

Chapter

Biomarkers in Wound Healing

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Abstract

Biomarkers are transforming the study and management of wound healing by providing quantifiable indicators of tissue repair, prognosis, and therapeutic response. This chapter brings together existing insights on biomarkers associated with various stages of wound healing. It highlights progress in their detection and measurements and assesses their practical application in tracking progression, informing treatment decisions, and aiding drug development. This chapter emphasizes the integration of recent discoveries, showcasing how biomarker research is transforming wound healing from mere descriptive observation to predictive, precision-based methodologies.

Keywords: wound healing, biomarkers, detection, wound biomarkers, clinical application

1. Introduction

Wound healing is an intricate, dynamic, and highly regulated process involving various cellular and molecular events interactions that work together to restore tissue integrity after an injury [1]. This intricate process is divided into four orderly phases, namely hemostasis, inflammation, proliferative, and remodeling. Any disruption or delays in these phases can lead to impaired healing. This results in chronic wounds or excessive scarring, which pose considerable clinical and economic challenges worldwide [2]. Recently, there has been an increasing focus on discovering biomarkers that can offer insight into wound healing status, forecast outcome, and inform therapeutic interventions [3].

Biomarkers are generally characterized as quantifiable indicators for biological processes, disease states, and response to medical treatment [4]. In wound healing, biomarkers present opportunities to function as diagnostic tools, prognostic indicators, or predictors of therapeutic effectiveness [5]. The role of biomarkers goes further than simply indicating the biological condition of a wound. They offer significant potential in the stratification of patients, allowing clinicians to customize interventions to specific wound characteristics and systemic conditions [6]. Recent progress in molecular biology, proteomics, and metabolomics has greatly broadened the range of potential biomarkers in wound healing [7]. This includes inflammatory, growth factors, components of the extracellular matrix (ECM), matrix-degrading enzymes, and new genomic and proteomic signatures. Despite these advances, biomarker discoveries are still difficult to apply in clinical practice due to assay standardization, temporal viability, and patient factors like age, comorbidities, and nutritional status [8].

2. Key biomarkers in wound healing

Wound healing is governed by a precisely coordinated sequence of cellular and molecular processes, each defined by the temporal expression of certain biomolecules. Biomarkers linked to these events provide mechanistic healing understanding and diagnostic and prognostic significance. The primary biomarkers in each stage of wound healing are delineated below, emphasizing their biological functions and clinical significance.

2.1 Hemostasis stage biomarkers in wound healing

Important biomarkers of the hemostasis phase consist of platelet-derived growth factor (PDGF), thrombin, and fibrinogen/fibrin degradation products (FDPs) [9]. PDGF, released from activated platelets, promotes the recruitment and proliferation of fibroblast and smooth muscle cells. Wound healing fibroblast and fibrin activity are seen in **Figure 1**. Thrombin affects inflammation and angiogenesis *via* protease-activated receptors in addition to fibrin clot formation. Fibrinogen/FDPs indicate clot formation and breakdown, which may affect wound stability early on [10]. After the effective establishment of hemostasis and the release of first pro-healing mediators, the wound healing process advances to the inflammatory phase.

2.2 Inflammation phase biomarkers in wound healing

Following hemostasis, inflammatory mediators facilitate the recruitment of neutrophils, macrophages, and lymphocytes to the wound site. The inflammatory

