloidal fibroblasts demonstrate an altered response and abnormal sensitivity to TGF-b. These include production of higher levels of collagen, fibronectin, elastin, and proteoglycan. Keloidal fibroblasts also show aberrant responses when compared with normal fibroblasts to metabolic modulators, glucocorticoids, hydrocortisone and growth factors. In addition they produce elevated levels of PAI. TFG-b also has an auto-induction ability, this sustained expression further increases matrix synthesis. The finding that PDGF depleted mice loses myofibroblasts from tissues and that TFG-b does not induce myofibroblast proliferation in the absence of PDGF may indicate a key role of PDGF in in-vivo fibrogenesis. Other cytokines with fibrogenic activity which may play a role in the pathologic scarring include IGF-1, interleukin 1 and 4 which acts synergistically with TGF-b to induce fibroblast proliferation and matrix synthesis.

Clinical and therapeutic implications of biology of pathological Scarring

The myriads of interventional modalities that are employed in the clinical management of abnormal scarring underlie the heterogeneity, and varying phenotypical expressions of this phenomenon. The absence of a universally effective scar treatment modality reinforces the highly complexed evolutionary processes of fibrogenic healing in man. The increased knowledge and numerous advances in understanding of the process of scar formation has led to the introduction of new treatment methods. These methods are known to target different phases of wound healing process.

Interventions which target inflammatory phase include topical use of human recombinant IL-10 which has been shown to produce scarless healing in adult mouse wounds. (prevascal has been introduced by Renovo)

Intralesional and systemic steroid use have been shown to be of value in pathologic scarring as they reduce fibroblast proliferation, collagen synthesis, TGF-B expression, inflammatory mediators and alter glucocorticoids synthesis. Though interferons are known to be involved in the fibrotic healing by mediating the p53 apoptosis pathway leading to DNA alteration and degradation, addition of alpha-2b interferon has been shown to improve the efficacy of intralesional triaminolone in keloids treatment. Bleomycin and 5-Flourouracil have been shown to reduce fibroblast proliferation. Adriamycin has also been shown to decrease collagen alpha-light chain assembly. Verapamil and Captopril are phenylalkylamine Calcium channel blockers block synthesis of fibronectin, collagen, glucosaminoglycan and increase fibrolytic activity; they impair cytoskeleton activity and induce procollagenase activity. Retinoic acid has been shown to reduce tonofilament and kerato-hyaline synthesis. Other vitamin A derivatives have been shown to produce a marked reduction in human fibroblast proliferation by interfering with DNA synthesis in vitro. Retinoids have also been shown to exhibit an inhibitory effect on TGF-b1-induced type I collagen gene expression in human fibroblasts.

Imidazolquinolines are topical immunomodulators that are toll-like receptors (TLR) -7 and -8 agonists. Imiquimod is a topical immunomodulator that induces TNF-α and other interferon. It also alters the expression of apoptotic markers in cutaneous wound healing as well as inducing the production of anti-fibrotic cytokines. Tamoxifen decreases TGF-b activity and also induces an unbalanced growth factor activity. Sirolimus reduces TNF-α and gfi-1 expression in fibroblasts by inhibiting mTOR a mammalian receptor serine threonine kinase which plays an important role in cellular metabolic processes and translation rates.

Human recombinant forms (Avotermin) of TGF-B3 have been developed that reduce fibronectin, types -1 and -2 collagen synthesis. They are currently in use for cutaneous scar improvement.

Radiation, which markedly reduces fibroplasias, has been used as an adjunct to other forms of treatment, though there are concerns about its long term effects.

Several anti-TGF-b analogues have been developed and are undergoing clinical trials. These include the naturally occurring TGF-B binding proteoglycan decorin and mannose-6 phosphate an antagonist of TGF-B activation. Botulium toxin (BTA) has been shown to immobilise local muscles and reduce skin tension caused by muscle pull, as well as reduces micro-trauma and subsequent inflammation. This reduction in tensile forces apparently down regulates the key balance between fibroblast proliferation and cellular apoptosis. Early results of clinical trials on Botulinum toxin have been encouraging.

Silicone sheets have been known to be effective in minimizing cutaneous scarring. The mechanism of action has been largely unknown, though it is believed to improve scar hydration. It is thought that static electricity generated by friction activated silicone induces involution of hypertrophic scars and keloids. There is also appears a cellular mediated immunological scar remodelling process associated with silicone use.

Carbon dioxide and Argon lasers in resurfacing scars have been known to induce fibroblast proliferation, induce apoptosis, upregulate MMP13, and down regulate TGF-B. The use of Carbon dioxide and Argon lasers in resurfacing scars have been demonstrated to have an efficacy of over 75% in selected patients. In general laser therapies have given poorer results in darker skin races due to melanin acting as a competing chromophore.

Figure: Florid keloids in a middle aged African
An overview of biological basis of pathologic scarring; B.O. Mofikoya

Conclusion

The knowledge of foetal wound healing and correlations between animal and human experimental wound healing processes have helped greatly in understanding the biological basis of pathological scarring. Further insights into the mechanisms of pathologic scarring have led to the development of various treatment modalities for problematic scars. Although, these treatment regimens have been applied in different phases of wound healing, clinical outcome still remains unsatisfactory. Therefore further researches are needed to clarify many aspects of the mechanisms that bring about pathological scarring. It is hoped that these would open a new frontier in the management of clinically symptomatic scarring.

Abbreviations- ECM extracellular matrix , IGF insulin growth factor , IL interleukin, TGF transforming growth factor, PDGF platelet derived growth factor, COX cycloxygenase, PA Plasminogen activator, PAI Plasminogen activator inhibitor, TIMP tissue inhibitor of metalloproteinase1, MMP metalloproteinase, BMP Bone morphogenic protein, TAK-1 transforming growth factor activated kinase, SMADs-intracellular signalling proteins

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