Review: Hypertrophic Burn Scar Evolution and Management

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Hypertrophic Burn Scar Evolution and Management

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Abstract
Burn injury outcome has improved significantly in recent years in relation to survival and patient rehabilitation. However, scarring and its accompanying aesthetic and functional sequelae still remain a major problem. The burn injury is characterized by unique differences in the nature of tissue trauma, the pathophysiologic response to that trauma and the molecular events that impact on the evolution of scar formation in these injuries. Some nuances in the burn injury profile have direct influence on scar outcome but have not been concentrated on in the past when designing treatment regimens for scar control. These include the exposed nerve endings, stimulation of neuropeptide mediators, neurogenic inflammation, pruritis, mechanotension signaling and hydration. A composite device for scar control in burn injuries should involve a multimodal approach that incorporates strategies for control of these contributing factors. A protective, hydrative, tension relieving device is predominant among the requirements, with substance impregnation being a secondary possibility in future renditions.

Introduction
Burn injury outcome has improved significantly in recent years in relation to survival and patient rehabilitation. However, scarring and its accompanying aesthetic and functional sequelae still remain a major problem. Hypertrophic scarring can functionally and symptomatically (pruritis, pain) impact on the patients’ quality of life. The identification of molecular events occurring in the evolution of the burn scar has increased our knowledge of this aspect of the injury; however, as yet this has not translated into significant effective burn scar limitation modalities.

Incidence and progression
The incidence of hypertrophic scar (HTS) occurrence following burn injuries has been reported in a range varying from 32-94%. Hypertrophic scars usually develop within one to three months after injury, usually manifesting about 4 weeks from complete reepithelialization. Treatment of these cases was estimated to cost at least $4 billion per annum in the US alone in 2005. In a study of 703 patients, pathologic scarring was diagnosed in 540 patients (77%); 310 had hypertrophic scars (44%); 34, contractures (5%); and 196, hypertrophic-contracted scars (28%). The hypertrophic characterization was observed at a median of 23 days after reepithelialization and lasted 15 months (median). 153 hypertrophic scars regressed within 1 year and underwent short-term evolution (38%). The same percentage became normotrophic within 2 years (intermediate-term evolution), and 98 remained active for many years (24%) (long-term evolution). Thus remodeling changes to reactive hypertrophic scar character takes 1 to 2 years to complete it course.

Pathophysiology
Although there is a genetic susceptibility to HTS and keloids among certain groups (e.g., people of African and Asian descent), the pathophysiologic background of most cases of HTS involves an overactive proliferative phase of wound healing. The stage is set for HTS during the phase of conversion from immature healing provisional extracellular matrix (ECM) to the mature healed scar. During this process the interaction of cellular components of the healing wound and the structural components of the ECM determine the scar outcome. These interactions are influenced by mechanical factors, bioburden, inflammatory stimulation and the ongoing signaling communication between keratinocytes, fibroblasts, collagen, fibronectin and the various combinations of integrins and ligands.

