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Surgery and Wound Healing. Modern Mechanisms, Protocols and Techniques

EDITURA UNIVERSITĂŢII DIN ORADEA

2026

Mircea Ioan Şandor, Ioan Lucian Borza, Maur Sebastian Horgoş, *Surgery and Wound Healing. Modern Mechanisms, Protocols and Techniques*, Oradea: Editura Universităţii din Oradea, 2026.

ISBN 978-606-10-2509-1

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SUMMARY

Preface

The wound healing process is one of the most complex and fascinating biological reactions of the human body, a fine balance between inflammation, regeneration and tissue remodeling. Although the physiology of these processes has been known for centuries, many aspects of how cellular responses, molecular factors, and therapeutic interventions interact to restore tissue integrity remain poorly elucidated.

Over the past few decades, advances in regenerative surgery, biotechnology, and laser medicine have opened up new horizons in the treatment of acute and chronic wounds. From platelet-rich plasma (PRP) therapies to smart controlled-release dressings, the modern approach to wounds involves an interdisciplinary vision in which the surgeon, biologist, pathologist, and regenerative medicine specialist collaborate toward the same goal: fast, safe, and functional healing.

This book aims to synthesize the clinical, experimental and scientific experience accumulated in recent years in the field of wound surgery, integrating the results of our own research with the latest evidence from the international literature. The book is structured to provide a complete overview of the mechanisms, protocols, and techniques involved in tissue healing, from fundamental cellular processes to cutting-edge technologies used in clinical practice.

We dedicate this paper to all fellow surgeons, residents, students and researchers who, through perseverance and passion, contribute to improving the quality of life of patients with chronic or complex wounds. This book is

not only a theoretical synthesis, but also an invitation to reflection and innovation towards a wound surgery guided by science, empathy and responsibility. The Authors

General Introduction

Wound healing is a biological process essential for survival that involves a coordinated sequence of cellular and molecular mechanisms aimed at restoring the structural and functional integrity of tissues. In modern surgical practice, wound management goes far beyond simply closing a skin gap, it requires a deep understanding of local pathophysiology, systemic factors, and technologies that can optimize tissue regeneration.

In the context of the escalating prevalence of chronic diseases (diabetes, venous insufficiency, peripheral ischemia), the aging of the population and the emergence of resistant bacterial strains, the treatment of chronic wounds has become a major challenge of current medicine. The management of these patients requires a multidisciplinary approach, in which classical surgery is combined with biotechnology, immunology and tissue engineering.

This paper is structured in seven chapters that address:

1. the biological and molecular mechanisms involved in tissue healing,
2. modern diagnostic and clinical assessment tools,
3. evidence-based treatment protocols,
4. emerging regenerative techniques,
5. specific surgery of complex wounds,
6. and, finally, the future prospects of research and practice in the field.

Each chapter combines theoretical concepts with practical experience gained in surgical practice, including clinical cases, validated therapeutic protocols, and observations from clinical-experimental research.

The purpose of the volume is twofold: on the one hand, to provide a complete guide for practitioners who deal with complex wounds on a daily basis, and on the other hand, to provide a solid basis for future research in regenerative surgery and tissue biotherapies.

In a constantly changing medical world, wound surgery is continually redefining itself, and understanding the underlying mechanisms of healing is key to therapeutic progress.

Chapter 1. Biological and Pathophysiological Foundations of Wound Healing

1.1. Classification of wounds: acute, chronic, atone, infected [1–20]

1.

Wound healing is a complex, dynamic, and coordinated process that aims to restore the structural and functional integrity of the skin and other injured tissues. Depending on the etiology, evolution and mechanisms involved, wounds can be classified into several categories, the most important of which are: acute, chronic, atone and infected wounds.

1. Acute wounds

Acute wounds occur as a result of a unique and well-defined traumatic aggression (cuts, punctures, burns, surgery, etc.), and their healing follows a normal physiological course, in a relatively short period of time (generally 7–21 days).

Main features of acute wounds:

1. Sudden presentation and clear delineation of the edges;
2. Short-term local inflammation;
3. Fast healing, with minimal scar tissue formation;
4. Low risk of complications, if infection is not associated.

The biological processes involved in the healing of acute wounds include hemostasis, inflammation, tissue proliferation and remodeling**.**

5. Chronic wounds

Chronic wounds are lesions that do not follow the normal healing process or in which healing is delayed beyond 6–8 weeks. They usually occur on a pre-existing pathological terrain – for example, venous insufficiency, diabetes, arterial ischemia or prolonged pressure.

Features:

1. Persistence of local inflammation;
2. Imbalance between degradation and synthesis of the extracellular matrix;
3. Poor circulation and reduced oxygenation of the tissues;
4. Possible chronic microbial contamination.

Typical examples include: venous ulcers, arterial ulcers, bedsores, and diabetic foot.

1. Atonic Wounds

Atonic wounds are a particular form of chronic wound, characterized by the lack or slowing down of the formation of granulation tissue. In these lesions, the regeneration process is stagnant, the wound edges are often thin and pale, and the surface is covered with a serofibrinous or necrotic exudate.

Favouring factors:

1. Local circulatory disorders;
2. Nutritional deficiencies (especially protein, zinc, vitamin C deficiency);
3. Persistent infections;
4. Inadequate local care.

The treatment of atonic wounds aims to stimulate the formation of granulation tissue by local methods (debridement, modern dressings, negative pressure therapies) and to correct systemic factors.

5. Infected wounds

Infected wounds occur when pathogenic microorganisms multiply in the wound, exceeding the body's local defense capacity. The infection can occur in both acute and chronic wounds, and is one of the most important causes of delayed healing.

Clinical signs:

1. Erythema, local heat, edema, intense pain;
2. Purulent discharge and unpleasant odor;
3. Possible fever and general signs of infection.

Microscopically, infection causes tissue destruction, increased inflammation and inhibition of collagen synthesis. Treatment includes rigorous wound cleaning, local or systemic antibiotic therapy (depending on severity), and monitoring of progress.

1. 2. Stages of tissue healing: hemostasis, inflammation, proliferation, remodeling [21–40]

The tissue healing process is a complex biological mechanism, essential for restoring the structural and functional integrity of injured tissues. It involves a well-orchestrated sequence of cellular and molecular events that take place in four major stages: hemostasis, inflammation, proliferation, and remodeling (maturation). Although these phases are described separately for didactic reasons, in reality they partially overlap and interact dynamically, ensuring the efficient transition from injury to regeneration.

Hemostasis phase

Immediately after the injury, the body's priority is to stop the bleeding and stabilize the affected area. Hemostasis begins with reflex vasoconstriction of the arterioles and platelet activation, which leads to the

formation of the primary platelet plug. Platelets release growth factors (PDGF, TGF-β, VEGF) and adhesion molecules that facilitate the recruitment of inflammatory cells. In parallel, the coagulation cascade is activated, resulting in the formation of a fibrin clot that serves as a temporary matrix and physical barrier against microbial contamination. The clot has a dual role: hemostatic and biological, providing the initial environment for subsequent cell migration.

Inflammatory phase

A few hours after the injury, the inflammatory phase sets in, which eliminates cellular debris and pathogens and prepares the ground for regeneration. Neutrophils are the first cells to be recruited, followed by macrophages, which coordinate the transition to repair by releasing cytokines (IL-1, IL-6, TNF-α) and growth factors (VEGF, FGF, TGF-β). Inflammation is accompanied by vasodilation, increased capillary permeability, and migration of leukocytes into the interstitial space. Although essential for initiating healing, inflammation must be controlled because its persistence can lead to delayed healing or the formation of pathological scars.

Proliferative phase

This stage is characterized by the formation of granulation tissue, a tissue rich in endothelial cells, fibroblasts and new extracellular matrix. Under the action of growth factors, angiogenesis provides the supply of oxygen and nutrients, while fibroblasts synthesize collagen (especially type III), fibronectin and proteoglycans, contributing to the restoration of the matrix. In parallel, keratinocytes migrate and proliferate to re-epithelialize the wound surface. The coordinated activity among epithelial, endothelial, and mesenchymal cells drives the progressive reconstruction of tissue architecture.

Remodeling phase (maturation)

In the last stage, the granulation tissue is gradually replaced by mature scar tissue. Fibroblasts turn into myofibroblasts, which contract the edges of the wound, reducing its size. Type III collagen is replaced by type I collagen, which is more resistant, and the collagen fibers align according to mechanical tension lines. In this phase, vascular density decreases, and the scar acquires a firm consistency, with a tensile strength of about 70–80% of intact tissue. Remodeling can continue for months or even years, depending on the location and depth of the lesion, but also on the patient's metabolic status.

The tissue healing process is influenced by numerous systemic and local factors: age, nutrition, oxygenation, the presence of infection, comorbidities (diabetes, vascular insufficiency), and the way the wound is managed (debridement, moisture control, oxygen therapy, use of bioactive dressings). A detailed understanding of the stages of healing is fundamental for the application of modern therapeutic protocols, including regenerative therapies, cellular treatments or smart biopolymers, aimed at accelerating regeneration and minimizing scar formation.

1. 3. The role of growth factors and the extracellular matrix [41–55]

Tissue healing is a complex process, dependent not only on cellular activity, but also on the close interaction between molecular mediators and the extracellular matrix (ECM). This biochemical and structural interface regulates the migration, proliferation and differentiation of cells involved in the regeneration of injured tissue. The growth factors act as coordination signals, while the extracellular matrix provides the physical and biochemical support for the reconstitution of the tissue architecture.

1.3.1. Growth factors – key mediators of regeneration

Growth factors are signal proteins that modulate cellular behavior in the healing stages. They are secreted by platelets, macrophages, fibroblasts, endothelial and epithelial cells**,** and act by binding to specific receptors on the surface of target cells.

The main factors involved include:

1. PDGF (Platelet-Derived Growth Factor) – released early from platelets, stimulates fibroblast proliferation and collagen synthesis; it plays a central role in the transition from the inflammatory to the proliferative phase.
2. TGF-β (Transforming Growth Factor-beta) – promotes extracellular matrix synthesis, inhibits collagen degradation and regulates myofibroblast differentiation. It is essential for tissue remodeling and scar formation.
3. VEGF (Vascular Endothelial Growth Factor) – induces angiogenesis, favoring the creation of new capillaries that provide oxygenation and nutrition to the granulation tissue.
4. FGF (Fibroblast Growth Factor) – stimulates the proliferation of fibroblasts and keratinocytes, accelerating re-epithelialization.
5. EGF (Epidermal Growth Factor) – activates epithelial regeneration and keratinocyte migration, contributing to the restoration of the superficial layer of the skin**.**
6. IGF-1 (Insulin-like Growth Factor-1**)** – stimulates protein and collagen synthesis, being involved in muscle and tissue recovery.

These growth factors do not act in isolation, but in a system of interdependence and feedback. For example, VEGF and FGF act synergistically in angiogenesis, and TGF-β modulates receptor expression for other growth factors. Imbalances in the secretion or signaling of these molecules can lead to delayed healing, excessive fibrosis or abnormal scarring**.**

Extracellular matrix – the biological skeleton of regeneration

The extracellular matrix (ECM) is the three-dimensional structure in which cells carry out their regenerative activity. It is composed of structural proteins (collagen, elastin), adhesion glycoproteins (fibronectin, laminin) and proteoglycans that form a complex network.

The main roles of the MEC in healing include:

1. Mechanical and architectural support – provides the necessary substrate for cell migration and granulation tissue formation.
2. Growth factor reservoir – MEC stores and releases bioactive molecules (e.g. TGF-β, FGF) in a controlled manner, prolonging their action over time.
3. Regulation of cell signaling – through cell-matrix interactions (via integrins), ECM influences the polarization, differentiation and metabolic behavior of cells.
4. Remodeling coordination – controlled degradation of MEC by metalloproteinases (MMPs) allows collagen reorganization and transition to mature scar tissue.

In the proliferative phase, the provisional ECM is rich in type III collagen and fibronectin, elements that favor fibroblast migration. In the remodeling phase, it is replaced by type I collagen, which is more resistant and better organized.

The interaction between growth factors and the matrix

A defining feature of the healing process is the two-way dialogue between cells, growth factors, and ECM. Growth factors influence the expression of matrix proteins, and the structure of the matrix, in turn, regulates the sensitivity of cells to these signals. For example, TGF-β stimulates collagen synthesis, but also the activity of MMPs, maintaining the balance between matrix deposition and degradation.

This interaction is crucial in modern regenerative therapies, where biomaterials and synthetic matrices produced by bioengineering are used to mimic the physiological properties of natural ECMs and deliver growth factors in a controlled manner. Systems based on hydrogels, native collagen or autologous fibrin have demonstrated increased efficiency in stimulating accelerated healing and reducing the risk of hypertrophic scarring.

Overall, the coordination between growth factors and the extracellular matrix determines the quality and speed of tissue regeneration. An in-depth understanding of these mechanisms enables the development of innovative therapeutic strategies, such as platelet-rich plasma (PRP) applications, mesenchymal stem cell tissue engineering, and smart biomaterials that gradually release growth factors. These approaches open up promising prospects for regenerative surgery, chronic wound medicine, and personalized tissue reconstruction.

1. 4. Disorders of the tissue repair process – from persistent inflammation to fibrosis [56–65]

Tissue healing is a delicate balance between destruction and regeneration processes. When this balance is disturbed, repair no longer follows the physiological course, and the resulting tissue becomes structurally and functionally abnormal. Disorders can occur at any stage of

the process, but the most common pathological causes are persistent inflammation, insufficient formation of new tissue and excessive deposition of extracellular matrix, which leads to fibrosis.

Persistent inflammation – causes and consequences

Prolonged inflammation is one of the main causes of poor healing. Normally, the inflammatory reaction is self-limited: macrophages switch from a proinflammatory phenotype (M1) to a reparative (M2) phenotype, facilitating the transition to the proliferative phase. When this switching mechanism fails, proinflammatory cytokines (IL-1β, TNF-α, IFN-γ) remain elevated, maintaining a destructive microenvironment.

Common causes include chronic infections, tissue ischemia, foreign bodies, metabolic disorders (diabetes), irradiation, or autoimmune diseases. The affected tissue shows chronic inflammatory infiltrate, continuous enzymatic degradation and the formation of a fibroinflammatory granuloma. The persistence of inflammation blocks cell regeneration, and fibroblasts become overactive, secreting excessive collagen and proteoglycans.

Delayed healing and progressive necrosis

When the early phases of healing are ineffective, the regeneration process is prolonged, leading to chronic wounds. They are characterized by local hypoxia**,** deficiency of growth factors and endothelial dysfunction, which prevents the formation of new vessels. In addition, high oxidative stress affects mitochondria and inhibits the proliferation of keratinocytes and fibroblasts.

A typical clinical example is diabetic ulcers, where microangiopathy and peripheral neuropathy prevent re-epithelialization. Under these conditions, the balance between the degradation and synthesis of the

extracellular matrix is lost, and the tissue remains stuck in a state of „low-intensity inflammation", with no progress towards the remodeling phase.

Fibrosis – the final result of pathological repair

When the inflammatory process is not resolved, the body tends to „seal" the injured area by forming dense tissue rich in collagen – a phenomenon known as fibrosis. This is not a genuine regeneration, but a substitute repair.

Fibroblasts, chronically stimulated by TGF-β, IL-13 and angiotensin II, transform into contractile myofibroblasts that produce type I and III collagen at an accelerated rate. Their excessive activity, combined with the inhibition of metalloproteinases (MMPs), loss of normal architecture.

Fibrosis can affect multiple organs – liver, lungs, heart, kidneys or skin – and is a major cause of chronic organ failure. In the skin and muscles, it translates into hypertrophic or keloid scars, with local stiffness and dysfunction.

Cellular imbalances and aberrant signaling

Healing disorders involve not only inflammatory mediators, but also abnormalities of cell-matrix communication. Aberrant signaling via the Wnt/β-catenin**,** Notch, or TGF-β/Smad pathways keeps fibroblasts in a continuous proliferative state. At the same time, insufficient apoptosis of inflammatory cells and reduced autophagy contribute to the accumulation of cellular debris and oxidized proteins.

Thus, a vicious circle is installed between inflammation, oxidative stress and fibrogenesis, in which the tissue is no longer able to return to its homeostatic state. This pathological pattern is found in chronic wounds, post-radiotherapy fibrosis and in some autoimmune diseases (scleroderma, cutaneous lupus).

Modern Therapeutic Perspectives

The approach to healing disorders is currently oriented towards modulating the tissue microenvironment, rather than simple symptomatic treatments.

Modern strategies include:

1. use of TGF-β and PDGF inhibitors to control fibrogenesis;
2. antioxidants and selective anti-inflammatory agents (e.g. IL-1β inhibitors, NLRP3) to reduce chronic oxidative stress;
3. cell and recombinant growth factor therapies to rebalance the regeneration process;
4. Smart biomaterials (hydrogels, collagen, chitosan) capable of regulating the controlled release of mediators.

In parallel, recent research is exploring the role of microRNAs and mesenchymal stem cells in limiting persistent inflammation and stimulating functional regeneration.

Disorders in the tissue repair process reflect an imbalance between destruction and reconstruction. Persistent inflammation, hypoxia, oxidative stress and excessive activation of fibroblasts are key links that transform a physiological process into a pathological one. The understanding of these mechanisms provides the foundation for modern regenerative therapies, oriented not only towards wound closure, but towards the complete restoration of tissue function.

1. 5. Immunological and microbiological peculiarities in chronic wounds [66–75]

Chronic wounds are pathological conditions in which the normal healing process is interrupted, and the damaged tissue remains in a

persistent inflammatory phase. This dysfunction results from a complex interaction between immunological, microbiological and metabolic factors, which maintain a microenvironment unfavorable to regeneration. In contrast to acute wounds, which follow an orderly sequence of events, chronic wounds are characterized by continuous inflammation, bacterial colonization, imbalance between cytokines and excessive degradation of the extracellular matrix.

Local immunological disorders

In chronic wounds, the local immune system fails to transition from the inflammatory to the reparative phase. Macrophages remain predominantly in the pro-inflammatory M1 phenotype, secreting large amounts of IL-1β, TNF-α, and IL-6, which perpetuate inflammation and degrade the extracellular matrix by activating metalloproteinases (MMPs) such as MMP-2 and MMP-9. Normally, conversion to the M2 phenotype (anti-inflammatory and repairing) should stimulate angiogenesis and collagen synthesis, but in chronic wounds this transition is blocked.

Also, neutrophils persist abnormally long in the lesion, releasing proteolytic enzymes (elastase, collagenase) and reactive oxygen species that cause secondary lesions. This proteolytic activity degrades growth factors and compromises matrix integrity, thereby preventing cell migration.

In parallel, regulatory T lymphocytes (Treg) and dendritic cells show reduced activity, limiting local immune control. This disruption of the adaptive immune response favors the persistence of inflammation and tolerance to resident bacteria.

The role of microbiota and bacterial biofilm

The microbiological component of chronic wounds is dominated by biofilm formation, a three-dimensional community of microorganisms encased in a protective polysaccharide matrix. This biofilm acts as a

physical barrier against phagocytes and antibiotics, allowing bacteria to survive under stress.

The most commonly isolated species include Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis and Proteus mirabilis, often in polymicrobial combinations. These bacteria secrete virulence factors that:

1. stimulates the secretion of pro-inflammatory cytokines;
2. induce apoptosis of epithelial and fibroblastic cells;
3. alters local pH and tissue oxygenation.

