Mechanobiology of scarring

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ABSTRACT

The mechanophysiological conditions of injured skin greatly influence the degree of scar formation, scar contracture, and abnormal scar progression/generation (e.g., keloids and hypertrophic scars). It is important that scar mechanobiology be understood from the perspective of the extracellular matrix and extracellular fluid, in order to analyze mechanotransduction pathways and develop new strategies for scar prevention and treatment. Mechanical forces such as stretching tension, shear force, scratch, compression, hydrostatic pressure, and osmotic pressure can be perceived by two types of skin receptors. These include cellular mechanoreceptors/mechanosensors, such as cytoskeleton (e.g., actin filaments), cell adhesion molecules (e.g., integrin), and mechanosensitive (MS) ion channels (e.g., Ca^{2+} channel), and sensory nerve fibers (e.g., MS nociceptors) that produce the somatic sensation of mechanical force. Mechanical stimuli are received by MS nociceptors and signals are transmitted to the dorsal root ganglia that contain neuronal cell bodies in the afferent spinal nerves. Neuropeptides are thereby released from the peripheral terminals of the primary afferent sensory neurons in the skin, modulating scarring via skin and immune cell functions (e.g., cell proliferation, cytokine production, antigen presentation, sensory neurotransmission, mast cell degradation, vasodilation, and increased vascular permeability under physiological or pathophysiological conditions). Mechanoreceptor or MS nociceptor inhibition and mechanical force reduction should propel the development of novel methods for scar prevention and treatment.

During the growth and development of the human body, the skin expands to cover the growing skeleton and soft tissues and is constantly subjected to extrinsic and intrinsic mechanical forces. These extrinsic forces include skin-stretching tensions (e.g., due to body movement) and external stimuli (e.g., scratch). Intrinsic forces include extracellular matrix (ECM) tension by the underlying skeletal growth, and fluid shear force and hydrostatic and osmotic pressures by the extracellular fluid (ECF).

Following skin injury, the mechanophysiological conditions are drastically changed by wound healing, granulation tissue formation, wound contraction, and epithelialization.[1] Coagulation and inflammation cause edema and blood circulatory alterations in the skin and wound,[2] thereby impacting the ECF-based mechanophysiology. Moreover, the proliferative and remodeling phases, which start within 1 week of injury and can continue for months, cause granulation tissue formation and wound contraction by myofibroblast activity.[3] These mechanophysiological alterations of the injured skin considerably influence the degree of scarring.[1] Here, we analyze the mechanisms of scarring mechanobiology, with the goal of developing new strategies for scar prevention and treatment.

CELLULAR AND TISSUE RESPONSES TO MECHANICAL FORCES ON CUTANEOUS WOUNDS

Mechanical forces, including stretching tension,[4] shear force,[5] scratch,[6] compression,[5] and hydrostatic[7] and osmotic[8] pressures, can be perceived by cellular mechanoreceptors[9]/mechanosensors[10] (Figure 1) and/or nerve fiber receptors (including mechanosensitive [MS] nociceptors[11]) that produce the somatic sensation of mechanical force (Figure 2). Cellular mechanoreceptors include the MS ion channels (e.g., Ca^{2+}, K^{+}, Na^{2+}, and Mg^{2+}),[9,12–14] cytoskeleton (e.g., actin filaments),[15] and cell adhesion molecules (CAMs) (e.g., integrins)[16] (Figure 1). Skin resident cells are attached to the ECM via CAMs, and the cytoskeleton is connected to MS ion channels and CAMs.[17] When the ECM is distorted by mechanical forces such as skin tension, the cytoskeleton is altered and MS ion channels are activated.[17] In contrast, ECF-based pressure cannot activate MS ion channels through cytoskeletal alteration, as hydrostatic pressure impacts ion inflow but not cell shape.[18] Cells convert mechanical stimuli into electrical signals through mechanoreceptors, thereby accelerating cell proliferation, angiogenesis, and epithelialization through various mechanotransduction pathways. In particular,

CAM	Cell adhesion molecules
CGRP	Calcitonin gene-based peptide
ECF	Extracellular fluid
ECM	Extracellular matrix
HSs	Hypertrophic scars
MS	Mechanosensitive
RCT	Randomized-controlled trial
SNP	Single nucleotide polymorphism
TGF	Transforming growth factor

