PERSPECTIVE ARTICLE

Cellular/extracellular matrix cross-talk in scar evolution and control

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ABSTRACT

The principles of scar evolution and control are recognized and defined. Further clarity has been shed on these principles with the elucidation and elaboration of the sequence of events occurring at a molecular level. Cellular cross-talk among structures in the cell cytosol, in the cellular nucleus, and outside the cell within in the extracellular matrix is continuous and controlling in nature. This interaction or ''dynamic reciprocity'' takes place via a series of signals, ionic messenger shifts, protein activation, and receptor transactions. The described principles are now able to be defined in terms of cellular/extracellular matrix interactions and the identification of the cross-talk involved in scar evolution and maturation presents the possibility of influencing the ''wording'' of this cross-talk to improve scar outcome. The principles of mechanostimulation and scar support, hydration occlusion, controlled inflammation, and collagen/extracellular remodeling are discussed with possible interventions in each category.

PRINCIPLE I: MECHANOSTIMULATION AND SCAR SUPPORT

Support of the fresh scar was one of the earliest principles espoused for scar management. Multidirectional vector forces applied to a fresh scar were observed to produce hypertrophy.[1,2] Sommerlad and Creasy[3] compared the effect on stretching of four techniques of wound closure and studied the microarchitecture of stretched scars. They concluded that with little tension across a scar itself, the mechanically weak collagen bond withstands the forces on it resulting in a narrow scar and the scar collagen remains aligned along the scar. If, however, the tension across the scar is sufficient to overcome the bond when sutures are removed, the longitudinal fibers separate and new collagen is laid down haphazardly across the scar. Observations made by Elliot et al.[1] regarding presternal scars showed that not only was hypertrophy common, but there were significant regional differences, with a tendency to scar hypertrophy overlying the body of the sternum, especially in females. ''The multi directional tension may over stimulate the fibroblast causing it to produce excess collagen which is the main constituent of the scar.''[1]

These original observations can be explained by current molecular research. Cellular functions are directly influenced by the extracellular matrix (ECM) environment. Signals emanating from the ECM initiate cellular functions including morphogenesis, differentiation, angiogenesis, and remodeling.[4] The signaling comes about as the ECM has the ability to modify the cell resulting in adhesions and changes in cellular shape. These changes create sensitivity to mechanical tension, which transmits to the cell initiating fibroblast stimulation, procollagen formation, and myofibroblast differentiation. This is a result of the proliferation of smooth muscle cells and fibroblasts accompanied by increased gene expression of ECM proteins procollagen and tropoelastin.[5,6] Thus, specific focal adhesions at the cellular surface allow mechanical tension generated in the system to be transduced to the cytoskeletal network.[7]

The integrin family of proteins constitutes the most abundant receptors mediating the interaction of cells with their surrounding ECM. Ligands bind to the extracellular domains of integrins, and integrin cytoplasmic tails connect via linker proteins to the cytoskeleton. By linking the outside environment with the cellular inside, they transduce signals in both directions from the outside in and the inside out.[8–10] Changes in mechanical stiffness and release of growth factors from inflammatory cells activate fibroblasts to form contractile actin fibers (myofibroblasts), which then attach via fibroblast integrins to the ECM.[11,12] Myofibroblasts have long been associated with wound repair and fibrosis.[13] The myofibroblasts bring about contraction and remodeling of wound and scar tissue and are thus key effectors in hypertrophic scars (HTSs). In the later stages of normal wound repair, myofibroblasts undergo apoptosis and disappear. Loss of mechanical tension, alterations in cell density or protease activity, and/or changes in the pattern of secreted growth factors are thought to contribute to cell death. Using collagen as three-dimensional structures, several groups have showed that applying tension to the matrix directly affects the biosynthetic capacities of fibroblasts.[4,14]

In the ECM, the integrins transmit signals often generated by important glycoproteins. One of the most important glycoproteins of the ECM is fibronectin, a major

product of dermal fibroblasts and myofibroblasts, which has a wide distribution in the dermis, in the dermal–epidermal basement membrane zone. Besides its structural role, fibronectin is the main cell adhesion molecule, modulating many cellular activities. After injury, fibronectin is initially deposited from blood plasma, and plays an important role in platelet function with the release of growth factors and cytokines.[15] Together with fibrin, it provides most of the provisional matrix in dermal wounds, guiding fibroblasts, and inflammatory cells to the site of injury. The formation of a stable collagen I/III fibrillar network is thought to depend on a preexisting fibronectin network through a mechanism involving integrins.[4,15] The relationship among fibronectin, fibroblasts, and myofibroblasts is integral to the balanced scar formation and wound contraction.

A second glycoprotein that has attracted much attention, particularly due to its presence in tissues with high tensile stress,[16] is tenascin-C. Tenascin-C expression is strongly up-regulated at the transcriptional level by mechanical stress. It is transiently expressed upon tissue injury, where it is specifically localized to the wound edge, and persistently up-regulated in fibrotic disease. Full-length tenascin-C promotes fibroblast migration within fibrin–fibronectin matrices, whereas specific fragments of tenascin-C inhibit fibroblast migration.[4,17] A balance in this glycoprotein is essential—its induction recruits fibroblasts into the wound while fragments resulting from its breakdown prevent excessive fibroblast infiltration.

The precise mechanisms by which different cell types transmit mechanical signals are not fully understood. They might involve stretch-activated ion channels, direct interactions between structural and signaling components, or activation of small GTPases.[18] Many cooperative interactions exist between integrins and growth factor signaling. In particular, fibroblast to myofibroblast conversion and α-smooth muscle actin (α-SMA) expression crucially depend on a combination of mechanical tension and transforming growth factor (TGF)-β.[19] Thus, in scarring, generation of tension can induce myofibroblast formation. Similarly, collagen synthesis in fibroblasts is induced by the mechanical tension. In this case, TGF-β is induced by tension, which in turn activates collagen synthesis via the classic pathways.[20] In addition, fibronectin is induced by the application of cyclic strain to fibroblasts. In parallel, many proteases are down-regulated, whereas protease inhibitors are up-regulated.[4]

Cellular mechanoreceptors are functionally related to membrane ion channels, particularly the calcium-transporting channels. Calcium influx and calcium-mediated intracellular signaling in fibroblasts as a response to mechanical stimulation has been observed in a number of studies.[18,21,22]

Smad signaling and, in particular, the amalgamation of Smad2, 3, and 4 within the nucleus is pivotal to TGF-β stimulation and maintenance.[23] Ligand (fibronectin, tenascin, TGF-β, etc.) binding leads to the activation of Smad2 and 3. After phosphorylation, Smad2 and 3 associate with Smad4 and migrate into the nucleus, where they bind to promoters and modulate transcription. Smad7 antagonizes TGF-β signaling by affecting the activity of the receptor complex decreasing the Smad2, 3, 4 entry into the nucleus and subsequent TGF-β stimulation.[23] Adhesion molecules or gap junction proteins, cadherins, connexins, can compete or interfere with Smad signaling; Cx43, in particular, has been found to displace Smad2, 3 from their microtubules making more available for amalgamation with Smad4 and translocation into the nucleus, promoting TGF-β production[24] (Figure 1).