For example, *Pseudomonas aeruginosa* produces phenagenic pigments and enzymes such as elastase, which degrade collagen and inhibit keratinocyte proliferation. *Staphylococcus aureus* interferes with immune signaling through superantigens, causing an exaggerated and resolution-resistant inflammatory response.

Biofilm is particularly difficult to remove, requiring repeated mechanical debridement and adjuvant antimicrobial therapies (solutions with silver, iodine, hyperoxygenated fatty acids or photodynamic therapies).

Metabolic microenvironment and hypoxia

Chronic hypoxia, commonly associated with peripheral ischemia, contributes to alterations in the immune response and the selection of anaerobic bacteria. In the absence of adequate oxygen, epithelial cells and fibroblasts reduce their proliferative activity, and macrophages shift their metabolism toward glycolysis, thereby generating local acidosis and accentuating oxidative stress.

This combination favors the maintenance of inflammation and the creation of an environment conducive to bacterial colonization. In addition,

persistent oxidative stress leads to oxidation of matrix proteins and reduced bioavailability of growth factors, significantly limiting the regeneration process.

Interaction between dysbiosis and immunity

The microbiota of healthy skin plays a protective role, maintaining local immune balance. In chronic wounds, this balance is disturbed, a phenomenon known as dysbiosis. A decrease in beneficial commensal bacteria (*Staphylococcus epidermidis*, *Corynebacterium spp.*) and a proliferation of pathogenic species.

This dysbiosis directly influences the expression of inflammatory genes and the synthesis of antimicrobial peptides. Recent studies have shown that polymicrobial biofilms generate a heterogeneous immune response, in which some macrophage subpopulations become tolerant and others hyperreactive, accentuating tissue destruction.

Therefore, understanding the relationship between the wound microbiome and the local immune system becomes essential for the development of targeted therapies — including the use of topical probiotics and enzymes capable of dislodging biofilms.

Therapeutic implications

The treatment of chronic wounds requires an integrated approach that combines infection control, modulation of the immune response and stimulation of tissue regeneration.

Modern strategies include:

1. combined local antimicrobial therapies (antibiotics, silver ions, hypochlorite solutions, ozone therapy);
2. topical immunomodulators (recombinant growth factors, anti-inflammatory interleukins, PRP);

1. emerging biological therapies – such as the use of bacteriophages against resistant species and microbiome-based therapies;

2. hyperbaric oxygen therapy and blue light phototherapy to reduce biofilm and stimulate cellular oxygenation.

The purpose of these interventions is to restore a balanced immune and microbiological environment that enables the transition to the proliferative phase and the formation of functional tissue. Chronic wounds are an example of the interdependence between immune dysregulation and microbial colonization. While the immune system remains locked in a destructive inflammatory response, the bacterial biofilm exploits the tissue's vulnerability, preventing regeneration.

The recognition of these immunological and microbiological particularities is fundamental for the application of personalized therapies, which restore the balance between defense and regeneration and prevent the irreversible chronicity of the lesions.

Summary – Biological and pathophysiological foundations of wound healing

Section	*Essential content*	*Processes/Factors involved*	*Pathological or clinical aspects*
1.1. Classification of wounds: acute, chronic, atone, infected	The main types of wounds, defined by evolution and etiology.	- Acute wounds – traumatic, surgical; - Chronic wounds – ulcers, bedsores, diabetic foot; - Atonic wounds – stagnant, without	- Normal healing (7–21 days) in acute wounds; - Delayed healing (>6 weeks) in chronic wounds; - Fibrinous/necrotic exudate in atone wounds;

		granulation; - Infected wounds – active bacterial colonization.	- Erythema, edema, secretion purulent in infected wounds.
1.2. Stages of tissue healing: hemostasis, inflammation, proliferation, remodeling	The physiological sequence of the healing process.	- Hemostasis: vasoconstriction, platelet activation, fibrin. - Inflammation: neutrophils, macrophages; cytokines (IL-1, IL-6, TNF-α). - Proliferation: angiogenesis, collagen III, re-epithelialization. - Remodeling: collagen I, wound contraction, scar maturation.	- Normal healing with restoration of tissue integrity; Imbalances between phases can produce abnormal scarring or slow healing.
1.3. Role of growth factors and the extracellular matrix (ECM)	Molecular coordination between mediators and tissue structure.	- Growth factors: PDGF, TGF-β, VEGF, FGF, EGF, IGF-1. - ECM: collagen, elastin, fibronectin, laminin, proteoglycans.	- Synthesis/degradation imbalance → delayed healing or fibrosis. - Therapies: PRP, stem cells, smart biomaterials.
1.4. Disorders of the repair process – from persistent inflammation to fibrosis	Causes and mechanisms of pathological healing.	- Chronic inflammation (IL-1β, TNF-α, IFN-γ), hypoxia, oxidative stress.	- Chronic wounds, ulcers, necrosis. - Skin fibrosis, hypertrophic/keloid scars.

		- Persistent activation of fibroblasts (TGF-β, IL-13).	- Imbalance between MMPs and collagen.
1.5. Immunological and microbiological peculiarities in chronic wounds	Immune-microbiota interaction in delayed healing.	- Predominantly M1 macrophages; persistent neutrophils; elevated MMP-2/9 levels. - Bacterial biofilm (S. aureus, P. aeruginosa); hypoxia, acidosis; dysbiosis.	- Perpetuated inflammation, matrix degradation, inhibition of epithelialization. - Requires combination therapies (antimicrobials, PRP, bacteriophages, hyperbaric oxygen).

Note: The table summarizes the structure of the chapter and the key relationships between the physiological and pathological mechanisms of wound healing.

Chapter 2. Clinical and Paraclinical Evaluation of Wounds

2.1. Principles of diagnosis and local assessment [76–95]

The tissue healing process cannot be understood and managed correctly without rigorous clinical and paraclinical evaluation of the wounds. The evaluation aims to determine the stage of the injury, the factors that influence it, and the risk of local or systemic complications. A correct analysis provides the basis for choosing the optimal therapeutic strategy, monitoring the evolution and adjusting the treatment. The evaluation of a wound should be seen as a continuous process, not a single act from the moment of the patient's presentation to the completion of healing.

History and general clinical background

The first stage of diagnosis consists of obtaining a detailed anamnesis, which includes the circumstances of the lesion (mechanism, time elapsed since the trauma, conditions of contamination) and the patient's general condition. It is important to identify systemic factors that can interfere with healing — diabetes, venous insufficiency, arteritis obliterans, immunodepression, malnutrition, corticosteroid or cytostatic treatments.

The history of surgeries, previous infections, as well as any allergic reactions to antiseptics or antibiotics are noted. These data guide the doctor on the risk of complications and the type of wound: acute, chronic, surgical, traumatic, ischemic or infectious.

Local examination – inspection and palpation

The local examination should be carried out in a sterile environment with good lighting and, ideally, after removing the previous dressing. The evaluation is carried out systematically, according to the principles of the TIME method (Tissue, Infection/Inflammation, Moisture, Edge):

1. T – Tissue (lesion tissue): the type of tissue present (necrotic, fibrinous, granulation, epithelial) is observed and the

proportion of each is noted. The black necrotic tissue indicates ischemia, the fibrinous yellow tissue signals infection, and the red-granular tissue indicates active healing.

2. I – Infection/Inflammation: signs of infection (edema, erythema, purulent exudate, fetid odor) or non-infectious inflammation are sought. The intensity of pain and the presence of abnormal secretions are assessed.

3. M – Moisture: an optimal level of humidity favors cell migration; excess causes maceration of the edges, and dryness leads to necrosis.

4. E – Edge: the appearance of the wound edges is observed — sharp, curved, undermined or hyperkeratotic. Irregular and thickened edges can signal stagnation in the healing process.

In addition to inspection, palpation provides information about local temperature, tissue consistency, and area sensitivity. An elevated temperature and pain on palpation suggest active infection, while cooling of the area may signal ischemia or necrosis.

Size and depth assessment

The dimensions of the wound are measured standardly, using a sterile planimeter or millimeter ruler: the length, width and depth are noted at each check. The total area (cm^2) or estimated volume (cm^3) can also be calculated. To monitor the evolution, it is recommended to periodically photograph under the same light and distance conditions.

The depth of the wound is determined with a sterile probe, noting the existence of underlying cavities, sinuses or drainage bags. Deep wounds or wounds with tunneling require careful exploration, as they can hide septic outbreaks.

Evaluation of exudate and odor

The exudate provides information about the stage of inflammation and the degree of infection:

1. serous, clear – normal healing;
2. serosanguinolent – capillary fragility;
3. purulent – bacterial infection;
4. hemorrhagic – trauma or vascular erosion.

Unpleasant odor is commonly associated with anaerobic infection or colonization with *Pseudomonas aeruginosa* (sweet, characteristic odor). It is recommended to take the exudate for bacteriological examination and antibiogram, before initiating empirical antibiotic therapy.

Evaluation of vascularization and sensitivity

An essential part of the diagnosis is the assessment of the local blood infusion. The following can be used:

1. hair refill test (normal <2 seconds);
2. ankle-brachial index (ABI) for the evaluation of peripheral ischemia;
3. vascular Doppler or transcutaneous oximetry to measure tissue oxygenation.

Skin sensitivity (tactile, painful, thermal) is also evaluated, as peripheral neuropathies, especially in diabetics, can mask serious lesions or extensive infections.

Classification of wounds

The classification of wounds facilitates the therapeutic decision. The most commonly used systems include:

1. NPUAP/EPUAP classification for bedsores (stages I–IV, depending on depth);
2. Wagner classification for diabetic ulcers;

3. Rutherford classification for critical ischemia;

4. Gustilo–Anderson classification for traumatic wounds with open fractures.

The choice of classification system depends on the etiology and the purpose of the evaluation (surgical, dermatological, vascular, metabolic).

Documentation and monitoring of progress

All clinical data must be documented in the wound evolution sheet, including daily descriptions, treatments applied, photographic images, and correlated biological parameters. This monitoring enables identification of cure stagnation, reinfection, or complications, allowing prompt adaptation of the therapeutic plan.

Rigorous clinical evaluation of wounds is the cornerstone of effective management. The integration of anamnesis, detailed local examination and modern measuring instruments allows for a complete, objective and reproducible diagnosis. In the next chapter, we will address paraclinical investigations — biological parameters, imaging and microbiological methods that complement clinical assessment and guide personalized treatment.

2.2. Clinical scores and digital wound monitoring tools [96–110]

The evaluation of wound evolution involves an objective, repeatable analysis of clinical parameters that reflect the healing process. In modern medical practice, this assessment is based on the use of standardized clinical scores and digital monitoring tools, which provide quantifiable data, reduce inter-observer variability, and facilitate longitudinal tracking of therapeutic response.

Clinical scores used in wound assessment

Among the most commonly used rating systems are:

1. **Bates-Jensen Wound Assessment Tool (BWAT)** – evaluates 13 parameters, including size, depth, edge, necrosis, exudate, color, edema, and epithelialization. The total score varies between 13 (wound healed) and 65 (severe wound), being considered one of the most sensitive tools for monitoring healing dynamics.

2. **Pressure Ulcer Scale for Healing (PUSH Tool)** – developed by the *National Pressure Ulcer Advisory Panel*, quantifies the size of the wound, the amount of exudate, and the appearance of the base tissue. It is easy to apply in clinics, providing a quick method of documenting progress.

3. **DESIGN-R Score** – a system used especially in Japan and Europe, which integrates seven dimensions (Size, Exudate, Infection, Granulation, Necrosis, Re-epithelialization and Depth – „Depth"), weighted to provide a total score of severity and progress over time.

4. **Wound Healing Index (WHI)** – based on predictive models and integrated clinical parameters, it is used especially for chronic ulcers, providing information on the probability of healing depending on the patient's general condition and lesion characteristics.

Table of the main clinical scores

Clinical Score	Evaluated parameters	Scale / Domain	Main advantages
BWAT	13 parameters: size, depth, edges, necrosis, exudate, etc.	13–65	Sensitive, objective, recommended for chronic wounds.

PUSH Tool	Size, exudate, base tissue.	0–17	Simple, fast, useful for clinical documentation.
DESIGN-R	Size, exudate, infection, granulation, necrosis, re-epithelialization, depth.	0–66	Detailed, internationally recognized score.
WHI	Clinical factors + characteristics of the wound.	Model predictiv	Estimate the probability of cure.

These standardized tools have the role of standardizing the documentation of evolution, allowing comparability between clinical trials and medical centers, as well as validating the effectiveness of topical or systemic treatments.

Digital monitoring tools

With the digitalization of medicine, wound monitoring has undergone a significant transformation. Digital platforms, mobile apps, and integrated 3D imaging systems have become essential components of modern wound management.

✓ **Fotogrammetria și analiza imagistică digitală** permit măsurarea precisă a suprafeței și volumului plăgii, folosind algoritmi de recunoaștere a marginilor și corecție a distorsiunii optice. Software-uri precum *eKare inSight*, *WoundZoom* sau *Swift Skin and Wound* oferă date obiective, arhivare automată și compararea imaginilor în timp real.

1. **Telemedicine and mobile apps** facilitate remote assessment, especially for patients with reduced mobility or in isolated areas. Doctors can receive standardized images and

automatic reports on the evolution of the wound, reducing the need for frequent visits to the clinic.

2. **Artificial intelligence (AI) and machine learning** are increasingly used to classify types of wounds and estimate the probability of cure. Machine learning models can analyze thousands of images to detect subtle changes in color, texture, or grain that are difficult to perceive clinically in the early stages.

3. **Smart sensors integrated into dressings** are an emerging innovation. They can monitor parameters such as temperature, pH, humidity level or concentration of volatile compounds in real time, providing predictive information about infection or stagnation of the healing process.

Benefits of integrating clinical scores and digital technologies

Combining traditional clinical assessment with digital tools brings major benefits:

1. increases the accuracy and consistency of the assessment;
2. allows for early intervention in case of stagnant healing;
3. facilitates forensic documentation and quality audit;
4. offers the possibility of integration into the electronic patient record and Big Data analysis for clinical research.

Therefore, standardized clinical scores, complemented by digital technologies, constitute a solid basis for comprehensive, objective and personalized monitoring of the wound healing process, in line with the trends of modern medicine based on data and precision.

2.3. Modern Imaging in Wound Evaluation: Ultrasound, Thermography, Coherent Optics [111–125]

The objective monitoring of the wound healing process has gone beyond the stage of exclusively visual evaluation, based on inspection and two-dimensional measurements. Today, modern imaging techniques — ultrasound, thermography and optical coherence tomography — provide a three-dimensional and functional perspective on tissues, contributing to a detailed understanding of the processes of inflammation, angiogenesis and tissue regeneration.

Ultrasound in wound evaluation

Skin and subcutaneous ultrasound is a non-invasive, accessible and reproducible method used for the structural characterization of wounds. Using high-frequency probes (20–50 MHz), the following can be evaluated:

1. The thickness of the affected tissues, the delimitation of the anatomical planes and the depth of the wound;
2. The degree of edema and the presence of fluid collections, abscesses or seromas;
3. The state of the vascular network**,** by color Doppler ultrasound, which highlights neovascularization and local perfusion;
4. Monitoring of grafts and flaps, where ultrasound allows early assessment of integrity and blood flow.

Recent studies have demonstrated a close correlation between increased Doppler flow and the proliferative phase of healing, making ultrasound a valuable tool for predicting wound progression and the risk of ischemic complications.

Ultrasound in wound evaluation follows the structural, vascular and evolution parameters of the healing process, providing information that is impossible to obtain only by visual inspection.

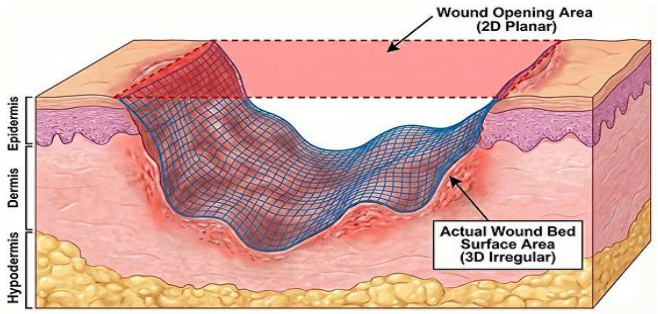

Fig. 1.1. Ultrasound in Chronic Wounds
(https://www.venturemedical1.com)

Here is what it aims concretely:

1. Wound size and depth
    1. It measures the thickness and extent of the lesion in depth.
    2. It allows the evaluation of the volume and the delimitation of the affected tissue planes.
    3. It identifies areas of necrosis or fibrosis that may delay healing.

2. Presence of fluid collections
    1. It highlights seromas, hematomas or subcutaneous abscesses.
    2. It helps differentiate between sterile inflammation and active infection.

3. Can guide puncture or drainage.

3. Vascularization and local perfusion

1. By color Doppler, the following are evaluated:
2. neovascularization in the proliferative phase;
3. perfusion of marginal tissues;
4. signs of ischemia or thrombosis.

Increased blood flow is associated with active regeneration, and reduced flow can signal delayed healing.

4. Evolution of grafts and flaps

Ultrasound allows monitoring the viability of skin or muscle flaps.

Ischemia, edema or dehiscence are detected early.

5. Evaluation of healing phases

1. In the inflammatory phase: increased echogenicity and diffuse edema.
2. In the proliferative phase: hyperechoic structures (collagen, granulation) appear.
3. In the remodeling phase: reduction of edema, emergence of uniform architecture.

In short, ultrasound follows the internal dynamics of the wound, allowing a quantitative and qualitative evaluation of tissue regeneration, with the major advantage of being non-invasive, repeatable and radiation-free.

Infrared thermography

Thermography uses infrared radiation emitted by the surface of the skin to determine the distribution of local temperature. In the context of

wound management, this method provides information about the circulatory state and the inflammatory process, without direct contact with the patient.

1. Hyperthermic areas indicate active inflammation, infection, or intense vascularization;
2. Hypothermic areas may signal ischemia, incipient necrosis, or delayed healing.

Thermography is useful in the evaluation of diabetic ulcers, burns, and chronic wounds, providing a thermal profile that can anticipate progression before changes are clinically visible. Also, the integration of thermography into digital platforms allows the comparative analysis of successive images and the automatic identification of risk areas through AI algorithms.

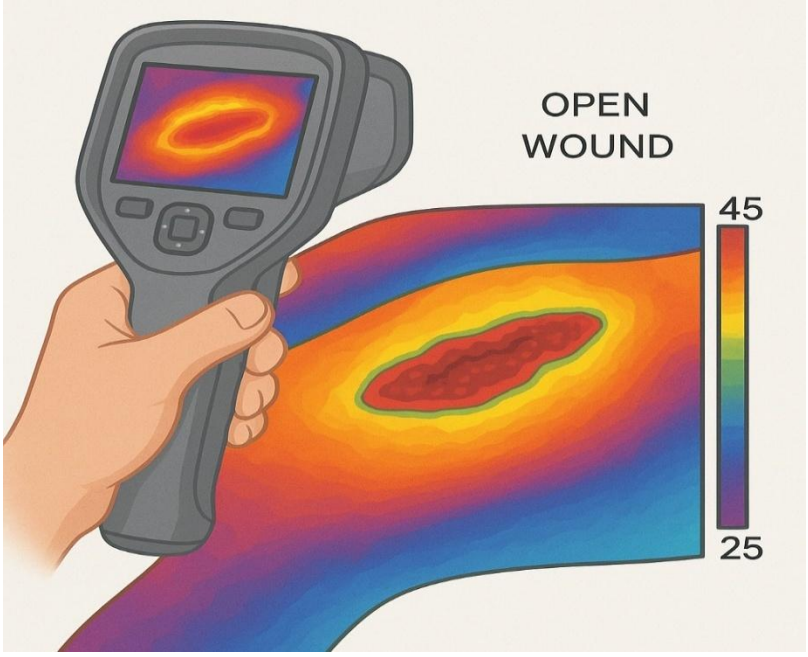

Fig. 1.2. Thermography in Wounds (original illustration)

Optical coherence tomography (OCT)

Optical coherence tomography is an advanced imaging method, based on interferometric light reflectometry, which provides micrometer-resolution images of skin structures. This can be considered the equivalent of "optical histology" in vivo, allowing the visualization of the layers of the skin without biopsy.