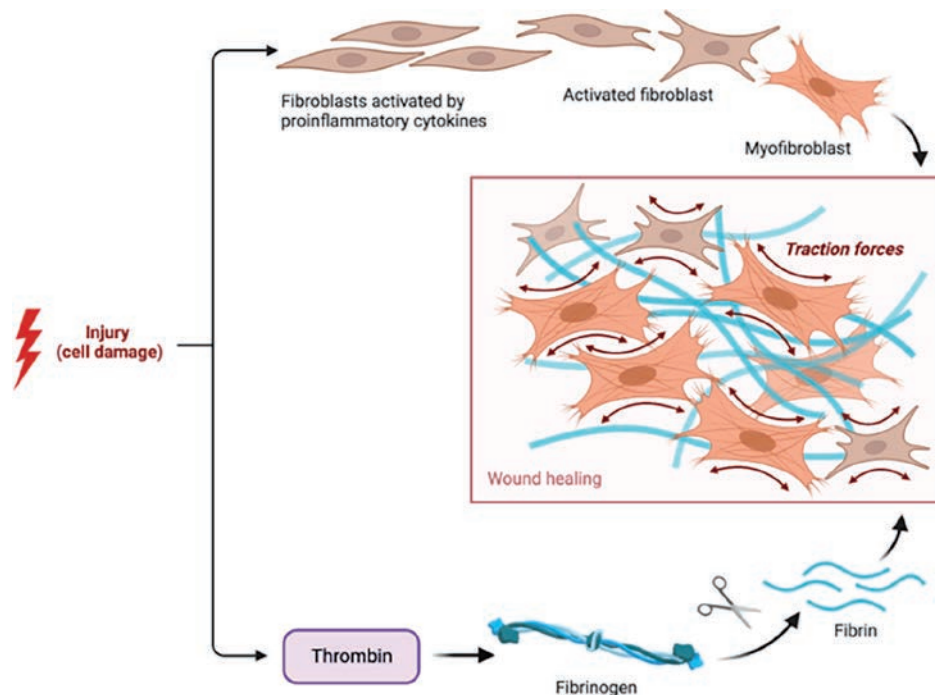


Figure 1. Fibroblast and fibrin activity in wound healing. Fibroblasts migrate into the wound area, deposit ECM, and contract the tissue, while fibrin creates a temporary scaffold that stabilizes the clot and facilitates cell infiltration.

phase plays a crucial role in healing, aiding in the clearance of debris and pathogens from the wound area and preparing the groundwork for tissue repair [11]. Biomarkers associated with inflammation play a vital role in mediating and regulating these processes. Signaling proteins like cytokines and chemokines indicate inflammation. Interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) are the important biomarkers that govern the inflammatory response [12]. These pro-inflammatory cytokines play a crucial role in regulating leukocyte recruitment, activating endothelial cells, and modulating the acute phase response. C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to IL-6 and functions as a systemic indicator of inflammation [13]. This protein also facilitates the control of the local inflammatory response by enhancing the recruitment and activation of immune cells.

High mobility group box 1 (HMGB1), a nuclear protein, serves as a danger-associated molecular pattern (DAMP) upon extracellular (EC) release, promoting inflammatory signaling [14]. Monocyte chemoattractant protein-1 (MCP-1) draws in monocytes that differentiate into macrophages, which are crucial for the removal of necrotic tissue [15]. Prostaglandins and leukotrienes, functioning as lipid mediators, play a crucial role in the inflammatory phase. Prostaglandins, produced by the action of cyclooxygenase enzymes, contribute to vasodilation and the increase of vascular permeability. Leukotrienes, generated *via* the lipoxygenase pathway, are involved in leukocyte chemotaxis, aiding the movement of immune cells to the injury site [16]. Matrix metalloproteinases (MMPs) are enzymes that degrade ECM components and facilitate the removal of damaged tissue. MMPs (MMP-2 and MMP-9) are involved in remodeling processes and help in the release of further growth factors that support the healing process. Their activity must be carefully controlled by the tissue inhibitor of metalloproteinases (TIMPs) to avoid excessive tissue damage [17].

Overproduction of reactive oxygen species (ROS) can damage tissue, yet controlled production is necessary for pathogen eradication and cellular communication. Neutrophils and macrophages produce ROS to eradicate pathogens and trigger additional immune response [18]. Antioxidants maintain equilibrium by reducing oxidative stress. Cellular part neutrophils are the first-wound responders, followed by monocytes/macrophages, which remove debris and secrete factors that switch the wound from inflammatory to proliferative.

2.3 Proliferation phase biomarkers in wound healing

The proliferation phase, which occurs between days 3 and 10 after injury, includes active tissue regeneration, angiogenesis, fibroblast proliferation, ECM deposition, and re-epithelialization [19]. Biomarkers indicate and impact these biological events, indicating wound healing progression and potential treatment targets. At this point, numerous biomarkers play a vital role in coordinating cellular functions that support tissue repair and regeneration. Growth factors are undoubtedly the most crucial indicators during the proliferative phase. Important biomarkers associated with proliferative phase include growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factors (FGF-2), PDGF, transforming growth factor- β (TGF- β), cytokines (IL-6 and IL-10), cellular proliferation markers (Ki-67, α -SMA), ECM component and remodeling enzymes (collagen III, MMP-2, and MMP-9), and angiogenesis markers (CD31) [20, 21].

VEGF plays a vital role in the proliferation of endothelial cells and the formation of new capillaries. EGF signals cell proliferation and migration by attaching to the

EGF receptor (EGFR) on target cells, promoting tissue regeneration. FGF-2 promotes the proliferation of fibroblasts and stimulates collagen synthesis. PDGF attracts and stimulates fibroblasts, which play a crucial role in producing collagen and ECM components vital for structural repair. TGF- β governs ECM synthesis and immunological regulation. Types I and III are structural proteins suggestive of ECM remodeling. IL-6 and IL-10 are essential in facilitating communication between immune cells and cells responsible for tissue formation. They increase fibroblast and endothelial cell activity to reduce inflammation and promote proliferation.

