Review Article

Comprehensive Histopathological and Immunohistochemical Insights into Wound Healing from Cellular Dynamics to Translational Applications

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Abstract | Wound healing represents a highly orchestrated and dynamic biological process characterized by intricate cellular and molecular interactions that aim to restore tissue integrity after injury. This review provides an in-depth analysis of histopathological and immunohistochemical methodologies utilized in evaluating wound repair, highlighting their translational relevance and clinical implications. The wound healing cascade is traditionally delineated into four sequential yet overlapping phases: Hemostasis, inflammation, proliferation, and remodeling. Each phase exhibits distinct cellular responses and extracellular matrix (ECM) modifications essential for successful tissue regeneration. The accurate selection of animal models, predominantly rodents and porcine species, remains pivotal in elucidating fundamental mechanisms underlying wound repair and assessing therapeutic efficacy. However, it is imperative to recognize species-specific differences that may influence experimental outcomes and translational applicability. Recent advancements in histopathological assessment techniques, including quantitative morphometry, immunohistochemistry, and digital imaging, have significantly enhanced the precision of wound characterization. This precision instills confidence in the accuracy of our understanding of wound healing processes. These methodologies facilitate detailed identification and quantification of critical cellular components involved in healing processes, notably macrophages, fibroblasts, and endothelial cells. Dysregulated interactions among these cellular entities contribute to impaired healing outcomes observed in pathological conditions, including diabetes mellitus, chronic inflammatory disorders, and infection-related lesions. Emerging therapeutic strategies leveraging nanotechnology platforms, stem cell-based interventions, and advanced biomaterials have shown considerable promise in promoting effective wound repair. This highlights the potential for significant advancements in wound healing shortly. Evaluating histopathological parameters and molecular biomarkers remains essential for monitoring therapeutic responses and guiding targeted intervention development. Integrating cutting-edge imaging modalities with omics-based analytical approaches holds significant potential for bridging experimental findings with clinical practice. This underscores the importance of multidisciplinary collaboration in advancing wound healing research. Future research endeavors should prioritize such collaboration to translate mechanistic insights into innovative clinical strategies, ultimately enhancing wound management outcomes.

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Introduction

Overview of wound healing as a dynamic process: Wound healing is a highly orchestrated and dynamic biological process involving a coordinated series of cellular and molecular events to restore tissue integrity following injury. This sophisticated process depends on precisely regulated interactions occurring at both cellular and molecular levels. This process is typically divided into four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Ridhanya, 2019).

Each phase contributes uniquely to restoring tissue structure and function, and disruptions in these phases can lead to abnormal or chronic wounds. Histopathological evaluation is pivotal in understanding these phases, providing detailed insights into tissue architecture, cellular composition, and extracellular matrix (ECM) dynamics during wound repair (Tuca *et al*., 2024).

Experimental models in wound healing: Bridging basic and clinical research

Laboratory animals, particularly rodents and pigs, have been indispensable in advancing our understanding of wound healing. These animal models offer a controlled environment to dissect the fundamental tissue repair mechanisms and identify critical histological and molecular changes during healing. These models allow researchers to study the histopathological and molecular mechanisms underlying tissue repair in a controlled environment (Parnell, 2013; Tuca *et al*., 2024). However, the choice of animal model significantly influences the translational relevance of findings, as species-specific differences in wound healing mechanisms can impact the interpretation of results (Nuutila *et al*., 2016; Parnell and Volk, 2019). Therefore, careful selection and validation of animal models are essential to ensure that experimental outcomes can be effectively translated to human clinical scenarios.

Advances in histopathological and immunohistochemical techniques for wound evaluation

Recent advancements in histopathological techniques, including quantitative morphometry, immunohistochemistry (IHC), and advanced imaging technologies, have significantly transformed the field. These cutting-edge methods enable researchers to obtain high-resolution, quantitative data that elucidate the spatial and temporal dynamics of wound healing. Consequently, these innovations facilitate more precise and objective evaluations of wound healing, allowing for a deeper understanding of the complex biological processes involved (Li *et al*., 2022; He *et al*., 2024). They have yielded critical insights into the roles of essential cellular components, such as macrophages, fibroblasts, and endothelial cells, as well as the molecular pathways that regulate inflammation, angiogenesis, and extracellular matrix (ECM) remodeling (Hassanshahi *et al*., 2022; Zhang *et al*., 2023). By correlating specific histopathological findings with functional outcomes, these advancements offer the potential to identify novel therapeutic targets and biomarkers for improved wound management.

This review seeks to deliver an in-depth analysis of the histopathological and immunohistochemical assessment of wound healing in laboratory animals. It will provide a comprehensive overview of current methodologies and discuss how these approaches contribute to our understanding of the healing process. The analysis will focus on the classification of wound healing stages, the histological markers involved, and the methodologies utilized for evaluation. Additionally, the discussion will address

the advantages and limitations of commonly used experimental models, the factors influencing histopathological outcomes, and the implications of these findings for pharmacological and toxicological research. Ultimately, this review aims to bridge the gap between experimental research and clinical application, highlighting the translational potential of histopathological insights in wound healing.

Mechanisms of wound healing

Hemostasis and inflammation: the initial phases of repair: Wound healing commences immediately after injury with the hemostasis phase. During this stage, rapid vasoconstriction, coupled with platelet aggregation, leads to the prompt cessation of bleeding, thereby establishing a stable environment for subsequent repair processes. This initial phase is brief, typically lasting only a few hours, and is essential to limit blood loss and prepare the wound bed for the influx of repair cells. Following hemostasis, the inflammatory phase ensues. This phase is marked by rapidly recruiting immune cells, primarily neutrophils and macrophages, to the wound site (Ridhanya, 2019). These cells perform critical functions: they clear debris and pathogens through phagocytosis and release pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These cytokines help remove damaged tissue and stimulate angiogenesis, ensuring the developing tissue receives an adequate blood supply (Huang *et al.*, 2023). Although the inflammatory phase usually extends over several days, its exact duration varies according to the severity and nature of the wound (Lodhi and Vadnere, 2017). Importantly, any dysregulation in this phase, especially an imbalance between pro- and anti-inflammatory cytokines, can result in chronic inflammation and impair the healing process (Table 1, Figure 1) (Cioce *et al.*, 2024).

Proliferation and tissue regeneration: fibroblast activity and angiogenesis

The proliferative phase is pivotal in tissue regeneration and is characterized by forming granulation tissue, a key element in repairing and rebuilding the wound. During this phase, fibroblasts proliferate and migrate into the wound bed, synthesizing and depositing ECM components, predominantly collagen, which provide a structural framework for new tissue (Nurrahmah *et al.*, 2019). Concurrently, re-epithelialization occurs as keratinocytes from the wound margins migrate across the wound surface, thereby re-establishing the epidermal barrier (Ridhanya, 2019). Fibroblasts also differentiate into myofibroblasts, critical for wound contraction and size reduction (Monika *et al.*, 2021). The duration of this phase may extend over several weeks and is influenced by factors such as the wound's size, anatomical location, and the occurrence of complications. Research further underscores the importance of an adequate oxygen supply in tissue regeneration; for example, hyperbaric oxygen therapy has been shown to enhance keratinocyte differentiation and promote epidermopoiesis (Table 1, Figure 1) (Dan Dimitrijevich *et al.*, 1999).