OCT can be assessed:

1. Thickness of the epidermis and dermis around the edges of the wound;
2. Degree of re-epithelialization and granulation tissue formation;
3. Collagen structure and fiber organization during tissue remodeling;
4. Progression of angiogenesis**,** by Doppler-OCT or OCT angiographic variants (OCTA).

This technique proves particularly useful in experimental research, in the monitoring of growth factor treatments, lasers or cell therapies, providing images up to 2 mm deep, with a resolution of 5–10 μm.

The main clinical roles of OCT are:

1. **Evaluation of the skin microstructure**
    1. It allows you to observe the layers of the epidermis and dermis, clearly delineate the edges of the wound and appreciate the depth of the lesion.
    2. It provides an image similar to an "optical biopsy", useful for monitoring the regeneration process.
2. **Monitoring of re-epithelialization**

1. OCT detects the formation of new epithelial tissue on the surface of the wound before it is visible to the naked eye.
2. It helps the doctor to assess **whether the healing process is active or stagnant**.

3. **Collagen and granulation tissue analysis**
   1. It highlights the degree of organization of collagen fibers and the extracellular matrix, essential indicators of healthy healing.
   2. It can differentiate between a young, active regenerative tissue and a fibrous, scarring tissue.

4. **Vascularization evaluation (by OCT-Angiography)**
   1. OCT Doppler or angiographic variants provide information about blood flow and angiogenesis, helping to diagnose ischemia or microcirculatory complications.

Multimodal integration of imaging technologies

In modern clinical practice, an integrated approach combining ultrasound, thermography, and OCT can provide a complete functional map of the wound:

1. Ultrasound provides structural and vascular information;
2. Thermography highlights metabolism and inflammation;
3. OCT allows microstructural analysis of regeneration.

By correlating these data, the clinician can more accurately establish the healing phase, the effectiveness of the therapy and the risk of local

complications. In addition, the digitization of images allows them to be integrated into artificial intelligence platforms for predictive analysis and clinical decision support.

Modern wound imaging marks a fundamental transition from subjective clinical evaluation, based on visual observation and the doctor's experience, to objective, quantifiable and personalized monitoring of the healing process. By integrating high-resolution ultrasound, infrared thermography and optical coherence tomography (OCT) technologies, it is possible not only to detail the structure and function of lesion tissues, but also to analyze microvascularization, the degree of inflammation and the depth of tissue damage. In modern clinical practice, the multimodal approach to wound assessment is an indispensable tool for complex diagnosis and monitoring of healing processes.

The combination of ultrasound, thermography and optical coherence tomography (OCT) allows the creation of a three-dimensional functional map of the wound, which combines structural, vascular, metabolic and microstructural information into a coherent and complete picture of the biological state of the lesion tissues.

Ultrasound provides a detailed insight into the internal morphology of the wound, the degree of edema, the presence of fluid collections, as well as local vascularization. By using the color or power Doppler mode, capillary flows and tissue perfusion can be quantified, aspects that reflect the regenerative capacity and epithelialization potential. This non-invasive method allows for an in-depth evaluation, difficult to obtain by direct clinical examination, providing objective data on tissue viability and integrity of anatomical planes.

Infrared thermography brings a metabolic complement to this information, by highlighting the temperature distribution at the level of the

skin surface. Hyperthermic regions signal active inflammatory processes or early infections, while areas of hypothermia may suggest ischemia, necrosis, or local circulatory deficiency. This method has a crucial role in identifying early physiological imbalances, before the visible clinical manifestation, allowing preventive therapeutic intervention. Thermography, by periodically repeating measurements, provides a dynamic indicator of the metabolic evolution of the wound, reflecting in real time the biological response to treatment.

Optical coherence tomography (OCT) completes this picture through microstructural analysis of tissues. With a resolution comparable to that of optical histology, OCT allows visualization of epidermis and dermis stratification, detection of re-epithelialization processes and quantification of collagen fiber density in tissue remodeling phases. The major advantage of the method lies in its non-invasive and repeatable character, which allows dynamic tracking of histological transformations without the need for invasive biopsies. By integrating these three techniques, the clinician can obtain a global and predictive picture of the biological behavior of the wound, identifying both the potential for healing and the risks of stagnation or recurrence.

These methods provide essential information for strategic treatment planning, by adapting therapeutic interventions in stages according to the evolutionary stage of the wound. Thus, based on imaging data, the optimal time for the application of regenerative therapies (PRP, laser, controlled oxygenation) or for the introduction of negative pressure therapy (VAC) can be decided, in situations that require stimulation of neoangiogenesis and drainage of exudate. At the same time, thermal and structural analysis guides the choice of smart dressings – hydrogels, collagen matrices, silver

or bioactive polymer dressings – depending on local humidity, exudation and bacterial load.

Moreover, these monitoring tools allow the monitoring of healing dynamics to be monitored in real time, through serial assessments that correlate morphological, functional and thermal parameters. In this way, the therapeutic approach can be adjusted quickly and precisely, depending on the biological changes that have occurred: increased vascularization, decreased inflammation, acceleration of epithelialization or formation of mature granulation tissue. Through the constant feedback provided by these methods, the decision-making process becomes predictive, personalized and based on objective data, transforming wound management from a reactive act to a proactive one.

Overall, ultrasound, thermography and OCT are not only complementary diagnostic techniques, but tools for therapeutic guidance and integrated monitoring, which optimize the treatment plan, reduce the duration of hospitalization and increase the success rate of healing.

This complex and interdisciplinary approach, which combines imaging analysis with modern regenerative interventions, represents one of the most promising directions of regenerative surgery and personalized medicine in the treatment of chronic wounds.

2.4. Histological and molecular analyses of the lesion tissue [126–135]

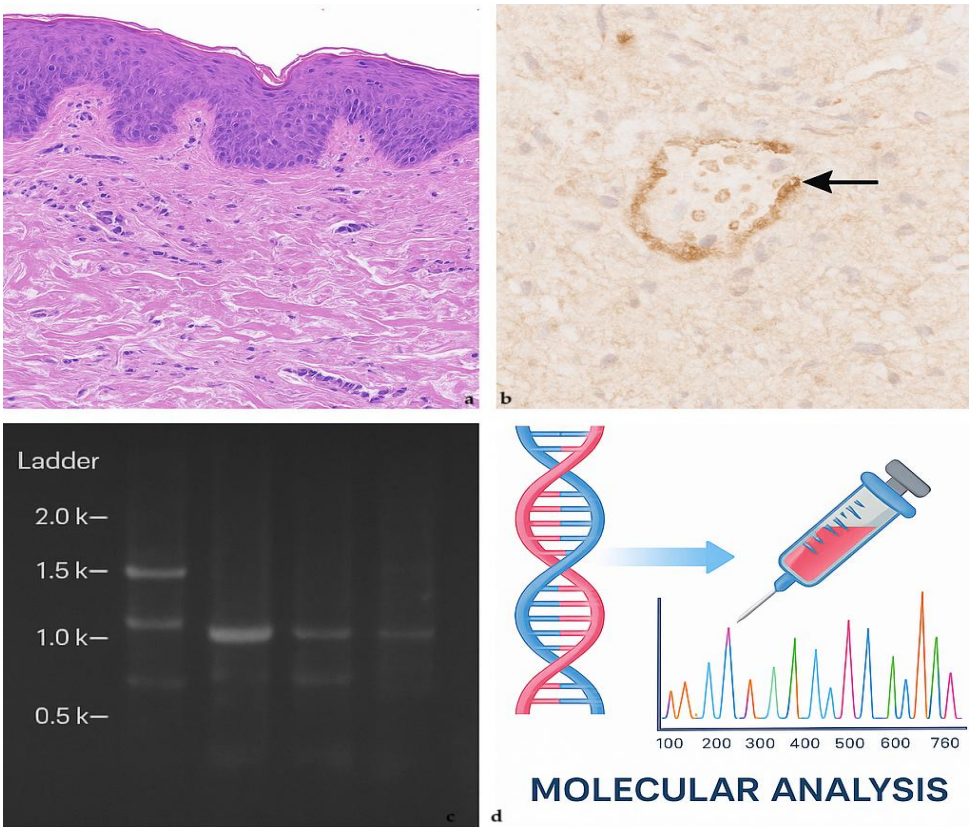

Fig. 1.3. Classical Histology (Histological Specimen)

a) Classical histology (Hematoxylin–Eosin – H&E staining)

This is a histological section of normal or healing skin, stained H&E.

1. The epidermis (upper layer, deep purple) has epithelial cells arranged in an orderly manner.
2. The dermis (light pink) contains collagen fibers and dispersed inflammatory cells.
3. In healing wounds, active fibroblasts, new vessels and granulation tissue can be observed.

Goal: to identify the degree of re-epithelialization, inflammation and organization of collagen.

b) Immunohistochemistry – CD31 marker for angiogenesis

The image shows immunohistochemical staining for CD31, a marker specific to endothelial cells that line blood vessels.

1. The brown areas highlight newly formed capillaries, a sign of the process of active angiogenesis.
2. The arrow indicates a group of endothelial cells positive for the CD31 marker.

Purpose: evaluation of perfusion and local vascularization in the proliferative phase of healing.

c) Molecular analysis – DNA electrophoresis gel (PCR)

It is an agarose gel used to separate DNA fragments obtained by polymerase chain reaction (PCR).

1. The horizontal lines represent DNA fragments of different sizes.
2. The "Ladder" column is the reference scale for fragment sizes (in kilobases).

Purpose: to confirm the expression of some genes involved in healing processes (e.g. VEGF, IL-1β, MMP-9).

d) Schematic molecular analysis – genetic and protein profile

This symbolic image illustrates molecular analysis and bioinformatics:

1. The → DNA strand represents the extraction of genetic material from injured tissue.
2. The syringe and the multicolored graph → signify *ELISA*, *Western blot* or *RNA sequencing* analyses, through which gene and protein expression levels are measured.

Purpose: to identify molecular pathways involved in inflammation, angiogenesis and regeneration.

The evaluation of wound healing is not limited only to clinical inspection or imaging, but also involves the detailed analysis of histological and molecular changes in the lesion tissue. These investigations allow the understanding of the cellular and biochemical mechanisms that govern the processes of inflammation, regeneration and tissue remodeling, providing essential information for the development of advanced therapies.

1. Classical histological analysis

Histological examination of tissue fragments taken from the wound is the standard method of evaluating cell architecture and extracellular matrix.

The main parameters evaluated histologically include:

1. Epithelial integrity – the degree of re-epithelialization and thickness of the newly formed epidermis;
2. Inflammation – density of leukocyte infiltrate (neutrophils, macrophages, lymphocytes);
3. Angiogenesis – the number and caliber of newly formed vessels, observable by special stains (e.g., CD31, CD34);
4. Collagen deposition – orientation and maturity of collagen fibers (Trichrome Masson staining);
5. Fibroblasts and myofibroblasts – involved in the contraction and remodeling of the wound.

The usual stains (Hematoxylin-Eosine, Trichrome Masson, PAS, Van Gieson) allow the general morphological characterization, while immunohistochemistry provides specific information about the expression of cellular and protein markers involved in healing.

2. Immunohistochemical analysis and specific markers

Through immunohistochemistry, key proteins involved in the healing phases can be detected:

1. CD31, CD34, VEGF – angiogenesis markers;

2. α-SMA (alpha-smooth muscle actin) – a marker for myofibroblasts;

3. Ki-67 – indicator of active cell proliferation;

4. TGF-β1 and Collagen I/III – involved in post-inflammatory remodeling and fibrogenesis;

5. IL-1β, TNF-α, MMP-8, MMP-9 – inflammatory and enzymatic markers associated with matrix degradation.

These data help to differentiate between a physiological and a pathological cure (e.g. the formation of hypertrophic scars or chronic wounds).

3. Molecular analysis

Modern molecular biology technologies offer the opportunity to investigate the expression of genes and signaling pathways involved in regeneration.

The methods used include:

1. PCR and RT-qPCR (real-time quantitative PCR) – for quantifying the expression of genes involved in inflammation, angiogenesis and remodeling (e.g. IL1B, MMP9, VEGFA, COL1A1, TGFB1);

2. Western blot and ELISA – to determine the protein levels corresponding to these markers;

✓ Microarray and RNA-Seq sequencing – for comprehensive transcriptomic profiling of the affected tissue, identifying new genes and pathways associated with healing.

Molecular analyses enable the early detection of dysfunctions in the healing process, even before clinical manifestations appear, and can be used to personalize treatment.4. Histomolecular Correlation and Clinical

ApplicationsThe correlation of histological and molecular data provides a comprehensive view of the regeneration process:

Level of analysis	Principal parameter	Metodă	Clinical relevance
Histologic	Organization of collagen, angiogenesis	H&E, Tricrom Masson, CD31	Healing stage, vascularization
Imunohistochimic	Expression of inflammatory and proliferative markers	IL-1β, TNF-α, Ki-67, α-SMA	Identification of the inflammatory/proliferative phase
Molecular	Gene expression (VEGF, MMP, TGF-β)	PCR, ELISA	Predicting Healing and Therapeutic Response
Level of analysis	Principal parameter	Method	Clinical relevance

Thus, histo-molecular analysis is not only descriptive, but has prognostic and therapeutic value. It allows the selection of patients who would benefit from specific regenerative therapies (e.g. growth factor treatments, cell therapies or biomimetic dressings).

Histological and molecular analyses are a fundamental tool in the research and management of complex wounds. They provide a deep

understanding of the cellular and molecular interactions that define healing success, allowing for the development of personalized and evidence-based therapeutic strategies.

2.5. Biochemical and immunological indicators of the healing process [136–145]

The wound healing process is the result of a complex succession of cellular and molecular events, regulated by precise biochemical interactions between inflammatory factors, cytokines, proteolytic enzymes and signaling molecules. The evaluation of biochemical and immunological indicators provides objective information about the stage of healing, the degree of inflammation and the quality of tissue regeneration.

1. Biological phases and correspondence of biochemical markers

Wound healing goes through three main phases: inflammatory, proliferative and remodeling, each characterized by a specific biomarker profile.

Phase	Period	Leading indicators	Physiological role
Inflammatory	Days 1–4	IL-1β, TNF-α, IL-6, CRP, Acute phase proteins	Initiation of the inflammatory response, leukocyte recruitment
Proliferative	Days 4–14	VEGF, TGF-β1, FGF, MMP-8,	Angiogenesis, extracellular matrix synthesis,

		MMP-9, fibronectină	re-epithelialization
Remodeling	>14 days	Collagen I/III, TIMP-1, IL-10, α-SMA	Organization of collagen, wound contraction, reduction of inflammation

This molecular dynamic reflects the balance between pro-inflammatory and anti-inflammatory processes, determining the quality of healing.

2. Inflammatory markers

Inflammatory indicators are essential for assessing the intensity and duration of the inflammatory response, especially in chronic or infected wounds.

1. Interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α): stimulates the expression of metalloproteinases (MMPs) and the degradation of the extracellular matrix. Persistently high levels can hinder the transition to the proliferative phase.
1. C-reactive protein (CRP): systemic indicator of inflammation; increased values may signal secondary infection or prolonged inflammation.
2. Interleukin-6 (IL-6): has a dual role — pro-inflammatory in the initial phases, anti-inflammatory in the late phases, contributing to the activation of hepatocytes and the synthesis of acute phase proteins.

3. Markers of proliferation and angiogenesis

In the proliferative phase, granulation tissue is formed under the influence of growth factors and angiogenic mediators:

1. Vascular endothelial growth factor (VEGF): stimulates the formation of new capillaries and local perfusion. Increased levels of VEGF correlate with effective healing.
2. Fibroblastic growth factor (FGF-2): promotes fibroblast proliferation and collagen synthesis.
3. TGF-β1 (Transforming Growth Factor Beta 1): regulates extracellular matrix deposition and myofibroblast differentiation.
4. α-SMA (alpha-smooth muscle actin): histological marker of myofibroblasts involved in wound contraction.

4. Proteolytic enzymes and inhibitors

The enzymatic activity of matrix metalloproteinases (MMPs) is a crucial indicator of the balance between extracellular matrix degradation and synthesis.

1. MMP-8 (neutrophilic collagenase) and MMP-9 (gelatinase B): are increased in the inflammatory phase, facilitating the cleansing of necrotic tissue.
2. TIMP-1 (tissue inhibitor of metalloproteinases): counterbalances the activity of MMPs; a high MMP/TIME ratio signals poor healing.
1. The MMP/TIME imbalance is characteristic of chronic wounds (diabetic, venous, ischemic).

5. Anti-inflammatory and immunomodulatory markers

In the late phases, the regulation of the inflammatory process and the limitation of fibrogenesis are ensured by anti-inflammatory mediators:

1. Interleukin-10 (IL-10): inhibits the synthesis of IL-1β and TNF-α, favoring the transition to the proliferative phase.

2. Adiponectin and resistin: involved in metabolic healing and local angiogenesis.

3. Immunoglobulins A and G (IgA, IgG): indicate local immune activity and tissue barrier integrity.

6. Systemic Biomarkers of Healing

In addition to local tests, serum parameters can provide a global picture of the body's response:

1. Acute phase proteins (CRP, fibrinogen, haptoglobin);

2. Metabolic indicators (glucose, lactate, zinc, vitamin C, albumin) influencing collagen synthesis and epithelial regeneration;

3. Circulating cytokines (IL-1β, IL-6, TNF-α) that can be quantified by ELISA or multiplex methods.

4.

Hint the biomarker	Main method	Example	Clinical relevance
Inflammatory cytokines	ELISA, Multiplex Assay	IL-1β, TNF-α, IL-6	Assessment of inflammation and infection
Growth Factors	ELISA, Western blot	VEGF, TGF-β1, FGF-2	Angiogenesis, tissue proliferation
Proteolytic enzymes	Zymography, ELISA	MMP-8, MMP-9	Degradation of the extracellular matrix
Enzyme inhibitors	ELISA	TIMP-1	Control of tissue remodeling
Anti-inflammatory markers	ELISA PCR	IL-10	Immune and anti-inflammatory balance

The determination of biochemical and immunological indicators provides an objective, quantitative and dynamic assessment of the healing process. By integrating them with imaging and histological data, deviations from physiological healing can be identified, the effectiveness of applied treatments can be evaluated, and personalized therapeutic strategies for acute or chronic wounds can be developed.

The wound healing process is the result of an orchestrated succession of cellular, biochemical and immunological mechanisms, which takes place in three main stages: inflammation, proliferation and tissue remodeling. Each stage is accompanied by quantifiable changes at the molecular level, reflected by the variation of biochemical and immunological markers in the serum, exudate, or lesional tissue. The analysis of these indicators allows the objective assessment of the progress of the cure, the identification of complications and the optimization of the applied therapy.

