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Review

Soft tissue mechanotransduction in wound healing and fibrosis

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ABSTRACT

Recent evidence suggests that mechanical forces can significantly impact the biologic response to injury. Integrated mechanical and chemical signaling networks have been discovered that enable physical cues to regulate disease processes such as pathologic scar formation. Distinct molecular mechanisms control how tensional forces influence wound healing and fibrosis. Conceptual frameworks to understand cutaneous repair have expanded beyond traditional cell-cytokine models to include dynamic interactions driven by mechanical force and the extracellular matrix. Strategies to manipulate these biomechanical signaling networks have tremendous therapeutic potential to reduce scar formation and promote skin regeneration.

Contents

1. Introduction

Human skin represents a complex biologic system that integrates different cell types within a highly organized matrix scaffold. It is constantly exposed to physiologic insults and must renew throughout life in response to myriad stressors [1]. Recent research has begun to elucidate the specific cell populations and molecular pathways that enable the tremendous regenerative potential of human skin [2]. Following trauma, a coordinated sequence of events follows to ensure that the injury site is expeditiously repaired. Hemostasis is initiated by clotting factors and circulating platelets while inflammatory cells are recruited, resulting in the continued secretion of cytokines to promote early inflammation. Both resident and circulating cell populations rapidly proliferate within the wound and promote the production of a "provisional matrix" of extracellular components. This matrix scaffold is remodeled for up to two years and results in the formation of a mature scar [1].

In humans, the formation of cutaneous scar varies considerably based on mechanism of injury, degree of damage, anatomic location, patient age, and genetic predisposition [3]. A fine, thin scar can be barely perceptible whereas exuberant fibrosis in the form of hypertrophic scar or keloid formation can result in significant dysfunction and disfigurement. The treatment of hypertrophic scarring alone in the United States is thought to cost over $4 billion annually [4]. Further, almost one half of deaths in the developed world can be attributed to some form of aberrant fibroproliferation (atherosclerosis, cirrhosis, pulmonary fibrosis) [5]. Thus, scar formation represents a substantial biomedical burden and an improved understanding of its underlying mechanisms may result in more effective therapies to ameliorate fibroproliferative disease.

The plasticity of skin (*e.g.* during pregnancy) has been observed since the beginning of man and anatomists have descriptively catalogued how intrinsic tension influences the cutaneous response to injury [6]. In clinical settings, scar formation is increased in wounds subjected to high mechanical force (*e.g.* sternotomies and wounds across joints) [7,8]. Conversely, offloading of physical force has been shown to significantly reduce pathologic scarring [9–11]. Taken together, these clinical findings indicate that human skin is exquisitely sensitive and responsive to mechanical cues. Almost all of the constituent cells in skin have been shown to respond

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to mechanical forces and these properties may dictate how skin and cutaneous wounds interact with the physical environment [12].

Mechanotransduction describes the conversion of mechanical forces to biochemical signals [13]. *In vitro* studies have shown that fibroblasts and keratinocytes (the predominant cell populations in skin) respond to a variety of physical stimuli including compression, stretch, and shear forces. Nearly all aspects of cell behavior can be mechanically modulated in experimental culture systems, suggesting that these interactions may also regulate complex tissue and organ function [14]. It is becoming increasingly clear that mechanotransduction pathways underlie a broad range of human diseases [15,16] and a more thorough understanding of these molecular mechanisms may provide insight into pathologic scar formation and fibrosis.

2. Biomechanical models of fibrosis

Biomechanical systems have been developed to investigate the role of physical forces on cell behavior *in vitro*. One of the most common models employs a deformable substrate onto which cells are seeded. This platform can then be stretched or compressed using automated systems that create dynamic mechanical environments. Fibroblasts have been extensively studied using these systems and it has been well-described that mechanical tension can regulate the expression of matrix and inflammatory genes potentially involved in scar formation [17–19]. Further, mechanical forces can induce fibroblast production of collagen, alpha-smooth muscle actin (a marker of human hypertrophic scarring and myofibroblasts) and pro-fibrotic chemokines, strongly suggesting that wound fibrosis can be modulated by fibroblast mechanosensing [20]. Keratinocytes are also mechanically responsive and have been studied using strain systems [21–23]. Researchers have shown that strain promotes keratinocyte proliferation (a feature of hypertrophic scars) [24] and migration *in vitro* [25]. However, two-dimensional environments do not accurately reflect *in vivo* behavior and overlook important paracrine crosstalk between skin compartments.

