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Dynamic Reciprocity in the Wound Microenvironment

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Abstract

Here, we define dynamic reciprocity (DR) as an ongoing, bidirectional interaction amongst cells and their surrounding microenvironment. In the review, we posit that DR is especially meaningful during wound healing as the DR-driven biochemical, biophysical and cellular responses to injury play pivotal roles in regulating tissue regenerative responses. Such cell-extracellular matrix interactions not only guide and regulate cellular morphology, but cellular differentiation, migration, proliferation, and survival during tissue development, including e.g. embryogenesis, angiogenesis, as well as during pathologic processes including cancer diabetes, hypertension and chronic wound healing. Herein, we examine DR within the wound microenvironment while considering specific examples across acute and chronic wound healing. This review also considers how a number of hypotheses that attempt to explain chronic wound pathophysiology, which may be understood within the DR framework. The implications of applying the principles of dynamic reciprocity to optimize wound care practice and future development of innovative wound healing therapeutics are also briefly considered.

Keywords

dynamic reciprocity; chronic wounds; extracellular matrix

INTRODUCTION

Normal wound healing is characterized by a well-coordinated, progressive series of events designed to restore the barrier function and mechanical integrity of the skin. Like other developmental processes and tumor growth, wound healing involves interactions between cells and their microenvironment, of which the extracellular matrix (ECM) is the primary component.[1–3] It is largely through these interactions that cells are directed to differentiate or dedifferentiate, proliferate or remain quiescent, and assume the architecture and function of the skin versus that of some other organ.[1, 4]

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More than 25 years ago, it was noted that interactions between cells and the ECM occur both ways – that is, they are reciprocal.[5, 6] Moreover, it was noted that these interactions were dynamic, continuously changing in response to cues from the microenvironment.[5, 6] These observations were collectively termed "dynamic reciprocity" indicating the ongoing, bidirectional interactions between cells and the ECM (Figure 1).

DR conceptually encompasses many types of cell-ECM interactions, which embrace a number of fields of basic and clinical study, which include developmental biology and the pathobiology of human disease. Indeed, DR is likely to be relevant during tissue development, reparative and regenerative processes, and human pathogenesis, including embryogenesis, angiogenesis, lactogenesis, cancer, and wound healing. Within each of these basic and clinical research domains, there have been numerous reports that reflect upon the biochemical and biomechanical effects that cells and their interacting surrounding microenvironments or ECM have on tissue or organ-specific responses during human development or disease. Reciprocally responsive biochemical and mechanochemical interactions between cells and ECM during wound healing and angiogenesis have been demonstrated and discussed by a number of authors.[7–17] These interactions represent integral features of DR,[9, 10] which link a deepened understanding or awareness of how cell-matrix interactions modulate cellular responses to injury and wound healing in vivo.

One goal of this review is to explore the relevance of DR as it relates to the wound microenvironment. First, we briefly review the history of DR and then turn to a consideration of specific examples of DR during each stage of wound healing. It is our contention that the concept of DR also provides a framework within which to understand the processes that are disrupted in chronic wounds and how these influence subsequent interactions in the wound microenvironment. Therefore, a second goal of this review is to re-consider some hypotheses that attempt to explain chronic wound pathophysiology and how these might be understood within the framework of DR. We end with a consideration of the implications of DR for clinical wound healing and future therapeutic development.

History of Dynamic Reciprocity

The phrase "dynamic reciprocity" was coined by Bornstein and colleagues in 1982 to describe the effects of the ECM on endothelial cell function.[6] They noted that endothelial cells secreted macromolecules that continually modulated cellular behavior and, presumably, this in turn influenced the identity and quantity of secreted macromolecules.[6] Although, at the time, it was not known how the ECM influenced cells, they hypothesized that these macromolecules may interact with the internal cytoskeleton "to regulate cellular shape and movement and affect metabolic function by, for example, modulating ionic fluxes or protein phosphorylation/dephosphorylation."[6]

In the same year, Bissell and colleagues published a review elaborating on the concept of DR and presenting a model by which the ECM could affect gene expression.[5] In this model, the ECM was believed to influence cells via transmembrane receptors that interacted with the cytoskeleton to eventually alter the pattern of gene expression.[5] This paper also outlined evidence demonstrating the importance of the ECM for cell shape and function, as well as maintenance of the differentiated state.[5] The emphasis in Bissell's model was that "the influence of ECM on the cell, both during the developmental process and in established tissues, appears to evolve continually." Thus, the ECM was believed to affect the cell, which in turn altered the composition and structure of the ECM through synthesis or degradation of ECM components that then influenced the cell, and so on.[5]

The model that Bissell and colleagues proposed in 1982 was supported by the discovery and characterization of integrins several years later.[18] Today, integrins are recognized as multi-domain receptors whose extracellular portion interacts with ECM molecules and whose intracellular portion interacts with signaling proteins (e.g., kinases) and adaptor proteins that link to the cytoskeleton (Figure 2).[2, 19] These interactions in part regulate this DR through gene expression, protein synthesis, actin organization, cell polarity, differentiation, proliferation, migration, and survival. Integrin binding also modulates the signaling by several other receptor mechanisms, including syndecans, growth factors, and cytokines.[20] Cells and the ECM also interact via additional mechanisms such as discoidin domain receptors, hyaluronan receptors, and cell surface proteoglycans, although the integrin pathways are the best characterized to date. Conversely, cells regulate the distribution and affinity of integrins for their matrix ligands. Interactions between cells and the ECM via integrins have been described as *inside-out* and *outside-in*, which describe their mutual influence.