In the initial phase of the inflammatory response, the body activates non-specific defense pathways, releasing pro-inflammatory mediators and enzymes that trigger the wound cleansing phase. The monitoring of these parameters provides an image of the metabolic and redox balance of the lesion microenvironment, being useful in adjusting the local treatment (antioxidants, oxygen therapy, PRP).

Wound healing involves a succession of immunological events controlled by cytokines, chemokines and growth factors, which modulate the migration and activation of the cells involved (neutrophils, macrophages, lymphocytes, fibroblasts).

The most important immunological indicators are:

1. Interleukin-1β (IL-1β) – stimulates macrophage activation and fibroblast proliferation; Excessive levels may indicate persistent inflammation.
2. Interleukin-6 (IL-6) – regulates hepatic synthesis of inflammatory proteins (CRP, fibrinogen); It grows in infected and chronic wounds.
3. Tumor necrosis factor alpha (TNF-α) – the main mediator of acute inflammation; In excess, it inhibits epithelialization and promotes tissue necrosis.
4. Interleukin-10 (IL-10) and TGF-β (Transforming Growth Factor Beta) – anti-inflammatory cytokines that modulate the immune response and initiate the proliferative phase, favoring collagen synthesis and angiogenesis.

The ratio between pro- and anti-inflammatory cytokines reflects the immune balance of the wound, and deviations may signal a poor evolution or chronicity of the process.

In the proliferative phase, tissue regeneration is orchestrated by a series of growth factors released locally from platelets, macrophages and stromal cells. These mediators stimulate cell proliferation, extracellular matrix synthesis, and angiogenesis:

1. PDGF (Platelet-Derived Growth Factor) – stimulates fibroblast mitogenesis and endothelial cell recruitment.
2. VEGF (Vascular Endothelial Growth Factor) – induces the formation of new capillaries and improves tissue oxygenation.
3. FGF (Fibroblast Growth Factor) – promotes the proliferation of keratinocytes and collagen synthesis.

4. EGF (Epidermal Growth Factor) – accelerates re-epithelialization and wound closure.

Determining the levels of these factors in wound exudate or peripheral serum allows assessment of the effectiveness of regenerative therapy (PRP, stem cells, biostimulating lasers) and provides valuable prognostic information.

The final phase of healing is marked by the reorganization of the extracellular matrix, a process controlled by a balance between matrix metalloproteinases (MMPs) and their inhibitors (TIMP).

1. MMP-1, MMP-8 and MMP-9 – enzymes that degrade collagen and elastin, facilitating tissue remodeling; persistently increased levels indicate excessive degradation and delayed healing.
2. TIMP-1 and TIMP-2 – inhibitors of MMPs, responsible for the stabilization and maturation of the scar.
3. The MMP/TIME ratio is an essential indicator of the quality of the repair process, often being used in experimental and clinical studies to evaluate the effectiveness of topical or regenerative treatments.

Currently, research is moving towards the development of miniaturized biosensors integrated into smart dressings, capable of detecting variations in these markers in real time. Dynamic measurement of pH, temperature and cytokine concentration or MMP in wound exudate allows immediate adaptation of treatment and prevention of complications.

Thus, biochemical and immunological analysis becomes an essential component of personalized wound healing medicine, providing an accurate tool for monitoring the biological response and optimizing therapy.

Chapter 3. Modern Mechanisms and Protocols in the Treatment of Wounds

3.1. Debridement: mechanical, enzymatic, surgical and biological principles, methods [146–155]

Debridement is one of the essential steps in preparing the wound bed, serving to remove necrotic tissue, foreign bodies, and fibrinous material that prevent healing. By removing these physical and biological barriers, the local bacterial load is reduced and optimal conditions are created for tissue regeneration and progressive epithelialization.

The primary goal of debridement is to restore an environment conducive to healing by:

1. removal of necrotic tissues and adherent crusts;
2. reducing the risk of infection;
3. stimulating the formation of healthy granulation tissue;
4. facilitating the action of topical and systemic treatments;
5. correct visual assessment of the extent of the lesion.

The debridement process must be individualized according to the etiology of the wound (traumatic, ischemic, diabetic, pressure), the general condition of the patient and the degree of local vascularization. In general, a phased approach is pursued, combining safety with therapeutic efficiency.

Methods of debridement

1. Mechanical debridement

It is the most widely used method, based on the physical removal of non-viable tissues. It can be achieved by:

1. washing with antiseptic solutions under controlled pressure (pulsatory irrigation);
2. mechanical removal with sterile gauze or impregnated compresses;
3. the use of hydroactive dressings (hydrogels, polyurethane foams) that promote natural autolysis;
4. the saline jet method or with negative controlled pressure systems, which allow atraumatic cleaning.

The advantage of this method lies in its simplicity and applicability in various clinical settings, but it can be painful and affect viable tissue if not performed carefully.

2. Enzymatic debridement

It is based on the use of proteolytic substances (collagenase, papain, bromelain) that selectively dissolve necrotic components without harming healthy tissue. It is indicated in chronic, atone wounds with a thick fibrin layer.

Advantages: selectivity and precise process control. Limitations: slow action and reduced efficiency in the presence of infection or thick crusts.

Common examples of preparations: collagenase ointments or enzymes combined with topical antibiotics, applied under occlusive dressings to maintain local moisture.

3. Surgical debridement

It is the method of choice for wounds with extensive necrosis, deep infections or septic risk. It is performed under local or general anesthesia, using surgical instruments (scalpel, scissors, curette). The surgeon precisely

removes devitalized tissues until capillary bleeding occurs — a sign of tissue viability.

This method allows you to:

1. exploring the actual extent of the lesion;
2. collection of samples for culture and antibiogram;
3. immediate reduction of bacterial load.

However, it requires sterile conditions and experienced personnel and is contraindicated in poorly vascularized areas or in hemodynamically unstable patients.

4. Biological debridement

A modern method, based on the use of sterile larvae of *Lucilia sericata* (green meat fly), which selectively digests necrotic tissue. Larvotherapy has proven effective for diabetic ulcers and chronic refractory wounds, and it also helps reduce the microbial load by secreting antibacterial enzymes. Advantages: selective action, lack of mechanical trauma, granulation stimulation.

Disadvantages: psychological discomfort for the patient and higher costs.

Comparison of the main methods of debridement

Types of debridement	Mechanism of action	Advantages	Limitations / Risks	Main indications
Mechanical	Physical removal of necrosis	Fast, affordable	Painful, non-specific	Contaminated, superficial wounds

Enzymatic	Proteolytic dissolution	Selective, atraumatic	Slow, pH-sensitive	Chronic, diabetic wounds
Surgical	Controlled excision	Efficient, complete	Invasive, requires a sterile environment	Extensive necrosis, deep infections
Biological	Sterile larvae – selective digestion	Eco-friendly, antibacterial	Costs, discomfort	Refractory ulcers, chronic wounds

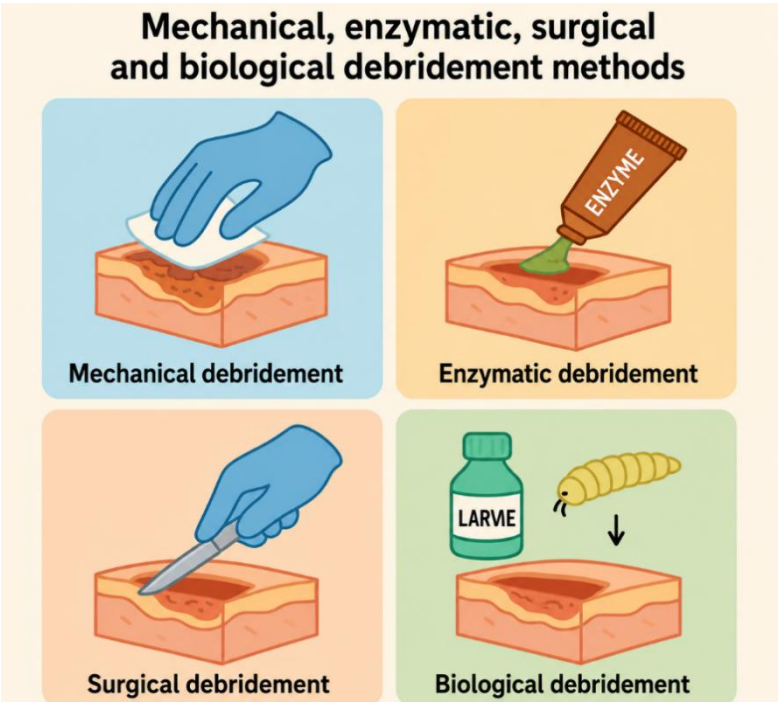

Fig. 3.1. Debridement Methods (original illustration)

Correct and periodic debridement is the essential prerequisite for effective healing. In modern practice, the combined approach (mechanical

+ enzymatic or surgical + biological therapy) provides superior results, reducing healing time and the risk of infectious complications. The choice of method depends on the peculiarities of the wound, the availability of resources and the experience of the medical team.

3.2. Infection Control and Biofilm Management [156–165]

Infection control is an essential component of modern wound treatment, as the presence and persistence of microorganisms in the lesion can transform an acute wound into a chronic one, delay the healing process and generate systemic complications. In recent decades, with the deepening of microbiological studies, the decisive role of the bacterial biofilm as the main factor of resistance and chronicity of local infection has been highlighted.

1. The concept of infection in wounds

Wounds can be colonized, contaminated, or infected, depending on bacterial density and host reaction.

1. Contamination is the transient presence of microorganisms without significant replication.
2. Colonization involves bacterial multiplication without clinical signs of infection.
3. Infection involves tissue invasion, systemic inflammatory reaction and delayed healing.

The most commonly involved microorganisms are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus spp.*, and *Klebsiella pneumoniae*. In chronic wounds, these bacteria often coexist in the form of biofilm, protected by a polysaccharide extracellular layer that gives them resistance to antibiotics and the local immune response.

2. Biofilm: mechanism and clinical implications

The biofilm is an organized community of microorganisms embedded in an extracellular matrix consisting of polysaccharides, proteins and extracellular DNA. This layer adhering to the wound surface acts as a biological barrier, limiting the penetration of antimicrobial substances and protecting bacteria from phagocytosis.

The formation of the biofilm goes through four stages:

1. Adhesion of bacteria to the surface of the wound (in the first hours);
2. Maturation of microcolonies (24–48 h);
1. Development of the protective extracellular matrix**;**
1. Release planktonic bacteria**,** which can colonize other areas of the wound.

This process explains the frequent recurrences of chronic infections and their resistance to conventional treatments. Studies have shown that bacteria in biofilm can be up to 1000 times more resistant to antibiotics compared to planktonic forms.

3. Infection Control Strategies

Effective infection management in a wound requires a multimodal approach, combining topical, systemic therapies and mechanical interventions.

a. Wound cleansing and debridement

Removing necrotic tissues and purulent secretions is the first step in reducing the bacterial load. Debridement (mechanical, surgical, enzymatic or biological) exposes viable tissue and favors the access of antiseptics and antibiotics to the infectious focus.

b. Topical antiseptics

Modern antiseptic solutions play a central role in biofilm control. Commonly used substances include:

1. Polyhexanide (PHMB) – effective and well tolerated locally, with antibacterial and antifungal action;
2. Octenidine – active on a broad spectrum of bacteria, including MRSA;
3. Povidone iodine-based solutions – effective in acute infections, but irritating in extensive chronic wounds;
4. Chlorhexidine – used in the initial phase, but contraindicated in deep wounds due to the cytotoxic potential;
5. Colloidal silver solutions – used in smart, slow-release dressings with bacteriostatic and anti-inflammatory effect.

c. Systemic antibiotic therapy

It is recommended only in the presence of systemic clinical signs (fever, leukocytosis, extensive erythema, lymphangiitis). Empirical treatment should then be adapted on the basis of the antibiogram. The most common classes used include:

1. broad-spectrum penicillins and β-lactamase inhibitors,
2. third-generation cephalosporins,
3. carbapeneme,
4. fluoroquinolone,
5. and, in cases with multidrug-resistant germs, linezolid or vancomycin.

It is essential to limit the duration of systemic treatment to the minimum necessary in order to avoid the selection of resistant strains.

d. Modern anti-biofilm technologies

Recent research has introduced innovative methods in biofilm control:

1. The use of dispersing enzymes (DN-ase, proteases) for the destruction of the extracellular matrix;
2. Antibacterial phototherapy (laser or LED with specific wavelengths) that destabilizes the biofilm;
3. Silver, copper or zinc oxide nanoparticles, capable of penetrating the biofilm and inhibiting bacterial DNA synthesis;
4. Bioactive dressings impregnated with antimicrobial compounds and controlled release, which maintain a local humid environment, but not conducive to bacterial proliferation.

4. Monitoring and evaluation of treatment effectiveness

The evolution of the wound should be monitored clinically and paraclinically:

1. reduction of secretions and unpleasant odor;
2. decrease in local inflammation;
3. the appearance of healthy granulation tissue.

In persistent cases, it is recommended to collect samples for culture and antibiogram, supplemented by molecular tests (PCR) to identify biofilm and multidrug-resistant strains.

1. Holistic approach

Fig 3.2. Therapeutic Approach (original illustration)

Figure created by the authors for educational purposes

The therapeutic approach to chronic and complex wounds is not limited to local treatments, but involves a holistic view of the patient, in which healing is seen as an integrative process, dependent on the metabolic, immunological, nutritional and psychological balance of the body. Effective wound healing involves not only direct actions on the lesion, but also the restoration of systemic functions that support tissue regeneration.

1. Metabolic balance and control of comorbidities

The correction of metabolic imbalances is a fundamental condition for the progress of healing. Conditions such as diabetes, chronic venous insufficiency, obliterating arteriopathy or cardiovascular diseases alter microcirculation, oxygenation and tissue trophicity, favoring chronic inflammation and delaying epithelialization.

A rigorous control of blood glucose, adjustment of antihypertensive treatment and maintenance of an optimal tissue infusion are essential

measures to restore the biological environment favorable to regeneration. In addition, balancing endocrine functions and lipid status contributes to maintaining an adequate inflammatory response and reducing local oxidative stress.

2. Nutritional support and protein intake

Nutrition plays a decisive role in all phases of the healing process. Deficiencies in protein, vitamins (A, C, E), zinc, iron and essential fatty acids affect collagen synthesis, enzymatic activity and immune response. Patients with chronic wounds require a high-protein and high-calorie diet with amino acid supplements (arginine, glutamine) and antioxidant micronutrients that accelerate angiogenesis and fibroblast proliferation. In severe cases, nutritional support can be administered enterally or parenterally to ensure a constant supply of nutrients and prevent muscle catabolism.

3. Optimizing immune status and reducing chronic inflammation

An effective immune system is essential for fighting infection and controlling local inflammation. Immune imbalances, such as those that occur in elderly, immunocompromised or polymedicated patients, can prevent the natural transition from the inflammatory phase to the proliferative one.

The controlled administration of immunomodulators, antioxidant supplements (vitamin D, selenium, coenzyme Q10) and selective anti-inflammatory treatments help to restore the balance between proinflammatory and repairing cytokines (IL-1β, TNF-α vs. IL-10, TGF-β).

4. Assessment of psychological and social factors

Healing a chronic wound is often a protracted and frustrating process for the patient. Pain, discomfort, social isolation and anxiety can reduce compliance with treatment and indirectly affect tissue regeneration. A

holistic approach includes psychological counseling, family involvement and emotional support, to maintain the patient's motivation and prevent the onset of depression associated with chronic illness. Studies have shown that stress-induced increased cortisol levels have negative effects on the immune response and collagen synthesis, prolonging healing time.

5. Physical therapy, mobilization and pain management

Early mobilization and stimulation of peripheral circulation have beneficial effects on oxygenation and lymphatic drainage. Adapted physical therapy and light exercises contribute to the prevention of venous stasis and the improvement of skin trophicity. In parallel, pain control through analgesics, laser therapy or transcutaneous electrical stimulation (TENS) is indispensable for maintaining compliance and reducing the body's physiological stress.

6. Summary of the integrative approach

Overall, the healing of a wound cannot be separated from the overall health of the patient. Therapeutic success depends on the harmonization of local and systemic interventions, in a personalized, multidisciplinary protocol — involving the surgeon, nutritionist, physiotherapist, psychologist and wound care nurse.

Through this integrated approach, the patient is seen in his or her biological and psychosocial totality, and treatment becomes an active process of restoring the general balance, not just healing an injury. The result is faster, more stable tissue regeneration with minimal risk of recurrence, confirming that modern wound medicine needs to be deeply interdisciplinary and patient-centered.

Mechanisms of biofilm formation and control in wounds

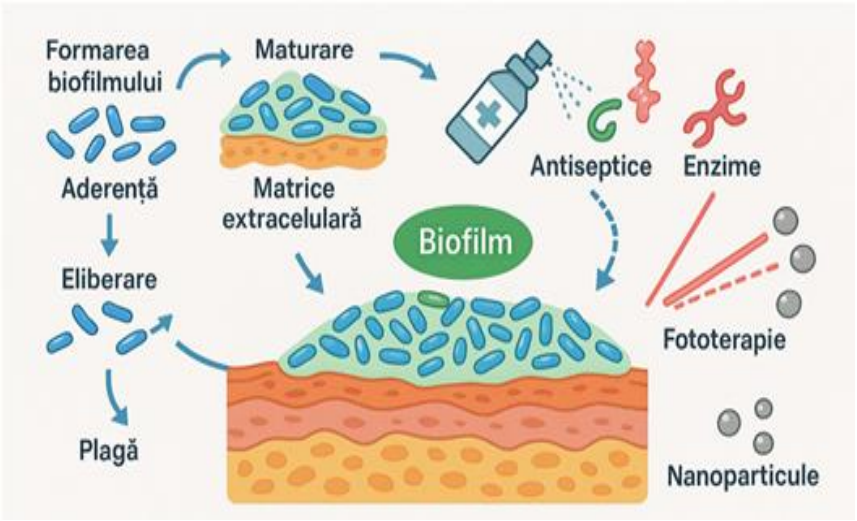

Fig. 3. 3. Mechanisms of biofilm formation and control in wounds (original illustration)

3.3. Negative pressure therapy (VAC) – indications and clinical results

Negative pressure therapy (VAC – *Vacuum-Assisted Closure*) is one of the most effective modern technologies for managing complex chronic and acute wounds. This method, originally introduced in the 1990s, uses a controlled suction system to create subatmospheric pressure in the wound, stimulating natural healing processes and reducing the risk of infection.

Principles and mechanism of action

The VAC system consists of an assembly consisting of a porous dressing (polyurethane foam or polyvinyl-alcohol), a drain tube connected to a vacuum device and an occlusive layer that ensures tightness. After applying the dressing, the pump generates a constant or intermittent negative pressure (between –75 and –125 mmHg), causing:

1. evacuation of exudate and detritus from the wound;

2. reduction of interstitial edema, which improves tissue perfusion;

3. stimulation of fibroblast proliferation and angiogenesis;

4. formation of granulation tissue and acceleration of epithelialization.

By maintaining a moist but sterile environment, VAC promotes healing in optimal conditions, preventing excessive drying and bacterial proliferation.