Ki-67 and α -SMA are implicated in two aspects of tissue healing: cellular proliferation (Ki-67 + fibroblast/endothelial cells/keratinocytes) and mechanical contraction and ECM deposition (α -SMA + myofibroblast, wound tightening, and collagen deposition) [19]. Angiogenesis markers (CD31) reflect functional maturity and growth factor activity in neovascularization and reflect structural angiogenesis. Collagen and MMPs provide essential structural support and act as a framework for the formation of new tissue. Proteoglycans and glycosaminoglycans, essential components of the ECM, are crucial for enabling cell signaling and establishing a hydrated environment that supports cell proliferation and migration.

2.4 Remodeling stage biomarkers in wound healing

The remodeling or maturation phase is the concluding and longest stage of wound healing. It starts 21 days after injury and can persist for months or years, depending on the wound and patient. This phase involves ECM maturation, collagen cross-linking, and a decrease in cellularity. Key biomarkers include tissue inhibitors of metalloproteinases (TIMPs), hydroxyproline, and lysyl oxidase (LOX) [21].

Collagen type I, a primary structural protein that replaces type III collagen, enhances wound tensile strength. Meanwhile, collagen type III, an early form of collagen, undergoes gradual degradation, indicating the transition into the remodeling phase [22]. MMP-1, MMP-2, MMP-9, and MMP-13 degrade ECM components for appropriate ECM remodeling. TIMP-1 and TIMP-2 regulate MMP activity to avert excessive ECM breakdown.

TGF- β 1 facilitates the differentiation of fibroblast into myofibroblasts, enhances ECM production, and contributes to scar formation. α -SMA is a hallmark of myofibroblasts that facilitate wound contraction and matrix remodeling. Fibronectin is an ECM glycoprotein that plays a crucial role in matrix construction and cellular adhesion throughout the remodeling process. Decorin modulates collagen fibrillogenesis and TGF- β activity. LOX crosslinks collagen and elastin fibers, enhancing matrix stability and tensile strength. VEGF decreases during the remodeling phase; however, some wounds may still exhibit residual angiogenic activity.

During the remodeling stage, biomarkers include collagen I, MMPs, TIMPs, and TGF- β 1 demonstrating inflammation resolution, balanced matrix remodeling, and tissue integrity restoration [19]. However, disruptions in these processes can result in impaired healing. When the biomolecular milieu of normal wound repair is disrupted by extended inflammation, increased protease activity, and inadequate growth factor signaling, the wound may become chronic [23]. **Table 1** summarizes the key biomarkers of each phase of wound healing. Understanding the biomarker profile change from normal remodeling to chronic wounds is crucial for identifying pathological processes and developing targeted therapies to enhance healing.

Phase	Key biomarkers	Role/function	Reference
Hemostasis	PDGF, thrombin Fibrinogen/fibrin	Initiate clot formation, platelet aggregation, and provision of matrix formation	[9, 10]
Inflammation	IL-1, TNF- α , IL-6, CRP, MPO	Pro-inflammatory cytokines and enzymes that recruit immune cells, clear debris, and prevent infection	[12–15]
Proliferation	VEGF, FGF, TGF- β , KGF, collagen type III	Stimulate angiogenesis, fibroblast proliferation, ECM deposition, and re-epithelialization	[19, 20]
Remodeling	MMPs (MMP-1, MMP-9), TIMPs, collagen type I, TGF- β (persistent)	ECM remodeling, collagen maturation, regulation of protease activity, and scar formation	[19–23]

Table 1.
Wound healing and key biomarkers.

3. Chronic wounds biomarkers

Inflammation, poor angiogenesis, and increased proteolytic activity persist in chronic wounds such as pressure ulcers, diabetic foot ulcers, and venous leg ulcers. Chronically elevated levels of IL-1 β , TNF- α , and IL-6 indicate persistent inflammation [19]. Decreased VEGF and PDGF levels lead to compromised angiogenesis and impaired cellular migration, while elevated bacterial DNA and endotoxin levels are indicative of biofilm presence and underlying infection [24]. The continuous presence of these mediators hinders tissue regeneration, exacerbates tissue damage, prolongs inflammation, and obstructs advancement to the proliferative phase. Chronic wounds, accompanied by persistent inflammation, exhibit compromised angiogenesis, and altered ECM remodeling. Insufficient blood vessel formation due to low VEGF and other angiogenic factors prevents tissue regeneration by preventing oxygen and nutrition delivery [19].