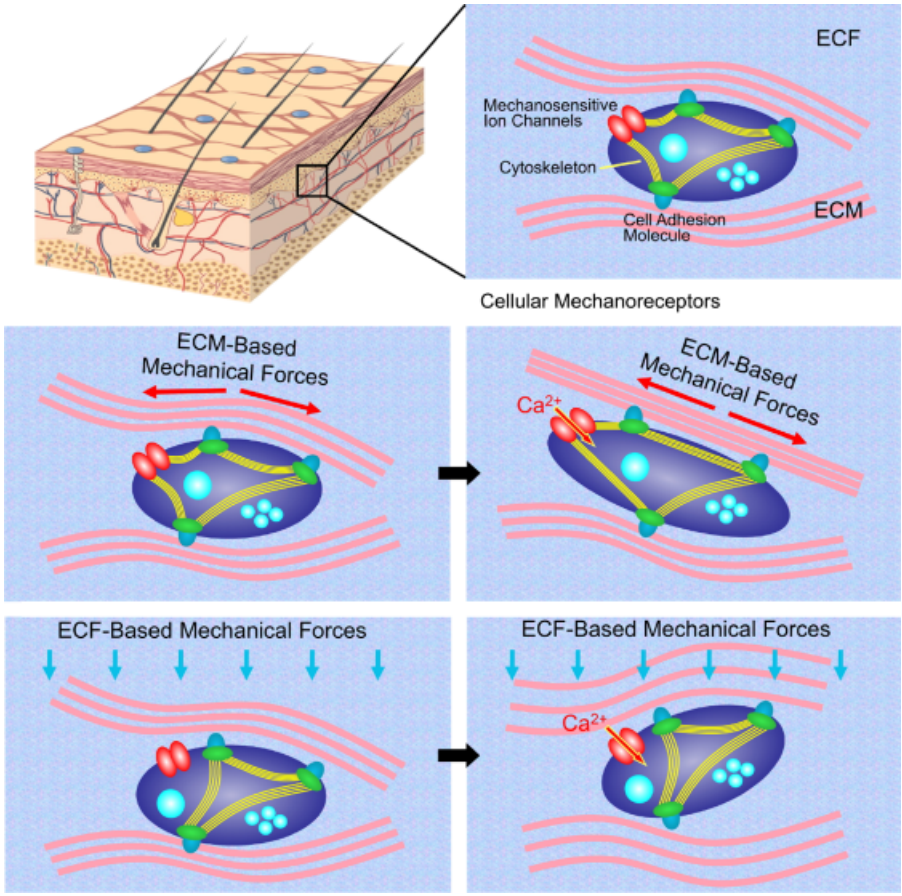

Figure 1. Cellular mechanoreceptors. At the cellular level, ECM-based mechanical forces such as stretching tension, shear force, and scratch, and ECF-based mechanical forces such as compression, hydrostatic pressure, and osmotic pressure can be perceived by cellular mechanoreceptors/mechanosensors, including the cytoskeleton (e.g., actin filament), cell adhesion molecules (e.g., integrins), and mechanosensitive ion channels (e.g., Ca^{2+} channel). ECM, extracellular matrix; ECF, extracellular fluid.

transforming growth factor (TGF-β)/Smad, integrin, mitogen-activated protein kinase G protein, tumor necrosis factor/NF-κB, Wnt/β-catenin interleukin, and calcium ion pathways have been the subject of extensive research in cutaneous scarring. TGF-β is particularly involved in the way scar tissue reacts to mechanical forces. Supporting this is that keloid-derived fibroblasts subjected to mechanical force in the form of equibiaxial strain produce more TGF-β1 and -β2 than normal skin-derived fibroblasts.[19] Another study has shown that stretching a myofibroblast-derived ECM in the presence of mechanically apposing stress fibers immediately activates latent TGF-β1, and that compared with relaxed tissues, stressed tissues exhibit increased activation of Smad2/3, which are the downstream targets of TGF-β1 signaling.[20]

G proteins are additional membrane proteins that modulate mechanotransduction pathways.[1] Mechanical stimulation alters the G protein conformation, leading to growth factor-like changes that initiate secondary messenger cascades and initiate cell growth.[1] Calcium ion MS channels are involved in phospholipase C activation, which can lead to protein kinase C activation and subsequent epidermal growth factor (EGF) activation.[1] These mechanotransduction pathways are thought to be associated with cutaneous scarring as a cellular response.

