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Mechanoregulation of Angiogenesis in Wound Healing

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Significance: Mechanical forces are important regulators of cell and tissue function. Endothelial cells proliferate in response to tissue stretch and the mechanical properties of the environment direct capillary sprouting and growth. As the vascular network is a key factor in physiology and disease, control of the vascularity by means of mechanical forces could lead to the development of innovative therapeutic strategies.

Recent Advances: Increased understanding of mechanobiology has stimulated translational research and allowed the development and optimization of clinical devices that exploit mechanical forces for the treatment of diseases, in particular in the field of wound healing. Stretching in distraction osteogenesis and tissue expansion induces neogenesis of well-vascularized tissues. In micro-deformational wound therapy, micro-mechanical distortions of the wound bed stimulate cell proliferation and angiogenesis by stretching resident cells to improve healing of difficult wounds. Relief from tension antagonizes proliferation and angiogenesis in primarily closed wounds allowing for better scar quality.

Critical Issues: The integration of mechanobiology into traditional cell biology and pathophysiology in general is not yet complete and further research is needed to fill existing gaps, in particular in the complexity of *in vivo* conditions.

Future Directions: Still largely unexplored approaches based on mechanical perturbation of the micro-/macro-environment can be devised to overcome the limits of current strategies in a broad spectrum of clinical conditions.

SCOPE AND SIGNIFICANCE

MECHANICAL FORCES ARE important regulators of cell and tissue biology. This review explains our current understanding of how mechanical stimuli affect cell and tissue biology with a focus on angiogenesis related to wound healing.

TRANSLATIONAL RELEVANCE

Basic science studies show that cell function is related to cell shape. By changing the mechanical environment through application of external forces, cell function can be

altered. As there are many forces and methods to apply forces, many opportunities exist in clinical medicine to apply these principles to a variety of disease states.

CLINICAL RELEVANCE

Clinical devices based on mechanical principles include tissue expansion, distraction osteogenesis, skin stretching devices, scar reduction devices, ultrasound, tissue induction products, and vacuum related wound healing devices. Common to each device is a method of

application of external forces to alter a biological response.

INTRODUCTION

Our bodies are constantly subjected to a variety of mechanical forces including pressure, compression, gravity, osmosis, surface tension, shear, and tension (Fig. 1).^{1,2} In addition, other biophysical forces such as electrical, magnetic, or electromagnetic forces have been shown to have biological effects.

The ability of mechanical forces to influence cell proliferation has long been known. For centuries, women in Ethiopia have used large plates to generate large lower lips and women in Thailand have used rings to lengthen their necks. Pregnancy and obesity result in excess skin accumulation as part of physiological processes. In the 19th century, Julius Wolff described the effect of external mechanical forces on the development of bone.³ Ilizarov used these principles to develop the principle of distraction osteogenesis.⁴

The biological processes underlying these observations are now being described. Cell stretch increases the proliferation rate both *in vitro* and *in vivo*; in 1968, Lorber and Milosky showed an increase of tritiated thymidine-labeling of the epidermal cells of the skin of living rats stretched by the insertion of steel pins.⁵ MacKenzie described

the increase of the proliferative rate of epidermidis stimulated by friction,⁶ which had been also observed by Bullogh and Laurence in 1959.⁷ Stretching of the skin by tissue expansion in a guinea pig was reported by Francis and Marks to induce an increase of the number of cells in mitosis seen in histologic sections.⁸ Stretching of fibroblasts cultured on a fabric mesh increases the mitotic rate.⁹ Squier, implanted a spring in the back of mice showing *in vivo* a hyperplastic response in stretched mouse skin. Later, they demonstrated a transformation of fibroblasts in myofibroblasts under mechanical stresses.^{10,11} A rapid increase in DNA-synthesizing epithelial cells after mechanical stretching was observed also *in vitro* by Brunette.¹²

DISCUSSION

Basics of mechanobiology

Several hypotheses have been advanced to explain how tissues and cells sense and respond to mechanical forces. Mechanical stress can open stretch-activated ion channels, distortion can activate cell membrane force-receptors that regulate internally controlled chemical signal cascades, or can induce the release of autocrine/paracrine growth factors.^{13–16} These observations suggest that different pathways of mechanical control of cell activity may coexist.¹⁷ However, cells not only sense active mechanical stimulation, but also sense

Figure 1. Mechanical forces in cell biology. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

the degree of cell stretch, which is less easily explained by these hypotheses.