An added factor that may be of importance when dealing with mechanical stress and mechanostimulation is that of connective tissue growth factor (CTGF). CTGF levels are transiently increased for several days following dermal injury; however, in HTS, this increase appears to persist.[25] CTGF appears to "partner" TGF-β in its fibrotic role. TGF-β potently stimulates CTGF possibly by encouraging its ligand–receptor binding in activated cells stimulating the formation of fibronectin, collagen, and tissue inhibitors of metalloproteinase 1 (TIMP1).[25] Prolonged expression of CTGF thus promotes matrix accumulation and there appears evidence that mechanical stress is an important inducer of this expression. CTGF (and indirectly mechanical stress) may become an important target for HTS prevention as it has direct fibrotic effects in conjunction with TGF-β. This is in contrast to manipulation of TGF-β, which is far more unreliable because of its varying physiological roles—reducing TGF-β and collagen stimulation may aid HTS but if the timing is not ideal, delayed wound healing may result. Thus, CTGF may be a more reliable target.

Mechanotension between cells stimulates the formation of myofibroblasts with α-SMA. Cadherins appear to play a significant part in this reaction. Without tension, the production of myofibroblast phenotype can be induced in vitro by plating human dermal fibroblasts at low density (LD). Upon reaching confluence, the LD-plated cells express α-SMA within stress fibers. In contrast, few cells express α-SMA when those same fibroblasts are plated at high density.[26] Additionally, a significant reduction in cadherins and α-SMA was observed when confluent LD-plated myofibroblasts were covered with a collagen lattice for 24 hours.[26] This has clinical significance with the emergence of biologic dressings including collagen matrices and dermal replacements. Cadherins appear to maintain tension between neighboring cells, which induces α-SMA expression in stress fibers. Cell contact with collagen reduces cadherin expression and subsequent myofibroblast phenotype induction.[26]

In summary, it has become obvious in recent years that the ECM and its extension to intracellular structures is not a haphazard conglomeration of molecular structures. Rather the ECM, its glycoproteins, ligands, integrins, and the cytoskeleton form a finely tuned syncytial-like structure, defined in shape and tension and extremely sensitive to mechanical stimuli. This allows the process of signaling to occur across a continuum between keratinocytes and fibroblasts with intervening adhesion molecules connexins (Cx43, in particular), cadherins, and a multiple variety of ligands, ligand-binding stimulating proteins (CTGF), integrins, MAP kinase signaling pathways, and other ECM cytokines. The term mechanotransduction refers to the mechanism by which physical forces are transduced into biomolecular responses in cells. The first step is the sensing of a mechanical stimulus. The keratinocyte appears to be an important initiator to the process and contrary to previously held thoughts about the keratinocyte, it may well

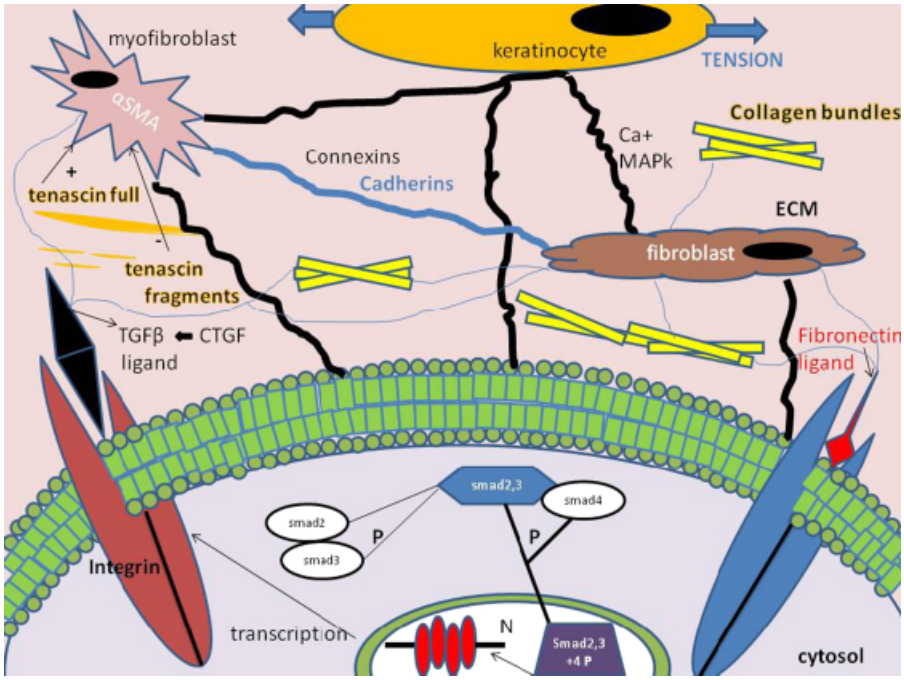

Figure 1. Mechanotension is transmitted through the cellular network setting off a process of intra- and extracellular signaling. The tension-related signals (Ca^{+} channels) are transmitted cell to cell via connexins, cadherins, and integrins, then bind with ligands such as fibronectin, TGF-β (reinforced by CTGF). The message from this union is transferred into the cell cytoplasm where phosphorylation of Smad2, 3 units amalgamates with Smad4 and translocates into the nucleus. Here they bring about transcription and message encoding relaying new instructions via integrins, ligands (TGF-β) to stimulate collagen formation, myofibroblast transformation, and matrix accumulation. TGF, transforming growth factor; CTGF, connective tissue growth factor.

be the controller of much of the cross-talk taking place between itself, the ECM, and the dermal fibroblasts (Figure 1).

It has been shown that mechanosensitive ion channels are able to transform a physical signal into an ion flux.[27,28] These signals reach the extracellular glycoproteins, primarily fibronectin and tenascin-C, which act as ligands and associate with integrins transmitting signals through to the cytosol. In the cytosol, signaling continues through Smad isoforms and the like moving into the cell nucleus, affecting transcription, and causing the activation (or inhibition) of growth factors (primarily TGF-β in conjunction with CTGF), which results in proliferation (fibroblasts, procollagen, tropoelastin), differentiation (myofibroblasts), collagen synthesis, and wound contraction. Once the wound is in the shortened contracted phase, with increased collagen production, even if the mechanostimulation ceases, it is difficult if not impossible to reverse the situation. This is especially so if the mechanostimulation occurs repeatedly and intermittently, each time stimulating further collagen production. The control of mechanostimulation and support of the early wound is thus essential to the process of scar control particularly in large wounds subjected to multidirectional vector forces. In short, mechanical interaction of the cell with its surroundings causes changes in cell morphology and biological signaling that ultimately leads to functional adaptation and may result in pathological scarring.