Remodeling and scar maturation: collagen reorganization and ECM dynamics

The final stage of wound healing, known as the remodeling or maturation phase, involves the reorganization and maturation of the ECM. In this phase, the initially abundant type III collagen in granulation tissue is gradually replaced by the stronger type I collagen, resulting in a more durable and functionally stable tissue (Santosa *et al.*, 2022). This complex process is mediated by matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), which together regulate the balance between collagen synthesis and degradation (Fadriquela *et al.*, 2020; Pilloni *et al.*, 2023). The remodeling phase may last months or even years, forming mature scar tissue (Table 1, Figure 1) (Nahar *et al.*, 2006; Nurrahmah *et al.*, 2019).

Pathological implications in wound healing

Impaired healing: Molecular and cellular dysfunctions: A fundamental aspect of wound healing research is the direct comparison between normal and impaired healing, which is crucial for elucidating the cellular and molecular mechanisms underlying delayed recovery and informing the development of targeted therapeutic strategies. For instance, studies on diabetic mice have demonstrated that impaired wound healing is characterized by prolonged inflammation, diminished collagen synthesis, and defective angiogenesis, all of which contribute to delayed tissue repair and an increased risk of chronic wounds (Park *et al.*, 2014; Lu *et al.*, 2023).

Histopathological analysis of diabetic wounds revealed a significant reduction in vascular density, accompanied by a pronounced downregulation of angiogenesis and inflammatory regulation genes, including CCND1, ENO1, HIF1, and SERPINE1.

These findings emphasize the pivotal role of endothelial cell dysfunction in impaired wound healing and highlight potential therapeutic targets for enhancing healing outcomes in diabetic patients (Figure 2) (Lu *et al.*, 2023).

Chronic wounds: Persistent challenges in tissue repair

Chronic wounds, characterized by prolonged healing time and impaired tissue repair, often exhibit unique histopathological features. These wounds typically show persistent inflammation, diminished angiogenic activity, and elevated matrix metalloproteinases (MMPs) that actively degrade the extracellular matrix, thereby hindering effective tissue regeneration (Schilrreff and Alexiev, 2022).

Chronic wounds are often associated with persistent inflammation, which hinders tissue repair and predisposes the wound to further complications. Histopathological analysis of chronic wounds has revealed the presence of excessive granulation tissue, extensive fibrosis, and altered collagen deposition patterns, all of which contribute to the chronic nature of the wound (Schilrreff and Alexiev, 2022). Moreover, the dysregulation of immune responses characterized by the persistence of pro-inflammatory macrophages alongside a downregulation of anti-inflammatory cytokines plays a significant role in perpetuating chronic inflammation (Barman and Koh, 2020).

Histopathological examination of chronic wounds can reveal the presence of excessive granulation tissue, mark fibrosis, and altered collagen deposition patterns, which collectively indicate an aberrant healing process. Moreover, identifying bacterial colonies and dense inflammatory cell infiltrates through histological analysis provides critical insights into how infection and chronic inflammation contribute to wound persistence and deterioration (Schilrreff and Alexiev, 2022).

Chronic wound-associated lesions, such as pyoderma gangrenosum (PG) and Marjolin's ulcer, exhibit histopathological characteristics crucial for accurate diagnosis and management. PG, a rare and challenging ulcerative dermatitis, is histologically marked by extensive epidermal and dermal necrosis accompanied by a prominent neutrophilic infiltrate, underscoring its inflammatory nature (Scafati *et al.*, 2015). Marjolin's ulcer, a malignant transformation arising secondary to burn injuries and chronic wounds, typically reveals features of squamous cell carcinoma on histological examination, emphasizing the risk of malignant degeneration in long-standing wounds (Mohammadi *et al.*, 2013). The rarity of PG underscores the need for specialized knowledge and expertise in its diagnosis and management. Histopathological analysis of these lesions provides valuable insights into the complex interplay between infection, persistent inflammation, and impaired wound healing, which are key factors in the pathogenesis of these conditions. Advanced imaging techniques, such as digital morphometry and molecular analysis, further enhance the evaluation of chronic wounds and infection-associated lesions by offering high-resolution, quantitative data that support more accurate diagnosis and facilitate targeted treatment strategies (Figure 3) (Mojumdar *et al.*, 2021; Kientzy *et al.*, 2024).

Infectious agents and their impact on wound healing

Bacterial infections represent a major impediment to wound healing. Bacterial toxins and proteases directly damage the ECM, disrupting the wound's structural integrity and hindering tissue repair (Lecron *et al.*, 2022). The resulting consequences range from delayed wound closure to the development of chronic wounds such as diabetic foot ulcers and surgical site infections (Goldufsky *et al.*, 2015).

Viral infections can also significantly impair wound healing. Viruses such as herpes simplex virus (HSV) and human papillomavirus (HPV) can establish persistent infections in the skin, leading to chronic lesions and impaired epithelialization. As these viruses replicate within keratinocytes, the primary cells of the epidermis, they disrupt critical processes of cell proliferation and migration necessary for wound closure (Lecron *et al.*, 2022).

Fungal infections, particularly those caused by Candida species, are another critical factor in impaired wound healing, especially in immunocompromised individuals. These fungi provoke chronic inflammation, compete with host cells and tissues for vital nutrients, and form biofilms that enhance resistance to treatment (Lecron *et al.*, 2022; Ge and Wang, 2023). The effects include extended healing times and an elevated risk of systemic infection, underscoring the need for advanced diagnostic approaches and targeted antifungal therapies.

Non-infectious systemic diseases and healing dysregulation

Non-infectious diseases, especially chronic conditions,

exert systemic effects that negatively impact wound healing. These systemic effects include impaired circulation, immune dysregulation, and metabolic imbalances (Sorg *et al.*, 2017; Zhao, 2023).

Diabetes mellitus is a leading cause of impaired wound healing, frequently resulting in chronic, non-healing ulcers, particularly in the lower extremities (Stachura *et al.*, 2022; Armstrong *et al.*, 2023). Hyperglycemia, a hallmark of diabetes, induces endothelial dysfunction, which results in reduced angiogenesis and impaired blood flow to the wound site. Additionally, the resultant hypoxia and accumulation of metabolic waste products further impair cell proliferation and ECM formation, ultimately hindering tissue repair (Catrina and Zheng, 2016).

Diabetic foot ulcers (DFUs are a common complication of diabetes, characterized by a triad of impaired angiogenesis, persistent inflammation, and altered ECM remodeling. Histopathological studies have revealed that DFUs exhibit a reduced vascular density and a downregulation of key genes related to angiogenesis and inflammation, including CCND1, ENO1, HIF1, and SERPINE1. These findings underscore the critical role of endothelial cell dysfunction in impaired wound healing and suggest potential therapeutic targets to improve healing outcomes in diabetic patients (Lu *et al.*, 2023).

Obesity is associated with chronic systemic inflammation and impaired circulation, contributing to delayed wound healing (Zhao, 2023). Adipose tissue releases pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which disrupt the normal inflammatory phase of healing. This chronic inflammatory state hinders the resolution of inflammation, leading to prolonged healing times (Zhao, 2023).