Judah Folkman made early observations on the relationship of cell shape and cell function. He noted in tumor growth that cell shape was a critical factor in determining whether cells would proliferate or stay quiescent (Fig. 2).^{18,19}

A conceptual framework that better encompasses the complexity of the different mechanotransduction pathways was described by Donald Ingber as tensegrity.^{1,20} Central to this theory is the observation that the cytoskeleton system transmits forces and interconnects cell structures. The focal adhesions complexes are specific membrane molecules that anchor the cell to the extracellular matrix (ECM) or other cells. The main component of focal adhesions complexes are transmembrane proteins (such as integrins), which internally function as anchors for the cytoskeleton. Other proteins such as protein kinases and stress-activated channels that can act as alternative force receptors also gather at focal adhesions. The cytoskeleton is built by filaments and tubules that vary in size and stiffness, some acting more like uncompressible struts, others as elastic strings (Fig. 3). The cytoskeleton has the capacity to store energy distributed throughout its various components in an equilibrium of tensile and compressive forces. The energy stored in the cytoskeleton derives from the balance of multiple forces, those applied from the outer environment (ECM and other cells), those produced inside the cell (actomyosinic apparatus and cytoskeletal remodeling) and osmotic and gravitational forces, which are thus effectively integrated. Additional external or internal forces alter the established state of

Figure 3. According to the tensegrity theory, cells are multimodular tensegrity structures. Stiff microtubules behave as rigid struts, while microfilaments and intermediate filaments behave as elastic elements, and the equilibrium of tensile and compressive forces between these elements maintains the three-dimensional integrity of the structure (tensegrity = tensional integrity). The cytoskeleton is anchored at the inner side of focal adhesions; membrane receptor complexes for the extracellular matrix and nearby cells behave as preferential pathways for mechanical force transmission. Energy is stored and generated by cytoskeletal equilibrium. Forces altering this balance change the global shape of the cell and move and deform all inner organelles and molecules anchored to the cytoskeleton, thus affecting cell function. The nucleus itself, as other structures of the cell, is a modulus inside the cell built according to tensegrity, and mechanical forces can directly influence DNA inside it via the cytoskeleton and nuclear envelope deformation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

cytoskeletal elements with a shifting of the reciprocal position transmitted globally inside the cell. The shape and relative position of all molecules and organelles connected to the cytoskeleton are affected, changing the likeliness of reactions to occur and providing a mechanism to transform resting

Figure 2. Cell function both *in vitro* (a) and *in vivo* (b) is regulated by its shape. (a) Cells that cannot elongate tend toward apoptosis; increasing stretching stimulates differentiation and induces cells to proliferate. Reprinted with permission from Ingber.¹ (b) Also *in vivo* tension is a pro-proliferative stimulus, up to a breaking point at which excessive tension damages tissues. Reprinted with permission from Pietramaggiore *et al.*² To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

tension and its perturbations into biochemical signals. Further, the isolated nucleus itself behaves as a structure built according to tensegrity principles, and its inner framework is in close interaction with the cytoskeleton via anchorage to the nuclear membrane. Mechanical stimuli can be directly transmitted to the DNA influencing gene transcription.²¹ Cells then adapt to new states of mechanical stress by activating internal and external mechanisms that establish a new equilibrium. For example, a new steady state can be achieved by cytoskeleton reorganization or by secreting proteins such as matrix-metalloproteinases that modify the surrounding ECM or producing new ECM structural molecules.