The concept of DR is well established in cell biology, where it continues to provide a basis for research into the mechanisms of ECM-cell interactions.[1–3] This research has been instrumental in establishing the ECM as a signaling entity rather than simply an inert scaffold and support structure for cells.[1, 21, 22] These non-structural properties of the ECM are best demonstrated by the functions of matricellular proteins, such as the thrombospondins, SPARC and hevin, tenascins and periostin, which influence cell function in numerous ways.[3] Over the years, DR has been expanded to encompass interactions between cells and their entire microenvironment, which in addition to the ECM, includes adhesive signals, paracrine signals such as growth factors derived from neighboring cells, and systemic cues.[1]

The parallels between wound healing and other types of tissue development – both normal and pathological – are numerous. In particular, angiogenesis is integral to all of these processes and has been suggested as an organizing principle underlying wound healing, tumor formation, diabetic retinopathy, and selected other conditions.[23] Angiogenic abnormalities are characteristic of both tumors[24] and chronic wounds.[25] Additionally, similar cytoskeletal mechanisms mediate epithelial cell migration in embryogenesis and normal, acute wound healing[26] and both processes require the coordinated activities of cytokines and growth factors, cell-cell interactions, and cell-ECM interactions.

Dynamic Reciprocity During Normal Wound Healing

Given the parallels between wound healing and other types of tissue development, it is reasoned that wound healing is also subject to the principles of DR.[5] In the following sections, we consider examples of DR that occur across the wound healing stages. Rather than providing a comprehensive review of wound healing biology, this section uses examples in an attempt to establish that cells and ECM are changed by their interactions with one another, and the orderly procession of these changes, which occur through a multitude of different signaling pathways, results in wound healing. In a subsequent section, we consider how alteration in the sequence, magnitude, or timing of these changes may contribute to stalled wound healing and/or the formation of aberrant tissue (e.g., fibrin cuffs).

Hemostasis

Hemostasis begins after the disruption of blood vessels, which leads to a series of events designed to halt blood loss. Events in this phase of healing include vasoconstriction, formation of a platelet plug, and coagulation, and involve cells responding to changes in the ECM and vice-versa.

Vascular damage leads to the extravasation of blood components and the exposure of ECM proteins such as collagen, fibronectin, laminin, and the matricellular protein thrombospondin-1.[27] Platelets bind to exposed collagen via integrins of the β_1 and β_3 families,[28] as well as to the glycoprotein (GP) VI immunoglobulin superfamily.[27] This binding initiates an intracellular signaling cascade that activates platelets, causing them to degranulate and release a multitude of chemokines and other soluble mediators that increase intracellular calcium, promote reorganization of the cytoskeleton, and activate $\alpha_{IIb}\beta_3$ integrins via cytoskeletal proteins (Table 1; Figure 3).[27]

Blood-derived fibrinogen and von Willebrand factor (VWF) then bind to activated $\alpha_{IIb}\beta_3$ integrins, promoting connections between platelets and the formation of a platelet plug (thrombus).[27] Fibrinogen is concurrently converted into fibrin by thrombin as the final step in the coagulation cascade. Fibrin then polymerizes to form a clot that is further stabilized by factor XIII. The fibrin clot minimizes blood loss and, together with bound fibronectin, serves as a provisional matrix that incorporates adherence sites for cells, modulates cell function, and serves as a reservoir for growth factors, proteases and protease inhibitors.[29]

Many of the proteins released by platelets are chemoattractants and/or mitogens for neutrophils, fibroblasts, monocytes/macrophages, and smooth muscle cells (Figure 3). Among these is platelet-derived growth factor (PDGF), of which several isoforms bind to heparan sulfate proteoglycan (HSPG), heparin, and other glycosaminoglycans present on cell surfaces and in the ECM.[30, 31] Heparin has been found to potentiate PDGF-α receptor phosphorylation, to increase the PDGF-induced activation of mitogen-activated protein (MAP) kinase, and to enhance chemotaxis of Chinese hamster ovary cells.[32] Platelets also store and release transforming growth factor (TGF)-α and TGF -β, which affect proliferation and matrix metabolism, respectively.

Inflammation

The next phase in healing is the inflammatory phase, which is characterized by the sequential influx of immune cells that, among their other activities, remove bacteria, debris, and devitalized tissue. Platelet-derived cytokines are chemotactic for neutrophils, which in addition to their proteolytic and oxidative functions, initially upregulate factors necessary for the extravasation and chemotaxis of other immune cells and inflammatory responses.[33] Monocytes bind to adhesion molecules on the luminal surface of activated endothelium and then utilize integrins to migrate from the blood into the wound and bind to ECM proteins (Table 1). ECM binding also enhances their phagocytic capacity[34], resulting in increased degradation of ECM fragments (Table 1; Figure 3). Monocyte binding to ECM proteins also induces their differentiation into macrophages and upregulates the production of growth factors such as PDGF-B and TGF-α.[35, 36] Activated monocytes and macrophages produce and release thrombospondin-1, which is chemotactic for macrophages[37], and this effect correlates with the activated angiogenesis demonstrated by these cells.[38] As this phase progresses, it is critical that macrophages remove apoptotic neutrophils, as failure to do so results in reduced release of active TGF-β1 and a subsequent reduction in myofibroblast differentiation and wound contraction.[39] This function of macrophages is dependent on the binding of β_2 integrin to ECM proteins.[39] TGF-β also stimulates fibroblasts to synthesize collagen, fibronectin, hyaluronic acid, thrombospondins 1 and 2, tenascin-C, and other proteins,[40–42], in addition to increasing the expression of integrins that bind collagen, fibronectin, and vitronectin[43, 44].

Among the circulating, marrow-derived mononuclear cells that are recruited during inflammation, there are several populations of cells that express characteristics of granulation tissue such as collagen expression or endothelial growth factor receptors. The early wound infiltrate includes cells of the hematopoietic lineage (CD45+; fibrocytes) and

the mesenchymal lineage (CD45−, CD44+; mesenchymal progenitor cells) that can transiently contribute to ECM production within the provisional matrix.[45–47] Unlike resident (skin) fibroblasts, these cells also have significant effector functions.

Migration and Proliferation

The proliferative phase is characterized by the formation of granulation tissue – new blood vessels, macrophages, fibroblasts, and loose connective tissue[10] – as well as early wound contraction and re-epithelialization.