PRINCIPLE II: SCAR HYDRATION AND OCCLUSION

Hydration of the scar surface is the basis of action of 90% of scar management systems in the market. Most oils (tissue oils), lotions, and creams have beneficial effects on scars primarily on the basis of their hydrative capacities.[29–31] Normal skin has a mature stratum corneum characterized by minimal transepidermal water loss (TEWL). Dehydration of the stratum corneum initiates signaling inducing keratinocytes to produce cytokines, which activate dermal fibroblasts to synthesize and release collagen. Excessive collagen production leads to abnormal scarring.[32,33] This would be the short explanation for the beneficial effects observed with hydration of the scar surface. However, the process has more associated subtleties—if the hydration is increased to the point of occlusion, further benefit is gained (silicone gel, sheeting); this hydrative/occlusive benefit was thought previously to be initiated by a direct effect on fibroblasts. It would now appear that the keratinocyte on the surface of the skin is well capable of orchestrating and initiating the signaling events that culminate in fibroblast TGF-β stimulation of collagen production or cessation. The pro- or antifibrotic status has been shown to directly link up with the hydration, or more particularly the occlusive state of the keratinocyte.[34,35] This has major impact on scar management modalities and cosmetic/cosmeceutical formulation as in many cases therapy may be directed superficially with an expectant result in the deep dermis where the impact is desired.

Occlusion of a healing closed wound results in hydration of surface keratinocytes. The hydration directly affects the differentiation of keratinocytes, with active-type keratinocytes releasing cytokines and growth factors within the ECM.[32,34] The background wound milieu and eventual appearance of the scar are dependent on a constant competitive challenge between proinflammatory and profibrotic agents and their antagonists. Proinflammatory cytokines with scar-producing effects predominantly involve interleukin (IL)-1β, with less input from IL-6, -8, and

-10[35]; tumor necrosis factor (TNF)-α, although potently proinflammatory, has been shown to be antifibrotic with suppression of collagen production showed in a number of studies.[35–37] Additionally, TNF-α stimulates protease (matrix metalloproteinase [MMP]) expression resulting in collagen breakdown and wound matrix remodeling.

Controlling the fibrotic element of wound healing is the backbone of scar control. Critical to this process is the balance of the major players. TGF-β is a major controlling growth factor in the process—the balance of TGF-β1 and -β2 profibrotic isoforms with the TGF-β3 antifibrotic isoform has attracted much attention with major efforts directed toward increasing the TGF-β3/TGF-β1 ratio.[38,39] This ratio is directly affected by the Smad signaling process with a similar picture of Smad3, 4 stimulating TGF-β1 production and Smad7-stimulating TGF-β3 production.[40–43] Thus, control of scarring would ideally involve reduction of proinflammatory cytokine mediators and stimulation of antifibrotic growth factors predominantly TGF-β3.

Occlusion hydration results in a decreased activation of keratinocytes resulting in a decreased production of IL-1β (and probably other cytokines yet to be identified), an increased production of antifibrotic TNF-α,[32–34] and an increase in TGF-β3 via stimulation of Smad7 signaling mechanism.[38–43] This results in ECM remodeling with less inflammation, decreased collagen production, and balanced protease activity collectively encouraging scar maturation.

PRINCIPLE III: MANAGING INFLAMMATION

Inflammation is a necessary sequence in wound healing. However, exaggerated inflammation appears to be the central problem in most chronic (and many acute) diseases, be it cardiac, vascular, diabetic, or arthritic. HTS is no exception to this issue—excessive inflammation results in exaggerated scars.[44,45] Managed inflammation is a sought after principle in scar management. Work on fetal wounds suggests that a very mild inflammatory response may underlie the scarless healing observed.[46,47]

Injury precipitates the activation of clotting and the complement cascade resulting in the release of vasoactive mediators and cytokines. The provisional clot and its constituents attract and release inflammatory cells—neutrophils, macrophages, and T-lymphocytes, as well as epithelial cells, endothelial cells, mast cells, and fibroblasts to the wound site.[48] Mast cells respond to monocyte chemotactic protein-1 and TGF-β1, -β2, -β3 and within the wound, release mediators (histamine, proteoglycans, proteases, platelet-activating factor, arachidonic acid metabolites) and cytokines, including TGF-β and IL-4. Mast cells and the complement cascade are responsible for mediators that stimulate vasodilation. Kinins, complement factors, and thrombin increase capillary permeability, which facilitates the extravasation of proteins into the wound site.[49] Subsequently, neutrophils and macrophages debride the wound and release several proinflammatory cytokines, some of which also are responsible for matrix production.

Multiple cytokines are involved in the inflammatory process, but some key modifiers of the scarring process need further elaboration. The TGF-β family, platelet-derived growth factors (PDGF), and epidermal growth factors (EGF)[44,50] stimulate fibroblast proliferation and matrix production, and induce leukocyte attraction. Leukocytes, in turn, reinforce fibroblast activity by acting through the TGF-β family, fibroblast growth factors (FGF), vascular endothelial growth factors (VEGF), prostaglandins (PG),[47] and Smad activation.[51,52] As previously discussed, increased levels of TGF-β1 and -β2 as well as decreased levels of TGF-β3 have been associated with HTS through inflammatory cell stimulation and fibroblast proliferation.

Exaggerating the inflammatory phase increases the concentration of potential profibrotic cytokines like TGF-β, PDGF, and IL-4. PDGF is produced by platelets early in the wound healing cycle and appears to be directly linked to HTS formation.[53] IL-4 has recently been identified as a major precursor to HTS and may be more potent than TGF-β at mediating fibrosis.[53]

Histamine is capable of enhancing the formation of collagen by fibroblasts in vivo,[54–56] and is significantly elevated in the plasma of patients developing HTS compared with age-matched normal volunteers.[57] Allied to this, patients in pain (particularly burns patients) secrete substance P, a neuropeptide, which induces mast cells to release more histamine contributing to HTS formation.[58] Mast cells are able to promote proliferation of fibroblasts by the release of TGF-β1, TNF-α, and IL-4. This indicates that mast cells may play a role in HTS formation via different mediators.

Macrophages produce proinflammatory cytokines, including IL-1α, -1β, -6, and TNF-α, which are not only responsible for the control of inflammatory cell adhesion and migration but also stimulate the proliferation of keratinocytes and fibroblasts.[59] Macrophages can possibly initiate HTS formation by the production of TGF-β, PDGF, FGF2, and IGF-1, stimulating fibroblasts to produce excess collagen.[60,61] IL-4 appears to be strongly profibrotic too,[53,62] with increased levels being found in HTS[63,64] and other fibrotic tissues.[62] In contrast to this, a similar cytokine IL-10, produced by macrophages and mast cells, is antiinflammatory and capable of inhibiting the synthesis of many proinflammatory cytokines including interferon (IFN)-γ, IL-1, and TNF-α. Fetal healing, which is normally scarless, reverses to scarred healing in IL-10-deficient mice, which suggests that IL-10 is necessary for scarless fetal wound repair.[65]

Apoptosis of the granulation tissue begins after wound closure, affecting cells in a consecutive fashion. p53 is an apoptosis-related marker. The p53 tumor suppressor acts as a transcription factor and has a central function in controlling apoptosis.[66] As the inflammation process declines, p53 levels increase. Dysregulation of cellular apoptosis is thought to play an important role during the formation and the development of unfavorable scars and overexpression of p53 appears to accompany HTS.[67]