Autoimmune diseases: Autoimmune conditions, such as rheumatoid arthritis and systemic lupus erythematosus, impair wound healing due to immune dysregulation. In these conditions, the immune system mistakenly attacks healthy tissues, resulting in chronic inflammation and tissue damage. This persistent inflammatory state disrupts the normal healing cascade, leading to delayed wound closure and increased infection susceptibility. The impaired immune response further compromises the body's ability to combat these infections, resulting in more severe complications (Zhao, 2023).

Vascular diseases: Peripheral artery disease (PAD) and venous insufficiency significantly reduce blood flow to the wound site, depriving the tissue of essential oxygen and nutrients. This hypoxic environment and the accumulation of metabolic waste products impair cell proliferation and ECM formation, ultimately leading to chronic venous or arterial ulcers (Catrina and Zheng, 2016; Sorg *et al.*, 2017).

Histopathological markers of wound healing

Histological markers are pivotal in elucidating the cellular and molecular mechanisms underlying wound healing. They are essential for assessing the healing progression, identifying dysregulated pathways, and evaluating the effectiveness of therapeutic interventions (Table 2).

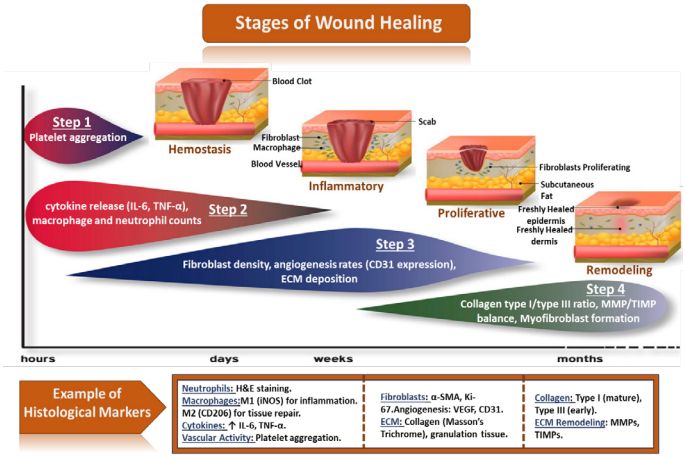

Figure 1: *Wound healing phases and example of histological markers in each phase.*

Table 1: *Classification of wound healing stages.*

Stage	Description	Key cellular and molecular events
Hemostasis and inflammation	Initiation of repair immediately after injury.	Platelet aggregation, vasoconstriction, cytokine release
Proliferative phase	Granulation tissue formation and angiogenesis.	Fibroblast proliferation, VEGF release, collagen deposition
Remodeling and maturation	Scar tissue formation and ECM reorganization.	Collagen type III replaced by type I, MMPs and TIMPs regulation

Table 2: *Histological markers in wound healing.*

Marker type	Key markers	Function
Inflammatory markers	Macrophages (CD206, iNOS)	Inflammation regulation
Proliferative markers	Fibroblasts (α-SMA), Ki-67	Cell proliferation and tissue repair
Angiogenesis markers	VEGF, CD31	Blood vessel formation
ECM remodeling markers	MMPs, TIMPs	Collagen degradation and synthesis regulation

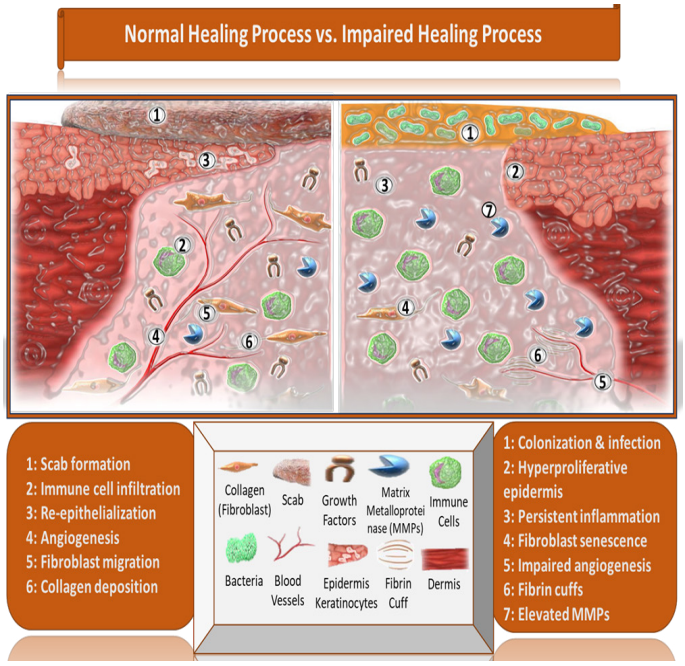

Figure 2: *Comparison of normal healing process vs. impaired healing process.*

Inflammatory phase markers: Macrophage polarization and cytokine expression

Inflammation represents a critical phase in the wound healing process, marked by the recruitment of immune cells, including neutrophils, macrophages, and lymphocytes, to the site of injury (Hassanshahi *et al.*, 2022; Sun *et al.*, 2024). During this phase, inflammatory cells coordinate the clearance of pathogens and debris, setting the stage for subsequent repair. Notably, macrophages exhibit a dual functionality in this context, transitioning from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype as the healing process advances (Huang *et al.*, 2023; Zhang *et al.*, 2023). M2 macrophages are known to secrete anti-inflammatory cytokines, such as IL-10, which mitigate inflammation and promote tissue repair and regeneration (Miron *et al.*, 2015; Vargas *et al.*, 2024). Immunohistochemistry (IHC) has been instrumental in distinguishing between these macrophage phenotypes. For instance, the mannose receptor CD206 is a reliable marker for M2 macrophages, while inducible nitric oxide synthase (iNOS) is typically associated with M1 macrophages (Nurrahmah *et al.*, 2019; Littig *et al.*, 2022). Studies have shown that an imbalance between M1 and M2 macrophages can lead to chronic inflammation and impaired healing (Huang *et al.*, 2023). Furthermore, treatments that modulate macrophage polarization, such as strong acidic electrolyzed water (StAEW), have demonstrated potential in reducing pro-inflammatory cytokines and enhancing tissue repair (Fadriquela *et al.*, 2020).

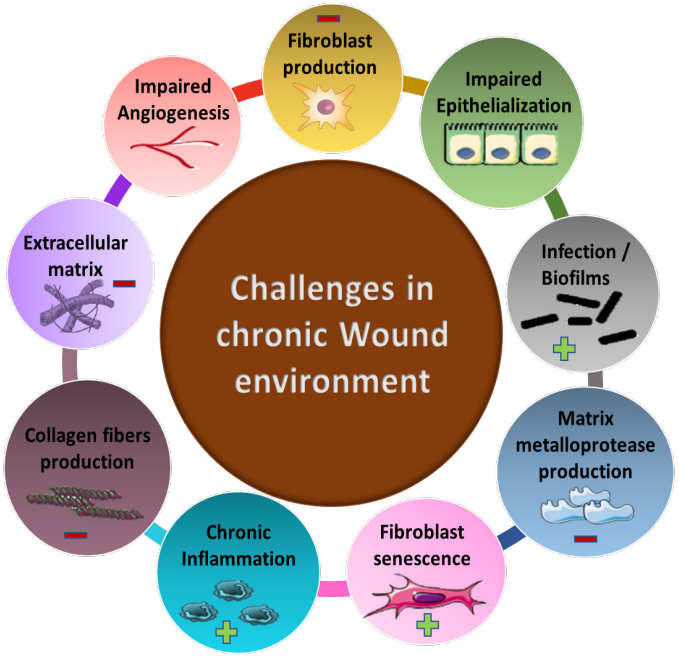

Figure 3: *Challenges in the chronic wound environment.*

Additionally, neutrophils are crucial in the initial inflammatory response, as they release reactive oxygen species and proteases to eliminate debris and pathogens (Miron *et al.*, 2015; Brownhill *et al.*, 2021). The abundance and distribution of these inflammatory cells can be visualized using histological staining and quantified with advanced image analysis techniques, providing objective metrics of the inflammatory response (Goto *et al.*, 2021).