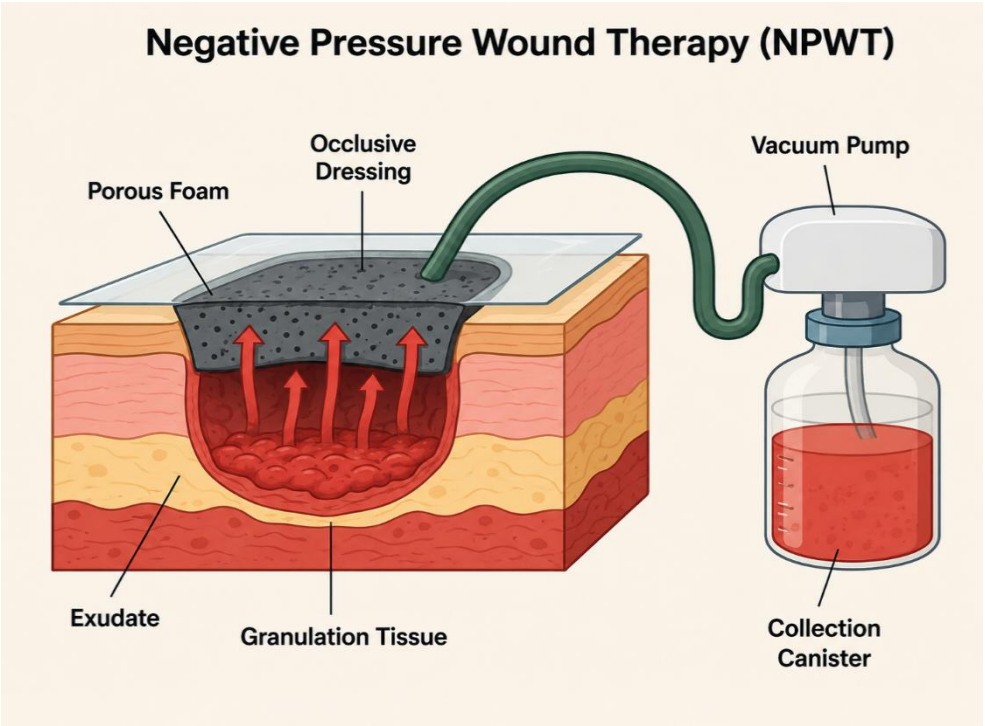

Fig. 3.4. VACUUM Therapy (original illustration)

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Clinical indications

VAC therapy is indicated in a wide range of surgical and traumatic conditions:

1. chronic atone wounds (venous, diabetic, arterial ulcers);
2. postoperative wounds with dehiscence or infection;
3. traumatic wounds with loss of substance;
4. pressure ulcers and pressure ulcers;
5. wounds with abundant exudate or fistulas;
6. wounds after surgical debridement or after excision of necrosis.

In surgical practice, VAC is frequently used as an intermediate step between radical cleansing and permanent coating with skin graft or flap.

Relative contraindications and precautions

1. Wounds with necrosis not completely removed;
2. Untreated infections or active osteomyelitis;
3. The presence of tumor tissue in the wound;
4. Ne-identified fistulas;
5. Active bleeding or exposed vessels.

The application of the therapy should be carried out by trained personnel, and the negative pressure is adjusted according to the location, the type of wound and the patient's tolerance.

Clinical results and proven benefits

Clinical experiences and comparative studies have shown significant benefits of VAC therapy compared to conventional treatments:

1. Reduction of healing time by up to 40–60%;
2. Acceleration of granulation tissue formation from the first 5–7 days;
3. Decreased pain intensity and discomfort;
4. Reduction of exudate and local odor;

5. Reduction of the need for daily dressings and the duration of hospitalization.

These effects are comparable to the results observed in clinical trials conducted on patients with atonic wounds and chronic ulcers, where the application of modern tissue stimulation methods led to a significant improvement in symptomatology ($p < 0.001$) and a reduction in pain intensity in the first 7–21 days of treatment. Also, in patients treated with VAC, a rapid improvement in general condition and a decrease in the need for systemic antibiotic therapy were observed, as a result of effective local control of the infection and drainage of exudate.

Practical considerations and application parameters

Parameter	General recommendation
Level of negative pressure	-75 to -125 mmHg
How to apply	Continuous or intermittent (5 min active / 2 min pause)
Duration of therapy	7–21 days, depending on the course of the wound
Dressing type	Polyurethane or hydrophobic foam (depending on secretion)
Frequency of dressing change	At 48–72 hours or at the onset of odor/saturation

Monitoring includes daily assessment of the volume of secretions, the appearance of the granulation and any signs of local discomfort. In patients with chronic wounds, a favorable evolution is generally observed after the first week of treatment.

Histological results and mechanisms of regeneration

At the microscopic level, an increase in the density of new capillaries, a reduction in inflammatory infiltrate and a reorganization of collagen were observed.

The mechanical effect of negative pressure favors:

1. migration of fibroblasts to the edge of the wound;
2. deposition of the extracellular matrix;
3. acceleration of the re-epithelialization process.

These observations are consistent with data obtained from clinical trials in patients with atonic wounds, where accelerated healing was associated with better vascularization and a decrease in the incidence of secondary infections.

In modern clinical practice, the treatment of chronic wounds involves a combination of complementary procedures – from mechanical cleaning and topical antiseptics, to advanced methods such as vacuum-assisted closure (VAC), active or passive oxygenation, and the use of bioactive dressings.

Practical considerations

1. VAC can be applied both in the hospital environment and at home, using portable devices.
2. In post-surgical wounds, early use of VAC reduces the risk of dehiscence and infection.
3. The association with local antiseptic therapies (dressings impregnated with silver or iodine) increases antibacterial effectiveness.

This method thus proves to be essential in the treatment of complex wounds, offering a safe and predictable alternative to classic healing techniques.

3.4. Hyperbaric oxygen therapy and topical oxygenation [173–178]

Oxygen plays an essential role in tissue repair processes, being indispensable for collagen synthesis, fibroblast proliferation, leukocyte activity and angiogenesis. In chronic wounds, local hypoxia is one of the main factors that slow down healing and favor bacterial colonization. Oxygen therapy – either administered systemically or locally – aims to restore the balance of tissue oxygenation and stimulate regenerative processes.

Hyperbaric Oxygen Therapy (HBOT)

Hyperbaric oxygen therapy consists of inhaling pure oxygen (100%) at pressures higher than normal atmospheric pressure (1.5–3 absolute atmospheres) in a hyperbaric chamber. This environment causes a significant increase in the amount of dissolved oxygen in the plasma, which favors its diffusion in the hypoperfused areas.

Mechanisms of action

1. Increasing partial oxygen pressure in ischemic tissues, stimulating fibroblast proliferation and collagen synthesis;
2. Reduction of edema by reflex vasoconstriction;
3. Stimulation of angiogenesis and formation of new capillaries;
4. Potentiation of the bactericidal effect of neutrophils;
5. Inhibition of anaerobic toxins**,** especially in *Clostridium perfringens infections*;
6. Improvement of leukocyte function and local energy metabolism.

Through these effects, hyperbaric therapy reduces inflammation and accelerates epithelialization, especially in chronic refractory wounds.

Therapeutic indications

1. diabetic and ischemic ulcers;
2. chronic atonic wounds;
3. pressure ulcers;
4. gas gangrene;
5. post-surgical cutaneous necrosis;
6. extensive burns and skin transplants with vascular distress.

In these cases, hyperbaric oxygen therapy is frequently associated with modern local treatments (e.g., hydroactive dressings or VAC) to optimize tissue oxygenation and drainage.

Contraindications and precautions

1. untreated pneumothorax;
2. uncontrolled epilepsy;
3. pulmonare obstructive severe;
4. acute upper respiratory tract infections;
5. intolerance to increased pressure (barotraumatic sinusitis).

Therapy should be applied under medical supervision, with close monitoring of pressure, duration of exposure and frequency of sessions (usually 60–90 minutes, 5–6 sessions per week).

Topical oxygenation

Topical oxygenation involves the direct application of oxygen to the wound, either in gaseous form, or through oxygenated solutions or portable continuous diffusion devices.

This method is based on the same physiological principle – increasing local oxygenation – but is applied non-invasively, being especially useful in superficial ulcers and chronic wounds with marginal hypoxia.

Advantages:

1. easy, non-invasive application;
2. low costs;
3. the possibility of combining with bioactive or antiseptic dressings;
4. reduction of inflammation and stimulation of re-epithelialization.

Limitations:

1. low efficiency in deep wounds or with extensive necrosis;
2. the need to maintain a perfect tightness of the device;
3. varying efficacy depending on the level of oxygen diffused and the duration of application.

In clinical trials, patients treated with hyperbaric or topical oxygen therapy showed a significant reduction in wound size **and an** acceleration of healing by 30–50% compared to conventional treatments. A decrease in inflammatory markers and an increase in the density of new capillaries was also observed at the histological level.

3.5. The role of systemic and topical antibiotics – strategies for rational use [179–184]

The use of antibiotics in the treatment of infected wounds is an essential pillar of modern medicine, contributing significantly to reducing mortality and morbidity associated with severe tissue infections. However, the uncontrolled and empirical administration of these drugs has led, in recent decades, to the emergence and spread of multidrug-resistant bacterial

strains, which complicate therapeutic management and increase the risk of systemic complications. In this context, the therapeutic approach must be rational and personalized, guided by microbiological evidence and susceptibility tests, to ensure the effectiveness of the treatment and limit the selection of resistant bacteria. The modern treatment strategy integrates the judicious use of systemic and topical antibiotics into a comprehensive plan that includes rigorous debridement of necrotic tissue, control of microbial biofilm, maintenance of a moist healing environment, and proper application of advanced dressings. Such a multimodal approach helps to speed up the healing process, reduce the duration of hospitalization and prevent infectious recurrences.

Clinical and bacteriological evaluation of the wound

Antibiotics are administered only in cases with clinical signs of infection (erythema, edema, purulent discharge, fever, severe pain). Collection of samples for culture and antibiogram is mandatory before initiating systemic treatment.

1. Choosing antibiotics according to the microbial spectrum

1. *Staphylococcus aureus* (including MRSA): oxacillin, cefazolin, vancomycin, linezolid.
2. *Pseudomonas aeruginosa*: piperacillin-tazobactam, ceftazidime, ciprofloxacin.
3. *Enterobacteriaceae*: cefalosporine de generația a treia, carbapeneme.
4. *Enterococcus spp.*: ampicillin, vancomycin.

2. Optimal duration of treatment

In general, systemic antibiotic therapy is maintained for 7–14 days, adjusted according to clinical response and culture results.

3. Monitoring of adverse effects and bacterial resistance

It is recommended to avoid prolonged empirical therapy and alternate classes of antibiotics in recurrent cases.

Topical antibiotics

Topical application allows to achieve an increased local concentration with minimal systemic toxicity. The most commonly used substances are:

1. mupirocin (active on *Staphylococcus aureus*);
2. gentamicin and neomycin (broad spectrum, but risk of sensitization);
3. bacitracin (bactericidal action on Gram-positives);
4. silver sulfadiazine (useful in burns and chronic infected wounds);
5. combinations with polymyxin B or metronidazole for anaerobic wounds.

Advantages of topical application

1. direct action on the wound;
2. reduction of the total systemic dose;
3. decrease the risk of side effects;

✓ compatibility with bioactive dressings.

Limitations

1. limited penetration into deep tissues;
2. reduced efficiency in the presence of mature biofilm;
3. risk of contact dermatitis with prolonged use.

Modern rational use strategies

1. association of topical antibiotics with anti-biofilm agents (dispersing enzymes, modern antiseptics);

2. periodic rotation of antibiotic classes to prevent resistance;

3. microbiologically guided, not empirical, use;

4. avoiding the concomitant administration of antibiotics with the same mechanism of action;

5. educating the patient for adherence to treatment.

The table below summarizes the main modern procedures used in the treatment of acute and chronic wounds, highlighting for each one the mechanism of action, the predominant clinical indications, the therapeutic advantages and the main limitations. Through this comparative presentation, an integrated vision is outlined on how the different interventions from debridement and infection control to advanced therapies such as VAC or oxygen therapy can be selected and rationally combined, depending on the biological particularities of the wound, the general condition of the patient and the therapeutic objectives pursued. This analytical perspective facilitates the personalized adaptation of clinical management, contributing to the optimization of the healing process and the reduction of postoperative or evolutionary complications.

Procedure	***Mechanism of action***	***Main indications***	***Advantages***	***Limitations***
Debridement (mechanical, enzymatic, surgical, biological)	Removal of necrotic tissue and cellular debris to stimulate the formation of granulation tissue.	Acute, chronic, atonic or infected wounds.	It reduces bacterial load, stimulates regeneration and facilitates the action of topical treatments.	It can be painful, risk of damage to viable tissue; Variable efficiency depending on the method.

Infection Control and Biofilm Management	Reduction and prevention of bacterial colonization through antiseptics, antibiotics and biofilm destruction.	Chronic infected wounds, ulcers, bedsores, postoperative wounds.	It decreases the risk of systemic infection, improves the local response to treatment.	Microbial resistance, difficulty in penetrating mature biofilm.
Negative Pressure Therapy (VAC)	Application of a controlled negative pressure that evacuates exudate and stimulates granulation.	Chronic atone wounds, bedsores, postoperative wounds, extensive trauma.	It accelerates healing, reduces secretions and edema, decreases the risk of infection.	Requires special equipment, higher costs, contraindicated in untreated necrosis.
Hyperbaric oxygen therapy and topical oxygenation	Increase tissue oxygenation to stimulate angiogenesis and collagen synthesis.	Diabetic ulcers, bedsores, ischemic wounds, post-surgical necrosis.	Improves perfusion and regeneration, anti-inflammatory effect, accelerates epithelialization.	Limited access to hyperbaric chambers, reduced efficiency in deep wounds (topical).
Systemic and topical antibiotics	Elimination of bacterial infections by systemic or local action guided by microbiologics.	Infected wounds with local or systemic signs of inflammation.	Broad spectrum, rapid effect, possible combined administration with local treatments.	Risk of bacterial resistance, side effects, reduced efficiency in dense biofilms.

Chapter 4. Regenerative and Biostimulating Techniques

4.1. High-intensity laser therapy (HILT) – histological principles and effects [185–192]

General principles of HILT therapy

High-Intensity Laser Therapy *(HILT*) is a modern method of biostimulating treatment, with deep action on the injured tissues. In contrast to low-intensity lasers (LLLTs), HILT uses increased energy (over 500 mW) and superior tissue penetration, allowing analgesic, anti-inflammatory and regenerative effects to be achieved simultaneously.

Its action is based on the principle of photobiomodulation, through which light energy is transformed into biochemical energy at the cellular level. Monochromatic and coherent laser radiation interacts with mitochondrial chromophores (especially cytochrome c oxidase), causing an increase in ATP synthesis, activation of cellular metabolism and intensification of reparative processes.

The biological effects depend on the wavelength, energy density, and duration of exposure. The wavelengths used in HILT range from 1064 nm (Nd:YAG) to 980 nm (laser diodes), chosen according to the depth of the target tissue.

Mechanisms of action

At the tissue level, HILT exerts a controlled photothermal effect, associated with photomechanical and photobiological changes that contribute to accelerating healing. The main mechanisms involved are:

1. Increasing local microcirculation through vasodilation and stimulating the release of nitric oxide (NO);
2. Reducing edema by increasing capillary permeability and activating lymphatic drainage;
3. Stimulation of fibroblast proliferation and synthesis of type I and III collagen;
4. Acceleration of peripheral nerve regeneration by activating axonal transport;
5. Decreased excitability of nociceptors, with a pronounced analgesic effect;
6. Anti-inflammatory effect by decreasing the expression of pro-inflammatory mediators (IL-1β, TNF-α, PGE_2).

Thus, HILT therapy combines the biochemical action of cell stimulation with visible clinical effects – reducing pain, improving mobility and accelerating the soft tissue healing process.

The parameters used vary depending on the depth and nature of the injury. In clinical practice, treatment is carried out with:

1. Wavelength: 1064 nm (Nd:YAG);
2. Output power: 5–12 W;
3. Durata impulsului: 150–200 μs;
4. Frequency: 10–40 Hz;
5. Energy density: 3–10 J/cm²;
6. Emission mode: pulsating or continuous, with dynamic application to the lesion area.

The treatment is carried out by direct contact with the skin, using a movable terminal piece, and the duration of a session varies between 5 and 10 minutes, depending on the size of the wound or the treated area.

Clinical effects observed

In clinical trials in patients with chronic and atone wounds, HILT therapy resulted in a significant reduction in pain intensity as early as the first 3–5 days of treatment, correlated with decreased inflammation and improvement of local trophicity ($p < 0.001$). It was also observed:

1. decrease in secretions and unpleasant odor of wounds;
2. appearance of granulation tissue after 5–7 sessions;
3. acceleration of re-epithelialization in the period of 14–21 days;
4. reduction of discomfort when mobilizing and peripheral sensitivity.

These results confirm the potential of HILT as an adjunctive therapy in the treatment of chronic wounds, bedsores and post-traumatic injuries.

Histological effects and tissue changes

At the microscopic level, the changes observed post-treatment highlight clear regeneration processes:

1. the presence of a superficial layer of young squamous epithelial cells;
2. proliferation of fibroblasts with elongated, cigar-shaped nuclei, indicating intense metabolic activity;
3. the appearance of collagen fibers organized parallel to the wound surface, indicating a solid support matrix;
4. increase in the density of new capillaries, confirming active angiogenesis;
5. reduction of inflammatory infiltration and normalization of tissue architecture.

These histological data, observed in the post-treatment examined preparations, demonstrate the profound biostimulation effect exerted by laser energy on the connective and epithelial structures.

Practical aspects and clinical protocols

In medical practice, HILT applies in several areas:

1. plastic and reconstructive surgery (chronic wounds, bedsores, skin grafts);
2. orthopedics (tendonitis, bursitis, muscle injuries);
3. dermatology (trophic ulcers, superficial burns);
4. medical rehabilitation (muscle contractures, neuralgia).

The treatment is well tolerated, non-invasive and without significant adverse effects, provided that the correct parameters are observed and direct application is avoided on exposed nerve or vascular structures.

Comparative results and clinical interpretation

Compared to conventional therapies (antibiotics, hydroactive dressings, VAC), HILT has demonstrated:

1. a reduction in healing time by up to 30–40%;
2. significant improvement of local mobility;
3. rapid analgesic effect, without the need for additional medication;
4. increase in scar quality (homogeneous structure, mature collagen).

Statistical analysis of clinical data revealed significant differences between treated and control groups, confirming the superiority of laser therapy in stimulating repair processes.

Laser therapy

A wound is considered **chronic** when it does not heal within a reasonable period of time, either due to a slow repair process or by recurrence of the injury after an apparently healed period. Chronic wounds occur as a result of disruption of one of the normal physiological processes of progressive healing, as a result of which the initial anatomical and functional structure of the tissue is no longer restored.

The term "chronic wound" was introduced into the medical literature in the 1950s to designate lesions that are difficult to heal or that do not follow the normal sequence of healing phases. Over time, its definition has been revised, with other names being proposed such as *difficult-to-heal wounds*, *complex wounds* or *non-healing wounds*. Currently, most sources define a chronic wound as a lesion that has not regained its anatomical and functional integrity after at least 3 months of evolution. However, there is still a lack of consensus on the duration and exact criteria of chronicity, which requires further research in this area. Most wounds normally go through the four physiological stages – hemostasis, inflammation, proliferation and maturation – but a significant percentage fail in one of these phases, leading to the appearance of a chronic wound with high morbidity and considerable costs for the medical system.