Collagen is synthesized, degraded, and reorganized during the remodeling phase, also a cytokine-mediated sequence. MMPs under cytokine control degrade collagen and other matrix proteins. MMPs also influence the ECM by regulating enzymes that process and digest cytokines and activate adhesion proteins in the matrix. TIMPs provide a counterbalance to the MMPs and disruption of this balance can lead to excess or insufficient matrix degradation and resultant tissue pathology.[68]

Fibroblasts generate new matrix in the granulation tissue. These fibroblasts degrade the provisional matrix via MMPs and respond to cytokine/growth factors by proliferating and synthesizing new ECM to replace the injured tissue with a connective tissue scar. Matrix synthesis begins within a couple of days, continuing for several weeks to months. TGF-β contributes to the fibrotic process by recruiting fibroblasts and stimulating their synthesis of collagen I, III, and V, proteoglycans, fibronectin, and other ECM components.[59,62] TGF-β concurrently inhibits proteases while enhancing protease inhibitors, favoring matrix accumulation. The progressive increase in TGF-β3 appears to be involved in cessation of matrix deposition.[68,69]

The background ECM constituents influencing scar control are a balance of proinflammatory and profibrotic agents. The inflammatory response is mediated by multiple cytokines and chemokines. Described earlier, one of the predominant cytokines involved in acute inflammation is TNF-α, which is activated via cleavage from the cell membrane by the TNF-α-converting enzyme (TACE).[69] One effect of TNF-α is stimulating the expression of MMP-9 by activating p38, MAPK, and NF-κB pathways. TIMP3 is one of the primary inhibitors of TACE; when TNF-α is high and TIMP3 lower, IL-6 is released and the inflammatory response is heightened. Thus, TIMP3 is important for regulatory control of inflammation.[69]

Like other proteolytic enzymes, MMP-9 is first synthesized as inactive proenzyme or zymogen. Activation of pro-MMP-9 is mediated by plasminogen activator (PA)/plasmin system. The regulation of MMP-9 activity is also controlled through TIMP3. MMP-9 expression is regulated by several cytokines and growth factors, including interleukins, interferons, EGF, NGF, basic FGF, VEGF, PDGF, TNF-α, and TGF-β. MMP-9's primary function is degradation of proteins in the ECM. It proteolytically digests decorin, elastin, fibrillin, laminin, gelatin (denatured collagen), and types IV, V, XI, and XVI collagen and also activates growth factors like pro-TGF-β and pro-TNF-α. Scarring is associated with a decreased MMP-9/TIMP1 ratio, which favors an accumulation of collagen.[70]

bFGF is another growth factor affecting the background ECM. bFGF appears to encourage MMP-1 activity and matrix clearance. It also accelerates angiogenesis, granulation, and epithelialization, thus being an overall positive stimulus to wound healing with diminished scarring.[71]

Although a complex array of inflammatory mediators and fibrogenic proteins have been described with multiple release and activation mechanisms, the process is fairly in order and involves constant cross-talk between keratinocytes and fibroblasts. The keratinocytes primarily monitor the events occurring at a superficial level related to contact with the external environment, while the fibroblasts react to these signals from the keratinocytes and to signals dictated by the status of the ECM and nature of the granulation tissue. After wounding, stored IL-1 is released by keratinocytes, which activates fibroblasts and adjacent keratinocytes, and attracts endothelial cells and lymphocytes to the injured area.[72] Macrophages release a host of interleukins and TNF-α, which potentiate the inflammatory cycle. Fibroblasts in turn secrete FGF7 and granulocyte–macrophage colony-stimulating factor (GM-CSF), to further activate keratinocytes.[73–75] The activation is sustained by growth factors like TNF-α, members of the EGF family (TGF-α, EGF, and HG-EGF), and IFN-γ from mast cells, monocytes, macrophages, and keratinocytes in the wound bed.[72,76]

The process of reepithelialization begins with the separation of keratinocytes from the basement membrane and subsequent mobility across the open wound. MMPs that will degrade collagen IV (MMP-9—gelatinase), collagen I (MMP-1—collagenase), and applicable subtypes are required. Now keratinocytes need to cut their way through the extracellular jungle of the provisional clot comprising fibrin, fibronectin, vitronectin, and collagen bundles present beneath the blood clot. For breakdown and degradation of the ECM in preparation for collagen ground substance maturity, MMPs are required that breakdown fibronectin, laminin, collagen III, and proteoglycans (MMP-3—stromalysin, MMP-2—gelatinase). PA induces transformation of plasminogen in plasmin that degrades the fibrin network.[77,78] To further crawl between the collagen bundles keratinocytes produce MMP-1 and -9.[70] Keratinocytes proliferate under the influence of multiple cytokines and growth factors. The conversation between keratinocytes and fibroblasts then becomes critical with fibroblasts, via TGF-β production, inducing inactivation of the keratinocytes—keratinocytes reply by switching off collagen production by fibroblasts.[79,80] This cross-talk between keratinocytes and dermal cells is constant until epithelialization is complete.[81] The activated state of keratinocytes normally ceases when the wound is reepithelialized, but in HTSs keratinocytes remain activated.[81,82]

In HTSs, the keratinocytes show increased proliferation and differentiation compared with normal scar keratinocytes,[82,83] inactivation does not take place and fibroblast production of collagen does not cease. How the keratinocytes remain activated is not known, but increased presence of epidermal Langerhans cells in HTS indicates that immunologic processes are involved.[64,84] An added influence on HT scar formation appears to be the site of wounding, more particularly the depth of wounding and components of the wound bed. In full-thickness wounds, the fibroblasts that populate the wound area are not only recruited from the surrounding dermis but also from other tissues such as the subcutaneous fat. These fibroblasts have been shown to possess increased expression of α-SMA, collagen types I and III, and TIMPs and decreased expression of MMPs in comparison with cultured dermal fibroblasts.[85] Furthermore, the cells show a collagen cross-linking profile comparable with bone collagen resulting in collagen fibers that are less accessible for proteases.[86] This kind of cross-linking is also seen in HTSs. This suggests that the myofibroblast from the subcutaneous fat may play a role in HTS formation,[85] explaining the observation of increased HT scar formation in full-thickness wounds.

Apart from the MMPs, additional mechanisms for clearing the ECM proteins exist. Proteasomes are very large protein complexes located in the nucleus and the cytoplasm.[86] The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis through their enzyme proteases. Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins.