The provisional fibrin matrix supports the ingrowth of cells by incorporating ECM proteins such as fibronectin and vitronectin, which interact with migratory cells via β_1, β_3, and β_5 integrins. Adhesive interactions permit fibroblasts and endothelial cells to migrate toward growth factors such as PDGF that are localized in the early wound environment.[29, 48] PDGF induces fibroblast proliferation and the expression of proteoglycans by fibroblasts, which, in conjunction with integrins, are required for fibroblast migration and binding to the provisional matrix.[49] Furthermore, bioactive lipids modulate cellular injury and reparative responses, including fibroblast and vascular cell proliferation. Indeed, lysophospholipid-stimulated cytoskeletal responses impact cellular adhesion, migration, and contraction.[50] In turn, lysophosphatidic acid interaction with ECM proteins have further been demonstrated to influence fibroblast migration rates in a laminin- and fibronectin-dependent manner. Indeed, the binding of fibroblasts to fibronectin stimulates the production of collagen, proteoglycans, and hyaluronic acid (Table 1).[51,52] In addition to modifying the mechanical environment, these molecules serve a host of functions, including cell and growth factor attachment, increased cellular motility (hyaluronic acid), and interactions with one another (e.g., proteoglycans may facilitate collagen fibrillogenesis).[10] Chemoattractant signals in the provisional matrix, including PDGF and TGF-β, activate cognate receptors on fibroblasts and other adherent cells. Sphingosine-1 phosphate interacts with PDGF to regulate vascular cell migration and with TGF-β to regulate MMP expression.[50] This growth factor signaling requires the cooperative engagement of integrins. Hyaluronan, an early addition to the provisional matrix, is recognized by CD44 receptors on migrating cells. Fibroblast binding to newly-deposited collagen via the β_1 integrin receptors facilitates migration and also stimulates the production of MMPs, which, by degrading matrix components, permit cell migration.[53]

Disruption of the epidermis and its association with the ECM of the basement membrane activates migration and proliferation of keratinocytes. With the MMP-driven breakdown of the hemidesmosomes, the anchor that held keratinocytes in place at basement membrane is disrupted, allowing keratinocytes to migrate.[54] Unlike mesenchymal and endothelial cells that migrate through a three-dimensional matrix, polarized epithelial cells migrate as sheets from the wound edge or remnant skin appendages. In order to migrate, the leading edge of the sheet detaches from the underlying basal lamina, where hemidesmosomes bind to laminin and type IV collagen through β_4 and β_1 integrins, respectively.[55, 56] The migrating keratinocyte relocates collagen-binding β_1 integrins from the lateral membrane surface to the basal surface to permit migration out over the newly-formed granulation tissue bed. Keratinocytes do not bind to fibrin-fibrinogen because they lack $\alpha_v\beta_3$ integrins; thus fibrin is anti-adhesive for these cells.[57] Instead, keratinocytes express integrin subtypes that bind to collagen I, fibronectin, tenascin-C, and vitronectin,[58] which guide their migration over granulation tissue. To resupply the advancing epidermal sheet, these cells rapidly proliferate just distal to the wound margin under the influence of growth factors.

MMPs and other enzymes are important in generating active ligands. For instance, heparin-binding epidermal growth factor (Hb-EGF) is initially bound to the membranes of monocytes/macrophages, T cells, and keratinocytes, where it is released by ectodomain

shedding in response to MMPs.[59] Hb-EGF is abundant in acute porcine wound fluid 1–3 days after injury, where it binds to heparin or heparan sulfate and is mitogenic for fibroblasts, smooth muscle cells, and epithelial cells.[59, 60] The ectodomain shedding of Hb-EGF bound to keratinocyte membranes is required for keratinocyte migration.[61]

In humans, capillary sprouts begin to migrate into the wound by day 4 post injury. This process depends on the degradation of existing basement membrane by MMPs and other enzymes,[62] as well as on the presence of fibrin, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, and other factors.[63] Capillary sprouts initially express $\alpha_v\beta_3$ integrins that allow them to bind to fibrin-fibrinogen.[63] As endothelial cells migrate into the wound, integrin expression is spatiotemporally controlled, with ECM adhesions being assembled and disassembled at various times and places.[64] By analogy with epidermal migration, endothelial sprouts advancing through the newly-forming ECM switch the types of integrins they express, leading to differences in adhesion dynamics, cytoskeletal organization, and signaling pathway activation over the course of angiogenesis.[64]

FGF, a key player in the regulation of angiogenesis, is an example of a growth factor that must be bound to the ECM to exert its effects. FGF is made by multiple types of cells (e.g., macrophages during the inflammatory phase and fibroblasts and endothelial cells during the proliferative phase) during wound healing, but FGF interacts with heparin-like moieties in the ECM and on the plasma membrane in order to stimulate target cells throughout the phases of wound healing. Heparanase, an enzyme that cleaves heparan sulfate to liberate bound growth factors from the ECM, has been found to accelerate wound healing primarily through an enhanced angiogenic response.[65] FGF molecules, either secreted from cells or liberated from the ECM by heparanase, are stabilized by heparan sulfate fragments and escorted to the FGF receptors by cell surface HSPG to form a tetrameric complex leading to receptor dimerization and signaling (Figure 3).[66] Prevention of FGF-2 binding to heparan sulfate prevents its ability to support fibroblast growth and reduces binding to its cell-surface receptors.[67, 68]

MMPs also liberate angiogenic factors from the ECM proteins to which they are bound, and they unmask cryptic sites in ECM components or generate bioactive ECM fragments that can bind to cells via growth factor or other receptors and initiate signaling pathways.[62, 69–71] Endothelial cells express $\alpha_v\beta_3$ integrin, which binds to fibrin, fibronectin, vitronectin, and vWF, and mediates endothelial cell adhesion and migration.[63] Matrix components regulate the temporal sequence of endothelial cell proliferation, with laminin inducing a higher proliferation rate than collagen IV.[72] PDGF-B is also mitogenic and chemotactic for pericytes – cells that surround capillary tubes where they promote new capillary growth and uniform structure. As angiogenesis progresses, endothelial cells form tubes, which involves the recruitment of pericytes in response to endothelial cell-derived FGF-2 and PDGF.[73] Thrombospondins are potent modulators of angiogenesis.[74]