At the same time, chronic wounds show increased levels of proteolytic enzymes, especially MMPs, which break down structural proteins and growth factors within the ECM. The heightened proteolytic activity compromises tissue integrity and hinders the cellular migration and proliferation necessary for effective wound healing. These pathological features create a poor wound environment, hindering healing and increasing the risk of infection, chronic pain, and amputation [24]. Angiogenesis is an essential component of the development of new blood vessels, enabling the reestablishment of oxygen and nutrient supply to repair tissue. PDGF and VEGF are critical pro-angiogenic factors that aid in the stabilization of newly formed vessels and the proliferation and migration of endothelial cells. In chronic wounds or certain clinical situations, low PLDGF and VEGF levels directly inhibit the angiogenic process, limiting wound bed vascularization and tissue repair [25].

In addition to angiogenesis, these growth factors play a vital role in cellular migration, especially for fibroblasts and keratinocytes. Fibroblasts generate components of the ECM and collagen, while keratinocytes contribute to re-epithelialization. A potent fibroblast chemoattractant, PDGF plays a critical role in cell proliferation and migration, whereas VEGF primarily indicates angiogenesis by promoting endothelial cell proliferation and new vessel formation. The compromised regeneration environment is further intensified by persistent wounds or infected tissue, which often

harbors high concentrations of bacterial DNA and endotoxins. Endotoxins, especially gram-negative bacteria's lipopolysaccharides (LPS), stimulate host immunological responses *via* toll-like receptors (TLRs) [26]. An elevated bacterial DNA load, usually detected *via* molecular assay, indicates ongoing bacterial colonization or infection. Biofilms develop a structured, self-protective matrix that makes bacteria resistant to immune clearance and antimicrobial therapies, resulting in a persistent infection source.

Biofilms worsen the detrimental wound environment by continuously generating planktonic bacteria, endotoxins, and diverse virulence factors that sustain local inflammation [27]. The presence of microbial products may exacerbate tissue injury, impede wound healing, and trigger pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6. The prolonged inflammatory environment hinders growth factor production and reparative cell recruitment and efficacy, sustaining chronic wounds [19].

Moreover, endotoxins found in the biofilm microenvironment can directly suppress the expression of PDGF and VEGF, worsening the issue of impaired angiogenesis. Studies show that bacterial components and persistent inflammation can impede the growth and movement of endothelial cells, leading to insufficient development of the vascular network [19, 28]. Reduced angiogenesis, impaired fibroblast migration, and continuing microbial stimulation cause hypoxia, necrosis, and exudate in non-healing wounds. A significant result of infections linked to biofilms is the disturbance of the ECM remodeling process [29]. Biofilms and their related endotoxins lead to elevated levels of MMPs while concurrently reducing TIMPs, causing unregulated degradation of the ECM. This undermines the framework crucial for cell migration and angiogenesis, thereby hindering the healing process. Without a strong ECM framework, endothelial cells and fibroblasts cannot migrate or multiply efficiently, resulting in persistent tissue damage [30].

In wounds affected by biofilm and lacking growth factors, therapeutic strategies present significant challenges. Conventional antibiotics struggle to penetrate biofilm matrices, and reduced pro-angiogenic signaling lowers the efficacy of growth factor-based treatments [31]. This emphasizes the need to combine anti-bacterial biofilm-disrupting agents with pro-angiogenic or ECM-stabilizing treatments to revitalize regenerative wounds. Lower PDGF and VEGF levels, along with higher bacterial DNA and endotoxin, indicate a severely impaired wound healing environment with disturbed angiogenesis, inadequate cellular migration, and biofilm-related infection [32]. Addressing these interconnected pathological features requires a complex, multifaceted therapeutic approach aimed at restoring vascularization, managing inflammation, and eliminating biofilms to ultimately promote effective tissue regeneration.

4. Clinical application of wound healing biomarkers

The importance of biomarkers in clinical setting is considerable, as they serve as key diagnostic indicators. Specific biomarkers can differentiate between acute and chronic wounds by highlighting the unique cellular and molecular activities associated with each type. Therapeutic monitoring involves evaluating therapy efficacy such as alterations in MMP levels subsequent to debridement. Non-healing wounds are frequently associated with elevated concentrations of pro-inflammatory cytokines and proteases such as MMPs, whereas their expeditious resolution is indicative of acute wound healing [33]. Doctors can tailor treatment to patients' biological

wounds using this differentiation. Prognostic biomarkers complement diagnosis. By observing specific markers and their levels over time, healthcare professionals can predict healing outcomes and identify patients who might be at risk for insufficient recovery. Persistently high levels of inflammatory mediators or reduced concentrations of growth factors (e.g., VEGF) could indicate a compromised healing process [19]. Identifying at-risk patients early allows for prompt and focused interventions, improving outcomes and reducing the likelihood of complications.