The degradation process yields peptides of about seven to eight amino acids long, which can then be further degraded into amino acids and used in synthesizing new proteins.[87] IL-1β was earlier described as being a potent inducer of the inflammatory response and subsequent HT scar formation, thus its control would be desirable in influencing scar outcome. IL-1β is a secreted protein that accumulates in the cytosol as an inactive precursor (pIL-1β) before processing and release of biologically active protein. One mechanism of control noted above involves hydration occlusion of the keratinocyte. Another source of control is via the proteasome that plays an important and previously unrecognized role in degrading and controlling the amount of biologically active IL-1β that is exported by activated monocytes.[88]

Added to this role, the proteasome also plays an important part in deactivating Smad3, 4 signals, stimuli to TGF-β1 production. TGF exerts its effects mainly through the receptor–Smad signal transduction pathway.[89–91] Ligand binding leads to the activation of the receptor complex, followed by phosphorylation of the receptor-activated Smad2 and 3. The phosphorylated R-Smads then associate with Smad4, and migrate into the nucleus, where they either bind directly to DNA or associate with other transcription factors to modulate the transcription of a large number of genes (Figure 1). Smad7, an inhibitory Smad (I-Smad), antagonizes TGF-β signaling by interfering with the activity of the receptor complex.[92,93] During recent years, the ubiquitin proteasome protein degradation system has emerged as an important regulation machinery of TGF-β signaling. Several components of TGF-β signal transduction, including both positive and negative transducers, are irreversibly turned over by this ''protein-destroying machine.'' In a steady state, Smad3 is constitutively degraded via the ubiquitin–proteasome pathway in the cytoplasm and in response to TGF-β, it is phosphorylated and translocated into the nucleus, where it is also degraded through the ubiquitin–proteasome pathway. This suggests that not only in response to TGF-β but also in a steady state, the level of Smad3 is regulated by the proteasome pathway.[89]

Collectively, TGF-β signaling is controlled by the proteasome both positively and negatively; degradation of Smad7 maintains the signal and production of TGF-β, whereas degradation of the activated receptor complex and R-Smads turns it off. Overexpression of Smad7 has been shown to antagonize TGF-β-mediated fibrosis, carcinogenesis, and inflammation, suggesting a therapeutic potential of Smad7 to treat these diseases.[89,90] Similarly, a therapeutic possibility to prevent HT scar formation is saturation with Smad7—this may be achieved by simultaneous stimulation of Smad7 expression with activation of proteasome degradation of Smad3, 4 signals. Certain plant phenols appear to achieve this goal.[94–96]

Finally, concerning the inflammatory response, a short time (1 hour) after wounding, the endothelial cell's cyclooxygenase-2 (COX-2) enzyme is activated to synthesize PGs. Metabolites and enzymes of the arachidonic acid cascade, including the COX-2 enzyme and its enzymatic product prostaglandin E_2 (PGE_2), are known to be critical mediators of the inflammatory response. Several studies have examined the COX-2 pathway and its part in the regulation of the inflammatory phase of cutaneous wound repair.[97–99] Inhibition of this inflammatory pathway has also been suggested to reduce scar formation.[97] Scarless fetal healing is known to proceed without a significant inflammatory response, which appears to be important for the lack of scarring. Research suggests that the COX-2 pathway is involved in scar production in fetal skin and that targeting COX-2 may be useful for limiting scar formation in adult skin.[99] The involvement of the COX-2 pathway in scar formation is further highlighted by the fact that increasing PGE_2 levels in scarless wounds results in the conversion of a scarless healing process into one of repair with the generation of a scar. It would appear that PGE_2 is involved through signaling cascades in modulating the phenotypical change of the fibroblast to the myofibroblast.[100]

Although the focus of much effort in HTS avoidance has been control of inflammation, immune reactions and recruitment of T lymphocytes, specifically $CD4^+$ T helper (Th) cells may be equally important. The cytokine expression related to these cells may be of two forms—Th1 or Th2 with completely different effects. If Th1 is stimulated, an antifibrotic effect is initiated—nitric oxide synthase is activated that promotes collagenase activity and matrix remodeling. Th2 stimulation, however, responds with production of IL-4, -5, -10, and -13 with a strong fibrogenic response. HTS fibroblasts appear to be subject to a Th2 response and is the subject of ongoing research.[53,101]

Recent studies have also revealed that circulating stem cells and monocytes have the potential to be transdifferentiated to a keratinocyte-like cell.[102,103] These cells exhibit antifibrotic properties and are able to induce up-regulation of MMP-1 in fibroblasts. In this regard, protein 14-3-3 (stratifin) seems to play an important role. It has been showed that these keratinocyte-like cells release stratifins via membrane vesicles called exosomes which induce a shift in the collagen/MMP balance of surrounding fibroblasts.[104] This release is probably related to the surrounding wound milieu and local environment, once again showing the importance of the ECM constituents and their potential effect of scar outcome. The exosomes are intimately linked and form part of the conversation of signals being transmitted via p38 MAPK mechanisms. Further studies should shed light on the exact mechanisms involved.

PRINCIPLE IV: REMODELING/COLLAGEN MATURATION

The final principle to be discussed that impacts on scar outcome is that of collagen fibrillar arrangement, maturation, and ECM remodeling. During the remodeling phase, myofibroblasts normally replace hyaluronic acid (HA) by proteoglycans such as decorin, which binds TGF-β1 and regulates collagen fibrillogenesis.[105] Decorin presents as a ''C''-shaped structure that impacts itself between collagen fibrils assuring uniform spatial arrangement of these fibrils. In HTSs, fibroblasts synthesize less decorin than normal dermal fibroblasts[57,105,106] and expression of decorin in burn scars is suppressed for about 12 months.[105] Decorin inhibits fibroblast proliferation and decreases TGF-β1 production and collagen synthesis in HTS fibroblasts,[57,105] emphasizing its possible role in HTS.

In normal skin, collagen fibrils are composed of both type I and III collagen—type III comprises almost 20% of the total amount of collagen.[107] It is thought that type III collagen plays a role in fibrillogenesis and determines the collagen fibril diameter.[108] During granulation tissue formation, type III collagen expression increases more than the type I expression, resulting in an altered ratio between the two collagen subtypes from 20 to 50% type III collagen.[109] During maturation of the scar, the ratio decreases again to normal levels. Thus, increased amounts of collagen III relative to collagen I depicts an immature scar. Ratios of both collagen subtypes remain high in HTSs.[110]

Collagen fibril molecular cross-links are important in determining their susceptibility to protease breakdown and remodeling. These cross-links are often determined by mechanical load on the tissue.[111] Thus, bone and cartilage are characterized by pyridinoline cross-links able to withstand major force—normal skin collagen does not have these cross-links. However, HTS collagen does have these cross-links,[112] which makes them less susceptible to breakdown by MMP-1.[113] Inhibition of this cross-linking pathway might improve wound healing by making the newly formed collagen more susceptible for degradation.

The mechanism of the formation of HTS still remains to be elucidated. However, the role of free radicals in HTS formation has been showed in normal human skin, at least in vitro.[114–116] Thermal injury and its subsequent HTS formation involve the activation of phagocytic cells and the release of free radicals.[117,118] Evidence that free radicals may be contributory to the increased formation of pyridinoline in HTSs following cutaneous thermal injury supports a role for therapeutic intervention using antioxidants. The antioxidant, catalase, was found to be effective in reducing the concentration of pyridinoline cross-links.[114] Thus, possible therapeutic interventions in preventing or deceasing pyridinoline cross-links include antioxidant agents that stimulate catalase production.