Dynamic and reciprocal interactions between cells and ECM are evident not only in the biochemistry of the wound microenvironment, but also in the biomechanical interactions that occur at the cell-ECM interface.[75] Cell shape is mediated by the tension generated when cells anchor to ECM via integrins, which are connected to the cytoskeleton. Cell tension and matrix elasticity interact to regulate cell differentiation. In combination with growth factor gradients, cell shape and tension generated through cell-ECM adhesions are critical determinants of cell migration.[76] Under homeostatic conditions in adults, the dynamic interactions between cell traction forces and resistance to these forces by the ECM strike a balance that maintains cell shape.[77] However, during tissue development, including wound repair, degradation of ECM disrupts mechanical tension and consequently cell shape, influencing cellular proliferation and migration.[75] The altered cell shape caused by changes

in the ECM then feeds back to modify cell behaviors such as growth, differentiation, motility, etc.[75]

Contraction and Remodeling

During the proliferative phase, fibroblasts that are initially bound to fibronectin via $\alpha_v\beta_5$ or $\alpha_v\beta_3$ integrins migrate and proliferate in response to PDGF, producing an ECM that is relatively sparse but enriched in hyaluronan and with relatively higher levels of type III collagen.[10] Under the influence of TGF-β and connective tissue growth factor (CTGF), collagen I becomes the predominant fibrous protein. In the continued presence of TGF-β1, a fraction of the maturing granulation tissue further differentiates into myofibroblasts (Table 1).[78, 79] It has been proposed that integrin-mediated myofibroblast contraction directly activates latent TGF-β1 from self-generated ECM, thereby restricting the progression of fibrosis to the area of mechanical stress.[80] Lysophosophatidic acid also enhances wound contraction via activation of G-protein-linked receptors and activation of the Rho and ROK pathways, eventually leading to increased phosphorylation of myosin light chain by impacting myosin phosphatase activity and actomyosin interactions.[50, 81]

One example of ECM-growth factor binding is the interaction between TGF-β and the protein components of decorin and betaglycan. TGF- β1 induces the synthesis of decorin and biglycan.[82] Binding of TGF-β1 to decorin, betaglycan, and biglycan inhibits its activity, suggesting a negative feedback loop [83]. In addition, the latent TGF-β binding proteins are closely related to the fibrillins, both of which can affect TGF-β bioavailability. TGF-β1 and 2 are involved in scar formation and TGF- β type II receptors are important in wound contraction.[84]

Myofibroblasts interact with collagen bundles and growth factors to contract the wound.[85] Macrophages, endothelial cells, and epidermal cells release MMPs that remodel the early matrix, while myofibroblasts replace it with the stronger collagen type I (Figure 3).[86] This newly deposited collagen is organized along the stress lines of the skin and shows increased tensile strength. Fibroblasts and myofibroblasts appear to continue to accumulate collagen until the compliance of the ECM reaches mechanical equilibrium with surrounding tissue. In hypertrophic scarring, there is an inappropriate response to tensional forces, leading to excess matrix deposition. The slow remodeling of collagen, including the formation of bundles and cross-links, progresses over a period of months to form a scar.

What Are the Problems In Chronic Wounds and How Can They Be Understood Within the Framework of Dynamic Reciprocity?

While most wounds heal in a timely and orderly pattern, the process can be stalled or halted in individuals with a variety of diseases or conditions, including diabetes mellitus, venous or arterial insufficiency, and immunosuppression, or following a period of immobility that leads to prolonged pressure. Chronic wounds may develop in these cases, potentially leading to pain, immobility, hospitalization, amputation, or even death.[87, 88]

Viewed in the context of DR, non-healing wounds fail to exhibit the normal sequence of actions and reactions between cells and ECM that characterizes acute wound healing. This is not to say that DR does not apply to chronic wounds; on the contrary, the highly organized nature of the ECM and cells evident in fibrin cuffs of venous stasis ulcers has led to the suggestion that this tissue is actively synthesized,[89] but perhaps inappropriately so. Additionally, the normal, sequential pattern of these interactions does not occur, and the disruption of these interactions – potentially at a variety of different points – leads to downstream effects on other cell-ECM interactions that ultimately delay or preclude healing.

In the following sections, we consider some of the disruptions in chronic wounds that may relate to non-healing and examine how they may fit into the framework of DR.

Disruptions due to disease state

No single hypothesis has emerged as a comprehensive explanation for why chronic wounds such as diabetic foot or venous leg ulcers fail to heal in a timely fashion. In fact, it has been suggested that more than 100 physiologic factors contribute to wound healing deficits in individuals with diabetes.[90] Diabetes-induced alterations in progenitor cell recruitment and homing or those that result from excessive nonenzymatic glycation of matrix proteins, would be expected to alter the dynamic and reciprocal interactions between cells and ECM that are necessary for wound healing. These changes also would be expected to have reverberating downstream effects that would interfere with the normal sequential interactions that characterize wound healing, thereby preventing timely wound closure. While many processes lead to the occurrence of chronic wounds, such wounds seem to share a number of characteristics regardless of cause. These include changes in cellular responsiveness, elevated proteolytic environments, and microvascular abnormalities. Table 2 lists some of the disease-related abnormalities observed in individuals with diabetes or venous insufficiency and/or animal models of these conditions, any or all of which may contribute to delayed wound healing.

Elevated proteases

Following observations of elevated levels of various MMPs in chronic wound fluid, it was hypothesized that these enzymes could be causing excessive degradation of ECM proteins and chronic tissue turnover that prevented the wounds from healing. In their 1993 study, Wysocki and colleagues showed that levels of MMP-2 and MMP-9 were increased by 5- to 10-fold in acute wounds, but were increased by another 5- to 10-fold in chronic wounds.[91] Both activated enzymes and proenzyme species were present. Numerous subsequent studies have documented the presence of elevated levels of various MMPs and decreased levels of tissue inhibitors of metalloproteases (TIMPs) in chronic wounds, or an imbalance between the levels of MMPs and TIMPs.[92–95] Other studies have found a correlation between elevated MMP levels and non-healing.[95–98]

In addition to degrading ECM, elevated levels of proteases would also be expected to degrade growth factors. The evidence indicates that chronic but not acute wound fluid rapidly degrades PDGF and TGF-β1 – effects that appear to be due to the activity of neutrophil elastase.[99]

The process of DR would predict that excessive degradation of the ECM would deprive cells of attachment sites and signals required for migration, differentiation, and proliferation. These consequences would interfere with the cell's response to the normal ECM interactions (e.g., increased or decreased protein synthesis), which would, in turn, prevent changes in the matrix composition needed for wound healing to progress. The degradation of growth factors by excess MMPs would also deprive the cell of critical signals. Finally, high levels of MMPs may be expected to cleave proteins with cryptic signaling sequences or shed ectodomains to generate active forms of some membrane-bound precursor proteins such as Hb-EGF. The flooding of these signaling components into the wound microenvironment, theoretically followed by their rapid degradation, may further interfere with the carefully orchestrated interactions of cells with their microenvironment that normally causes wound healing to progress.