Inflammatory and immune cells participate in creating an abnormal extracellular matrix accumulation with increased cellular activity. Langerhans cells, macrophages, and activated T cells disrupt normal wound healing and tissue remodeling. Additionally the release of
neuropeptides, primarily Substance P, with a burn injury is believed to modulate and promote a ‘neuroinflammatory’ response encouraging cellular proliferation and cytokine and growth factor production, leading to exuberant ECM production, hyperemia, and pruritus. Thus a prolonged inflammatory state, protracted (>25 days) healing time and increased bacterial bioburden commonly precede HTS. There is also no doubt that the deeper the burn, the more likely the HTS. It has been suggested that this may be related to anatomic differences related to dermal cones. The upper portion of the dermal cones contains the hair shaft, sweat duct, and the pilosebaceous unit. The lower portion contains the sweat glands, the deep aspect of the hair follicle, and the fat dome. First trimester and early second trimester fetal skin show no dermal cones. Early second trimester fetal skin has only immature skin appendages and early fat tissue deep to the dermis. This may relate to observed scarless healing in these developmental stages. The cones exist where hypertrophic scars are visible and do not exist where hypertrophic scar does not occur, and the cones are distorted in the vicinity of scar and are inflamed in burns. Hypertrophic scars were shown to occur in sites characterized by fat domes that perforate the ECM. By significantly reducing tension in widespread healing through rapid myosin ATPase from ‘contraction’ an abnormal occurrence of excess stiffened scar tissue primarily caused by myofibroblast action, Thus the early phase of wound contraction is independent of the myofibroblast presence and is brought about by inherent contractile elements within the fibroblast and by compaction of collagen due to the movement of water out of the ECM. By significantly reducing tension in widespread healing wounds, scarring can be minimized. Additional other growth factors/cytokines (such as connective tissue growth factor CTGF, IL-1, IL-6, IFN (interferon)-γ, IGF (insulin growth factor), NGF (nerve growth factor)) can influence α-SMA expression. Delayed re-epithelialization time also correlates with higher levels of α-SMA expression. Wang et al demonstrated in a porcine burn model that peak expression of α-SMA in most scars was around 3–4 weeks post-burn – a retained high level of α-SMA in some scars 2–3 weeks after wound closure and with minimal ongoing contraction, may represent pathological healing of these scars. This occurred in deep dermal burns in particular.

Thus in HTS myofibroblasts persist and are believed to cause further hypertrophy. Increased myofibroblast numbers in granulation tissue appear to follow increased mechanical tension in the ECM. In biopsies of a human burn scar model, Junker et al demonstrated that scars that were stretched and examined after 1 and 6 days showed an increase in the number of myofibroblasts after mechanical stimulation. This indicates that mechanical stimulation using stretching induces fibroblast to myofibroblast transdifferentiation.

Many cells are known to be mechanoresponsive. Cell surface integrin molecules are activated by mechanical forces, leading to increased fibroblast activity and collagen production. This results from intracellular signaling initiated from surface keratinocytes to collagen/fibronectin in the ECM with signals transmitted via smad system to the nucleus of the fibroblast resulting in nuclear transcription of a message that stimulates TGFβ or CTGF to stimulate fibroblast production of collagen or to transdifferentiate into a myofibroblastic phenotype. When overactive, this process results in HTS.

As noted, increased levels of TGF-β1 is commonly associated with HTS, but TGF-β2 as well as decreased levels of TGF-β3 have also been associated with HTS. Other than these specific cytokines other elements of the ECM are relevant. Remodeling of the ECM involves both the degradation and clearance of its components mediated predominantly by a balance of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). A decrease in the ratio of MMPs to TIMPs can cause fibrotic diseases by excessive accumulation of ECM components. Patients with HTS after burn trauma presented a significantly higher TIMP-1 concentration. The relative expression of MMP 2 was significantly higher in samples of proliferative hypertrophic scars after burn injury. Although MMP-2 activity was significantly elevated in hypertrophic scars, studies have shown a decrease in the ratio of MMPs to TIMPs systemically due to an increase of TIMP-1 in patients with burn injuries. Thus the physiological balance between matrix metalloproteinases and their endogenous inhibitors is disturbed after burn trauma. The elevated systemic TIMP-1 concentration might contribute to tissue fibrosis, leading to pathological scar formation.

From the above account it is likely that burn HTS evolution may have unique characteristics when compared to the sequence of scar evolution described in incisional scars. The previous principles that were described relating to the management of incisional scars – mechanotension, inflammation, hydration and influencing scar remodeling and maturation – clearly have significance but certain nuances related to the nature of the burn injury have added relevance. These nuances will be discussed below.
Risk factors

In a multivariate regression model reported by Stella et al., female sex, young age, burn of neck or upper limb, more than one surgical operation, and meshed skin graft were independent risk factors for HTS after burn injury. As noted above burn depth is an important risk factor with less skin appendages available for epithelial regeneration and a possible interplay of increased cytokines and myofibroblast stimulation by a deeper differing adipocyte cell type. Meshed skin grafts, also appear to have a profibrotic phenotypic pattern. A delay in reepithelialization increases the risk of wound infection and prolongs the inflammatory phase, consequently leading to scar abnormalities (S). The type of injury, wound size and depth, anatomic region, bacterial infection and mechanical tension on the wound are important as well. Deitch et al demonstrated that wound closure should be achieved within three weeks to reduce the risk for hypertrophic scar development.