Therapeutic implications for scar control—altering the "cross-talk" conversation

A focus of this paper is to deal with preventive strategies directed against exaggerated scarring. Once a HTS or a keloid scar is present, different strategies are necessary and are not in the scope of this paper (corticosteroids, compression therapy, radiation therapy, IFN, 5-fluorouracil, doxorubicin/adriamycin, bleomycin, verapamil, retinoic acid, imiquimod, tacrolimus, sirolimus, tamoxifen, pycnogenol, mitomycin C, AZX100, 585 nm pulsed dye laser therapy, etanercept—TNF-α inhibitor, etc.).

Presently, most therapies directed at scar control consist of strategies aimed at a single principle or the addition of a single cytokine in the hope that this will result in perfect healing and scar outcome. Having described the myriad of events occurring before scar resolution, it seems logical that monotherapy is unlikely to be effective. However, it is equally improbable that the entire web of factors that promote good scar resolution can be incorporated into a single therapeutic strategy. However, multimodality strategies provide many more avenues for influencing the cellular conversation and affecting the ultimate scar outcome.[119,120]

Table 1 lists the therapeutic agents that have been used in an attempt to influence each principle described above.

Mechanostimulation/scar support

Focal adhesions at the cellular surface allow mechanical tension generated in the system to be transduced to the cytoskeletal network. These changes create a sensitivity to mechanical tension that transmits to the cell via signaling (probably by opening Ca^+ channels) to glycoproteins, primarily fibronectin, which acts as ligand attaching to integrins transmitting the signal from the ECM into the cytosol.[18,21,28] From the cellular cytoplasm, Smad3, 4 signals are stimulated by this tension to form complexes that enter the nucleus initiating TGF-β1 stimulation, procollagen formation, collagen formation, fibroblast differentiation to myofibroblast, and wound contraction with excess collagen III. If the cycle is repeated sufficient times, particularly intermittently, the physical representation that results is the HTS.

Taping of the scar with microporous tape has proven effective in scar support in numerous publications.[121,122] Alternate therapies are theoretically possible to interrupt the process that is initiated by mechanostimulation. Based on the research noted above, one can extrapolate theoretic methods to influence scar mechanotension—these may include Smad7 inhibition of signaling to TGF-β, Cx43 antagonism, CTGF targeting, anti-Smad2, 3, 4 complex, decreasing cadherin activation, and more practically myofibroblast phenotype induction inhibition either by direct methods or indirectly by introducing collagen matrices to the wound where appropriate. Suffice it to say, simple support of the wound would appear to be the most logical strategy. This support is applied by taping the wound in a longitudinal direction rather than at right angles; we have also found that keeping the tape in place (including bathing with the tape) for 4–5 days or until spontaneous separation occurs substantially reduces the risk of adhesive sensitivity or tape stripping. The ability to apply active agents to the surface of the tape that have additive effects on scar reduction, such as those described in this paragraph, make the tape gel combination a very appealing modality.[119,120]

Other extraneous reports have begun to appear in the literature relating to agents that may influence mechanostimulation. Thus, botulinum toxin has been suggested as a mechanism of decreasing HTS based on its effect of reducing TGF-β1 in in vitro studies.[123] It is likely that the influence on mechanostimulation and the pathway described above may be the mechanism of improved scarring. It remains to be seen whether this is a viable practical and financial option in comparison with other modalities.

CTGF, working together with TGF-β, stimulates the formation of fibronectin, collagen, and TIMP1. Prolonged expression of CTGF thus promotes matrix accumulation and there appears evidence that mechanical stress is an important inducer of this expression. CTGF (and indirectly mechanical stress) may become an important target for HTS prevention as it has direct fibrotic effects in conjunction with TGF-β. Antisense inhibition of CGTF mRNA has proven effective in reducing myofibroblasts, collagen, fibronectin, and TIMP, and thus may prove to be a good future target for scar control.[25]

Table 1. Therapies directed at scar control (preventatives)

Principle	Agent	Proposed mode of action	References
Mechanosupport	Tape	Support of the scar decreasing mechanical stress Decreasing profibrotic signaling	Reiffel and colleagues[121,122]
	Botulinum toxin	Decreased movement and tension Decreased TGF-β stimulation	Xiao et al.[123]
	Antisense CTGF	Decreased TGF-β-induced stimulation of myofibroblast, collagen, fibronectin, TIMP usually induced by mechanical stress of the fibroblast	Sisco et al.[25]
Hydration/occlusion	Silicone Dimethicone	Decreased TEWL: decreased cytokines—IL-β, TNF-α; decreased TGF-β production of collagen	Chan and colleagues[124–127]
	Bulbine frutescens	As above	Widgerow et al.[119,120]
	Onion extract	As above	Draelos and colleagues[128–130]
	Oils	As above	Widgerow et al.[119,120]
	Skin substitutes/matrices	Appear to switch off mechanotension receptors via cadherins or other tension receptors	Ehrlich et al.[26]
Controlled inflammation	Human recombinant TGF-β3	Decrease matrix deposition Decrease collagen and fibronectin production	Lee and colleagues[131,132]
	Centella asiatica	Stimulation of TGF-β3, decreased TGF-β1; decreased matrix deposition including collagen and fibronectin; increased IL-10; decreased IL-6; Increased Smad7; decreased Smad2, 3, -4 complex iNOS, COX-2 inhibition	Capon and colleagues[133–147]
	Human recombinant IL-10	Decreased synthesis TGF-β	Bush et al.[148]
	TGF-β antibodies	Decreased collagen production, decreased TIMP, increased matrix breakdown	Choi and colleagues[149,150]
	Mannose-6-phosphate	Natural antagonist to TGF-β	Huang and colleagues[151,152]
	bFGF	Stimulates MMP-1; decreases myofibroblast transformation; increases angiogenesis	Bates and colleagues[153–155]
	Hepatocyte GF	Antifibrotic	Ono and colleagues[156–158]
	Antisense Cx43	Cx43 displace Smad2, 3 encouraging complex with Smad4 and TGF-β stim; antisense oppose this . . . conflicting evidence	Aoki and colleagues[159–161]
	Stratifin (14-3-3) (± aspirin)	Up-regulate MMP-1 release from fibroblasts	Abdollahi et al.[162]

	PDGF receptor inhibitors	Inhibit fibrogenesis	Mori et al.[163]
	PGE2	Inhibit myofibroblast differentiation	Parekh et al.[100]
	Interferon	Natural antagonist of fibrogenesis	
	Oleuropein	Proteasome stimulant; inhibitor COX-2; antioxidant; VEGF, MMP-2, -9, and -13 by reducing COX-2 levels	Huang and colleagues[95,96,164–168]
Collagen and ECM remodeling	*C. asiatica*	Regulation TGF-β to balance collagen III/I ratios; antioxidant against pyridinilone linkages	Zhang and colleagues[134,144]
	Oleuropein	Antioxidant—pyridinole cross-linking	Puel[168]
	B. frutescens	Decorin like tetrapeptides	Widgerow et al.[119,120]
	Recombinant human decorin	Anti-TGF-β1; decreased collagen production	Visioli and colleagues[169–171]

TGF, transforming growth factor; CTGF, connective tissue growth factor; COX, cyclooxygenase; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TEWL, transepidermal water loss; IL, interleukin; TIMP, tissue inhibitors of metalloproteinase; ECM, extracellular matrix; TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; PGE, prostaglandin E; FGF, fibroblast growth factor.