Biofilm formation

A biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface.[100] As a result of their structured community, biofilms are largely resistant to innate immune mechanisms and antimicrobial agents.[101] Furthermore, biofilms provide a continuous source of inflammation[101, 102]—one of the hallmarks of chronic wounds. Few studies have evaluated the extent of biofilm formation in chronic wounds. In one small study using molecular sequence analyses, 30 of 50 (60%) of chronic venous ulcers contained biofilm (although 40% did not); in contrast, only 1 of 16 (6%) acute wounds contained biofilm.[103]

From the perspective of the process of DR, the formation of wound biofilm may be expected to interfere with the effects of leukocytes and their subsequent interactions with the ECM that would normally lead to differentiation and release of chemokines and growth factors. For instance, because neutrophils cannot engulf and digest bacteria within the biofilm, they release large quantities of proinflammatory cytokines that lead to chronic inflammation.[101] Biofilm also inhibits the chemotaxis and degranulation of neutrophils[104] and phagocytosis by macrophages.[105] Inhibition of these processes and massive release of inflammatory mediators would be expected to interfere with the sequential series of events that characterize normal wound healing, resulting in aberrant leukocyte-ECM interactions.

Over the past few years, increasing attention has focused on the hypothesis that chronic wounds may fail to heal because they have developed biofilms,[101, 102] a concept that is supported by preclinical research.[106] However, evidence for a causal relationship between biofilm formation and delayed wound healing in humans is either inconclusive[107] or requires further analysis. For example, it will be important for wound care practitioners and wound repair scientists to clarify whether biofilms actually cause non-healing, chronic wounds or whether the chronic wound microenvironment renders all of these wounds or a select fraction thereof to become highly susceptible or conducive for biofilm development. Clearly, more work will be required before any causal relationships between bacterial biofilms and chronic wound are established.

Integrin switching

Over the course of wound healing, cells are stimulated to change the integrins they express, allowing them to bind to different ECM components or different parts of a single component that variously foster migration, differentiation, polarity, survival, and other properties (Figure 4).[108] As in development and morphogenesis, this process is clearly illustrated during wound healing, when successive interactions between matrix receptors and matrix ligands exert a critical influence on cell shape and movement.[109] In the resting epidermis, basal keratinocytes are anchored to laminin332 in the underlying basal lamina through $\alpha_6\beta_4$ integrin. Basal keratinocytes maintain lateral associations, in part, through $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins, which interact with E-cadherin. After injury, the area beyond the intact epithelial sheet has no basal lamina. It is initially covered by fibrin and plasma fibronectin, and subsequently by collagen and cellular fibronectin, as the provisional matrix matures into granulation tissue.

As basal cells at the wound margin assume a migratory phenotype, several key processes occur. The β_1 integrins shift from the lateral to the basal surface of the cell, where interactions with collagen occur. There is an increase in β_1 integrin and α_v integrins that can interact with fibronectin and other adhesive proteins.[109] Furthermore, there is an induction of the filopodial expression of $\alpha_v\beta_6$, which also participates in the activation of TGF-β.[110, 111] Keratinocytes at the leading edge of the wound beyond the limits of the basal lamina activate the expression of MMP-9 and MMP-10 (stromelysin 2), whereas the proximal

proliferating population that supplies the leading edge expresses MMP-3 (stromelysin 1) and a more distal, intact population expresses MMP-28/epilysin.[112, 113] These findings indicate that different MMPs are required for mobilization from the basal lamina versus advancement across the granulation tissue.[112, 113] Keratinocytes appear to require the ability to cleave collagen with MMP-1/MMP-13 at their trailing membrane edge in order to migrate across this substrate.[114]

As basal lamina components reappear under the advancing epithelial front, $\alpha_v\beta_1$ and $\alpha_v\beta_6$ expression disappears. Adherence to laminin resumes, and β_1 integrins resume a lateral location. Concurrently, underlying granulation tissue expresses MT1-MMP, an important activator of other MMPs and TIMPs, which modulate MMP activity.[54]

During angiogenesis, endothelial mobilization similarly requires disengagement of $\alpha_6\beta_4$integrin from laminin in the endothelial basement membrane. The engagement of the $\alpha_v\beta_5$ and $\alpha_5\beta_1$ integrins by their insoluble arginine-glycine-aspartic acid (RGD) ligands is required for the execution of the angiogenic signals from VEGF and FGF-2, respectively.

Integrin switching is also evident in fibroblasts. For instance, fibroblasts expressing $\alpha_5\beta_1$ or $\alpha_v\beta_1$ integrins both adhere to fibronectin, which leads to their rapid movement.[64] However, those expressing the former integrin migrate in a random and non-persistent fashion, whereas those expressing the latter integrin migrate in a highly persistent fashion.[64]

Deficits in integrin switching would be expected to interfere dramatically with wound healing,[108] and may be a reason that chronic wounds fail to heal. Several studies have found altered integrin expression in chronic wounds.[115, 116] In the mouse, deletion of α1 or α2 integrins produces subtle effects on wound healing. This may reflect the redundancy of the collagen-binding integrins. However, there are numerous factors that could lead to defects in integrin switching in chronic wounds, and thus this hypothesis may be consistent with a variety of other potential explanations.

Clearly, deficits in integrin switching would interfere with the DR that characterizes the process of wound healing. Lack of appropriate spatial and temporal integrin presentation and switching would interfere with cell-ECM interactions that guide cell migration and other processes. Changes in the integrin repertoire can lead to changes in the ECM,[19] which would be expected to further influence the dynamic and reciprocal interactions in the wound.