Wound bed preparation and debridement

The concept of *wound bed preparation* involves local management that favors natural healing or preparation for complementary methods (surgical or regenerative). This includes removing necrotic tissues, reducing local pressure, draining secretions, and optimizing the healing environment. Debridement aims to remove dead tissue, prevent infection, correctly visualize viable tissues, and facilitate the topical action of therapeutic agents.

Chronic wounds are often complicated by factors such as ischemia, necrosis, bacterial infections, and increased levels of matrix metalloproteinases (MMPs). These pro-inflammatory enzymes, together with the deficiency of growth factors, maintain inflammation and prevent the regeneration of the extracellular matrix. As a result, healing becomes slow and incomplete, and recurrences are common.

Peculiarities of atonic wounds

Atonic wounds, characterized by the lack of signs of active inflammation and the absence of neutrophil infiltrate, are frequently treated incorrectly. They do not heal spontaneously and require complex therapeutic strategies. The increased morbidity and costs of care justify the need for standardised prevention and treatment guidelines**.**

The most common chronic wounds of the lower limbs include arterial, diabetic, pressure and venous ulcers. The correct clinical diagnosis is based on a complete physical examination, evaluation of the location, size, depth, type of tissue and secretion of the wound, followed by vascular exploration.

Each plague must be evaluated individually, depending on the etiology, duration, location and level of microbial contamination. Traditional methods have often proved insufficient, which is why it is necessary to develop innovative diagnostic and therapeutic procedures**.**

Patients with chronic wounds experience not only pain and functional limitation, but also a major psychological and social impact. The introduction of modern technologies – such as laser therapy, VAC systems or hyperbaric oxygen therapy – is helping to reduce the duration of treatment and lower overall care costs.

The Role of Laser Therapy

Laser therapy is one of the most promising modern methods of stimulating regenerative processes. It uses **coherent infrared** radiation, applied to the injured areas, with the aim of accelerating the healing of soft tissues and relieving acute or chronic pain.

The physiological effects of laser therapy include:

1. stimulating local microcirculation;
2. improving lymphatic drainage;
3. reduction of inflammation and edema;
4. analgesic effect by decreasing the excitability of nociceptors.

By combining biostimulation with the photomechanical effect, laser therapy contributes to tissue regeneration while providing a non-invasive method of pain control.

High Intensity Laser (HILT)

High-intensity lasers produce deep photothermal, photomechanical, and photochemical effects, capable of penetrating to the deep layers of tissue. Through this mechanism, HILT therapy causes rapid pain relief**,** stimulates local metabolic processes and contributes to the functional recovery of the affected area.

Compared to low-intensity laser therapy (LLLT), HILT therapy enables larger surface area coverage and the delivery of a higher energy dose in a shorter time, achieving lasting effects on both the wound and the supporting tissues.

The laser used in the treatment was Nd:YAG (neodymium-yttrium-aluminum-garnet), with a wavelength of 1064 nm and a maximum power of 12 W, and can be applied both continuously and pulsatory, depending on the particularities of the lesion and the patient's local tolerance. The laser beam, emitted coherently and monochromatically, was directed onto the

lesion surface using a 30 mm applicator, which ensured a uniform distribution of energy over the entire treatment area. The duration of a therapeutic session was approximately 8 minutes and 20 seconds, a time considered optimal for inducing the biostimulator effect without the risk of tissue overheating.

During application, the photon energy emitted by the laser penetrates the depth of the tissue up to 3–5 cm, interacting with the cellular and extracellular components of the dermis. The photomechanical waves and the controlled photothermal effect cause capillary vasodilation, increased microcirculatory flow and stimulation of cellular metabolism, processes that lead to the activation of fibroblasts, the acceleration of collagen synthesis and the intensification of epithelialization processes. In addition, the stimulation of mitochondrial receptors causes the production of adenosine triphosphate (ATP) to increase, providing the energy necessary for tissue regeneration.

The laser therapy was applied uniformly to the surface of chronic wounds, in successive layers, maintaining constant contact between the applicator and the skin, to ensure optimal energy absorption. During the procedure, sudden movement or beam intersection was avoided to prevent overexposure.

Clinical inclusion criteria excluded patients with severe systemic or immunosuppressive conditions. Those treated frequently had cardiovascular and peripheral vascular comorbidities, such as hypertension, chronic ischemic heart disease, venous and valvular insufficiency, all under stable maintenance treatment. This selection enabled evaluation of the effectiveness of laser therapy under controlled conditions, without major pathological interference with the healing process.

The clinical effects observed following the application of the therapy included the reduction of local inflammation, the reduction of edema and pain, along with the acceleration of the formation of granulation tissue and the progressive restructuring of the extracellular matrix. Through its combined action – photothermic, photomechanical, and biochemical – the Nd:YAG laser has proven to be an essential tool in the regeneration of connective tissue and in improving the quality of healing, representing an important component in modern therapies for chronic wound healing. For comparison, the control group received exclusively drug treatment and antiseptic dressings. Both groups completed questionnaires on pain intensity, discomfort when walking, and quality of nighttime sleep. Harvested tissue fragments were fixed in 10% formalin and histologically analyzed after H&E staining.

The results obtained from laser therapy showed significant histological changes, reflecting the wound's transition from the inflammatory phase to the proliferative phase of healing. During treatment, areas of superficial necrosis were observed, characteristic of the process of biological cleaning of the wound, coexisting with tissue structures in active regeneration. The sub adjacent connective tissue was densely infiltrated with inflammatory cells and crossed by neoformation vessels, indicating intense angiogenic activity. In the same context, numerous macrophage cells loaded with hemosiderin were noted, a sign of phagocytosis of erythrocyte residues and the ongoing tissue remodeling process (**Figure 1a**).

After the completion of the therapy, the histological appearance of the wounds changed significantly, demonstrating an obvious progress in the regeneration process. The epidermis had areas of partial re-epithelialization, with squamous cells arranged in an orderly manner, and at the level of the

dermis there was an active proliferation of fibroblasts, characterized by spindle-shaped nuclei and elongated cytoplasm, a sign of intense synthetic activity.

The newly formed collagen fibers were aligned parallelly, compactly arranged, confirming the structural reorganization of the extracellular matrix and the resumption of the normal connective tissue architecture (**Figure 1b**). This morphological evolution confirms the biostimulating effect of laser therapy, which contributes not only to the macroscopic healing of the lesion, but also to the functional and histological recovery of the affected skin.

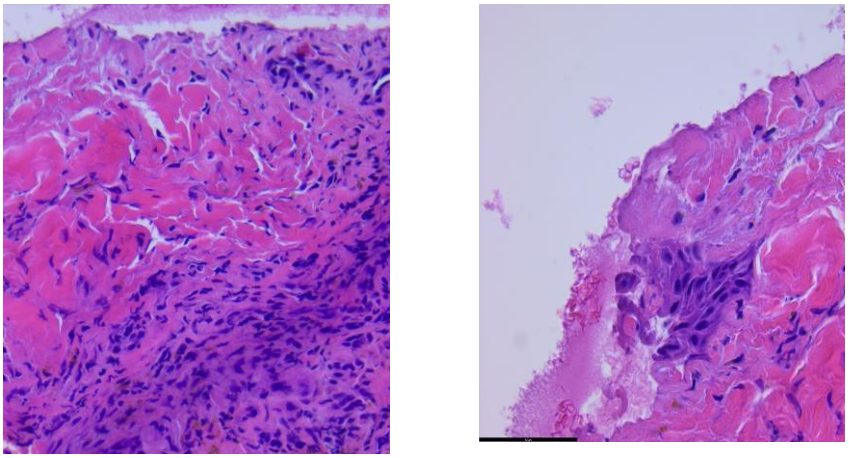


Figura 4.1 a. Histological Aspects of Laser Therapy

(a) During treatment – areas of superficial necrosis and incipient regeneration, with the presence of macrophages.

(b) After treatment – re-epithelialization layer and mature collagen fibers.

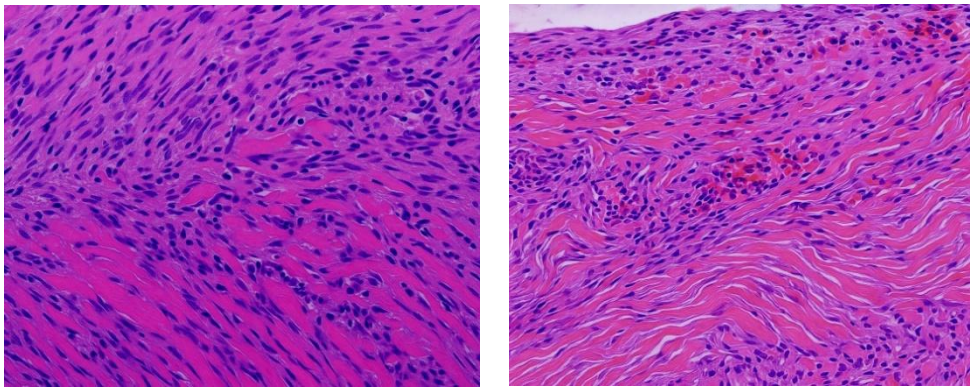
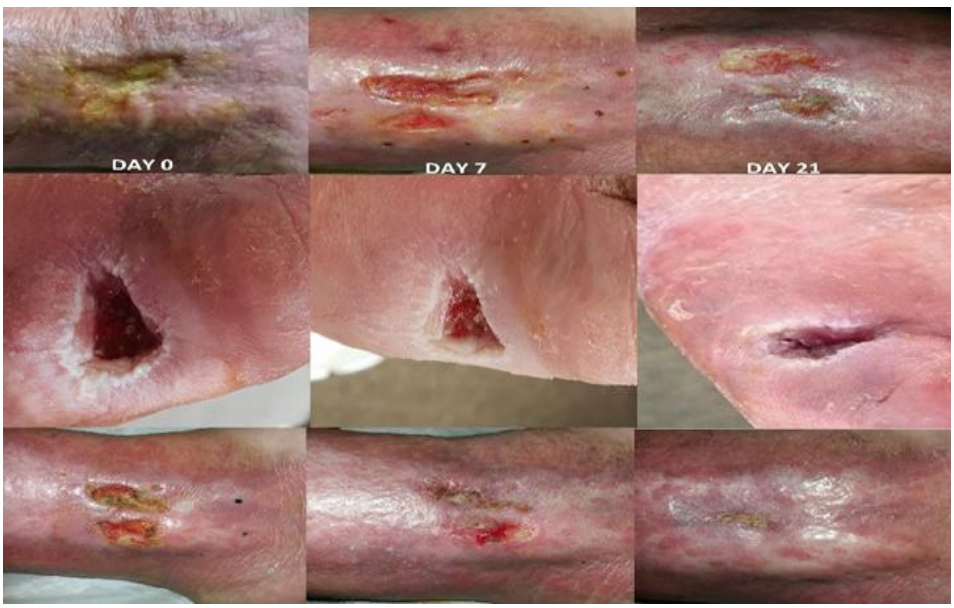


Figura 4.2 b. Histological aspects of Laser Therapy

At 90 days post-discharge, 75% of the patients treated with laser returned to the check-up; Of these, 70% did not require re-hospitalization, and 5% required a new procedure due to ulcer recurrence.

The clinical evaluation highlighted the decrease of purulent secretion, the reduction of inflammation and the acceleration of the epithelialization



process, confirming the major role of high-intensity laser therapy in the treatment of atone wounds.

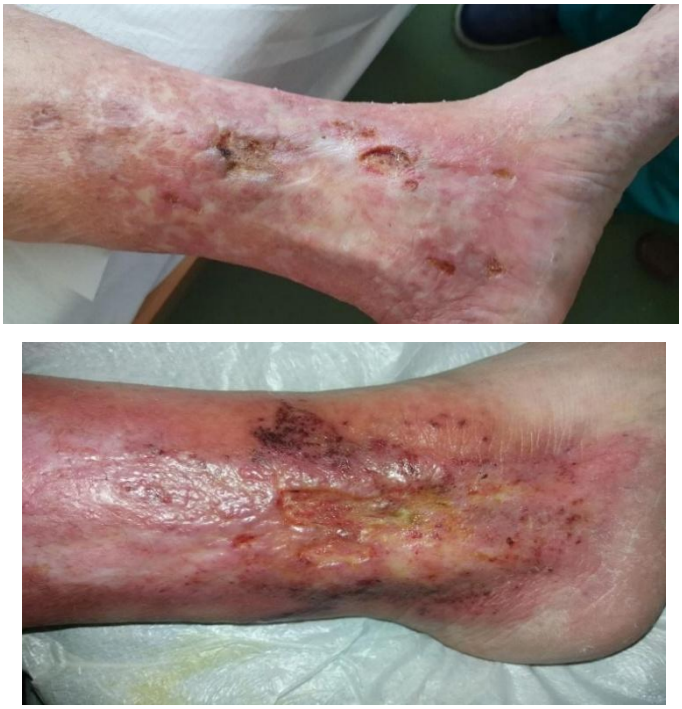


Fig 4.3. Evolution of Wounds Following Laser Therapy (personal collection)
Atonic wounds after laser therapy

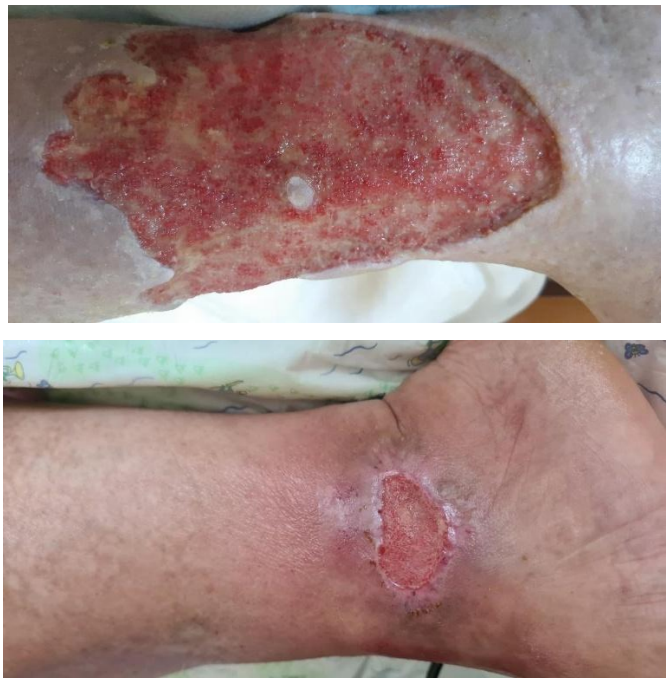


Fig 4.4. Evolution of Wounds Following Laser Therapy (personal collection)

4.2. Platelet-rich plasma (PRP) therapy – mechanisms of action and clinical protocols [193–198]

Chronic wounds are a complex medical problem that arise from disruption of one of the physiological processes involved in the progressive healing of tissues, resulting in incomplete restoration of anatomical and functional integrity. These are frequently complicated by factors that prevent healing— such as ischemia, necrosis, high bacterial load, or elevated levels of pro-inflammatory metalloproteinases. Wound healing is a dynamic process, supported by a multitude of cellular events that must be carefully coordinated to ensure the effective recovery of the injured tissue.

Although the human body has a remarkable regenerative capacity, it can be diminished by systemic conditions such as diabetes, chronic vascular insufficiencies or degenerative processes associated with old age. Under

these conditions, chronic wounds — especially venous ulcers, bedsores, and diabetic ulcers — are becoming a major therapeutic challenge and a priority area of research in regenerative medicine.

In this context, platelet-rich plasma (PRP) is one of the most promising autologous regenerative therapies. PRP contains an increased concentration of platelets, growth factors and cytokines directly involved in tissue repair, angiogenesis and local immunomodulation processes. By injecting PRP locally, the biological cascades responsible for fibroblast activation, collagen synthesis and capillary neoformation are stimulated, thus accelerating the healing process. In clinical trials dedicated to the treatment of chronic wounds, patient cohorts are frequently divided into two main groups, in order to allow objective comparison of the effectiveness of different therapeutic methods. In general, the study group comprises patients undergoing modern regenerative procedures – such as platelet-rich plasma (PRP) therapy, autologous cell therapy, or growth factor treatments – while the control group comprises patients treated with conventional methods, based on standard care, antiseptic dressings, and classic pharmacological treatment. The selection of participants follows strict inclusion and exclusion criteria to ensure comparability of results and the statistical validity of conclusions. As a rule, adult patients of both sexes with predisposing chronic conditions – such as diabetes, chronic obliterating arteriopathy or venous insufficiency with ulcers – and who express their informed consent to participate are included. On the other hand, patients with active wound infections, severe systemic pathologies (e.g. neoplasms, advanced renal or hepatic insufficiency, immunosuppressive conditions), recent traumatic injuries or lack of cooperation in complying with the protocol are excluded. This methodological approach allows to obtain a

homogeneous cohort, reducing confusing variables and facilitating the comparative analysis of the effects of therapy on the healing process.

Platelet-enriched plasma (PRP) is an autologous component obtained by centrifugation of venous blood, having a platelet content 3–5 times higher than basal plasma. These platelets release growth factors such as PDGF, TGF-β, VEGF and EGF, which stimulate fibroblast proliferation, angiogenesis and extracellular matrix formation – essential processes in tissue regeneration.

Stages of obtaining PRP

The preparation procedure is carried out under strict aseptic conditions, using certified medical kits, equipped with tubes provided with separator gel and anticoagulant (sodium citrates 3–4%). An average of 10–12 ml of venous blood is collected from each patient and then centrifuged for 5 minutes at 4000 rpm.

Centrifugation causes the blood to be separated into three distinct layers:

1. The upper layer, yellow-transparent in color, containing platelet-rich plasma (PRP);
2. The median layer, consisting of the separating gel, which delimits the plasma from the erythrocyte mass;
3. The lower layer, representing the erythrocyte mass itself.

The upper platelet-rich plasma fraction is gently mixed by slow movements for homogenization and then extracted with a sterile transfer device, being immediately prepared for therapeutic application.

Clinical application procedure

Before the administration of PRP, careful preparation of the wound bed is carried out, through surgical toilet and mechanical or chemical debridement, depending on the local peculiarities of the lesion. The application is usually performed on the fifth day of treatment, when the tissue environment becomes optimal for receiving growth factors.

The biological material is injected at the edges of the wound, at a distance of about 2 mm between the points of administration, and partially in the center of the wound, to ensure an even diffusion of the bioactive compounds. Local buffering is not performed to avoid premature absorption of the solution. After about 10 minutes, a simple sterile dressing is applied, designed to protect the area and prevent contamination.

The clinical evolution is reassessed at 14 days, with standardized photographic documentation and comparative analysis of the healing parameters: wound size, degree of epithelialization, reduction of exudate and relief of painful symptoms.

The analysis of the clinical evolution of the patients included in the research revealed significant differences between the study group, treated with regenerative therapies, and the control group, treated conventionally. Within the study group, most patients (76%) presented to the medical consultation accusing the presence of a chronic wound, with slow evolution and resistance to previous treatments, while 24% were hospitalized for other concomitant conditions, but also had chronic lesions associated with vascular or metabolic processes. In the control group, 63% of the patients had chronic non-epithelialized wounds as the main reason for presentation, the rest presenting for the associated pathologies that influenced the healing process.

Local pain was the dominant symptom in both groups, reported by approximately 80% of patients, with a mean intensity of 8-10 points on the

visual analogue scale (VAS). This increased level of discomfort has been correlated with persistent inflammation and abundant exudate in chronic wounds, characteristics commonly found in venous, arterial, or diabetic ulcers.