At the tissue level, sensory fibers act as mechanical stimuli receptors in the skin[11] (Figure 2). Mechanical stimuli are received by MS nociceptors, and signals are transmitted to dorsal root ganglia that contain neuronal cell bodies in the afferent spinal nerves. This results in neuropeptide release from the peripheral terminals of primary afferent sensory neurons, which innervate the skin and often contact epidermal and dermal cells. These neuropeptides can directly modulate the functions of keratinocytes, fibroblasts, and Langerhans, mast, dermal microvascular endothelial, and infiltrating immune cells.[21–23] Substance P (SP), calcitonin gene-based peptide (CGRP), neurokinin A, vasoactive intestinal peptide, and somatostatin are neuropeptides that effectively modulate skin and immune cell functions, including cell proliferation, cytokine production, antigen presentation, sensory neurotransmission, mast cell degradation, and vasodilation, and increase vascular permeability under physiological or pathophysiological conditions.[24,25] These proinflammatory responses are termed neurogenic inflammation.[26–28] SP and CGRP act through the neurokinin 1 receptor and CGRP1 receptor, respectively, and are synthesized during nerve growth factor (NGF) regulation.[21,24] Some have also suggested a relationship between burn and abnormal scars (e.g., keloids and hypertrophic scars [HSs]) and neurogenic inflammation/neuropeptide activities.[29–33]

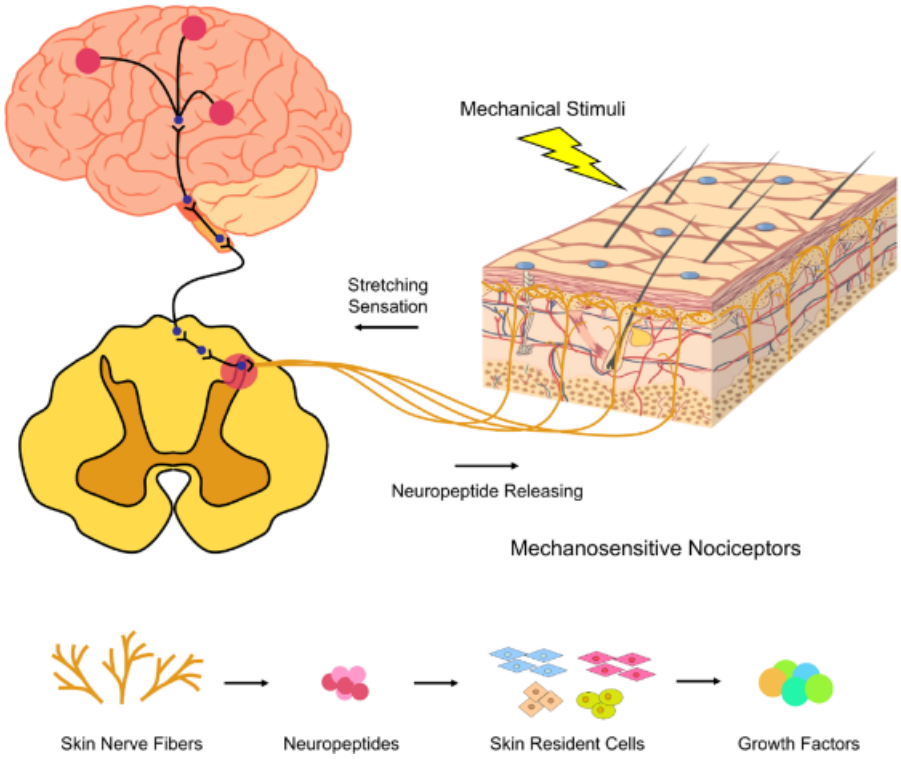

Figure 2. Mechanosensitive nociceptors. In the tissue-level response, sensory fibers act as receptors for mechanical stimuli in the skin. Mechanical stimuli are received by mechanosensitive nociceptors, and signals are transmitted to dorsal root ganglia that contain neuronal cell bodies in the afferent spinal nerves. Neuropeptides are thereby released from the peripheral terminals of the primary afferent sensory neurons in the skin, and modulate skin and immune cell functions, such as cell proliferation, cytokine production, antigen presentation, sensory neurotransmission, mast cell degradation, and vasodilation, and increase vascular permeability under physiological or pathophysiological conditions.