Alterations in specific proteins (other than proteases)—Alterations in many other proteins have been noted in chronic wounds. These include decreased levels of intact fibronectin, an increase in fibronectin degradation products,[117] a loss of type II TGF-β receptors on fibroblasts,[118] reduced levels of PDGF,[119] and dramatic down regulation of keratins in epithelial cells.[120] Chronic wounds also show upregulation of β_6 integrins, with overexpression in mice leading to spontaneous chronic wounds in approximately 20% of animals.[116] Venous or diabetic ulcers that heal slowly show decreased levels of intact fibronectin (Figure 5),[89] decreased levels of type II TGF-β receptors,[118] increased levels of inflammatory cytokines, and decreased levels of PDGF.[119]

In most of these cases, it is not known whether the alterations cause or are caused by changes in the wound microenvironment. Also in most cases, the alterations in these proteins may be compatible with one or more hypotheses as to why chronic wounds occur and/or why they fail to heal. The alterations in individual proteins described in this section may be a glimpse into disruptions that occur at one slice of time in the chronic wound – a piece of the overall disrupted sequence of DR in wound healing. Because alterations in each

of these proteins may be expected to have downstream effects, it is unclear whether replacement of any single protein would be sufficient to remedy the slowed healing in chronic wounds. That is, we may be unable to restore the normal pattern of DR with the addition or deletion of any single component that is altered in cells or extracellular fluid of chronic wounds. This hypothesis is supported by the variable effects of growth factors on chronic wounds.

Clinical Manifestations

Given the above data suggesting that normal DR is disrupted in many chronic wounds, various attempts have been made to re-set the "normal" processes. For example, some efforts to remove all or part of the ulcer through debridement to restore normality have been suggested,[121] although the quality of the data that supports this approach has been questioned.[122] Recent work proposes that intrinsic factors create a geometry of the wound as exemplified by biopsies taken from the edge of venous ulcers that heal back to their original position.[123] Cytokines, tissue, and cell therapies have been successful in improving healing in various wound types,[124–127] but, overall, results have been moderate.

What Are the Implications of Dynamic Reciprocity for Chronic Wound Healing and Future Therapeutic Development?

Multiple biochemical and structural abnormalities have been documented in chronic wounds.[89–91, 117] DR may help us understand how these abnormalities fit together and how disruptions in one part of the wound healing process may lead to disruptions in subsequent interactions that ultimately prevent chronic wounds from healing. That is, understanding how the sequential changes in the ECM lead to specific changes in cells that then lead to alterations in the ECM and so forth, forces us to think about how defects in the early stages of wound healing may have downstream effects that eventually preclude wound closure.

Viewed this way, many of the biochemical alterations noted in chronic wounds may be responses to lack of adhesion to (or detachment from) an ECM of specific structure and composition at the right time in the wound healing sequence. Of course, this may be due to the inability of cells to generate the ECM or ECM attachment sites (integrins or other receptors) appropriate to the particular point in the healing process. Even the over-production of MMPs in chronic wounds could be viewed as a reaction to inadequate attachment of cells to the specific variant of the ECM needed at that point, which would naturally maintain appropriate levels of enzymes. Alternately, the excess MMPs could be an attempt by cells to degrade ECM that is not matched to the expressed cellular adhesion sites. Ultimately, a better understanding of the dynamic, reciprocal processes that take place during wound healing has the potential to influence the development of improved diagnostics, as well as therapeutics.

In this article, we have used the phrase "dynamic reciprocity" as a point of departure to explain the pathophysiology of chronic wounds. However, it is important to recognize that DR does not, in itself, explain the pathophysiology of the complex phenomena and factors that result in delayed healing. Instead, we view this concept as a working construct that provides a framework within which to interpret data. It brings together discrete observations from different fields and will hopefully stimulate ideas as to why chronic wounds don't heal.

An example of the data that may be integrated under the rubric of DR is the interface between biochemical and biomechanical cell-ECM interactions. In addition to the widely appreciated biochemical interactions between cells and ECM, biomechanical interactions are increasingly recognized as key determinants of cell shape, polarity, and tissue architecture,

ultimately influencing cell differentiation, proliferation, motility, and survival.[75] An understanding of the combined influence of biomechanical and biochemical factors may be a cornerstone to the development of future tissue replacement products.

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List of abbreviations

CTGF	connective tissue growth factor
ECM	extracellular matrix
FGF	fibroblast growth factor
GP	glycoprotein
Hb-EGF	heparin-binding epidermal growth factor
HSPG	heparan sulfate proteoglycan
IL	interleukin
MAP	mitogen-activated protein
MMP	matrix metalloprotease
PDGF	platelet derived growth factor
RGD	arginine-glycine-aspartic acid
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloprotease
VEGF	vascular endothelial growth factor
VWF	von Willebrand factor

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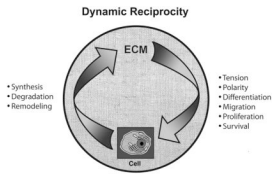

Figure 1.

DR between cells and ECM. Cells synthesize ECM components, and also degrade and remodel ECM, the latter events occurring through the production and regulation of matrix metalloproteases (MMPs) and other enzymes. The ECM regulates cellular tension and polarity, differentiation, migration, proliferation, and survival. The ECM consists of collagen, elastin, multidomain glycoproteins (eg, fibronectin), and proteoglycans and glycosaminoglycans; the exact composition of the ECM varies by tissue and by state of the tissue (eg, intact adult tissue, healing wound, cancer, etc.)