Hydration/occlusion

Hydration of the stratum corneum appears to result in reduced TEWL, reduced inflammatory cytokine release, and reduced TGF-β1 stimulation with direct impact on the scar outcome.[31,33,35] This would appear to be the modus operandi of many of the current scar therapies on the market today.

The most effective barrier to TEWL and stratum corneum breach is silicone, either in the form of sheeting or gels (dimethicone).[124–127] In addition, the gel derived from the plant bulbine frutescens has been found to be effective as a hydrating agent—the glycoproteins of this plant extract are large and remain on the surface of the skin long enough to produce effective hydration of the skin.[119,120]

A further novel patented approach to combining scar support and hydration/occlusion is by application of a gel including dimethicone silicone, hydrating agents, and inflammatory controlling components to the surface of the tape. This results in a saturated tape through which actives of the gel are absorbed creating an ideal occlusive dressing for managing scars.[120]

Onion extract, that is, extract of *Allium cepa* is being used as an antiscarring agent. The clinical studies have varied inconsistent reports of efficacy,[128,129,172] as have the in vitro studies. Although no major improvements were seen in HTS in the rabbit ear model[130] as opposed to asiaticoside,[94] realignment of collagen in a more orderly fashion was noted. This beneficial effect may well be occurring on the basis of hydration of the wound.

A multitude of antiscarring agents present in the marketplace are expected to have some beneficial scar outcome purely on the basis of their hydrative properties. These agents affect merely one aspect of scar control—hydration—as opposed to multimodality formulations aimed at dealing with a number of principles of scar control simultaneously.[120]

Managed inflammation

The inflammatory sequence in wound healing and scar formation presents a host of different possible pathways for manipulation. Many of them are interlinked and have common-end effects even if the pathways differ. Thus, the process of histamine release, substance P, nociceptors, mast cells likely share a common pathway of IL-4, -6, TNF-α, and TGF-β stimulation, which can be challenged by antihistamines. Although this provides a theoretic pathway for scar control, very little substantive work has been done on the basis of antihistaminic therapy and the major benefits at this stage appear to be symptomatic—pruritis, pain relief, and the like.[173,174]

Maturation of the inflammatory process involves a progressive increase in TGF-β3. This growth factor isoform appears to be involved in cessation of matrix deposition.[175] TGF-β3 reduces fibronectin and collagen deposition and is considered potently antifibrotic.[131] Increased levels are thus desirable in scar control strategies. This can be achieved indirectly via certain plant extracts (*Centella asiatica*) or directly derived via human recombinant TGF-β3—avotermin (Juvista, Renovo, Manchester, UK), which will be released in the future. This new medication has shown promise in a phase I trial and 2 phase II trials completed in the United Kingdom.[132]

Interleukins are intimately involved in the inflammatory process, predominantly not only as stimulators of inflammation controlling inflammatory cell adhesion and

migration but also stimulating the proliferation of keratinocytes and fibroblasts. The exception to this is IL-10, which is found in significant amounts in fetal scarless healing. It appears that IL-10 inhibits TGF-β synthesis. In IL-10-deficient mice, adding IL-10 reverts the process to scarred healing suggesting that IL-10 plays a significant role in scarless fetal wound repair.[57,65] This is in apparent conflict to research reported earlier which labels Th2 cells (including elaboration of IL-10) as profibrotic.[53,57,101] IL-10 has been developed by Renovo—Prevascar, a human recombinant IL-10 formulation. Preclinical experiments have showed that application of Prevascar to the margins of acute incisional wounds by intradermal injection decreases subsequent scarring.[148] Results are awaited in forthcoming years and resolution of this apparent contradiction in research is sought.

A natural target for reducing scarring would be TGF-β1, the trigger for collagen production and myofibroblast phenotype transformation. Various studies have used TGF antagonists and monoclonal antibodies with reasonable results.[149,150] Unfortunately, TGF-β plays pleiotrophic physiological roles with different effects occurring at different times. Thus, timing of targeting would be important, tends to complicate such therapy, and has impacted on these studies. Allied to this, mannose-6-phosphate has been identified as a natural antagonist to TGF-β. Trials are being conducted on its use as collagen inhibitors primarily in tendon repair.[151,152]

bFGF activates and stimulates the activity of MMP-1. It also stimulates angiogenesis, cellular differentiation, and impedes phenotypic fibroblast change to myofibroblast. In vivo and in vitro studies have shown encouraging results with intradermal injections appearing to optimize scarring in treated cases.[153–155]

Hepatocyte growth factor was identified as being important in the regeneration of hepatocytes.[156] Additionally it has angiogenic, angioprotective, and antifibrotic activities,[157,158] hence the interest in scar control. In vitro studies have been performed but at this stage more studies are awaited before formulating decisions on its efficacy.

Cx43 connexin protein studies on scarring have been confusing. As previously described, CX43 can displace Smad2, 3, from their microtubules making more available for amalgamation with Smad4 and translocation into the nucleus, promoting TGF-β production.[24] Application of Cx43 antisense oligonucleotides to skin wounds on mice was reported to reduce infiltration of inflammatory cells, accelerate healing, and reduce the overall area of granulation tissue formation after skin wounding in mice.[159] Subsequent work showed benefits of Cx43 antisense to reduced scarring after cutaneous thermal injury.[160] In addition, other reports showed enhanced expression of collagen and evidence of increased rates of granulation-tissue formation.[161] This conflict in outcome may again show the difficulty in attempting to influence TGF-β1 directly—these effects often being temporal and dependent on the status of the surrounding ECM.

The importance of PDGF and IL-4 in HTS production has been described. PDGF-receptor inhibitors can successfully prevent the development of fibrosis suggesting that inhibition of fibrogenesis, and not just inflammation, is critical to antifibrotic treatment.[163] Clearance of the ECM via MMP-1 induction is associated with stratifin, combination with acetyl salicylic acid (ASA), relying on its antiinflammatory effect, have shown promising results.[162] As with many agents described above, timing of intervention with various combinations is very important in HTS prevention. When intervening at the early postinjury stage, the aim is to limit ECM accumulation but not wound closure. In particular, apoptosis of myofibroblasts can be detected 12 days after wounding, and is believed to peak at day 20 in normal scar formation. Early intervention by agents such as ASA can be complicated by weakened stretched scars—thus defining the exact timing of intervention is important.