Proliferative activity and angiogenesis indicators: fibroblasts, myofibroblasts, and vascular density

The proliferative phase of wound healing is marked by the migration and proliferation of fibroblasts and

endothelial cells, essential for tissue regeneration and angiogenesis (Pensalfini and Tepole, 2023; Santoso *et al*., 2024). During this phase, fibroblasts not only synthesize and deposit ECM components primarily collagen but also form a robust scaffold that facilitates the formation of new tissue, while endothelial cells actively orchestrate the development of new blood vessels to ensure an adequate supply of vital nutrients and oxygen (Kyriakides *et al*., 1999; Sottile and Hocking, 2002). Key markers of fibroblast activity include α-smooth muscle actin (α-SMA), which serves as an indicator of myofibroblast differentiation and is critical for effective wound contraction, and Ki-67, a nuclear protein that denotes active cellular proliferation (Eggers *et al*., 2020; Pilloni *et al*., 2023).

Angiogenesis, a critical hallmark of the proliferative phase, is primarily regulated by vascular endothelial growth factor (VEGF), which plays a pivotal role in ensuring the efficient delivery of oxygen and nutrients to the wound site (Yari *et al*., 2020; Jin *et al*., 2024). This process is typically evaluated using markers such as VEGF and CD31, well-established indicators of endothelial cells. Additionally, quantitative analysis of these markers often performed using immunohistochemistry (IHC) combined with advanced image analysis software offers valuable quantitative insights into the extent of vascularization and the overall progress of tissue repair (Yari *et al*., 2020; Jin *et al*., 2024; Wong *et al*., 2009; Mohammadi *et al*., 2016). Moreover, studies have shown that treatments designed to promote angiogenesis, such as administering growth factors or stem cells, can enhance wound healing by stimulating neovascularization and accelerating tissue repair (Park *et al*., 2014; An *et al*., 2020).

Extracellular matrix (ECM) dynamics: Collagen typing and MMP/TIMP balance

The ECM is a critical component of wound healing, providing structural support and facilitating cell migration and proliferation. Collagen, particularly type I collagen, is the predominant ECM protein in the dermis and is crucial for wound strength and mature scar formation (Sottile and Hocking, 2002; Parekh and Hebda, 2017). Histological staining methods, such as Masson's trichrome and Picro-sirius red, are commonly used to assess collagen organization and density. These stains allow researchers to differentiate between collagen types, quantify collagen deposition, and objectively evaluate ECM remodeling over time (Yari *et al*., 2020; Alghabban *et al*., 2023; Possa *et al*., 2024; Santoso *et al*., 2024). Disruptions in the balance between collagen synthesis and degradation mediated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) can lead to impaired wound healing and excessive scarring (Fadriquela *et al*., 2020; Pilloni *et al*., 2023). Consequently, treatments targeting ECM remodeling, such as bioactive glass or specific growth factors, have shown promise in improving wound healing outcomes by optimizing collagen deposition and organization (Vargas *et al*., 2024).

Immunohistochemical assessment of cellular dynamics

Macrophage phenotypes: M1 vs. M2 and their impact on healing: Macrophages exist in various activation states, which are primarily categorized into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes (Huang *et al*., 2023; Sun *et al*., 2024). Immunohistochemistry (IHC) is a valuable technique for differentiating these phenotypes by targeting specific cell surface markers. For instance, CD206 (mannose receptor) is commonly used as a marker for M2 macrophages (Zhang *et al*., 2023), while iNOS (inducible nitric oxide synthase) is typically associated with M1 macrophages (Nurrahmah *et al*., 2019). Studies using IHC have demonstrated a dynamic shift in macrophage polarization during wound healing, with an initial predominance of M1 macrophages giving way to a gradual increase in M2 macrophages as the wound progresses towards resolution. This transition is critical for effective wound healing since M2 macrophages promote tissue repair and angiogenesis, whereas excessive M1 activity can perpetuate inflammation and impair healing (Huang *et al*., 2023; Sun *et al*., 2024; Zhang *et al*., 2023). Furthermore, the precise temporal and spatial mapping of M1 and M2 macrophages via IHC offers critical insights into the inflammatory microenvironment and its influence on wound healing outcomes. Additional studies have explored how various treatments modulate macrophage polarization by using IHC to assess changes in these phenotypes and correlate them with improved healing (Littig *et al*., 2022; Abou El-ezz *et al*., 2022; Sheng *et al*., 2023).

Fibroblasts and myofibroblasts and their impact on healing

Fibroblasts and myofibroblasts play a crucial role in the synthesis and remodeling of the ECM during the

wound healing process (Monika *et al.*, 2021). Fibroblasts produce collagen and other ECM components that form the structural scaffold necessary for tissue repair. At the same time, myofibroblasts, identified by their expression of α-smooth muscle actin (α-SMA), are key drivers of wound contraction. IHC enables researchers to differentiate between these cell types by targeting specific markers. For example, α-SMA is a definitive marker for myofibroblasts (Elbialy *et al.*, 2020), whereas markers such as CD40 can identify distinct fibroblast subpopulations with specialized functions (Littig *et al.*, 2022).

Endothelial cells and angiogenesis: Vascularization of wound tissue

Angiogenesis, forming new blood vessels, is crucial for supplying oxygen and nutrients to the wound site and facilitating tissue repair (Hou *et al.*, 2020; Zhang *et al.*, 2023). Endothelial cells, which line the interior surfaces of blood vessels, are the primary drivers of angiogenesis. IHC enables the identification and quantification of endothelial cells using markers such as CD31 (platelet endothelial cell adhesion molecule-1) and von Willebrand factor (vWF), which effectively delineate vascular structures (Zhou *et al.*, 2020; Zhang *et al.*, 2023; Yang *et al.*, 2021). Studies utilizing IHC have demonstrated that the density of blood vessels correlates with the rate of wound healing; impaired angiogenesis is associated with chronic wounds, while enhanced angiogenesis accelerates tissue repair (Hou *et al.*, 2020; Zhang *et al.*, 2023; Yang *et al.*, 2021).

Factors influencing histopathological outcomes

Histopathological outcomes in wound healing studies are affected by many factors, including experimental design, inherent characteristics of the animal model, and external factors such as age and nutritional status. A comprehensive grasp of these influences is crucial for accurately interpreting results and designing effective, clinically applicable therapies (Figure 4).