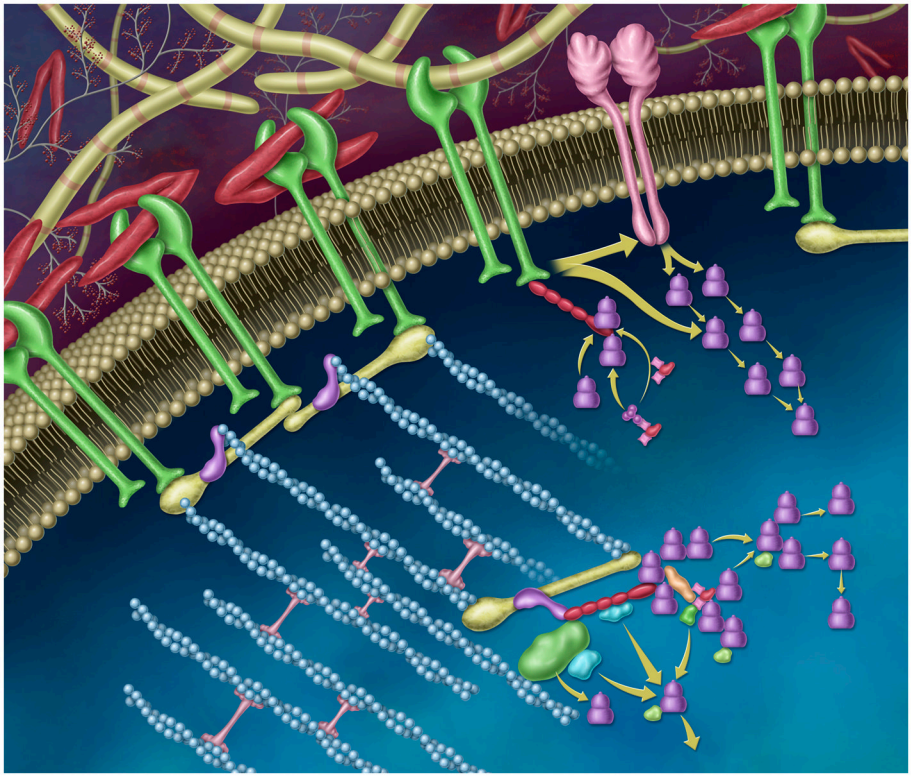

Figure 2.
Dynamic and reciprocal signaling through the integrin- and growth factor receptor-rich plasma membrane. In this stylized representation, integrins (membrane-spanning proteins shown in green) bind to extracellular matrix components such as fibronectin (red “v”s) and collagen (yellow striped rods). The cytoplasmic tails of the integrin receptor directly interacts with the cytoskeleton via talin (yellow), vinculin (purple), and filamentous actin, blue). Through these dynamic protein-protein interactions, mechano-chemical signaling cascades are initiated and propagated, which modulate cell adhesion, shape, polarity, cell proliferation and migration. These reciprocally-regulated interactions can influence gene expression via effector and adaptor pathways. Molecular components, here, include members of the focal adhesion complex, including paxillin (shown in red), Crk, Cas, and the focal adhesion kinase, FAK. FAK and src can signal ‘downstream’ via linked effector pathways (e.g. shown as green, blue, and purple shapes). Integrins can also laterally interact with growth factor receptors (membrane-spanning protein shown in pink) via the MEK1 pathway (shown as purple stacked cylinders).

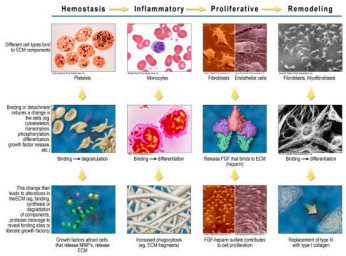

Figure 3.
Key cellular examples of DR at each wound healing stage. The left-most column summarizes a general mechanism that invokes DR, beginning with the binding of cell types to ECM components. This binding (adhesion) or reduced/altered adhesion then leads to changes in the cells, which in turn leads to changes in the ECM. Examples of DR are provided at each wound healing stage, using one or two cell types for the purposes of illustration.

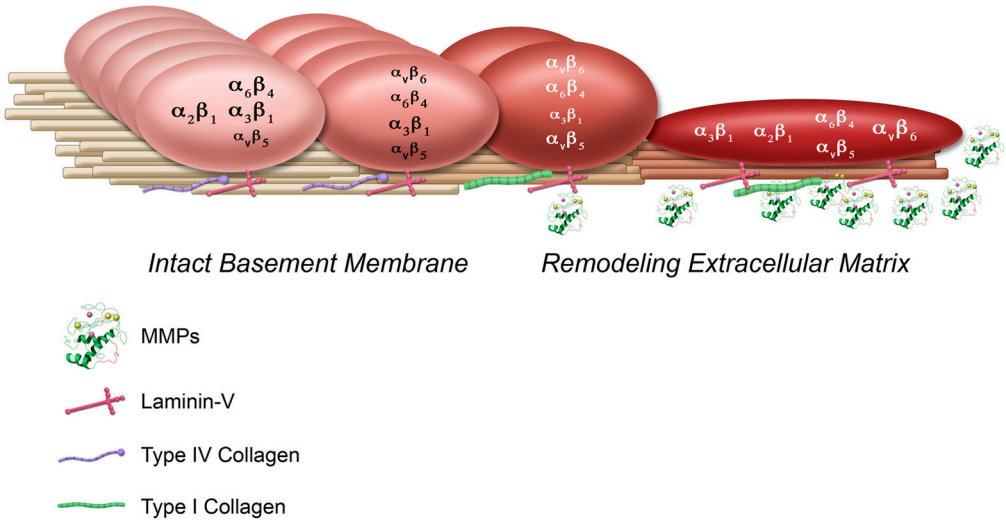

Figure 4.

Integrin switching helps mediate keratinocyte migration across wounded skin. In this graphic representation, keratinocytes are depicted as ovals containing the major integrin subunits they express, and the extracellular matrix is depicted as elongated brown cylinders. Intact keratinocytes bound to basement membrane are shown on the left and migrating keratinocytes at the wound edge are shown on the right. MMPs enable migration by breaking down the underlying basal lamina at the leading edge of the keratinocyte sheet, where the cells assume a flattened shape and express an array of integrins that permits migration across the newly-formed granulation tissue. The leading epithelial cells rearrange their distribution of β1 integrins to engage with type I collagen below the damaged/absent basement membrane.