Additional therapies that have been widely used with very little good evidence for their topical use include hydrocortisone, vitamin E, petrolatums, and oils. Potential problems that have been highlighted in publications about these agents include scar weakening, stretching, contact dermatitis, and hypersensitivities.[176–179]

Laser-assisted surgical healing (LASH) has been promoted for immediate use following surgery with reportedly good results.[180] Mechanisms for scar improvement have yet to elucidated, but here again practical financial realities have impact. The theory of heat shock protein activation and its effect on TGF-β still needs clarification.[180] One would also need to be cautious of superficial burns and the potential for infection in these cases. Lastly, it remains to be seen whether consistently good results can be obtained—thus far reports are conflicting.

Data reported suggest that low levels of COX-2 expression and PGE_2 may be necessary for the scarless repair of fetal skin. With the demonstration that COX-2 and its products enhance scarring, the availability of COX-2 inhibitors offers a potential way to mediate scar tissue production in adult skin.[97–99]

We have been particularly interested in plant extracts with effect on various phases of scar control. Triterpenic fractions of *C. asiatica* and phenolic extracts of olive oil, oleuropein, for example, have showed multiple beneficial properties in controlling fibrogenesis. These extracts have been showed to increase Smad7, increase TGF-β3, decrease TGF-β1, decrease COX-2, increase proteasome activation, and display potent antioxidant effects. The combination is therefore elegant in synergy toward scar control.[94–96,120,133–147,164–168]

Collagen remodeling

The sequence of collagen and ECM remodeling is important in initiating scar maturity. Collagen subtypes, ratios of collagen I to III, uniform arrangement of fibers within the fibrillogenesis sequence, cross-linking, and final wound strength are all important factors in the final scar outcome. During this phase, myofibroblast apoptosis occurs and decorin replaces HA as a dominant glycoprotein in the ECM with inhibitory and regulatory effects on TGF-β and collagen production.[169]

Changing ratios of collagen III to I are taking place (back to 20% collagen III from up to 50%) and fibrillogenesis is occurring constantly during the maturation phase. During this process, packaging of new fibers should be uniform and structured in a nonclumped moiety. The process of fibrillogenesis involves the conversion of procollagen to tropocollagen (nonhelical ends cleaved

off) to fibrils arranged uniformly by "spacers" preventing collagen clumping. These spacers are normally provided by decorin.[170] Decorin was found to inhibit cell proliferation and down-regulate TGF-β1 production in keloid fibroblasts. Furthermore, decorin had a down-regulatory effect on type I procollagen production.[171,181,182] Hence, there has been an active interest in decorin-like proteins with tetrapeptides akin to the decorin molecule (bulbine frutescens) for collagen remodeling and structural arrangement.[106]

Collagen cross-linking is important in this remodeling phase—linkages need to be susceptible to MMP breakdown to ensure balanced degradation and neosynthesis. Pyridinoline cross-links, not normally seen in skin, have been reported to occur in HTS. They are able to withstand major force and tend to be resistant to MMP-1 degradation.[112–114] This pyridinoline phenomenon has been linked to oxygen radical activity (especially in burns), thus antioxidants appear to have benefit in potential scar reduction. To protect the cells from oxidative damages by oxidants, produced during oxygen metabolism, an antioxidant system is used by aerobic organisms.[114] The main antioxidant agents such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid, and tocopherol are important for cellular protection, due to their ability to eliminate free radicals, such as reactive oxygen species.[114] There is an increasing interest in the biochemical functions of natural antioxidant extracts from vegetables, fruits, and medicinal plants, which can become candidates to prevent oxidative damage. Extracts of *C. asiatica* and oleuropein have showed significant antioxidant capacities.[144,168]

PRACTICAL PERSPECTIVES GLEANED FROM CURRENT RESEARCH

Although a major amount of information appears to be available which could be construed as confusing and directionless, this research has given us considerable direction in the practical design and application of wound control products. In broad terms, we have adopted the following principles and selected appropriate components for scar control according to these principles:

A multimodality approach to scar control is imperative—as with so many other physiological processes, circumstances are variable, and possible influences on outcome are multiple—thus a multimodality approach increases the odds of success.

Adopting the multimodal approach, we have concentrated on those principles that have good research background related to them—thus Support, Controlled inflammation, Adequate hydration, and Remodeling (SCAR) (maturation) of collagen are the all-encompassing areas where focus is needed.

We have used the patented application of porous tape applied to the scar surface with gel applied to the surface of the tape. The tape provides support while the components of the gel ensure hydration, controlled inflammation, and collagen remodeling (ScarScience™, LITHA Healthcare Ltd, Johannesburg, South Africa).

The gel components comprise extracts of the plant *C. asiatica* (collagen remodeling, signaling control, antioxidant), dimethicone (hydration), plant extract from bulbine frutescens (hydration and decorin effect), and oleuropein (antiinflammatory, antioxidant, proteasome stimulant). Centella and bulbine plants are propagated and organically grown in South Africa and the extraction process is rigidly controlled.

This combination is used until scar maturation (white appearance of scar, 3–6 months). Tape is left in place until spontaneous separation occurs (4–7 days); patients bathe with tape apply gel to the surface of the tape twice a day.[120] In areas of reduced tension, the process may exclude tape and gel alone may be used.

This approach has proven extremely successful in the prevention of HTSs in $> 80\%$ of cases and the creation of excellent scars in the majority of these cases.[120] Most importantly, the selection process has involved scientific motivations in all choices of components.

CONCLUSION

Cellular cross-talk forms the backbone of all wound healing processes—scar formation is no exception. New research has translated much of the "conversation" that takes place at a cellular level revealing that the structural and functional outcome of individual cells and the organ as a whole are determined by intricate signaling mechanisms from cell to cell, ECM to cell, and within the cells and ECM themselves.[183,184] The keratinocyte, previously thought to be a passive participant, has been highlighted as an extremely important orchestrator of the process with initiation and maintenance of the entire process being dictated by signals related to the status of the cell—this includes mechanotension and pressure on the cell and its attachments, the state of hydration of the surface cells, the stimulus to inflammation, and finally the remodeling and final touches to the maturing scar tissue.

This interwoven multitude of functions, often occurring simultaneously, makes a multipronged approach to scar control seem more logical than an attempt at targeting one component of the process. We have found the multimodality approach to scarring control to show significant benefits.[119,120] This approach allows us to elegantly combine agents active in relation to all the principles elaborated above. By using tape (in larger scars) to support more extensive scars and saturating the tape with agents actively hydrating, lubricating, controlling inflammation, and encouraging controlled remodeling, the action is multidirectional and more assured of success. This has been confirmed in clinical studies.[120] In smaller scars (including acne scars), gel alone is used. Although much has been revealed concerning this ongoing cellular cross-talk, the fluent conversation is yet to be unearthed. Future directions should take into account the micromolecular processes that influence scar outcome. Changing or controlling signaling at a molecular level interrupting this cross-talk between cells and ground substance may alter the "conversation" that influences scar outcome. Agents are available and need to be selected according to these desired actions. This "micromanagement" has brought us closer to understanding and translating the language of scar evolution and control.

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