During therapy, a progressive improvement in pain symptoms was observed, with greater improvement in the group that benefited from PRP and associated regenerative procedures. Assessments performed at regular intervals showed a significant decrease in pain from day 7 onward, and by day 10 of treatment, 30% of patients in the study group reported a return to normal mobility, compared with 20% in the control group, who continued to have moderate functional limitations.

This favorable development was accompanied by a noticeable decrease in local inflammation, a reduction in wound size, and an increase in granulation tissue in the treated area. Beyond the objective parameters, the patients in the study group reported a significant improvement in quality of life, expressed by decreased walking discomfort, improved sleep and reduced need for analgesics.

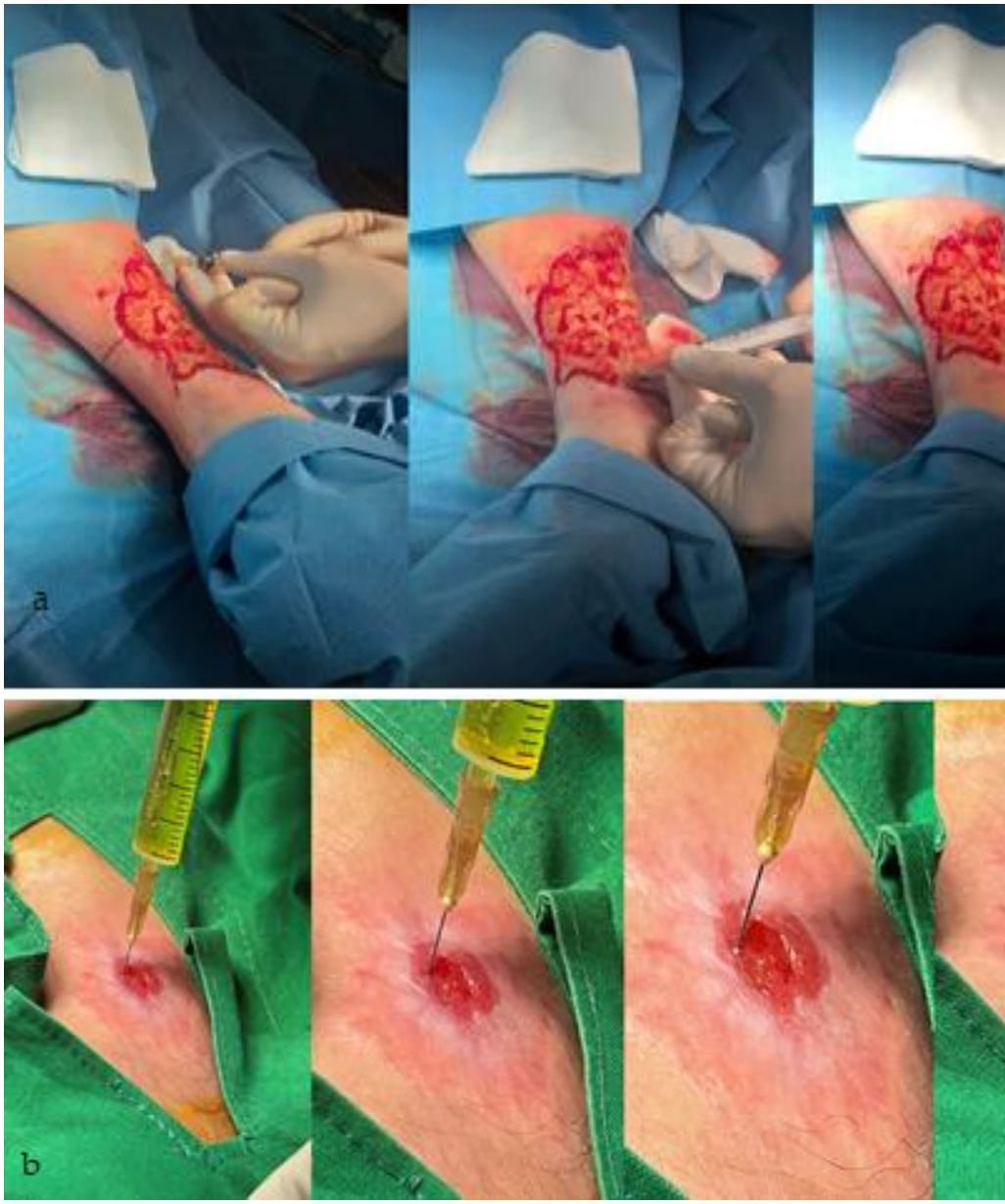


Fig. 4.5. Preparation of the wound bed before applying therapy; (b) Application of platelet-enriched plasma (PRP treatment).

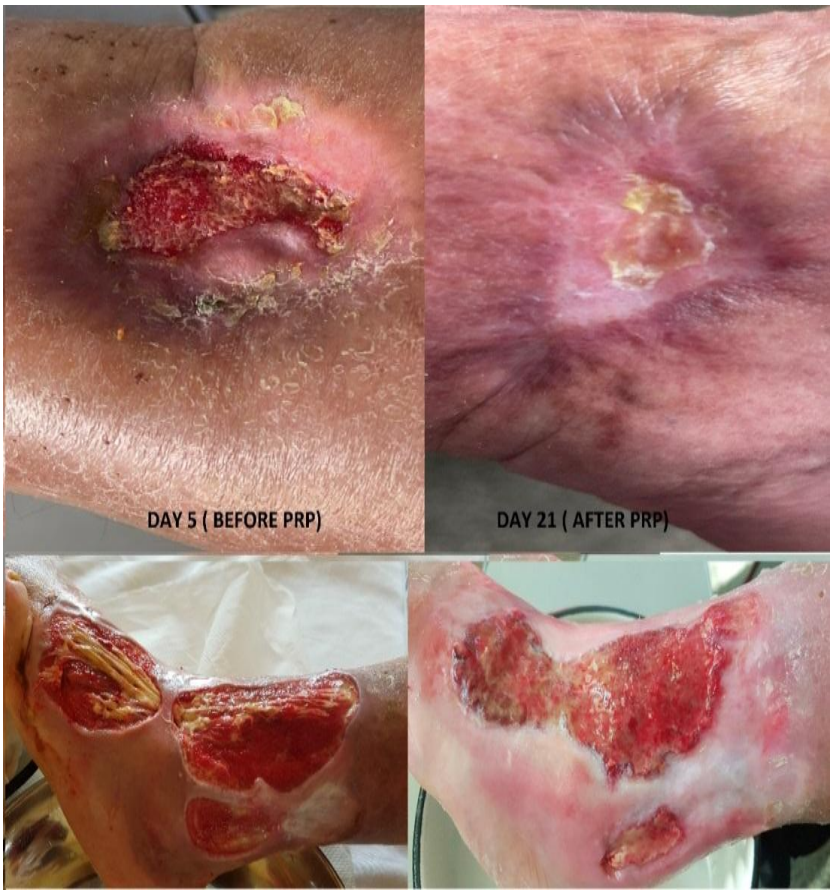


Fig. 4.6. Local course of the wound after platelet-rich plasma injection.

Authors' clinical archive, reproduced with informed patient consent.

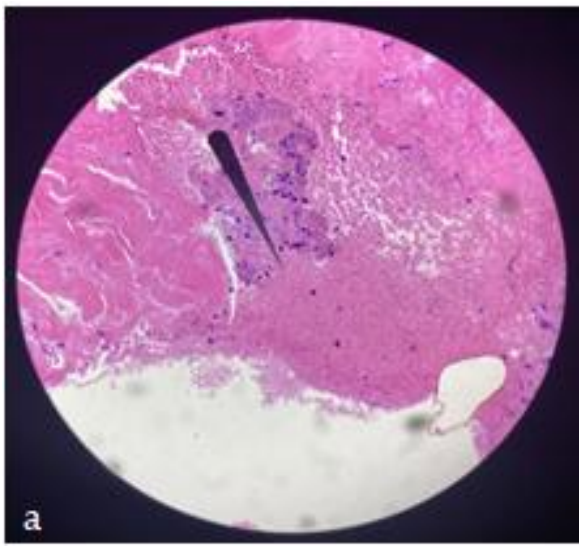


Fig 4.7. (Resident Laboratory Oradea)

(a) Ulcerated tissue with superficial necrosis and inflammatory infiltrate (HE 100×, before); (b) Tissue with active regeneration, presence of fibroblasts and organised collagen (HE 100×, after 21 days).

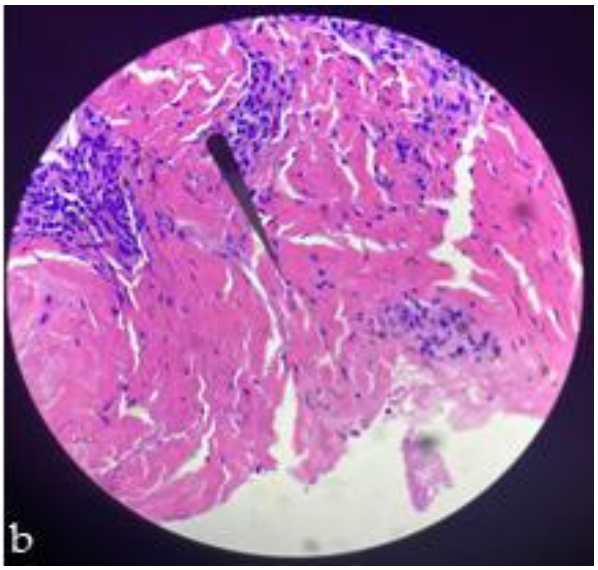


Fig 4.8. (Resident Laboratory Oradea)

b- A small area of residual necrosis, located superficially, can be observed, and numerous fibroblasts (cells with cigar-shaped nucleus)

are present underneath, which denotes an active phenomenon of regeneration. Numerous parallel collagen fibers can still be observed, which will support the healing process. HE 100X (after) (biopsy day 21)

The results obtained in numerous clinical and observational research highlight the essential role of platelet-enriched plasma (PRP) in stimulating tissue regeneration processes and accelerating the healing of atone wounds. Due to the increased content of biologically active growth factors – such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) – PRP promotes cell proliferation, collagen synthesis, angiogenesis and extracellular matrix restructuring.

Compared to conventional treatments, the application of PRP causes accelerated epithelialization, a significant reduction in local pain and an overall improvement in the clinical condition. Histologically, these changes translate into the activation of fibroblasts, the intensification of neoangiogenesis and the reorganization of collagen fibers, processes that support the rapid and functional recovery of the injured tissue.

The procedure stands out for its high safety profile, being an autologous, biocompatible and immunological risk-free method, as the biological material comes from the patient himself. In addition, its relatively low costs and ease of application recommend it as an affordable and effective solution in current clinical practice.

The figure illustrates the process of separation and collection of platelet-enriched plasma (PRP), performed after centrifugation of venous blood into tubes provided with separator gel and anticoagulant. Following centrifugation, the blood separates into three distinct layers – the erythrocyte mass at the base, the intermediate layer of separating gel and

the golden-yellow upper layer, corresponding to platelet-rich plasma, which contains an increased concentration of growth factors essential for tissue regeneration. This plasma fraction is carefully extracted, under sterile conditions, by means of a syringe, to be used immediately in regenerative treatments designed to stimulate the healing processes of chronic wounds.

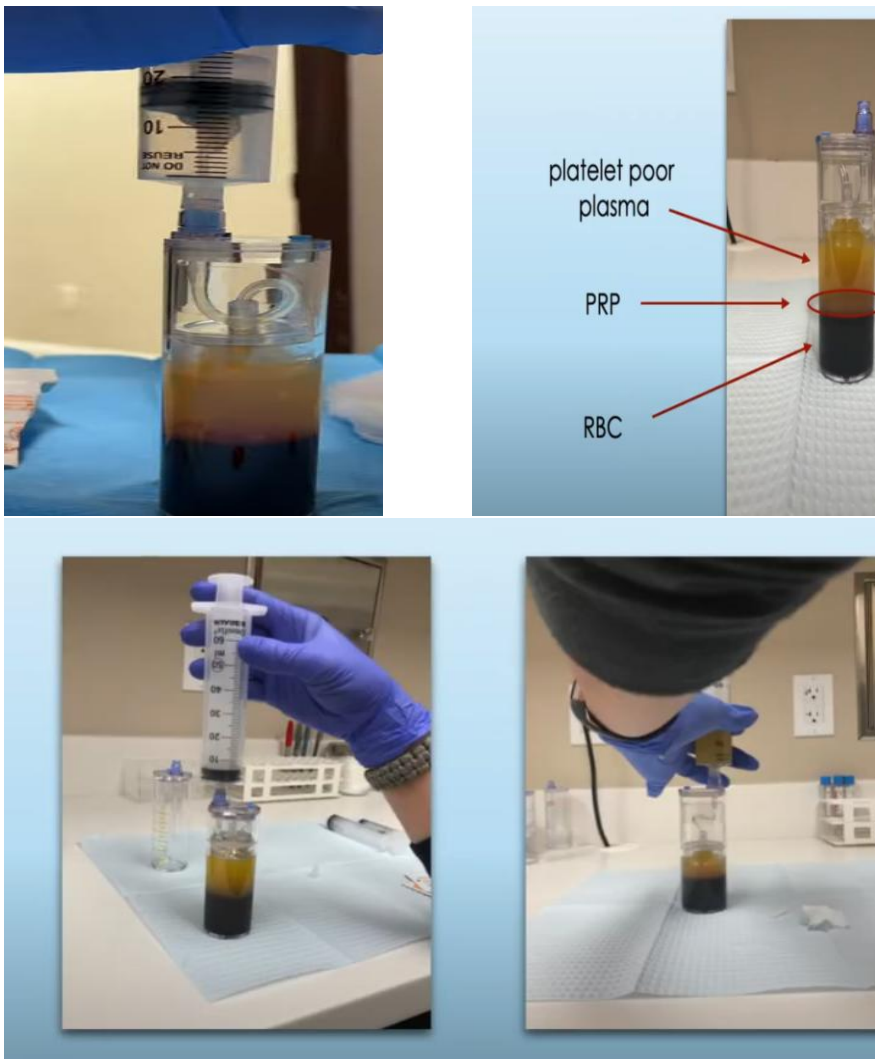


Fig. 4.8. Preparation of biological material for topical application

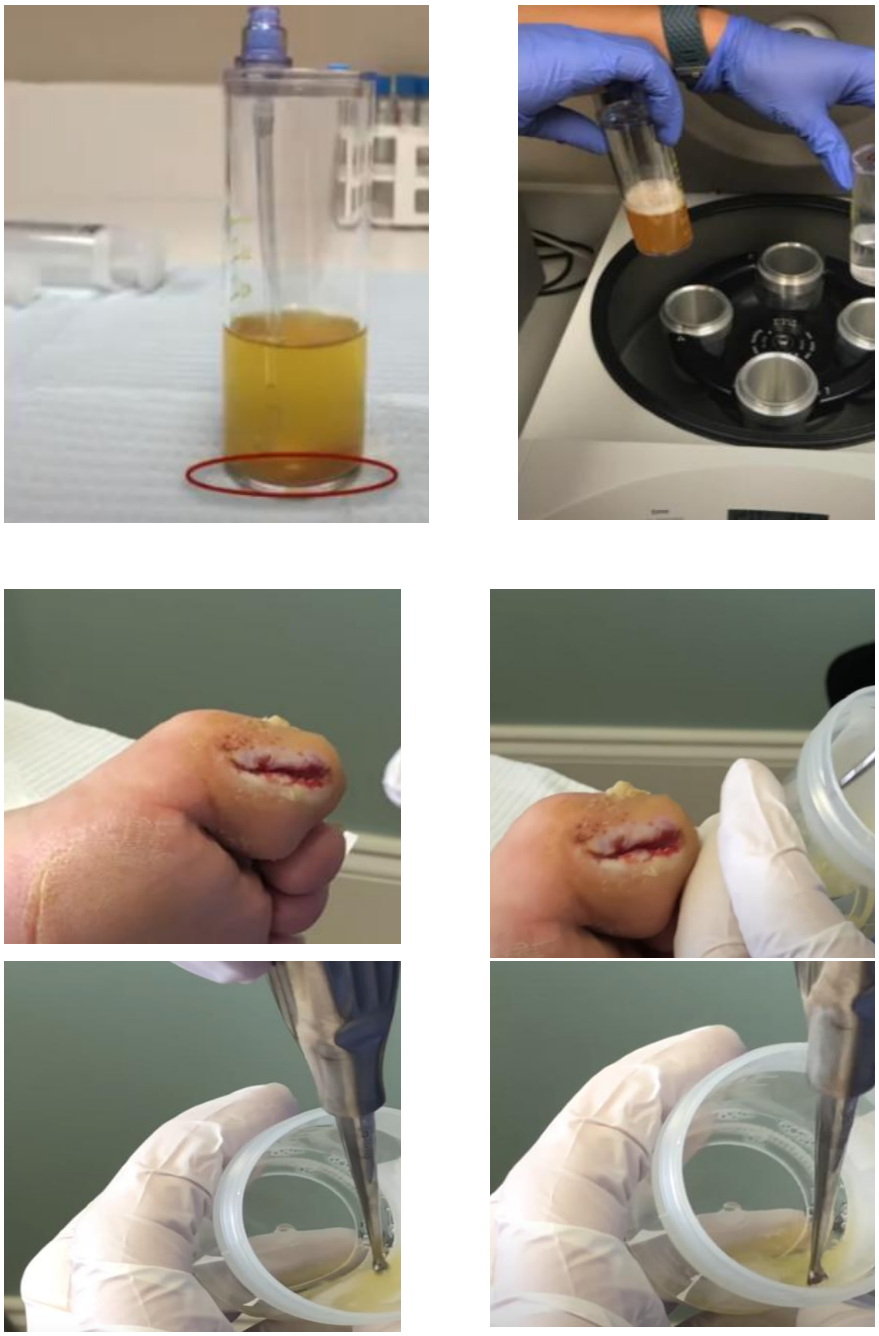


Fig. 4.9. Centrifugation, extraction, and topical application by dripping onto the wound

Authors' clinical archive, reproduced with informed patient consent.

The figure shows the steps in the application of platelet-rich plasma (PRP) in the treatment of chronic wounds, starting with the local preparation of the lesion area and continuing with the manipulation of autologous biological material under sterile conditions. After the wound toilet and selective debridement are performed, the PRP is activated and prepared for application, and is subsequently introduced locally in liquid or gelled form to stimulate angiogenesis, fibroblast proliferation, and epithelial regeneration, thereby accelerating healing and reducing the risk of infection.

The application of platelet-rich plasma (PRP) therapy is a modern, safe, and effective strategy for the management of chronic atone wounds. By activating endogenous repair mechanisms and stimulating microcirculation and tissue regeneration, PRP helps reduce inflammation, restore the skin's structural integrity, and shorten healing time.

The integration of this procedure into multimodal treatment protocols – along with debridement, oxygen therapy or negative pressure therapy – strengthens the complex and personalized approach to the chronic wound patient, confirming the clinical value and extended potential of PRP in modern wound surgery.

4.3. Autologous cell therapy and biological skin substitutes [199–202]

In recent decades, advances in the field of regenerative medicine have introduced autologous cell therapy and biological skin substitutes as modern alternatives to conventional skin grafts, providing viable solutions for tissue reconstruction and stimulation of endogenous healing processes. These methods are based on exploiting the regenerative potential of the patient's own cells and using compatible biomaterials that recreate a microenvironment conducive to regeneration.