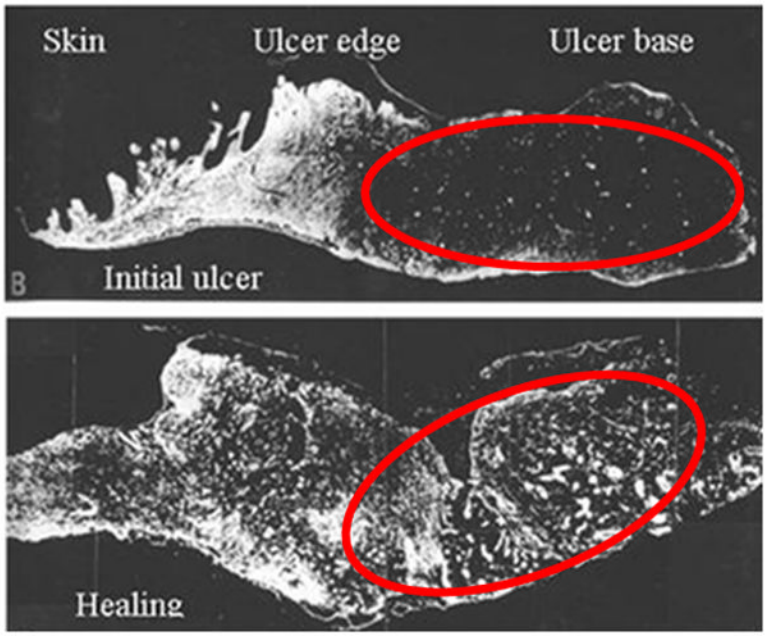

Figure 5.
Degradation of fibronectin in base of chronic venous ulcer (top photo) reverses with initiation of healing (bottom photo). Reprinted from Am J Pathol 1992, 141:1085–1095[89] with permission from the American Society for Investigative Pathology.

Table 1

Selected examples of dynamic reciprocity between ECM and cells during wound healing

Cells →	ECM →	Cells →	ECM →	Cells →
Hemostasis				
Platelets	Bind to exposed collagen via vWF	Platelets degranulate, release growth factors and bioactive lipids, and shift to functional fibrinogen receptors [128] ($\alpha_{IIb}\beta_3$ integrins)	Bind to fibrinogen	Platelet aggregation
Inflammation				
Neutrophils and monocytes bind to endothelium (selectins), integrins change from low to high affinity state, tight binding to endothelial cells, release of chemokines	Chemokines bind to heparan sulfate proteoglycans on endothelial cells	Bound chemokines stimulate expression of β_1 and/or β_2 integrins by neutrophils and monocytes [129]	Bind to fibronectin, collagen	Facilitates chemotaxis along cytokine gradient
Monocytes	Bind to fibronectin	Increases phagocytic capacity [34]	Increased degradation of ECM components and other debris	Transition from an inflammatory phenotype (M1) to constructive phenotype (M2)
Migration and Proliferation				
Fibroblasts	Bind to fibronectin	Stimulates production of MMPs [53]	MMPs degrade certain ECM components	Enhances cell migration
Endothelial cells	Bind to ECM via integrins	Integrins connected to cytoskeleton exert tension, maintain cell shape	Disruptions in ECM (e.g., degradation, increased density) alter tension and cell shape	Differentiation, proliferation, or migration (in presence of growth factors) [75]
Keratinocytes	MMPs dissolve hemidesmosomes (ECM attachments), cells detach from basement membrane	Cell shape changes, migration initiated, expression of specific integrins, [109, 130] phagocytosis of ECM debris, release of MMPs [130, 131]	Deposition of fibronectin, tenascin-C, laminin-5; cells bind via integrins [130]	Guide cell migration
Contraction and Maturation				
Fibroblasts	Bind to fibronectin via $\alpha_v\beta_5$ or$\alpha_v\beta_3$ integrins [79]	Differentiate into myofibroblasts in presence of TGF-β1 (possibly from self-generated ECM) [80]	Interact with collagen via β_1 integrins to contract and remodel the wound; Collagen and MMP expression modulated by mechanical tension	Myofibroblast and fibroblast levels decline in response to declining levels of growth factors and induction of CXCL2 cytokines[132]

ECM=extracellular matrix, MMPs=matrix metalloproteases, vWF=von Willebrand factor

Table 2

Selected Disease-Related Abnormalities That May Contribute to Delayed Healing of Diabetic and Venous Ulcers

Disease	Documented Abnormalities
Diabetes	Microvascular impairments and defects in endothelium [133]
	Pericyte degeneration and thickening of basement membrane in skeletal muscle capillaries and glomeruli due to an increased level of ECM proteins [133–135]
	Decreased vasodilatory response [136] and abnormal autoregulation [133] of microvascular endothelium
	Correlation between tissue oxygenation and ulcer healing [137]
	Altered blood flow and sensory deficits due to neuropathy [25]
	Deficits in the bactericidal action of granulocytes [138]
	Glycation of fibronectin that reduces collagen binding [139]
	Glycation of collagen and fibronectin that interferes with epithelial cell adherence [140]
	Glycation of albumin that leads to overproduction of certain growth factors [141]
	Effects in experimental animals: altered leukocyte infiltration and IL-6 levels in wound fluid during the late inflammatory phase,[142] differential regulation of 27 ECM genes compared with normal rats [143]
Venous Insufficiency	Microangiopathy, consisting of decreased capillaries, changes in capillary morphology, reduced oxygen content of skin, increased permeability of capillaries to low-molecular-weight substances, elevated subcutaneous flow, and diminished vascular reserve [144]
	Focally extravasated red blood cells and deposits of hemosiderin [89]
	Prominence of fibrin cuffs: organized structures around blood vessels containing fibrin, laminin, fibronectin, collagen, and tenascin C, as well as trapped monocytes, macrophages, and polymorphonuclear leukocytes[89]
	Prolonged (>12 month) presence of fibronectin, chondroitin sulfate, and tenascin [145] and impaired angiogenesis in wound fluid [146]
	Build up of fibrin slough in wound beds impairs keratinocyte attachment and migration due to lack of $\alpha_v\beta_3$ integrin receptors on keratinocytes that bind fibrin[57]
	Significant alterations in inflammatory cells, including more macrophages, B cells, and plasma cells than in chronic wounds [145]

ECM=extracellular matrix, IL=interleukin