Impact of experimental design on histological reproducibility

The experimental design significantly influences the reproducibility and interpretability of histopathological findings. Key factors include the type of wound model employed (e.g., excisional, incisional, or burn models), the size and anatomical location of the wound, the timing of tissue collection, and the methods used for histological processing (Nuutila *et al.*, 2016). For example, using a splinted excisional wound model in mice can reduce the confounding effect of wound contraction, thereby providing a more accurate representation of human-like healing (Fischer *et al.*, 2023). Similarly, variations in fixation methods, embedding media, and staining techniques can directly influence the quality and subsequent interpretation of histological sections. Standardized protocols and rigorous quality control measures are thus essential to minimize variability and ensure the reliability of the histopathological data (Nuutila *et al.*, 2016).

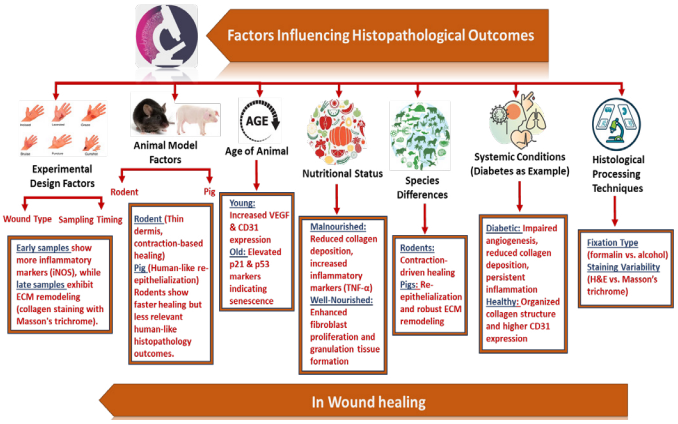

Figure 4: *Factors influencing histopathological outcomes in wound healing.*

Understanding the variability in experimental design is crucial for interpreting histopathological data in your research. Factors such as the type of wound model used, the wound size and location, and the timing of tissue collection can significantly alter the observed outcomes. For instance, a splinted excisional wound model in mice may better mimic human healing. However, the absence of standardized protocols can lead to inconsistencies in the histopathological findings, complicating cross-study comparisons and definitive conclusions.

Table 3: *Common experimental models for wound healing.*

Model type	Advantages	Limitations
Rodent models	Cost-effective, genetic manipulation available	Reliance on wound contraction, species differences
Porcine models	Human-like skin structure and wound healing	Expensive, logistical challenges
Rabbit models	Useful for corneal wound studies	Limited application in large-scale chronic wound studies
Canine models	Relevant for veterinary applications	High costs, ethical considerations

Table 4: *Techniques for histopathological assessment.*

Technique	Purpose	Example stains/Markers
Histological staining	Assess general tissue morphology	HandE, Masson's Trichrome
Immunohistochemistry (IHC)	Detect specific cellular markers	VEGF, CD31, α-SMA
Quantitative morphometry	Objective measurement of tissue components	Image analysis software
Advanced imaging	High-resolution 3D visualization	Confocal microscopy, SHG microscopy

Table 5: *Therapeutic impact of novel approaches.*

Therapeutic approach	Mechanism of action	Histopathological impact	References from document
Plant extracts and phytochemicals	Anti-inflammatory, antioxidant, and angiogenic properties	Enhanced fibroblast proliferation, collagen deposition, and reduced inflammation	Silymarin, Teucrium polium extract, Trichosanthes dioica
Nanotechnology-based therapies	Antimicrobial and anti-inflammatory effects	Improved wound closure, reduced inflammation, enhanced collagen organization	Silver and zinc nanoparticles
Growth factors (VEGF, EGF, PRP)	Angiogenesis and tissue regeneration	Increased granulation tissue formation and collagen deposition	PRP treatments, VEGF signaling modulation
Stem cell therapies	Cellular differentiation and immune modulation	Enhanced tissue regeneration and angiogenesis	Adipose-derived stem cells (ADSCs)
Advanced biomaterials (Hydrogels, Nanofibers)	Support for cell migration and ECM remodeling	Accelerated wound closure and improved granulation tissue	Chitosan-based hydrogels, polymeric nanofibers
Bioactive glass	ECM remodeling and angiogenesis promotion	Enhanced collagen organization and capillary formation	Bioactive glass applications
Ceramide 1-Phosphate (C1P)	Inflammatory modulation and fibroblast migration	Improved fibroblast activity and collagen deposition	Interaction with cPLA2 pathways
Strong acidic electrolyzed water (StAEW)	Anti-inflammatory effects	Reduced pro-inflammatory cytokines and improved tissue repair	StAEW treatments
Hyperbaric oxygen therapy (HBOT)	Enhanced oxygenation and keratinocyte differentiation	Improved epithelialization and angiogenesis	Keratinocyte differentiation studies
MicroRNA (miRNA) therapeutics	Gene expression modulation	Regulation of inflammation and ECM remodeling	miRNA studies (e.g., miR-21)

Influence of age, species, and nutritional status

Age, species, and nutritional status significantly affect wound healing and histopathological outcomes. For instance, aging is associated with impaired immune function, reduced collagen synthesis, and decreased angiogenesis, all contributing to delayed wound healing. The expression of senescence-associated biomarkers, such as p21 and p53, is altered during wound healing in older individuals compared to younger subjects.

Species-specific differences are also significant. For instance, rodents typically heal wounds predominantly through contraction, whereas human healing relies more on re-epithelialization and granulation tissue formation (Nuutila *et al.*, 2016). Such differences can confound histological data interpretation and affect animal studies translational relevance.

Furthermore, nutritional status is critical for providing the necessary substrates for tissue repair. Deficiencies in essential nutrients such as proteins, vitamins, and minerals can impair collagen synthesis, angiogenesis, and immune function, leading to delayed wound healing and altered histopathological features. Metabolic conditions, such as diabetes, further affect wound healing by altering growth factor levels and hypoxia-inducible factor (HIF-1) in the wound tissue; for example, studies on diabetic foot ulcers (DFUs) have revealed impaired angiogenesis and downregulation of genes such as CCND1, ENO1, HIF1, and SERPINE1, underscoring the role of endothelial cell dysfunction in impaired wound healing (Lu *et al.*, 2023).

Species-specific differences in wound healing mechanisms pose a significant challenge in interpreting histopathological data. The fact that rodents primarily heal through wound contraction, which is markedly different from the re-epithelialization and granulation

tissue formation observed in human wounds, is an intriguing aspect of the research (Nuutila *et al.*, 2016). These fundamental differences confound histological analyses and limit the direct translation of findings from animal models to human clinical settings, sparking further interest in the field.