Angiogenesis and mechanical forces

The process of angiogenesis is mediated by interactions with the ECM, in which endothelial cells activate, degrade the basement membrane, penetrate by new cell-matrix interactions into the existing ECM, and establish a new lumen directed by guiding cues. ECM chemistry is well studied, as composition affects type, density, and distribution of ligands exposed to cells. The ECM binds chemokines and growth factors that can either be shielded from or concentrated and exposed to cells, and forces acting on the matrix affect the structure of bound molecules, influencing their activation.²² Matrix stiffness is therefore a critical parameter that influences cell biological behavior and differentiation capacity. Endothelial cells self-assemble into capillary tubes and networks when seeded on compliant matrices such as Matrigel. On stiffer surfaces structural organization into tubes does not occur, and cells grow as monolayers.^{23,24}

By their cytoskeletal and stress-filament structure endothelial cells can sense differences and alterations in stiffness of the ECM, and tensile forces generated by nearby cells transmitted by the ECM. Differential stiffness-based reorganization of the cytoskeleton provides directional information by gradient that guide cells in their migration. This principle, for analogy with chemotaxis, is termed durotaxis.²⁵ It is a subset of mechanotaxis, the term encompassing direction of cell motility by mechanical cues in general.

Shear stress and circumferential stretch by blood flow on the endothelial surface of blood vessels are also recognized as regulators of vessels biology.^{26,27} Shear stress affects the release and the signal pathways of a number of factors, among which hypoxia inducible factor-1 alpha (HIF-1 α), vascular endothelial growth factor (VEGF), angiogenesis-related membrane receptors,²⁸ and

increases the expression of endogenous nitric oxide synthase contributing to vasculogenesis.²⁹ Stretch activated channels participate in stimulation of the release of nitric oxide (NO), a key vasculature regulator factor,³⁰ by shear stress.

In wound healing the perturbation of the flux by distortion of blood vessels, as occurs with matrix reaction to wounding, adds mechanical cues that potentially contribute to the initiation of capillary budding.

Mechanical stimulation can therefore affect blood vessels at multiple levels. Tension transmitted through the ECM and focal adhesions can induce multiplication of endothelial cells and guide the sprouting of new capillaries. In parallel, stretching of tissues can result in alterations of the blood flow, with temporary ischemia that activates in nearby cells the HIF-1 α pathway, which by releasing VEGF stimulates the proliferation of endothelial cells and formation of a richer vessels network. These different mechanically-initiated pathways cannot completely be separated *in vivo* and constitute a known difficulty to researchers approaching this field in human and animal models.

In addition to angiogenesis, formation of new vessels by sprouting from existing ones, vasculogenesis, which is the development of new vessels from circulating endothelial progenitor cells (EPCs), seems to be affected by mechanical forces. According to recent studies, shear stress stimulates immature EPCs with circulating phenotype to mature into adhesive EPCs,³¹ and the differentiation of mature EPCs into endothelial cells,^{32,33} via activation of mechanosensitive molecules and cytoskeletal rearrangement.³⁴

Mechanical signaling in wound healing

Wound healing is a complex orchestrated sequence of events that restores the integrity of disrupted skin. It is classically divided in four phases: hemostasis, inflammation, proliferation, and remodeling.³⁵ Angiogenesis supports new tissue growth by allowing adequate distribution of nutrients for new tissues to sustain its metabolic needs. It progressively proceeds by sprouting and elongation of new capillaries from the blood vessels of the intact tissues around the wound.