Therapeutic monitoring constitutes a crucial application of biomarkers. Clinicians can assess a wound's reaction to specific treatments by observing changes in biomarker levels before and after the intervention. An illustration of this is the evaluation of MMP levels following debridement. A decrease in MMP levels following debridement indicates a better healing environment, while persistently elevated levels may signal tissue deterioration and the need for further treatment changes [34]. Infection surveillance entails the prompt identification of microbial colonization or biofilm development. Examples encompass point-of-care (POC) assessments for protease activity in the management of chronic wounds. Assessment of serum CPR and procalcitonin levels guide antibiotic treatment in instances of infected wounds [35]. Effective wound management necessitates the early identification of microbial colonization or biofilm formation by antibiotic-resistant bacteria. Increased levels of biomarkers such as IL-6 may signal the initial onset of infection, enabling prompt clinical action before clear signs and symptoms develop [36]. This proactive approach can help prevent complications such as sepsis or worsening of wounds associated with chronic infections.

The incorporation of biomarkers into regular wound care enhances personalized treatment. The specific needs of a patient's wound can be addressed by doctors through customized treatment approaches to account for the unique biological obstacles that are present. A wound showing elevated protease activity could benefit from protease-modulating dressings, while one lacking sufficient angiogenic activity may require interventions focused on enhancing blood vessel formation [37]. This method, informed by biomarkers, enhances treatment precision and boosts the likelihood of attaining favorable outcomes. Moreover, biomarkers are crucial in clinical research and the development of innovative therapies. Biomarkers serve as surrogate endpoints in clinical studies, providing objective and measurable assessments of the biological response to experimental therapies. This accelerates the evaluation of new wound care products and techniques, ultimately providing innovative solutions to patients more effectively. Innovative biomarker research is uncovering new wound biology, enabling the opportunity to improve existing treatments and discover new therapeutic targets.

The growing availability of POC biomarker tests holds promises for improving the feasibility of biomarker-driven wound management in everyday clinical settings. Portable devices and rapid tests that identify specific biomarker levels at the bedside facilitate real-time decision-making, enabling doctors to promptly modify therapies based on immediate feedback. This innovation improves wound care efficiency and efficacy in outpatient, home care, and resource-limited settings. In many non-healing wounds, elevated levels of proteases, especially MMPs, can degrade essential ECM components and growth factors, thereby impeding the healing process [38]. POC diagnostics allow clinicians to quickly assess protease activity at bedside, providing rapid insights into the biochemical environment of the wound [39]. Upon identification of elevated protease activity, targeted interventions, such as protease-modulating dressings or enhanced debridement, may be promptly implemented. This prompt

enables the personalization of wound management, reducing the danger of prolonged inflammation and increasing the likelihood of tissue regeneration.

These markers help doctors identify localized inflammation from systemic infection and determine if antibiotics are needed [40]. This approach encourages responsible use of antimicrobials by reducing unnecessary antibiotic prescriptions and decreasing the risk of resistance. Furthermore, monitoring CPR and procalcitonin levels over time may reveal the patient's therapeutic response, allowing treatment plans to be adjusted based on objective, real-time biological data [41].

4.1 Introduction to emerging biomarkers and technologies in wound healing

Traditional biomarkers, such as cytokines, growth factors, and MMPs, have been thoroughly examined to assess wound development. The advent of improved molecular biology and high-throughput omics technology enables researchers to develop novel biomarkers that have enhanced sensitivity and specificity [42]. The new biomarkers improve comprehension of the molecular pathways in wound repair, offering prospects for breakthroughs in diagnostic, prognostic, and therapeutic techniques. MicroRNAs (miRNAs) are a category of small non-coding RNAs that regulate gene expression after transcription, and they are among the most significant discoveries. miRNA binds to complementary regions in messenger RNAs, leading to mRNA destruction or translation inhibition [43, 44]. Their involvement in numerous physiological and pathological processes has positioned them as crucial regulators in the wound healing process.

Various miRNAs are linked to distinct phases of wound healing, affecting processes such as cell proliferation, inflammation, angiogenesis, and remodeling of the ECM.