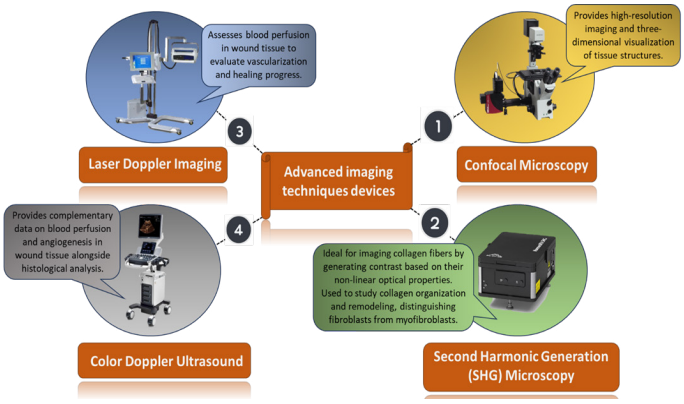

Figure 5: *Advanced imaging techniques devices.*

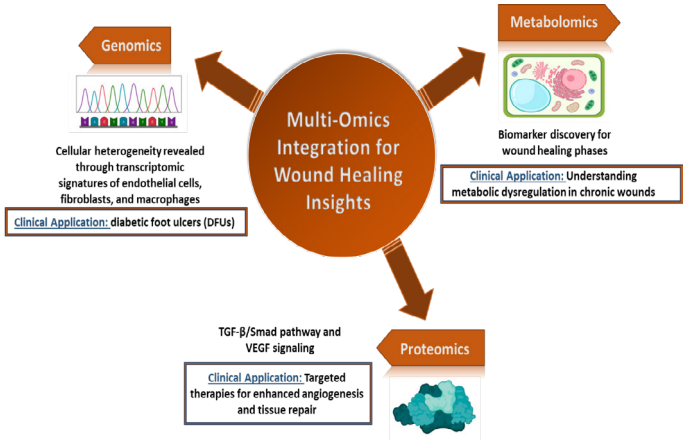

Figure 6: *Multi-omics integration for wound healing insights.*

Impact of systemic conditions

Systemic conditions, such as diabetes, aging, and nutritional deficiencies, are not just factors to be aware of but crucial aspects that researchers, clinicians, and students in the field should consider in their research. These conditions can significantly influence wound healing and histopathological outcomes. For example, diabetic wounds are characterized by impaired angiogenesis, reduced collagen synthesis, and persistent inflammation, which can delay healing and alter histopathological features. This understanding should drive a sense of responsibility and commitment to thorough research in the audience (Lu *et al.*, 2023; Nirenjen *et al.*, 2023) Similarly, aging is associated with reduced immune function and decreased collagen production, which leads to delayed wound healing (Chia *et al.*, 2021). These factors must be carefully considered when interpreting histopathological data and developing therapeutic strategies.

Experimental models in wound healing research

The selection of experimental models is paramount in wound healing research, significantly influencing the validity and translational relevance of the results. Laboratory animals, especially rodents and pigs, are commonly utilized because of their physiological similarities to humans and capacity to regulate experimental conditions effectively.

Rodent models: Advantages and limitations

Rodent models, including mice and rats, are the most commonly used animals in wound healing research due to their cost-effectiveness, ease of handling, and well-established genetic manipulation techniques (Nuutila *et al.*, 2016; Fischer *et al.*, 2023). These models allow researchers to study specific molecular mechanisms and test therapeutic interventions in a controlled environment (Parnell and Volk, 2019).

While rodent models are cost-effective, easy to handle, and allow for studying specific molecular mechanisms and therapeutic interventions, they have limitations. Their dependence on wound contraction as the predominant healing mechanism contrasts with the processes of re-epithelialization and granulation tissue formation characteristic of human healing. Researchers have devised advanced methodologies to overcome this limitation, but the inherent anatomy, physiology, and immunology differences between rodents and humans remain a significant concern. (Parnell and Volk, 2019).

Porcine models: Bridging translational gaps

Porcine models are increasingly recognized for their high concordance rate with human wound healing outcomes, making them particularly suitable for studying chronic wounds and evaluating the efficacy of new treatments (Parnell, 2013; Tuca *et al.*, 2024). The skin structure of pigs closely resembles that of humans, with similar epidermal and dermal thickness, hair follicle density, and collagen organization (Tuca *et al.*, 2024).

Standardized porcine models, such as the 1.2 mm-deep dermatome wound model, have been developed to enable reproducible and comprehensive wound healing studies. These models allow researchers to

perform histological analyses along the complete wound length, providing a more accurate representation of the healing process than traditional punch biopsy methods (Tuca *et al.*, 2024). Additionally, porcine models are particularly useful for studying chronic wounds and infection-associated lesions as they can mimic the complex pathophysiology of these conditions (Parnell, 2013).

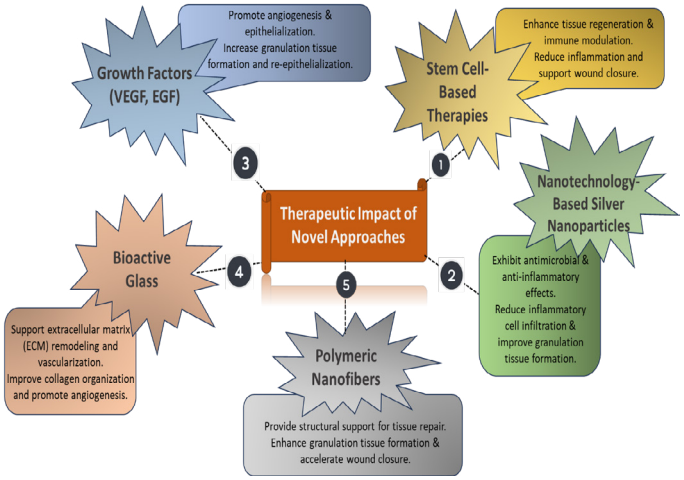

Figure 7: *Therapeutic impact of novel approaches.*

Alternative animal models for wound healing research

In addition to rodents and pigs, animal models, such as rabbits and canines, have been used in wound healing research. Rabbit models are particularly useful for studying corneal wound healing and skin regeneration, while canine models are valuable for evaluating the efficacy of treatments in veterinary medicine (Parnell and Volk, 2019). These alternative models offer unique advantages, such as larger wound sizes and the ability to induce chronic wounds, but they also pose practical challenges, including higher costs and limited availability (Parnell, 2013).

Techniques for histopathological wound healing assessment

Histopathological assessment is essential for evaluating the progression of wound healing and the efficacy of therapeutic interventions. This comprehensive approach enables researchers and clinicians to correlate microscopic tissue changes with functional outcomes in wound repair. Several techniques are employed to visualize and quantify histological markers, providing an objective and detailed understanding of the healing process (Table 4).

Histological staining and immunohistochemistry

Hematoxylin and eosin (H and E) staining is the most widely used technique for assessing general tissue morphology, including evaluating inflammation, cell density, and overall tissue architecture (Yari *et al.*, 2020; Jin *et al.*, 2024). Specialized stains such as Masson's trichrome, which is particularly useful for visualizing collagen deposition and organization, and Picrosirius red, which enables the differentiation between type I and type III collagen, enhance our ability to assess specific tissue components (Possa *et al.*, 2024; Santoso *et al.*, 2024). Combined with quantitative image analysis, these staining methods provide precise collagen content and organization measurements, which are critical for assessing wound healing outcomes (Wong *et al.*, 2009; Mohammadi *et al.*, 2016).

IHC is a powerful tool for detecting specific proteins and cellular markers within tissue samples. This technique allows for visualizing the spatial distribution of key molecules, such as VEGF, CD31, and α-SMA, thereby offering insights into cellular activity and signaling pathways during wound healing (An *et al.*, 2020; Jin *et al.*, 2024). IHC has been employed to investigate the impact of epidermal growth factor (EGF) on re-epithelialization, as well as to elucidate the role of VEGF in angiogenesis (Abbas *et al.*, 2017; Mallick *et al.*, 2022). Integrating IHC with quantitative analysis enables the evaluation of changes in protein expression and cellular activity throughout the healing process, offering critical insights into the mechanisms underlying therapeutic interventions (Hassanshahi *et al.*, 2022).