Current therapy and mode of action

In relation to surgery skin grafting does not prevent HTS, but sheet grafts or mesh grafts with a small expansion ratio results in a superior functional and cosmetic result. Dermal substitutes have been effective in minimizing HTS, contractures and increasing scar elasticity in acute burn wounds particularly in deep dermal burns, hand burns or those in flexion creases prone to contractures. After healing has occurred, massage, pressure therapies, steroids, and silicone dressings are frequently used to manage the scar burden in burn patients.

Pressure therapy

Pressure therapy has been the mainstay of HTS management for decades. The pressure modalities have varied from elastized garments and bandages to pressure earrings (lined with silicone). The pressure should be maintained between 24 and 30 mm Hg for at least 6 to 12 months to be effective. It would appear that the stabilizing of the scar unit and the limitation of outside vector forces on the scar may decrease the signaling induced by cellular mechanotransduction thus limiting the collagen formation and promoting ECM remodeling. Although the theory is compelling, proof of this hypothesis is still lacking. Additionally results are variable, treatment is expensive and patient compliance is poor. When used in conjunction with silicone sheeting a further advantage of surface hydration of the scar and its effect may be additive to the stabilization factor.

Silicone gel sheeting

Silicone gel sheeting is another widely used management option for HTS. Its mechanism of action has been suggested to be hydration and occlusion and multiple trials have supported its efficacy. The advantages of silicone therapy is that it improves the appearance and reduces the symptoms of the scars, it is easy to apply and painless. Silicone gel is easier to apply, can be used on more areas of the body and gives a higher patient compliance. It appears that it is important that the application starts from the second week at least until the third month of the postoperative period. 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. 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-b 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.

Corticosteroid

Intralesional application is not a primary treatment modality for patients with severe burns especially when the injury involves extensive surfaces. Prevention of HTS with this modality is not practical. Corticosteroids can reduce scar formation by affecting the collagen remodeling with a collagenase-like effect, decreasing fibroblast proliferation and the inflammatory phase of wound healing. Injections can be painful and may induce skin atrophy, depigmentation, crystalization and telangiectasias. Steroids, most commonly triamcinolone acetonide, can be injected intralesionally at 4–6 week intervals.

Pulsed dye laser has been proposed for HS treatment and it seems effective for the intense itching, whereas reduction of scar redness and height and textural improvement have not been proved. The mechanism by which laser achieves improved clinical outcome is still unclear. It probably influences the collagen remodeling phase and/or the angiogenesis.

Cryotherapy is thought to induce ischemic damage of the microcirculation resulting in cellular destruction and anoxia causing shrinkage of HTS. Intra-lesional cryotherapy by use of a cryoprobe causes maximizes cell destruction deep in the lesion. This is more directed at established HTS or keloids rather than at their prevention.

Surgery is also directed at ready established HTS, contractures or keloid scars. Z-plasty techniques used to relieve tension on the scar by changing its direction, tissue expansion and various flap designs are all used as methods to deal with established exaggerated scars.

Discussion – burn scar nuances

Our efforts at evidence based scar management have been primarily directed at incisional wound scars or those limited to smaller surface areas. The principles of management for these scars are directed toward hydration, controlled inflammation, collagen remodeling and mechanotension signaling factors. To a certain extent the management of burn scars involves similar principles but there are nuances to the burn injury that increase susceptibility to HTS and deserve special attention. These factors are important in designing a regimen of scar control in burn injuries.