CLINICAL EVIDENCE OF THE RELATIONSHIP BETWEEN MECHANICAL FORCES AND SCARRING

While appropriate amounts of intrinsic tension are necessary for wound closure,[34] an important factor in the degree of scarring after wounding is the extrinsic mechanical force. The balance of these forces plays a key role in heavy scar production (Figure 3). Mechanical forces promote the growth of fibroproliferative skin disorders such as HSs and keloids.[35] Thus, abnormal scarring should be studied from the perspective of the extreme example of excess wound healing in the skin.

Site specificity of keloids and HSs

Keloids and HSs may constitute two stages of a continuous disease, with only the chronic inflammation strength being different between them (Figure 4). Although distinguishing between a keloid and a HS remains imprecise,[36] with respect to hyalinizing collagen bundle formation, the inflammation of a keloid is much greater than that of an HS, and the inflammation of either is greater than that of a mature scar.[37] The inflammation strength reflects the degree of angiogenesis in and around the scar, including the redness of the scar itself and of the skin adjacent to the scar. Keloids display scar and adjacent skin redness; in contrast, redness on adjacent skin is not observed in HSs.[38] It has been suggested that these inflammatory features are closely related to the mechanical force sensitivity (Figure 4), although many other chronic inflammation triggers may be involved.[39]

According to a statistical study of more than 1,000 anatomic regions in Asian patients, keloids tend to occur at specific sites, including the anterior chest, shoulder, scapular, and suprapubic regions (Figure 5).[40] All of these sites are constantly or frequently subjected to mechanical forces, including skin stretching due to daily body movements. The anterior chest skin is regularly stretched by respiration and upper limb movements, the shoulder, and scapula skin by upper limb movements and body bending motions, and the lower abdomen and suprapubic skin regions by sitting and standing motions.

HSs can occur anywhere in the body, especially when a scar is long, wide, and located on a frequently moved joint. Long and wide scars can produce an imbalance of the skin stretching forces on adjacent scars and can sometimes cause scar contracture. Plastic surgeons divide scars and release contractures using geometrical plasties (e.g., z- and w-plasties) and small-wave incisions for scar and scar contracture treatments.[41] In contrast, heavy scars rarely occur on the scalp or the anterior lower leg[40] (Figure 5). Even in patients with keloids or HSs covering the entire body, heavy scars on the scalp or the anterior lower leg are rare.[40] The commonality in these sites is that the bones lie directly under the skin; consequently, the skin at these sites is rarely subjected to tension.[33] The site specificity of scar development suggests that mechanical forces may not only promote keloid/HS growth, but may also be a primary trigger for their generation.[33]

There is a possibility that a genetic predisposition to keloid exists, as suggested by a recent study of single nucleotide polymorphism (SNP).[42] In clinical situations, not only keloid but also HS patients sometimes have genetic