More complex *in vitro* systems have been designed to address some of these concerns. For example, fibroblast-populated collagen lattices (FPCL) are based on three-dimensional cell–matrix interactions that may reflect key aspects of dermal biology [26]. Fibroblasts seeded into these constructs contract and elongate to produce alterations in the lattice shape, allowing for real-time observation of mechanotransduction pathways in a controlled environment. Advances in nanotechnology and fluorescence resonance energy transfer (FRET)-based mechanosensors have allowed researchers to study physical interactions between cells and their substrates on a subcellular level [27,28]. Single cells can be mechanically manipulated using technologies including atomic force microscopy, traction force microscopy, and magnetic twisting cytometry [29–31], allowing a more nuanced exploration of the physical environment in biologic systems.

Studies using human skin and scar explants have shown that skin behaves as a dynamic viscoelastic material. Human pathologic scars are stiffer compared to unwounded skin, findings consistent with histologic analyses demonstrating increased collagen deposition and thicker collagen bundles [32]. In contrast, early gestation fetal mammalian skin (which does not form appreciable scar) contains thinner collagen fibers that exhibit very low levels of resting stress, suggesting a direct relationship between mechanical tension and scar formation [4]. However, explanted human scar specimens do not allow an investigation of early pathogenic mechanisms in fibrogenesis. To overcome this, several models of wound healing have been developed in small animals to study the effects of mechanical forces on skin behavior.

A servo-stretch device has been developed to investigate cyclical stretching of unwounded skin in mice [33]. In this model, the expression of inflammatory genes was upregulated by mechanical stimulation, and epidermal proliferation and angiogenesis were stimulated via ischemia-induced signals. Another cyclic stretch model using skin flaps in mice demonstrated increased endothelial cell proliferation and expression of angiogenic markers [34]. Finally, a tissue expansion model has shown that genes related to cell growth and proliferation can be activated by physical force [35]. All of these studies highlight the potential overlap between mechanotransduction and pro-fibrotic signaling.

Our laboratory has developed a mouse model of hypertrophic-like scarring based on the application of exogenous mechanical loads to incisions that normally heal with minimal scar [4]. Initial studies demonstrated alterations in apoptotic pathways in wounds under mechanical stress [36]. Subsequent research has focused on fibroblast-specific responses to mechanical force both *in vitro* and *in vivo*. Microarray analysis of scars in our mouse model implicated a role for aberrant cell–matrix interactions involving focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase involved in cell mechanotransduction [20]. Specific blockade of FAK in fibroblasts prevented the mechanical stimulation of chemokine signaling and collagen production *in vivo*, suggesting a fibroblast-mediated relationship between physical force and inflammation during wound healing. Further investigation has demonstrated the role of helper T cell signaling in sustaining a pro-inflammatory environment during scar formation [37]. Despite the significant insight gained by utilizing mouse models to study fibrotic disease, mouse skin is significantly different human skin which may limit its usefulness for translational experiments [38].

Large animal models in pigs have been developed to study pathogenic mechanisms in scar formation [39]. Pig skin is similar to human skin in several aspects including epithelial rete peg architecture and dermal microvasculature. Studies using pig models have demonstrated that mechanical stress can regulate collagen fibril thickness, blood flow and inflammatory neuropeptide release [40–44]. Our group has developed a pig model of scar formation based on intrinsic mechanical forces generated from the closure of elliptical wounds [9]. Scar fibrosis directly correlated with the amount of tension required to close the wound, similar to observations made in human wounds [7]. Scars under tension demonstrated greater cellularity, vascularity, and numbers of myofibroblasts, a phenotype which could be prevented by offloading the tension. Collectively, these preclinical studies suggest that mechanical forces critically dictate the degree of scar formation following injury.

3. Cellular mechanotransduction

A large body of work has begun to elucidate the specific molecular pathways that link physical force with fibrogenic responses. Five major overlapping cellular pathways have been described that can translate mechanical forces into biologic programs. These five pathways are integrin–matrix interactions, cytoskeletal strain responses, stretch ion channels, cell traction forces (CTFs) and G protein-coupled receptors (Fig. 1) [6,14,45,46]. The most well understood involve integrins which are transmembrane heterodimeric receptors consisting of α and β subunits that connect the extracellular matrix (ECM) to the intracellular cytoskeleton via cytoplasmic tails. They transmit physical forces from the external world and are a key component of focal adhesions, large macromolecular structures that mediate bidirectional crosstalk between cells and their ECM. Specific integrin receptors (*e.g.* $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 8$, $\alpha 3\beta 1$, and $\alpha 5\beta 1$) have also been shown to activate pro-fibrotic cytokine cascades, demonstrating their ability to modulate both physical and chemical signaling networks [47]. Although this