Itch/pruritis

Burn therapists’ analysis of burn patient symptomatology revealed that the most common and distressful complications in burn patients were abnormal appearance (75.2%), itch (73.3%), and pain (67.6%). Itching usually begins at the time of wound closure with maximal intensity at 3 months often continuing over a year or
more with significant impairment of quality of life. Through the years we have accepted that itching/pruritis is an accompaniment of the healing process. However it has become apparent, particularly in extensive scar areas, that pruritis needs to be controlled, not only to alleviate discomfort but to halt a cellular/molecular process that can contribute significantly to and as part of the HTS scenario. Itching has been identified as part a HTS syndrome but it is prudent to examine the possibility of itching contributing to HTS evolution. The cause of this itch phenomenon is not only related to histamine release but recent studies suggest a histamine-independent pathway for itch. This may occur via a direct activation of opioid receptors, which have recently been identified in the skin.

Previous studies have shown that itch can be caused by histamine, neuropeptides, mast cells. The mast cells release histamine, leukotrienes, prostanoids, proteases, growth factors, and cytokines, which in turn can excite and stimulate the surrounding neuropeptide-containing C-fibers which initiate or exaggerate neurogenic inflammation. A deep dermal burn is accompanied by an immediate loss of the cell membrane. As noted above SP levels in acute burn tissue were found to be low in keeping with the decrease/damage of nerve tissue.

Neuropeptides

Noxious stimuli – including burn injury – cause nerves to release neuropeptides such as SP and calcitonin gene-related peptide (CGRP) that are stored in the terminal endings of the sensory nerves. SP activity is regulated by a membrane-bound metallopeptidase known as neutral endopeptidase (NEP), which degrades substance P at the cell membrane. As noted above SP levels in acute burn tissue were found to be low in keeping with the decrease/damage of nerve tissue. This level rebounds at approximately two weeks after the burn often corresponding with the time of re-epithelialization and maintains a high level for a protracted period of time usually related to low NEP activity. This may induce an exuberant neuroinflammatory response. SP concentration has been found in significantly greater concentrations in HTS when compared with normal uninjured skin.

The interaction of neuropeptides with mast cells and leukocytes release histamine and various inflammatory mediators - this has been described above in the context of pruritis. However, neurogenic inflammation may play a role in the pathogenesis of HTS. Neuropeptides have a direct fibroplastic effect. In addition, Chin et al demonstrated that cyclical mechanical stretching of murine skin resulted in a significant increase of neuropeptides. Hypertrophic scar mast cells were found to release more histamine than normal skin mast cells after stimulation by substance P. Thus mast cells may play a role in hypertrophic scar formation via different mediators including histamine and substance P. Additionally mast cells are able to promote proliferation of fibroblasts by the release of TGF-β1, TNF-α and IL-4. It is likely that excessive neurogenic inflammation results in increased cellular proliferation, cytokine and growth factor stimulation and excess ECM deposition. Additionally the neuropeptide release results in hyperemia and pruritis.

Mechanotension and cell signaling

Fibroblasts migrate into the wound to produce and remodel extracellular matrix (ECM). They are usually present in the wound within 24 h and predominate by the tenth postoperative day and are primarily responsible for collagen biosynthesis and remodeling through their production of collagen, the major protein component of the ECM. This process involves direct interaction of cell with matrix by creating ‘focal adhesion’ (FA) points using specialized structures on the cell surface. Most cells require these adhesion points for survival. Termed anchorage dependence, these FA points facilitate communication between ECM and the cytoskeleton of the cell which is mediated through integrin cell surface receptors. One important point for tension reception is the keratinocyte. Mechanoreceptors in these cells (or neuropeptide receptors in nerve cells) initiate the transmission of intercellular and cellular matrix signals. The sensitivity to mechanical tension that transmits to the fibroblast cell via signaling (probably by opening calcium channels and connexons) to glycoproteins, primarily fibronectin (and collagen), which act as ligands attaching to integrins transmitting the signal from the ECM into the cytosol. 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 and ligands, primarily TGF-β (reinforced by CTGF) to stimulate procollagen formation, collagen formation, fibroblast differentiation to myofibroblast, and wound contraction with excess collagen type III collagen (see Figure 1). If the cycle is repeated sufficient times, particularly intermittently, the physical representation that results is the HTS. The differentiation into myofibroblasts involves fibronectin input and occurs in the wound by the fifth day. Although these myofibroblasts play an important role in wound contraction and closure, over expression is associated with excess scarring and fibrotic conditions. It is very likely that FA points serve as a integration centers for growth factors, cytokines and integrins involving signal channels between the cell and the ECM.
Thus cellular mechanoreceptors and membrane neuropeptide receptors are functionally related, both causing ion channels (calcium in particular) to mediate intracellular signaling in fibroblasts as a response to mechanical stimulation. Mechanoreceptor stimulation and collagen stimulation (transcribed via TGF-β etc) has been confirmed by many authors and is the area of intervention with the most promise,20-22,46 while neuropeptide receptor agonists have been proven to directly increase intracellular calcium and subsequent signaling confirming their involvement in this signaling mechanism.51