Table 2 provides an overview of emerging biomarkers in wound healing.

miR-21 is one of the most thoroughly studied miRNAs regarding wound healing. This chemical has shown the capacity to promote angiogenesis. By concentrating on and suppressing anti-angiogenic factors such as phosphate and tensin homolog (PTEN), miR-21 enhances the migration of endothelial cells and the development of capillary tubes [49]. Elevated levels of miR-21 in wound tissue correlate with improved neovascularization, suggesting its possible therapeutic function in promoting healing, especially in ischemic or chronic wounds [49]. Conversely, miR-29 plays a vital role in the regulation of fibrosis, an essential process in wound healing and remodeling [50]. This miRNA targets and inhibits the expression of various ECM components, including several collagens. The reduced expression of miR-29 has been

Type of biomarker	Illustrative biomarker	Essential functions in wound healing	Reference
MicroRNAs	miR-21, miR-29	Modulate angiogenesis, fibrosis, and inflammation	[43–45]
Exosomes	MSC-derived exosomes, Kera-exosomes	Regulate inflammation, stimulate angiogenesis	[46]
Metabolites	Lactate, amino acids, lipids	Exhibit tissue metabolism, hypoxia, and healing	[47]
Proteins	VEGF, TGF- β , MMP-9	Demonstrate chronicity, fibrosis, and angiogenesis	[48]

Table 2.
Overview of emerging biomarkers and their roles.

associated with heightened matrix deposition and fibrotic conditions. Modulating miR-29 may facilitate the equilibrium of matrix production and degradation throughout the wound remodeling process, potentially preventing pathological scarring or the continuation of chronic wounds [50]. **Figure 2** depicts the regulatory mechanism of miR-21 and miR-29 in the process of wound healing.

4.2 Exosomes and EC vesicles: Innate mediators of cellular interaction

Exosomes and other EC vesicles (EVs) play a crucial role in facilitating cell-to-cell communication within the wound microenvironment [51]. **Table 3** summarizes exosome-based wound diagnosis and therapy. Diverse cell types release these nanoscale vesicles, which transport a payload of proteins, lipids, and nucleic acids, including miRNAs. Exosomes derived from mesenchymal stem cells, keratinocytes, and immune cells are crucial in the wound healing process as they modulate inflammation, promote angiogenesis, and enhance tissue regeneration [57]. The diagnostic potential of exosomes is rooted in their capacity to mirror the molecular condition of the cells from which they originate. Exosomal content can show biomarkers that indicate wound disease, including acute, chronic, and healing, providing a non-invasive diagnostic approach. For therapeutic purposes, exosomes can be produced or extracted from specific cell types to deliver regenerative signals directly to the wound sites. Studies show that therapies using exosomes accelerate wound healing, reduce inflammation, and improve outcomes in tissue regeneration [57, 58].

4.3 Metabolic profiling: Mapping biochemical alterations in wounds

Metabolomics, involving the comprehensive analysis of small-molecule metabolites within biological systems [59], has attracted attention in the field of wound

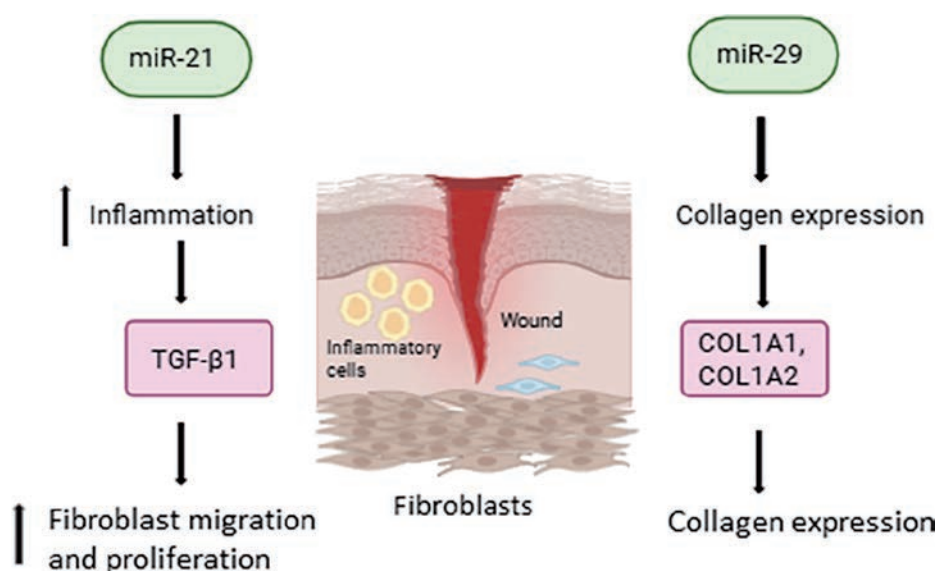


Figure 2. The regulatory mechanism of miR-21 and miR-29 in the process of wound healing. The diagram demonstrates the role of miR-21 in promoting angiogenesis. Conversely, miR-29 suppresses the deposition of the ECM by downregulating collagen gene expression in fibroblasts, thus influencing fibrosis and ECM remodeling.