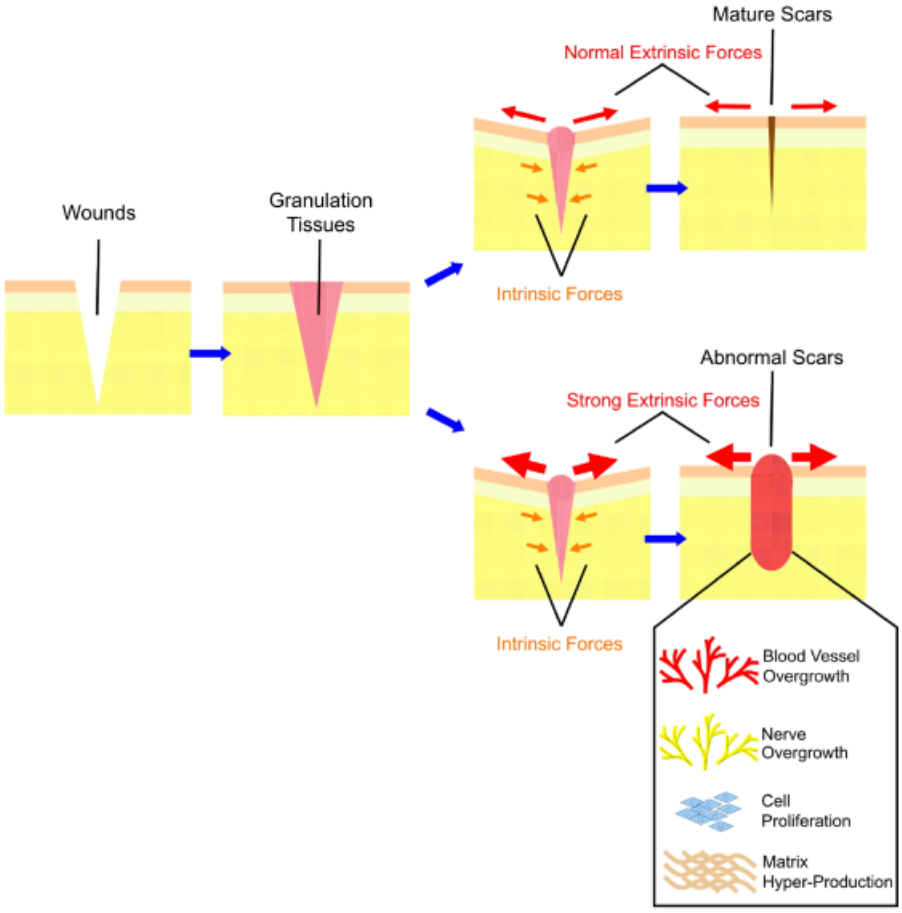

Figure 3. Relationship between mechanical forces and scarring. An appropriate intrinsic tension is necessary for incisional wound closure; however, extrinsic mechanical forces can lead to scarring. Scar formation is determined by the balance between these internal and external forces. In particular, strong extrinsic forces can result in the acceleration of angiogenesis, nerve growth, cell proliferation, and collagen hyperproduction, leading to abnormal (keloid and hypertrophic) scar formation.

predispositions; thus, the relationship between these SNPs and hypertrophic scarring should also be studied.

Relationship between scar growth and the direction of the stretching tension

HSs do not grow beyond the boundaries of the original wound, and thus only grow vertically. In contrast, keloids grow and spread both vertically and horizontally, similar in many respects to slowly growing malignant tumors. The direction of their horizontal growth results in characteristic shapes that depend on their location. For example, keloids on the anterior chest grow in a "crab's claw"-like pattern, whereas shoulder keloids grow in a "butterfly" shape. These patterns may reflect the predominant directions of skin tension at these sites.

Our previous finite element analysis of the mechanical force distribution around keloids[43] revealed high skin tension at the keloid edges and lower tension at the keloid centers. This result indicates why keloids generally stop growing in their central regions. Keloid expansion occurred in the direction of skin pulling, and the skin stiffness at the keloid circumference directly correlated with the degree of skin tension (Figure 6). These observations strongly support the notion that skin tension is closely associated with the pattern and degree of keloid growth. The growth pattern differences between HSs and normal scars from those of keloids may reflect differences in their responsiveness to skin tension (Figure 4).

BASIC RESEARCH ON THE RELATIONSHIP BETWEEN MECHANICAL FORCES AND SCARRING

Animal models of skin stretching

To accelerate skin growth, dermatogenesis, and wound healing, skin-stretching strategies and devices have been developed.[30,44–46] The optimal amplitude and waveform of skin tension may facilitate skin growth and expansion, but excessive tension can cause heavy scarring.[46] Static and periodic tensile force application to rat ears showed vascular remodeling and epidermal proliferation.[44] A gene chip analysis performed on this rat model suggested tissue-level hypoxia as a possible mechanism for the observed effects.[45] In addition, prior in vitro studies have shown that mechanotransduction mechanisms can stimulate cell proliferation[47] and angiogenesis.[48,49]