Mechanical forces have a multifaceted role in the wound healing process. The interruption of the skin causes skin retraction along anatomic vectors originally described by Langer.³⁶ This degree of stress within normal skin creates an elastic reaction of the ECM that communicates to the resident cell with forces that can—for example—activate the

transdifferentiation of fibroblasts into myofibroblasts. Further, mechanical alterations of the environment through wounding can activate proliferative and migratory pathways, bringing cells to the wound bed between which endothelial cells that follow stiffness gradients into the wound margins. The new ECM deposited in the wound, initially the avascular fibrin clot and then the granulation tissue, possesses specific mechanical properties. These mechanical properties contribute to the mechanotactic guiding of cells toward the wound in addition to orchestrating their activities and differentiation. The wound matrix exhibits well-known chemical, mechanical, and density gradients from the surface to deeper, intact tissue. This process is mutually dynamic and subject to multiple feedback mechanisms, first of which is that cells secrete a new matrix and modify the existing one. Restoration of tissue continuity reduces the healing-conductive mechanical conditions of the environment so that stimuli of cell migration and proliferation are suppressed.

In vivo models of mechanical control of angiogenesis

Some clinical conditions and devices are paramount to how mechanical control of angiogenesis can be employed in clinical fields.

Skin stretching. Application of tensile forces has been shown to generate new skin in both tissue expansion and distraction osteogenesis. Tensile forces applied to skin, thin the skin according to its viscoelastic properties while stimulating cells to proliferate, with minimal inflammatory response.^{37–39} While the normalization of dermis is described to take up to 2 years, epidermal and vascular proliferation can be seen after a few hours and is generally accomplished within a few weeks.

In particular, an increase in vascularity of the tissue is reported, which has proven useful to obtain an increase of the survival of larger skin flaps.^{40–42} Our group studied the effects of tension on intact skin in animal models. By continuously stretching rat ears, we could show by intravital microscopy an increasing vessels diameter starting from day 2 and doubling by day 4. Analysis of the vessel network by corrosion casting showed intense remodeling and a reorientation of the vessels along the main axis of tension (Fig. 4). Stretch also induced a significant increase in vascular surface area on histological sections, dependant on both the intensity of force and the pattern of stimulation.² In a model of unidirectional tensile mechanostimulation of the dorsal skin of mice we confirmed a pro-angiogenic effect of skin stretching with two–five to three–five-fold increase in vessels density 2 days after either continuous or cyclical (2 min on/1 min off) stimulation for up to 4 h.⁴³ The stimulation was accompanied by an elevation of gene expression for *HIF-1 α* , consistent with the expected presence of some degree of hypoxia during stretch basing on the observations gathered from gene chip analysis performed on rat ear stimulated samples.⁴⁴ Expression of *VEGF* and other downstream growth factors that can in part account for the angiogenic response was also elevated.⁴⁵

External volume expansion. External volume expansion (EVE) is a type of device that exerts a three-dimensional (3D) stretch on the skin and subcutaneous tissue by creating vacuum under a rigid dome. Clinically, these devices are employed as noninvasive tissue expanders to enlarge breasts and have been recently used to prepare the recipient site for fat grafts. Fat grafts without pre-expansion are affected by high percentages of graft loss due to multiple factors, the principal being

Figure 4. *In vivo*, tensile stimulations affect endothelial cells and induce proliferation and remodeling of blood vessels. The figures represent scanning electron microscopy visualization of resin casts of the vasculature of rat ears, without stimulation (sham), or 72 h after either continuous tension at 0.50 N for 2 days or cyclical (2 h on, 1 h off) at 0.50 N for 8 h. Notice heterogeneity of caliber and increased curvature in mechanically stimulated vessels, early signs of vascular remodeling. Reprinted with permission from Pietramaggiore *et al.*²

inadequate graft to vascular network ratio and excessive interstitial pressure after injection of the fat, which leads to fat necrosis because of insufficient nutritional support in the early days when vascular connections are not yet established and graft cells survive on diffusion from surrounding tissues. The clinical experience with pre-expansion of the breast prior to fat grafting demonstrated more consistent fat survival.⁴⁶ MR scans showed that pre-expanded breasts appeared to have a richer vascular network.⁴⁷ In a miniaturized animal model for EVE we demonstrated that continuous 3D stretch increases blood vessel density and cell proliferation.⁴⁸ Increased vascular density could be seen even just 2 days after a single EVE stimulation for 2 h.⁴⁹ By the pimonidazole hydrochloride method and by hyperspectral imaging we showed how this effect on the vascular network was likely the combined effect of direct tension applied to vessels and of a transitory state of ischemia.