Exosome source	Diagnostic function	Therapeutic function	Reference
Mesenchymal stem cell-derived exosomes	Analyze cargo (miRNAs, proteins) to reflect inflammatory or regenerative wound microenvironments	Facilitates angiogenesis, decreases inflammation, increases fibroblasts, keratinocyte proliferation	[52]
Keratinocyte-derived exosomes	Assess epithelial cells status and re-epithelialization advancement of <i>via</i> molecular composition	Enhance migration and proliferation of keratinocytes, facilitating better wound closure	[53]
Endothelial cell-derived exosomes	Evaluate endothelial-specific biomarkers to evaluate angiogenic activity and vascular remodeling	Stimulate the formation of new blood vessels, aiding in tissue perfusion and repair	[54]
Immune cell-derived exosomes (e.g., macrophages)	Assess the polarization of immune responses (M1 vs. M2 phenotypes) in wounds	Modulate inflammatory responses to encourage a shift from pro-inflammatory to regenerative phenotypes	[55]
Fibroblast-derived exosomes	Reflect ECM remodeling processes and signaling associated with fibrosis	Improve the deposition of the ECM, increase collagen production, and strengthen wound tensile strength	[56]

Table 3.

Exosome-based wound diagnosis and therapy. Exosomes from different cell types can be used as diagnostic biomarkers for wound status and therapeutically to improve tissue regeneration and modulate inflammatory responses.

research. Wound exudates consist of a variety of metabolites that reflect local metabolic processes [60]. **Figure 3** shows a procedure for metabolomic analysis of wound exudates. By profiling these metabolites, researchers can identify alterations in metabolic pathways occurring at different stages of wound healing or chronic, non-healing wounds. Alterations in amino acid, lipid, and energy metabolism have been linked to impaired healing processes [61].

Identifying unique metabolomic signatures associated with healing or chronic conditions offers valuable clinical insights. Specific metabolites, such as increased lactate or altered amino acid ratios, may signify continued inflammation or hypoxia in the wound [62]. Metabolic profiling helps us understand wound biochemistry and anticipate healing outcomes, allowing us to tailor treatment to the wound's metabolic state. To develop a more thorough system-level comprehension, it is important to analyze upstream regulators, primarily proteins. Thus, proteomics functions as a supplementary platform, providing insight into the functional effectors that influence these metabolic alterations.

Wound healing entails dynamic, stage-specific changes in metabolic and proteomic patterns. Metabolomics measures biological activity, while proteomics reveals protein expression, alteration, and location that affects these processes, providing a more complete mechanistic insight [63].

4.4 Proteomics analysis in wound research

Genomic and metabolomic research is enhanced by proteomics, which reveals dynamic changes in protein expression, alterations, and interactions in injured tissues [64]. A summary of protein biomarkers found from proteomic analysis of wound