Using a sophisticated servo-controlled device to stretch murine dorsal skin, stretched samples had upregulated

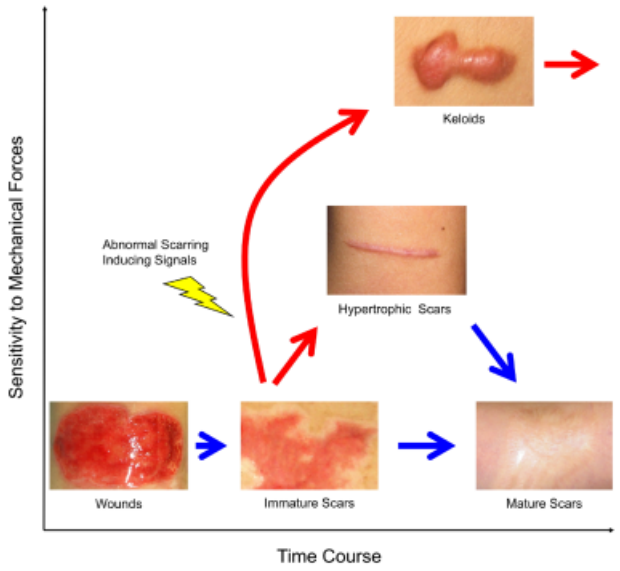

Figure 4. Relationship between scar type and mechanical force sensitivity. In general, wounds gradually progress from immature to mature scars. If the period defined by immature scarring is long or if strong signals (e.g., mechanical forces, infection, or immune reaction) exist, then these immature scars will form abnormally. Although hypertrophic scars (HSs) naturally progress to mature scars, keloids rarely progress following formation. It remains unclear whether keloids and HSs are stages of a continuous disorder, but keloids appear to be more sensitive to mechanical forces than HSs, and both are more sensitive than normal scars.

epidermal proliferation and angiogenesis.[46] Real-time RT-PCR revealed that EGF, NGF, vascular endothelial growth factor, and TGF-β1 were more strongly expressed in cyclically stretched than in statically stretched skin.[30,46] This cyclical stimulation also significantly increased skin neuropeptide accumulation, while the corresponding peptide receptors were down-regulated.[30] This study showed that neuropeptides are produced in resident skin cells. Although neuropeptide release from the peripheral nerve fiber terminals was not shown, this study did prove that neuropeptides are associated with the process of skin stretching.

Construction of an HS animal model using mechanotransduction

Many authors have attempted to construct suitable animal models of heavy scars using mice, rats, and rabbits; however, these models, especially for keloids, seem to be driven more by an acute inflammatory response than by chronic inflammation, leading to immature scar formation.[38] An HS mouse model based on mechanical force loading showed that scars subjected to tension exhibit less apoptosis, and that inflammatory cells and mechanical forces promote fibrosis.[50] These findings support the well-established notion that mechanical forces strongly modulate cellular behavior in the scar.

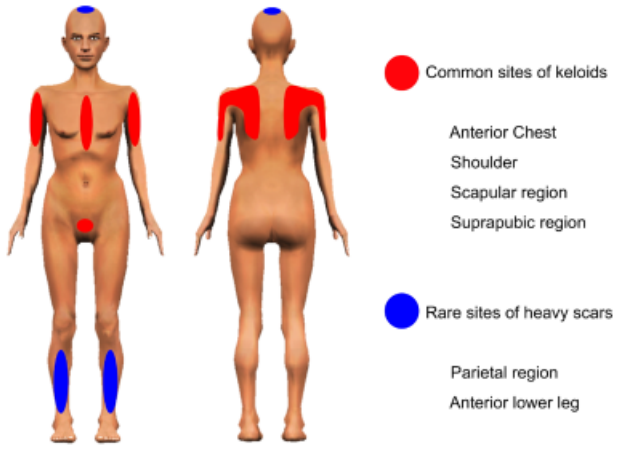

Figure 5. Site specificity of scars. Keloids tend to occur at specific sites, such as on the anterior chest, shoulder, scapula, and suprapubic region. All of these sites are constantly or frequently subjected to mechanical forces, including skin stretching, according to body movements. In contrast, hypertrophic scars can occur anywhere on the body, particularly when a scar is long, wide, and/or located on movable joints. Even when abnormal scars cover other portions of the body, they rarely occur on the scalp or the anterior lower leg, where the bones lie directly under the skin and the skin is rarely subjected to tension. The site specificity of scar development suggests that mechanical forces may both promote keloid and HS growth and trigger their generation.