Quantitative analysis: Morphometry and image-based metrics

Morphometric analysis is critical in evaluating wound healing by providing precise, quantitative measurements of tissue structure and cellular components. This approach involves capturing high-resolution images of stained tissue sections and utilizing specialized software to assess parameters such as cell density, collagen deposition, and immunohistochemical staining intensity (Mohammadi *et al.*, 2016). Histomorphometry, a key technique in this field, enables objective evaluation of healing progress by measuring epithelial thickness, granulation tissue formation, and inflammatory cell infiltration (Hassanshahi *et al.*, 2022). These quantitative assessments are essential for comparing normal and impaired wound healing and determining the efficacy of therapeutic interventions (Aziz *et al.*, 2023).

Digital morphometry and AI-driven image analysis

Digital morphometry, with its unparalleled precision and objectivity, is indispensable for analyzing experimental models' wound areas and other quantitative parameters. The combination of digital photography and image analysis software allows for highly precise measurements of wound size, shape, and other key features, essential for tracking healing progression over time (Kientzy *et al*., 2024).

Integrating artificial intelligence (AI) and deep learning methods has further advanced digital morphometry by automating the analysis of histological images. For instance, deep learning approaches have been developed to predict wound healing progress based on collagen fiber analysis, achieving up to 82% accuracy in classifying six distinct stages of healing (He *et al*. 2024). This innovative approach leverages the unique capabilities of deep learning models to capture subtle variations in collagen fiber features, addressing the limitations of traditional methods that primarily focus on spatial characteristics (He *et al*. 2024).

Moreover, the application of transfer learning and interpretability techniques, such as Layer CAM, has significantly enhanced the accuracy and interpretability of these models. This combination of digital morphometry and AI-driven analysis holds significant promise and instills optimism for the future, improving the efficiency and accuracy of wound area measurements and predicting healing trajectories (He *et al*. 2024).

Cutting-edge imaging technologies

Recent advancements in imaging technologies have significantly transformed histopathological assessment, allowing for more accurate and objective evaluations of wound healing. Confocal and second harmonic generation (SHG) microscopy offer high-resolution imaging capabilities and enable three-dimensional visualization of tissue structures (Figure 5) (Guan *et al*., 2009; Acosta *et al*., 2023). SHG microscopy, in particular, is highly effective for imaging collagen fibers due to its ability to generate contrast based on the non-linear optical properties of collagen. This makes it an invaluable tool for examining collagen organization and remodeling during wound healing (Acosta *et al*., 2023). Additionally, complementary imaging techniques such as laser Doppler imaging and color Doppler ultrasound facilitate real-time evaluation of blood perfusion and angiogenesis, offering a more comprehensive assessment when used alongside traditional histological methods (Salih *et al*., 2020; Li *et al*., 2022).

Modern imaging technologies, such as two-photon excited fluorescence microscopy (TPEF) and laser speckle contrast imaging (LSCI), have revolutionized the field of wound healing research. TPEF, combined with immunofluorescence staining, facilitates three-dimensional reconstruction of blood vessel structures, thereby providing detailed insights into the angiogenic processes occurring during wound healing. This non-invasive technique also enables longitudinal monitoring of vascular network dynamics, allowing researchers to track changes in blood vessel density and morphology throughout the healing process (Li *et al*., 2022).

LSCI, on the other hand, quantifies blood perfusion in wounds, offering additional quantitative data on microcirculatory changes critical for assessing wound healing. Together, these imaging modalities deliver a comprehensive and quantitative evaluation of both angiogenesis and perfusion, improving the overall accuracy of wound healing assessments (Li *et al*., 2022).

Integration of molecular and histological techniques

Combining histopathology with molecular biology approaches: Integrating molecular techniques, such as quantitative polymerase chain reaction (qPCR) and single-cell RNA sequencing (scRNA-seq), with traditional histopathological analysis has provided a more comprehensive understanding of the molecular mechanisms underlying wound healing. For example, qPCR analysis performed alongside histological and immunohistochemical evaluations can yield critical insights into the expression profiles of key genes involved in wound repair, such as VEGF, collagen, and matrix metalloproteinases (MMPs) (Jeon *et al*., 2024).

scRNA-seq, on the other hand, allows for a detailed characterization of cellular heterogeneity within the wound bed. It reveals distinct transcriptomic signatures for various cell types, including endothelial cells, fibroblasts, and macrophages, essential for understanding impaired healing in conditions like diabetic foot ulcers (DFUs). This integrated approach bridges the gap between molecular biology and histopathology, enabling a more nuanced

interpretation of how cellular and molecular changes impact wound healing outcomes (Lu *et al.*, 2023).

Integration of multi-omics approaches

Integrating multi-omics approaches, such as genomics, proteomics, and metabolomics, with histopathological analysis revolutionizes wound healing research. These cutting-edge techniques allow for a comprehensive interrogation of the molecular landscape underlying tissue repair, enabling the discovery of novel therapeutic targets and biomarkers (Figure 6) and sparking excitement about the potential discoveries.

For example, single-cell RNA sequencing (scRNA-seq) has been used to characterize cellular heterogeneity within the wound bed, revealing distinct transcriptomic signatures for various cell types, including endothelial cells, fibroblasts, and macrophages. This high-resolution approach has provided valuable insights into the molecular mechanisms responsible for impaired wound healing in conditions such as diabetic foot ulcers (DFUs) (Lu *et al.*, 2023).

Similarly, proteomic analysis has been employed to identify key proteins and signaling pathways involved in wound healing, such as the TGF-β/Smad and VEGF signaling pathways, critical for orchestrating tissue repair and angiogenesis (Abbas *et al.*, 2017; Pan *et al.*, 2024). These discoveries have directly contributed to developing targeted therapies to modulate these pathways, enhancing tissue repair and regeneration.

Applications in pharmacological and toxicological studies

Histopathological evaluation is a cornerstone of pharmacological and toxicological studies, providing essential insights into the efficacy and safety of therapeutic interventions. By examining tissue architecture, cellular composition, and molecular markers, researchers can objectively assess the impact of various treatments on wound healing and identify potential adverse effects (Table 5, Figure 7).

Evaluating novel therapeutics: From plant extracts to nanotechnology

Histopathological analysis is critical for evaluating the efficacy of novel therapeutics, including plant extracts, phytochemicals, and nanotechnology-based approaches. For instance, studies on silymarin, a flavonoid extracted from Silybum marianum, have demonstrated its ability to enhance fibroblast proliferation, promote granulation tissue formation, and stimulate angiogenesis in a rat model (Aliabadi and Farahmand, 2009). Similarly, research on *Trichosanthes dioica* extract has shown that it improves wound contraction and reduces epithelialization time in albino rats, as evidenced by detailed histopathological analysis (Nurrahmah *et al.*, 2019).

Nanotechnology-based approaches, such as silver and zinc nanoparticles, have been extensively investigated for their potent antimicrobial and anti-inflammatory properties, which help prevent wound infections and support tissue regeneration. Histopathological studies further indicate that when these nanoparticles are appropriately sized and concentrated, they can significantly reduce inflammation and enhance collagen deposition, ultimately leading to improved wound healing outcomes (Ghorbanpour and Wani, 2019; Mallick *et al.*, 2022).