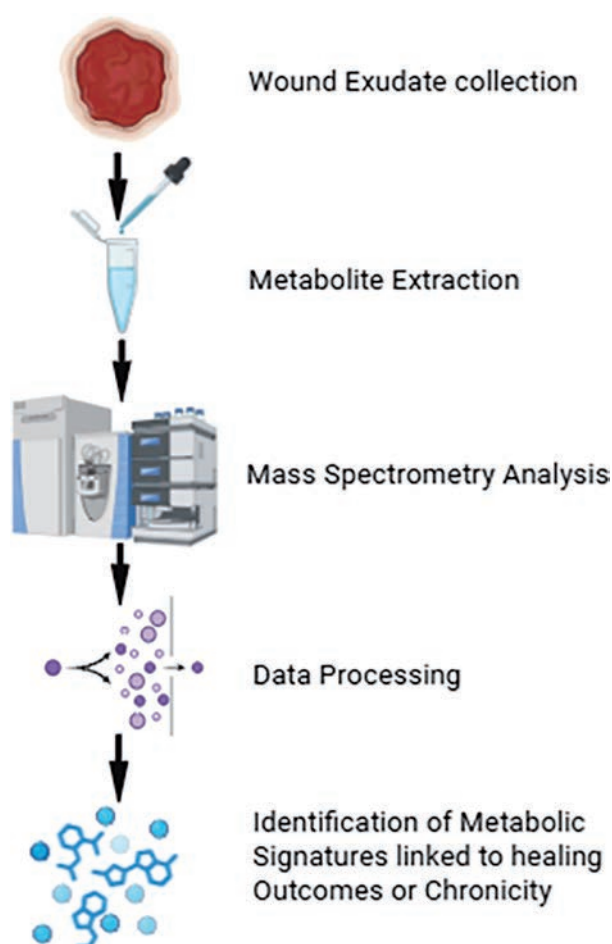


Figure 3.
 Procedure for metabolomic analysis of wound exudates. The diagram illustrates the sequential steps involved in metabolic analysis, encompassing wound exudate collection, metabolite extraction, mass spectrometry, data processing, and the identification of metabolic signatures linked to healing outcomes or chronicity.

tissue and exudates is provided in **Table 4**. Wound exudates, tissue, and cellular secretomes serve as ample sources for proteomic analysis. Advanced mass spectrometry and bioinformatics can identify protein signatures that indicate chronic wound problems or healing stages. Proteomic analysis has uncovered a range of proteins whose expression levels correlate with effective healing or persistent inflammation. For instance, active tissue regeneration is linked to high amounts of fibronectin, VEGF, and TGF- β , while chronic wounds are linked to higher MMP-9 and inflammatory mediators [70]. Understanding these protein patterns can help clinicians evaluate wound health and identify appropriate treatments.

Metabolic profiling and proteomic analysis have become significant methods in enhancing our understanding of the intricate biochemical environment present in both healing and non-healing wounds. Metabolic profiling provides vital information about the wound environment and systemic factors that affect repair by mapping metabolic pathways such as amino acids, energy, and oxidative stress [71]. In addition, proteomics helps uncover mediators and therapeutic targets by characterizing

Protein	Functional role in wound healing	Connection to healing or chronicity	Reference
VEGF	Encourages the formation of new blood vessels and the growth of endothelial cells	Increased in actively healing wounds; reduced in chronic or ischemic wounds	[65]
TGF- β	Promotes fibroblast activation, ECM deposition, and scar formation	Increased in both standard healing and fibrotic, hypertrophic scar tissue	[66]
MMP-9	Breaks down ECM components and regulates remodeling	Significantly increased in chronic, non-healing wounds leading to ECM degradation	[67]
Fibronectin	Facilitates cell adhesion, migration, and matrix organization	Increased during the initial and proliferative stages of acute wound healing	[9, 10]
IL-6	Mediates inflammatory response and the recruitment of immune cells	Increased during acute inflammation, consistently elevated in chronic wounds	[68]
Collagen I and III	Provides structural foundation for tissue repair and scar formation	Impaired healing or excessive fibrosis results from dysregulated deposition	[22]
S100A8/A9 (Calprotectin)	Regulates inflammation and supports antimicrobial defense	Heightened levels in chronic wounds, indicating ongoing inflammation	[69]

Table 4.
Overview of protein biomarkers found from proteomic analysis of wound tissue and exudates.

proteins involved in inflammation, angiogenesis, ECM remodeling, and immune control. Collectively, these high-throughput, systems-oriented methods are transforming wound research by identifying novel biomarkers, developing tailored therapy regimens, and improving clinical outcomes in acute and chronic wound management.

5. Conclusion

Biomarkers are essential in regulating and monitoring the intricate, interconnected stages of wound healing, starting with inflammatory markers such as IL-6, TNF- α , and neutrophil elastase during the hemostasis and inflammation phases, progressing to growth factors like VEGF and PDGF in the proliferation stage, and including ECM-related proteins such as collagen I, MMPs, and TGF-B1 in the remodeling phase. In chronic wounds, the ongoing elevation of inflammatory cytokines, the imbalance of MMP/TIMP ratios, and the disruption of growth factor signaling interfere with the normal healing process, leading to delayed recovery. The clinical application of wound healing biomarkers facilitates the development of personalized treatment strategies, enabling the targeted therapies that specifically address deficiencies or excesses within the healing cascade. Metabolic profiling and proteomics provide thorough investigations of wound management, revealing biomarkers and therapeutic targets. Recognizing and focusing on phase-specific and chronic wound biomarkers presents significant opportunities for improvements in prognosis, diagnosis, and treatment within the realm of wound care.

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Conflict of interest

The authors declare no conflict of interest.

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