Micro-deformational wound therapy. The vacuum assisted closure device introduced by Argenta and Morykwas in 1997^{50,51} has dramatically changed how complex wounds are managed.⁵² This device was originally described as a porous, polyurethane foam filling the wound, covered with a seal and connected to a vacuum source. We have shown that when the pore structure comes in contact with the wound surface it induces wound micro-deformations.^{53,54} We believe an appropriate generic name for this technology is micro-deformational wound therapy (MDWT). The interface material can be altered by stiffness, pore size, pore thickness, and pore surface characteristics. Our theory would predict that a larger pore size would induce greater wound surface deformations that would induce more cell proliferation.⁵⁵

Our group investigated the relationship between mechanical forces, wound healing, and angiogenesis in MDWT through a combination of computational studies and *in vitro* and *in vivo* models.⁵² Saxena *et al.* analyzed by finite element modeling the expectable mechanical effects on tissues and cells imposed by suction through the stiff porous structure of foams. They showed this would result in a cobblestone-like deformation of the surface at a microscopic level that well-correlated with the clinical observations on wounds treated with MDWT and with the histological appearance. In sections, surface length was increased by 22% with MDWT treatment, which was predicted to result in stretching of the cells residing in the ECM within the range of forces that *in vitro* experiments demonstrated being pro-proliferative.⁵³ Lu *et al.* investigated *in vitro* the effects of microdeformation on fibroblasts cultured on a deformable fibrin surface.⁵⁶ Combined foam + suction treatment induced deformation of the matrix, cell proliferation, changes in cells morphology from bipolar to stellate, increase in expression of *bFGF* and *TGF- β* genes, as well as α -SMA, and collagen 1a1.

The relevance of mechanical stimulation in MDWT is highlighted by the findings of Chen,⁵⁷ who in a rabbit model observed at transmission electron microscopy initial modifications in the capillaries at wound edges with reorganization of the basement membrane and of the shape of endothelial cells after just 2 min of MDWT, with capillary budding starting after 30 min, and onset of endothelial proliferation at 24 h; a quick response consistent with a hypothesis of activation of mechanobiological pathways.

Erba *et al.* found by corrosion casting increased numbers of more physiologically oriented arcades of small vessels directed toward the surface in MDWT-treated wounds, compared with tortuous

Figure 5. Corrosion casting of the vessels of experimental wounds treated for 7 days with occlusive dressing (*left*) or micro-deformational wound therapy (MDWT) (*right*) in a diabetic mouse model. MDWT-treated wounds presented a richer network of small vessels with more physiological configuration. Modified and reprinted with permission from Erba *et al.*⁵⁸

and dilated vessels in control wounds (Fig. 5).⁵⁸ Expression of *HIF-1 α* and *VEGF* in MDWT-treated wounds was decreased in comparison with control wounds, suggesting a more physiological blood flow and more effective exchange in oxygen and metabolites in MDWT. They also reported a gradient of *VEGF* expression closer to wound surface, correlating with the observations in human wounds by Bassetto *et al.*, who reported concentration of angiogenic phenomena in the close vicinity to the wound-foam interface.⁵⁹ Further, the response to MDWT is stronger with cyclical stimulations,⁶⁰ and dependent on the pattern of cyclical stimulation.⁶¹

While in all of the above conditions a proliferation of blood vessels is beneficial, in scarring diminishing both the proliferative and angiogenic responses would be preferable.