CLINICAL MECHANOBIOLOGY STRATEGIES FOR SCAR PREVENTION AND TREATMENT

To limit skin stretching and external mechanical stimuli during wound healing/scarring, wounds or scars should be covered by fixable materials, such as tape, bandages, garments, or silicone gel sheets. A randomized-controlled trial (RCT) showed that tape fixation helped to prevent HS formation after a cesarean section in 70 subjects, with significantly less scar volume when paper tape was used.[49] Other RCTs have shown that silicone gel sheeting significantly reduces the incidence of HSs or keloids.[51,52] Our computer analysis of mechanical force conditions around scars showed that silicone gel sheeting reduces tension at the scar edges,[53] suggesting an important mechanism for HS formation.

Fluid control may also help prevent and treat scars by inducing hydrostatic pressure gradients and shear forces that alter genomic expression through MS ion channels (Figure 1). Therefore, the control of ECF-based mechanical forces (fluid shear forces, hydrostatic pressure, and osmotic pressure) may be achieved through various devices or materials (e.g., vacuum-assisted closure,[2] wound dressings). The magnitude and balance of these force patterns must be further studied to develop sophisticated devices for scar prevention and treatment.

Based on the described relationships between scar formation and mechanobiology, several potential scar therapeutic approaches can be suggested. With respect to neurogenic inflammation, neuropeptide blockade using continuous local anesthesia may be effective for abnormal

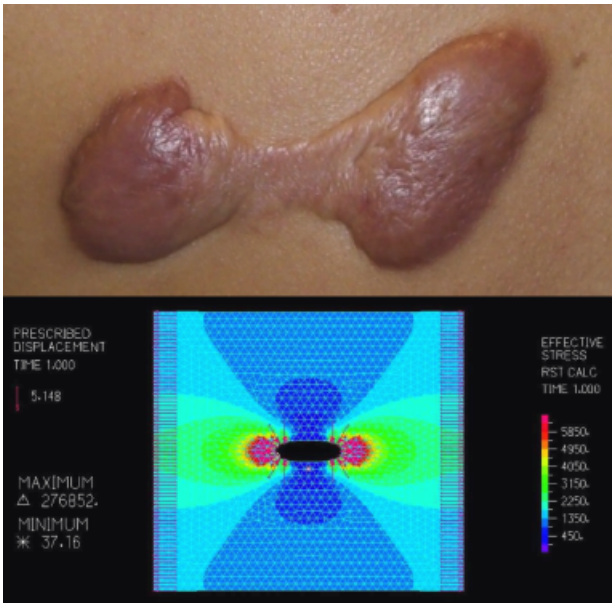

Figure 6. Finite element analysis of the mechanical force distribution around keloids. High skin tension (red) areas were observed at the keloid edges, while keloid centers were regions of lower tension, explaining why keloids generally stop growing in their central regions. Keloid expansion occurred in the direction of skin pulling, and the skin stiffness at the keloid circumference correlated directly with the degree of skin tension at the circumference. These observations strongly support the notion that skin tension is closely associated with the pattern and degree of keloid growth. Because hypertrophic and normal scars differ in their growth characteristics from those of keloids, these differences may reflect differences in the responsiveness to skin tension.

scar treatment. Peripheral nerve activity, including neuropeptide release, can be controlled via the central nervous system (Figure 2). Mechanoreceptors and neuropeptides can be inhibited, such as through ion channel, integrin, or neuropeptide receptor blockers. Indeed, calcium channel blockers are already in use for scar treatments,[54–56] where they have been shown to decrease ECM formation[57] and inhibit fibroblast and vascular smooth muscle cell proliferation.[58]

CONCLUSION

Understanding the mechanobiological environments of skin and wounds will be helpful in designing novel strategies for scar prevention and treatment, such as through mechanoreceptor or MS nociceptor control.

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