Assessing the safety of therapeutic interventions

Histopathological analysis is essential for evaluating therapeutic efficacy and assessing the safety of wound healing interventions. Studies on 5-fluorouracil (5-FU), a widely used chemotherapeutic agent, have demonstrated its potential to impair microvascular function and delay wound healing in rats. Examining wound tissues from 5-FU-treated animals revealed disrupted microvascular architecture and altered dye distribution, emphasizing the importance of considering macroscopic and microscopic parameters when assessing treatment effects on wound repair (Aszodi and Ponsky, 1985).

The biocompatibility of nanomaterials, such as polymeric nanofibers, has also been extensively evaluated using histopathological techniques. Research indicates that these materials promote re-epithelialization and tissue integration while eliciting minimal inflammatory responses, highlighting their potential as safe and effective wound dressings (Mallick *et al.*, 2022; El-Sherbeni and Negm, 2023).

Growth factors and biologics: Accelerating tissue regeneration

Growth factors and biologic treatments such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-rich plasma (PRP) have shown significant potential in promoting wound healing. Histopathological assessment of

these treatments has consistently revealed increased granulation tissue formation, enhanced angiogenesis, and improved collagen deposition, all of which indicate superior tissue repair (Abbas *et al.*, 2017; Mallick *et al.*, 2022).

For example, PRP, rich in growth factors, has been demonstrated to accelerate wound closure and improve the quality of regenerated tissue in animal models and clinical settings. Histological analysis of PRP-treated wounds has shown increased fibroblast activity and enhanced collagen maturation, underscoring its potential as a powerful therapeutic agent in wound management (Mallick *et al.*, 2022).

Therapeutic strategies and future directions

The impairment of wound healing by infectious and non-infectious diseases necessitates the development of effective therapeutic strategies. These strategies must simultaneously target the underlying causes of impaired healing while actively promoting tissue repair and controlling infection.

Targeting infectious agents: Antibiotics, nanoparticles, and phage lysins

Combating infectious agents is paramount in promoting wound healing. This approach involves diagnosing the infecting pathogen, assessing its susceptibility to antimicrobial agents, and implementing targeted treatment regimens (Becerra *et al.*, 2016; Rai *et al.*, 2017). Furthermore, developing novel antimicrobial agents, such as phage lysins (Cheng *et al.*, 2018) and Phyto-engineered gold nanoparticles (Boomi *et al.*, 2020), holds significant promise in overcoming the challenges of antibiotic resistance.

Addressing systemic factors: Comprehensive care for healing

Managing underlying systemic conditions is crucial for improving wound healing outcomes. In diabetes, maintaining strict glycemic control alongside interventions that target endothelial dysfunction and neuropathy is essential for effective healing (Vatankhah *et al.*, 2017). Similarly, in obesity, weight management and strategies to reduce chronic inflammation are key to enhancing tissue repair. For autoimmune diseases, therapies that modulate the dysregulated immune response are necessary to alleviate chronic inflammation and minimize tissue damage (Zhao, 2023). In vascular diseases, interventions such as revascularization procedures are vital to restore adequate blood flow and oxygenation to the wound site, promoting healing (Lavery *et al.*, 2016; Yang *et al.*, 2021). Additionally, using growth factors and other targeted therapeutic agents offers promising avenues to enhance aspects of the healing process (Raffetto *et al.*, 2020).

Therapeutic strategies for chronic wounds

Histopathological evaluation has been instrumental in guiding the development of therapeutic strategies for chronic wounds. For instance, applying growth factors such as VEGF and EGF has demonstrated promise in promoting angiogenesis and re-epithelialization, thereby accelerating wound closure (Mallick *et al.*, 2022; Abbas *et al.*, 2017). Similarly, stem cell-based therapies have shown potential in enhancing tissue regeneration and mitigating inflammation in chronic wound models (Nurrahmah *et al.*, 2019). Nanotechnology-based approaches, such as silver nanoparticles and polymeric nanofibers, have been explored for their dual ability to combat infection and promote tissue repair in chronic wounds. (Ghorbanpour and Wani, 2019; Mallick *et al.*, 2022). Histopathological studies have demonstrated that these nanomaterials can reduce inflammatory cell infiltration and enhance granulation tissue formation, improving healing outcomes (Ghorbanpour and Wani, 2019; Mallick *et al.*, 2022).

Advanced wound care strategies

Optimizing wound care is critical for achieving favorable healing outcomes. This includes appropriate wound bed preparation, which involves the debridement of necrotic tissue and the removal of bioburden to create a conducive environment for healing (Barrett, 2017). Select dressings that maintain a moist wound environment, effectively control exudate, and protect the peri-wound skin (Lavery *et al.*, 2016). Moreover, negative pressure wound therapy (NPWT) has effectively promoted granulation tissue formation and reduced edema, particularly in high-risk patients (Scalise *et al.*, 2016). Applying bioengineered skin substitutes, growth factors, and other advanced therapies further offers potential to enhance wound healing outcomes (Lavery *et al.*, 2016). Nanotechnology-based approaches, such as cerium oxide nanoparticles (Allu *et al.*, 2023) and collagen-nanoparticle composites (Grigore *et al.*, 2017), promise to reduce inflammation, promote angiogenesis, and combat infection.

Development of advanced biomaterials

The development of advanced biomaterials is an emerging trend in wound healing research. These novel materials, including bioactive glass, polymeric nanofibers, and hydrogels, are engineered to mimic the ECM, thereby providing a supportive scaffold that facilitates cell migration, proliferation, and differentiation (Mallick *et al*., 2022; Vargas *et al*., 2024). For example, chitosan-based hydrogels have been shown to accelerate wound closure rates and enhance collagen deposition in rat models, making them promising candidates for clinical applications in wound healing (Jeon *et al*., 2024). Similarly, bioactive glass has been utilized to promote angiogenesis and ECM remodeling, improving healing outcomes in diabetic wound models (Vargas *et al*., 2024).

Personalized medicine: Customizing treatment for optimal outcomes

Personalized medicine is an emerging approach that tailors treatment strategies to the unique characteristics of individual patients, such as genetic predisposition, comorbidities, and nutritional status. This approach uses genomic profiling, biomarker analysis, and patient-specific therapies to optimize wound healing outcomes. For example, studies on adipose-derived stem cells (ADSCs) have shown that these cells can be genetically modified to express therapeutic genes such as VEGF and TGF-β, enhancing their regenerative potential (Wang *et al*., 2024). Similarly, the formulation of patient-specific growth factor cocktails has been demonstrated to improve healing outcomes in chronic wound models by providing tailored therapeutic support (Mallick *et al*., 2022).

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Novelty Statement

This review presents a novel framework that integrates advanced histopathological techniques with omics-driven biomarkers to better understand the cellular dynamics in wound healing. This approach offers high-resolution insights into wound repair processes, enabling the development of personalized therapeutic strategies that target specific pathological microenvironments.

Author's Contribution

Mohamed R. Mansour, Nada A. Ashour, Aliaa M. Gaballah, Yara E. Elzoghby, Rania M. Elbatawy, Mostafa Shoraba, Salma Shoulah: Writing original draft preparation, writing review and editing.

All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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