Scarring. If tension is good for wound healing, compression might be beneficial to control scarring. Pathologic scarring, keloidal or hypertrophic, occurs by a number of factors but is exacerbated by tension.^{62,63} Wound healing should stop when a tissue gap is filled and re-epithelization is concluded. In some cases proliferation proceeds longer than needed leading to elevated scars (hypertrophic) or even scars whose tissue tends to overgrow into the nearby healthy skin (keloids). Together with a degree of genetic predisposition, a persistent inflammatory status has been called in as a causative mechanism, with particular emphasis on neuroinflammation.^{45,64} The fibroblast is considered the pivotal cell in pathologic scarring because of its role in matrix deposition and remodeling. Scar growth is also maintained by the presence and proliferation of a valid vascular network, pathologic scars in their active phase are red and richly vascularized, and the angiogenic-relevant VEGF pathway has been suggested as a potential target for therapy.⁶⁵ Neutralization of VEGF reduces scarring in animal models of wound healing,⁶⁶ and Interferon- α 2b has been shown to reduce hypertrophic scarring by inhibiting angiogenesis.⁶⁷ Scars are also hypoxic, have an excess of irregularly shaped endothelial cells,⁶⁸ and of inflammation that is a potent stimulator of angiogenesis. Therapies based on a mechanobiological approach are those proving most effective in the prevention and treatment of exu-

TAKE-HOME MESSAGES

- Mechanical forces are major regulators of cell and tissue physiology, and integrate with other biological signals such as soluble molecules or electromagnetic forces in determining cell function.
- Tension is broadly pro-proliferative *in vitro* and *in vivo*, and stimulates angiogenesis and vascular remodeling.
- Cells sense the mechanical characteristics of the environment and the interaction guides differentiation and migration: this process is called *mechanotaxis*.
- Gradients of substrate rigidity direct cell migration: it is a subset of mechanotaxis known as *durotaxis*.
- Blood flow applies shear stress and circumferential stretch that influence endothelial cells from the inner side of blood vessels *in vivo*.
- Mechanical loading can deform vessels, perturb blood flow, and create tissue ischemia to both directly affect cells and to activate angiogenic pathways such as HIF-1 α -VEGF.
- In wound healing, angiogenesis is activated by tensile forces developed at wounding, and is guided by gradients of stiffness of the matrix deposited in the wound.
- Microdeformation of the wound bed can be exploited to maintain intense cell proliferation and angiogenesis in poorly healing wounds, such as skin ulcers.
- Shielding from mechanical forces is a strategy to remove a stimulus to excessive cell proliferation and angiogenesis in linear wounds as prevention of excessive scarring.

berant scars. Traditional therapies such as taping, elastic garments, silicone sheets and gels, primarily act by compressing the scars at both a macro and micromechanical level. A promising preventive approach based on the same principles was recently developed by Gurtner *et al.*⁶⁹ A new mechanomodulating polymer was developed, that in experimental settings was used to overload or off-load closed wounds. Scar loading resulted in an enhanced pro-fibrotic phenotype of tissues, which markedly increase cell proliferation and blood vessel density. Shielding of the scar from mechanical stresses successfully antagonized the establishment of a pro-fibrotic environment, with normalization of vessels density. Developed into a human scar-prevention device, this system obtained positive results in a phase I study.

SUMMARY

Mechanobiology has allowed us to understand the importance of mechanical forces on cells in soft tissue applications. Application of these forces to tissues can alter cellular proliferation, angiogenesis, and inflammation. We look forward to learning new insights into this exciting field and translating these to clinical practice.

AUTHOR DISCLOSURE AND GHOSTWRITING STATEMENT

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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Abbreviations and Acronyms

α -SMA = alpha smooth muscle actin
bFGF = basic fibroblast growth factor
ECM = extracellular matrix
EPC = endothelial progenitor cell
EVE = external volume expansion
HIF-1 α = hypoxia inducible factor-1 alpha
MDWT = micro-deformational wound therapy
TGF- β = transforming growth factor beta
VEGF = vascular endothelial growth factor