**Hydration/Occlusion**

An occluded wound environment is thought to decrease the stimulation of damaged nerves (free nerve endings) and their neuropeptide release whereas a dry and unoccluded wound is more likely to be painful stimulating nociceptive nerve fibers releasing neuropeptides mediators.21 This is the basis of the use of long-term occlusive material such as silicone gel sheeting as a “sensory isolation” to the scar surface - decreasing neurogenic inflammation, leading to a reduction in scar tissue.21 Alternatively Akaishi et al22
demonstrated on a computerized model that silicone gel sheeting reduced the tension at the border between the scar and normal skin – this tension was transferred lateral edge of the silicone gel sheet. Silicone gel sheet with the hardness of normal skin was the most effective. It is likely that both effects – tension relief and sensory protection – are synergistic decreasing neuropeptide release and neurogenic inflammation.

**Cytokines and proteases**

TGF-β is one of the key mediators in the pathogenesis of HTS. It is a chemoattractant for fibroblasts, stimulates the deposition of collagen and ECM components, and can stimulate the transdifferentiation of fibroblast to myofibroblast. These events are mainly accomplished by the TGF-β/Smad pathway described above. HTS are associated with fibroblasts with increased TGF-β expression, decreased TGF-β3 expression, upregulated profibrotic Smad2 and decreased antifibrotic-effective Smad7 in contrast to normal skin-derived fibroblasts. In addition fibroblast growth factor 2 (FGF-2) and CTGF regulate scar formation process with TGF-β. FGF-2 appears to block TGF-β1 mediated myofibroblast activation while CTGF, supplements TGF-β activity and is associated with scar and fibrotic disorders. Additionally, enzymes are involved in scar formation. As described earlier, a physiological balance between matrix metalloproteinases and their endogenous inhibitors is disturbed after burn trauma. The elevated systemic TIMP-1 concentration might contribute to tissue fibrosis, leading to pathological scar formation.

**Possible solutions**

From the nuances particular to the burn injury wound, it is evident that multiple processes are occurring concurrently and that therapy directed at just one process is unlikely to be successful. Most of the present attempts at scar control are directed at one aspect or mediator and it is unlikely that this approach will succeed. We have previously described multimodal scar management regimens but these are primarily directed at incisional wounds or smaller open wounds. The burn injury however, presents unique pathophysiologic characteristics that warrant consideration when designing a scar management device.

From the discussion above it is apparent that successful management of the burn scar would need to accomplish the following ideals:

- Sensory protection of exposed nerve endings and surface hydration
- Itch prevention or control
- Stability of the wound/scar from mechanoreceptor stimulation or blocking of various signals in the TGF/smad pathway
- Down-regulating TIMP and increasing the activity of certain MMPs

Such a device would incorporate a hydrative/occlusive gel (silicone) serving as sensory protection from pain and itch, while simultaneously providing the necessary inherent tension to stabilize the area of the scar surface. Yagmur et al demonstrated that a silicone gel sheet with hardness greater than normal skin produced more side effects, such as dermatitis, desquamation, itching, and erythema, while that with the thickness between normal skin and half of that was the most effective when side effects were considered. Thus advanced products that are soft, gentle with inherent elasticity and correct silicone consistency and ‘tackiness’ would fulfill the criteria of sensitivity protection, hydration and tension relief (Figure 2).

Another possible step would be incorporation of a substance impregnated material into the device described above that would include substances able to interact at a molecular level influencing cellular signaling, inflammatory mediators and growth factor activity. Possible alternatives include:

- Relaxin has an insulin-like growth factor hormone reported to decrease collagen expression, increase MMP and decrease TIMP expression in a variety of in vitro and in vivo models; however, topical formulations still need to be perfected due to short residence time.
- Vanadate blocks protein tyrosine phosphatases and prevents the expression of αSMA within fibroblasts in full-thickness rat open wounds. It blocks sustained myosin ATPase activity, inhibiting the appearance of myofibroblasts, while leaving the rate and degree of wound contraction unaffected.
- PDGF secreted by macrophages during the proliferative phase of wound healing induces fibroblasts to produce and exocytose osteopontin. Osteopontin is an extracellular glycoprotein that connects integrins on cell surfaces to collagen within the extracellular matrix. This acts to promote cell adhesion and enhance cellular migration and enhances fibroplasia. Abolition of osteopontin (osteopontin antisense oligonucleotides) reduces the trafficking of both inflammatory cells and fibroblasts and also leads to a larger number of these cells dying by apoptosis.
- Halofuginone (HF) has been shown to inhibit fibrotic processes. HF is a low molecular weight quinazolinone alkaloid isolated from the plant Dichroa febrifuga that inhibits the Smad2/3-phosphorylation and stimulates the Smad7-expression in the TGF-β/Smad pathway. The inhibition of Smad3-dependant activity of collagen promoters leads to a reduction of the type I collagen (COL1A1) gene expression and to a blockade of the Smad3-mediated fibroblasts migration and proliferation. Zeplin (Z2) reports that the use of a silicone gel sheet with a modified HF-impregnated surface may provide a significant deceleration of scar development by...
normalization of the TGF-β1 induced and TGF-β1/Smad pathway mediated CTGF expression and collagen synthesis.56

- Centella asiatica, bulbine frutescens have been used successfully in scar management programs predominantly in incisional scars.12,14 The extracts from these plants have a similar effect on the TGF/smad pathway promoting decreased smad 3, increased smad 7 and increased TGFβ3 concentrations with hastened healing and scar maturation evident.13

- Neutral endopeptidase degrades substance P at the cell membrane.16 This may provide a therapeutic target for regulating inflammatory response in healing burn wounds and controlling HTS formation.16,58

- Other studied agents include interferons;60 5-Fluorouracil,61 bleomycin,62 anti TGF antibodies,63 TGF-β1 receptor inhibitor.60

The above cited agents are not meant to be an exhaustive list but merely examples of previously researched agents, some of which may have the potential for incorporation into a composite scar management device suited to burn injuries. The risk of including such agents includes skin sensitivities, drug interactions and possible regulatory restrictions due to ‘drug’ components. Additionally previous benefits of silicone sheeting combined with newer advances in mechanotransduction understanding would confer great advantages to a tensile scar management sheet alone.

Conclusion

The burn injury is characterized by unique differences in the nature of tissue trauma, the pathophysiologic response to that trauma and the molecular events that impact on the evolution of scar formation in these injuries. The areas that have been highlighted have direct influence on scar outcome but have not been concentrated on in the past when designing treatment regimens for scar control. The most important of these appears to be the control of mechanotransduction by scar stabilization. In addition the exposed nerve endings, stimulation of neuropeptide mediators, neurogenic inflammation, pruritis, and hydration should be considered. A composite device for scar control in burn injuries should involve a multimodal approach that incorporates strategies for control of these contributing factors.

A gentle, graded tension relieving, protective, hydrative device would satisfy many of the required criteria switching off signaling emanating from stress on the scar, itch, pain and extraneous sensitivities.

References