Autologous cell therapy

Autologous cell therapy consists of the collection, processing and reimplantation of cells from the patient's body, in order to restore damaged tissue structures. The most widely used cellular sources are mesenchymal cells (obtained from bone marrow, adipose tissue or peripheral blood) and autologous keratinocytes, which play an essential role in the re-epithelialization of chronic wounds.

The process involves isolating adult stem cells, multiplying them in sterile culture media, and applying them topically in the form of a cell suspension or biocompatible gel. These cells release paracrine factors that stimulate fibroblast proliferation, angiogenesis and collagen synthesis, thus accelerating the regeneration of dermo-epidermal tissue.

Compared to traditional therapies, autologous cell therapy offers the advantage of perfect biocompatibility, eliminating the risk of immune rejection and reducing the need to harvest wide skin grafts. In addition, its effect manifests itself not only locally, but also by modulating the inflammatory response, favoring the rapid transition from the exudative to the proliferative phase of healing.

Biological skin substitutes

Biological skin substitutes are advanced materials, natural or synthetic, designed to temporarily or permanently replace the dermo-epidermal structure and to support the regeneration process. They can be classified into:

1. Epidermal substituents (keratinocytes, epithelial matrix);

2. Dermal substitutes (collagen, hyaluronic acid, elastin);

3. Composite systems (cell combinations and biodegradable three-dimensional matrices).

The most widely used materials are collagen, fibroin, chitosan and decellularized extracellular matrix, which provide a three-dimensional architecture favorable to cell migration and the formation of new viable tissue. In clinical practice, substitutes can be soaked with PRP or colonized with autologous cells, forming so-called *bioactive constructs*, capable of accelerating epithelialization and reducing the risk of hypertrophic scarring.

A notable example is collagen matrices combined with mesenchymal stem cells, which have demonstrated a significant increase in vascularization and superior structural organization of regenerated tissue. Hyaluronic acid-based biomembranes are also widely used for maintaining a moist environment, controlling inflammation, and preventing secondary infections.

Advantages and clinical prospects

The integration of autologous cell therapies with biological skin substitutes has opened a new stage in regenerative surgery of complex wounds, combining molecular biology with tissue engineering. These approaches make it possible to:

1. reduction of healing time and hospitalization needs;

2. restoration of the functionality and elasticity of the skin;

3. decreased risk of infection and recurrence of wounds;

4. achieving a superior aesthetic result with minimal scarring.

Although the initial costs and the necessary infrastructure may be limitations, advances in biotechnology and 3D printing promise to expand these therapies into current clinical practice, with the possibility of customizing skin substitutes according to the type of lesion and the patient's biological profile.

Autologous cell therapy and biological skin substitutes mark a paradigm shift in chronic wound management and tissue reconstruction. By combining knowledge from the fields of cell biology, biomaterials and regenerative medicine, these technologies offer an integrative and personalized approach, capable of restoring not only the morphological integrity, but also the biological functionality of the skin.

4.4. Biomaterials, hydrogel and smart dressings for wound healing [203–206]

The evolution of chronic wound treatment has been profoundly influenced by the emergence of advanced biomaterials and smart dressings, which have revolutionized the concept of local care, moving from simple passive protection of the lesion to active intervention in the tissue regeneration process. These modern materials are designed to dynamically interact with the wound environment, regulate humidity, oxygenation and controlled release of bioactive substances, thus facilitating faster, safer and more aesthetic healing.

Biomaterials used in tissue regeneration

Biomaterials are biologically compatible physicochemical supports, capable of temporarily replacing skin structures and stimulating tissue regeneration. Depending on the origin and composition, they can be:

1. Natural – such as collagen, chitosan, fibroin, alginate or hyaluronic acid;

2. Synthetics – such as polyurethane, polylactide (PLA), polyglycolite (PGA) and PLGA copolymers.

Natural biomaterials have the advantage of excellent biocompatibility and controlled degradation, favoring cell migration and neoangiogenesis, while synthetic ones allow precise control of porosity, mechanical strength and release rate of bioactive substances.

In clinical practice, these materials are often enriched with growth factors, antibiotics, plant extracts or antimicrobial nanoparticles, forming bioactive structures that can prevent infection and accelerate re-epithelialization.

Hydrogel – ideal microenvironment for healing

Hydrogels represent a distinct class of biomaterials, characterized by a high water absorption and retention capacity, maintaining an optimal humid microclimate for granulation and epithelialization processes. Their three-dimensional structure, made up of networks of natural or synthetic polymers, allows the diffusion of oxygen and nutrients, while absorbing excess exudate.

Due to this property, hydrogels can be used as carriers for the controlled release of bioactive substances, such as:

1. growth factors (PDGF, VEGF);
2. molecule antimicrobee;
3. enzymes or autologous cells integrated into the matrix.

There are also thermoresponsive and photoreactive hydrogels, which change their structure according to local temperature or exposure to light, allowing an adaptive release of active compounds according to the needs of the wound.

By combining physicochemical properties with biological effects, hydrogels help reduce inflammation, stimulate angiogenesis and increase the speed of re-epithelialization, while providing a protective barrier against microbial contamination.

Smart dressings – a new generation of therapeutic solutions

Smart dressings are the latest innovation in wound management, based on the integration of biological sensors, shape memory materials and controlled release systems. Their purpose is no longer just to cover the wound, but to monitor and regulate local conditions in real time: humidity, pH, temperature and oxygen level.

Such dressings can detect infection early by changing color or transmitting signals to electronic devices, and some versions include microchips and wireless sensor networks, which provide continuous data on the evolution of the wound.

In addition to monitoring functions, smart dressings can automatically release antimicrobial, anti-inflammatory or regenerative substances, depending on biochemical changes in the local environment. In addition, their structure allows for selective gas exchange and maintaining a constant temperature, favorable to cellular metabolism.

Integrating biomaterials and digital technologies

Current trends are moving towards combining traditional biomaterials with digital and nanotechnological technologies, in order to obtain personalized products, adapted to the type of wound and the patient's needs. Examples include:

1. 3D printed dressings with architectures inspired by the structure of the skin;
2. bioactive matrices integrated with micro-sensors that continuously measure biological parameters;

3. nanohydrogels that react intelligently to variations in pH or temperature.

These innovations transform wound care into a dynamic, personalized and monitored process, where biomaterials are no longer simple physical supports, but active actors in tissue regeneration.

The use of biomaterials, hydrogels and smart dressings marks a revolution in chronic wound management, offering a scientific, personalized and integrated approach. These modern solutions contribute to creating an optimal biological environment for healing, reducing the risk of infection and improving patient comfort, integrating the principles of regenerative medicine with advanced technology. As research progresses, smart dressings and bioactive biomaterials are becoming not only therapeutic tools, but also diagnostic and monitoring platforms, capable of radically transforming the way tissue healing is approached in the 21st century.

4.5. Combined interventions: PRP + laser + controlled oxygenation [207–208]

The modern approach to chronic wounds is no longer limited to the use of a single therapeutic method, but is based on the synergistic integration of several complementary procedures, capable of acting simultaneously on different phases of the healing process. The combination of platelet-enriched plasma (PRP), high-intensity laser therapy (HILT) and controlled oxygenation is one of the most effective clinical strategies today, providing cumulative benefits on tissue regeneration, microcirculation and inflammation control.

Therapeutic reasons and mechanisms of action

Each of these therapies acts on a specific healing mechanism, and their combination allows the amplification of the overall biological effect:

1. PRP provides growth factors (PDGF, VEGF, TGF-β, EGF) that stimulate fibroblast proliferation, angiogenesis and collagen synthesis;
2. The high-intensity laser acts through photonic biostimulation, increasing cell metabolism, local blood flow and tissue oxygenation;
3. Controlled oxygenation, either hyperbaric or topical, improves the diffusion of oxygen in tissues, favoring epithelialization processes and inhibiting anaerobic bacterial growth.

The interaction between these methods generates a synergistic effect, in which laser-induced cellular activation increases the sensitivity of the tissue to growth factors in PRP, and the additional oxygen supplied locally optimizes the energy use of the cells involved in regeneration.

Combined clinical protocol

The integrated therapeutic protocol involves a logical succession of stages, so that each intervention amplifies the effects of the other:

1. Preparation of the wound bed by mechanical or enzymatic debridement, followed by antiseptic toilet;
2. Application of high-intensity laser (HILT), in controlled doses (1–3 W/cm²), to stimulate microcirculation and reduce local inflammation;
3. Administration of PRP, immediately after irradiation, by perilesional injection and topical application, ensuring uniform diffusion of biological factors;

4. Controlled oxygenation (hyperbaric or topical), applied subsequently, which favors the oxygenation of the newly formed capillaries and the maintenance of a sterile humid environment.

The treatment is repeated at intervals of 7–10 days, depending on the size of the wound and the evolution of the regeneration process, being monitored clinically and imaging (serial photos, thermography or high-resolution ultrasound).

Observed biological and histological effects

The combined application of PRP, laser and controlled oxygenation leads to a significant acceleration of regeneration processes, with:

1. rapid formation of well-vascularized granulation tissue;
2. increasing the density of fibroblasts and organized collagen fibers;
3. reduction of chronic inflammation and exudate;
4. reducing pain and improving local mobility.

Histologically, a dense network of new capillaries is observed, the activation of fibroblasts and keratinocytes and the reorganization of the extracellular matrix into ordered layers, indicating a functional, not only morphological, regeneration of the tissue.

Clinical advantages and prospects

PRP + laser + controlled oxygenation combination therapy brings a number of major advantages:

1. shortening the total healing time by up to 30–40%;
2. reducing the frequency of secondary infections;
3. improving the aesthetic appearance of the treated area;

4. increasing patient comfort and reducing the need for additional surgeries.

This therapeutic strategy also has a positive psychological impact, by relieving pain and restoring the functionality of the affected area, which significantly contributes to increasing the patient's quality of life.

The combined PRP + laser + controlled oxygenation interventions define a new paradigm in the treatment of chronic wounds, based on the synergy between biostimulation, cell regeneration and optimization of the local environment. By coordinating the activation of angiogenesis, collagenogenesis, and epithelialization, this multifactorial approach offers superior results compared to isolated therapies.

From a current clinical perspective, such protocols can be considered an emerging standard in regenerative surgery, with the potential to redefine therapeutic strategies for chronic and atonic lesions.

Comparison of modern regenerative therapies in the treatment of chronic wounds Therapies	Main mechanism of action	Biological effects	Clinical advantages	Limitations / Considerations
PRP (Platelet Enriched Plasma)	Release of growth factors (PDGF, VEGF, TGF-β, EGF) that stimulate cell proliferation and angiogenesis	Activation of fibroblasts, formation of the extracellular matrix, increase of collagen synthesis	Accelerated, autologous, biocompatible healing, minimal immunological risk	Requires rapid collection and processing, variability in platelet concentration
High Intensity Laser (HILT)	Photonic biostimulation,	Reducing inflammation,	Rapid anti-inflammatory	Requires specialized

	increasing cell metabolism and microcirculation	stimulating collagenasis and angiogenesis	and analgesic effect, improves local oxygenation	equipment and trained personnel
Controlled oxygenation (hyperbaric / topical)	Increase partial pressure of oxygen at the tissue level, improve oxidative metabolism	Stimulating epithelialization, inhibiting anaerobic bacteria, supporting angiogenesis	Increases regenerative capacity and resistance to infections	Limited access to hyperbaric chambers, contraindications in patients with pulmonary or cardiac conditions

Chapter 5. Modern Perspectives and Research Directions

5.1. Nanotechnologies and controlled release systems of active substances [185–192]

Over the past two decades, nanotechnology has become one of the most promising frontiers of regenerative medicine and chronic wound therapy. At the interface between physics, chemistry, biology and materials engineering, it offers the possibility to act directly on tissue microstructure and cellular processes, through intelligent systems for the controlled release of bioactive substances, which can modulate the inflammatory response, accelerate regeneration and prevent recurrent infections.

General principles of nanotherapy applied in wound healing

Nanotechnology uses particles between 1 and 100 nanometers in size, whose large surface area and high chemical reactivity give them unique properties of interaction with cells and biomolecules. These nanoparticles can be made up of organic (biodegradable polymers, lipids, proteins) or inorganic (zinc oxide, silver, gold, silicon, hydroxyapatite) materials, being designed to transport active agents to the injured tissue in a precise and controlled way.

By altering their surface properties — electrical charge, hydrophobicity, pH sensitivity or temperature — these particles can be programmed to release therapeutic substances only into the specific biological environment of the wound, characterized by increased acidity, oxidative stress and high enzymatic activity. This specificity of action ensures superior therapeutic efficacy and minimizes systemic side effects.

Types of nanotechnology systems used in wound therapy

1. Metal nanoparticles (Ag, ZnO, CuO, Au): these exert strong antimicrobial effects, acting on bacterial membranes and blocking microbial replication. In addition, zinc and gold oxide nanoparticles exhibit

anti-inflammatory and antioxidant properties, reducing local oxidative stress and stimulating fibroblast proliferation.

2. Polymer nanocapsules and nanospheres: Made from materials such as PLGA (polylactic-co-glycolic acid), chitosan or alginate, they function as transport vehicles for antibiotics, growth factors, antimicrobial peptides or natural antioxidants. They allow the slow and steady release of active substances over a period of several days, maintaining a stable therapeutic concentration at the wound level.

3. Electrospun nanogels and nanofibers: These flexible three-dimensional structures are used as bioactive supports that combine the physical barrier effect with local pharmacological action. Nanofibers can be impregnated with drugs, enzymes, or stem cells and integrated into smart dressings that react to stimuli such as temperature, pH, or humidity.

4. Liposomes and niosomes: These nanoscalar lipid vesicles are ideal for transporting fragile molecules such as growth factors, vitamins or antioxidant substances, protecting them from premature degradation and ensuring targeted delivery to epithelial and fibroblast cells.

Intelligent controlled release systems

The integration of nanotechnology with biological sensors and responsive materials has led to the development of intelligent controlled-release systems that can regulate the dose and rate of drug administration based on the wound's local physiological parameters.

Relevant examples include:

1. pH-sensitive nanocomposite dressings, which release antibiotics only into the acidic environment of infected wounds;

1. thermoresponsive hydrogels, which become permeable to the increase in local temperature and promote the diffusion of anti-inflammatory substances;

2. photo- and electrostimulable systems, in which light energy or weak electric current triggers the controlled release of growth factors.

These technologies offer the possibility of a monitored and self-regulating healing, in which the dressing material becomes an active participant in the therapeutic process, not just a passive support.

Clinical Applications and Demonstrable Benefits

Recent clinical studies have shown that the use of dressings based on silver or zinc nanocomposites significantly reduces the bacterial load and accelerates the formation of granulation tissue, especially in diabetic ulcers and ischemic wounds. In addition, growth factor-impregnated nanofibers (EGF, VEGF) have been shown to be effective in stimulating rapid epithelialization, reducing overall healing time by up to 40%.

Another emerging field is that of nanocarriers combined with platelet-rich plasma (PRP), which allow prolonged release of growth factors and amplification of the regenerative effect. At the same time, current research is exploring the bioconjugation of nanoparticles with specific antibodies or peptides, in order to direct the therapeutic action precisely to the injured cells.

Although promising, the large-scale clinical application of nanotechnologies is still limited by issues related to biocompatibility, toxicity and standardization. A careful assessment of the interactions between nanoparticles and the immune system, and their potential for accumulation in target organs, is required.

The future of the field is shaping up toward hybrid systems that combine nanomaterials with artificial intelligence and biomedical sensing to create autonomous dressings capable of detecting, responding, and reporting in real time on the evolution of the healing process. Also, personalized nanomedicine will allow the adaptation of the composition of

the nanodressings according to the genetic, microbiological and immunological profile of the patient, transforming wound treatment into an individualized and predictive process.

Nanotechnologies and controlled-release systems represent one of the most innovative directions in contemporary regenerative medicine. They offer the possibility of a precise, self-regulating and minimally invasive treatment, with proven benefits in reducing infections, accelerating regeneration and increasing therapeutic efficiency. By combining these technologies with biological therapies (PRP, stem cells, laser), wound surgery is gradually transforming into a predictive, intelligent and integrated field, oriented towards complete healing and functional tissue restoration.

5.2. Artificial Intelligence in Image Analysis and Cure Prediction [193–198]

Artificial intelligence (AI) has become an indispensable tool in contemporary medicine, including in wound surgery, offering new possibilities for automated analysis, early diagnosis, and prediction of healing evolution.

By using machine learning algorithms and convolutional neural networks (CNNs), AI-based analysis systems can interpret complex wound images obtained through clinical photographs, thermography, ultrasound or OCT. These algorithms are able to recognize morphological and chromatic patterns associated with different healing stages, automatically quantify the size, depth and degree of vascularization of the lesion, and distinguish infected wounds of the uninfected ones.

AI-based predictive models use longitudinal analysis of serial images and clinical data (age, comorbidities, treatments applied), to estimate the likelihood of complete cure, time required, and risk of recurrence. In this way, the clinician can adjust the therapeutic plan in a proactive manner, avoiding stagnation of the regeneration process or the application of ineffective treatments.

The emerging applications of artificial intelligence in the field of surgery and wound therapy are aimed at developing integrated diagnostic, monitoring and intervention systems, based on smart dressings equipped with multiparametric micro-sensors and AI processing modules. These innovative devices combine the principles of nanotechnology, microelectronics and algorithmic analysis to measure, interpret and react in real time to physiological changes in the wound.

Thus, micro-sensors integrated into the dressing structure can continuously monitor essential parameters such as local temperature, pH, humidity level, mechanical pressure and concentration of dissolved oxygen in the exudate. The values of these parameters directly reflect the biological stage of healing: the increase in temperature may signal an inflammatory or infectious process, the decrease in pH indicates the accumulation of acidic metabolites, and variations in humidity influence the degree of epithelialization and the efficiency of hydroactive dressings.

The data obtained is transmitted wirelessly to a central digital platform, where an artificial intelligence algorithm analyzes the evolution of each parameter, identifies deviations from normal and issues automatic alerts to the clinician or patient, in case of signs of infection, ischemia or stagnation of healing. Some prototypes already include deep learning neural networks capable of learning the characteristic patterns of each patient, thus adapting the treatment according to the individual biological response.

At a later stage of development, these systems will be able to operate autonomously, triggering the controlled release of therapeutic substances (antibiotics, growth factors, anti-inflammatory agents) based on data transmitted by the sensors — a concept known as a "self-regulating active dressing." Through this approach, treatment becomes dynamic, adaptive and personalized, reducing manual interventions and the risk of human error.

The integration of AI in wound management also paves the way for remote patient monitoring. By connecting smart dressings to mobile apps or cloud hospital platforms, the clinician can remotely track the evolution of each wound, compare serial images, and analyze physiological parameter curves. This continuous surveillance system allows for early interventions, reduced outpatient visits and optimized care costs. In addition, the patient takes an active role in managing their own recovery, being automatically notified when the dressing needs to be changed or when deviations from the normal healing process are detected.

In the near future, the combination of artificial intelligence, nanotechnology, and the medical Internet of Things (IoMT) will lead to the emergence of a global network of interconnected devices capable of simultaneously collecting and analyzing millions of chronic wound datasets from various regions. This huge volume of information will allow the collective learning of algorithms, the generation of increasingly accurate predictive models and the identification of the most effective therapeutic protocols for each type of lesion and patient category.

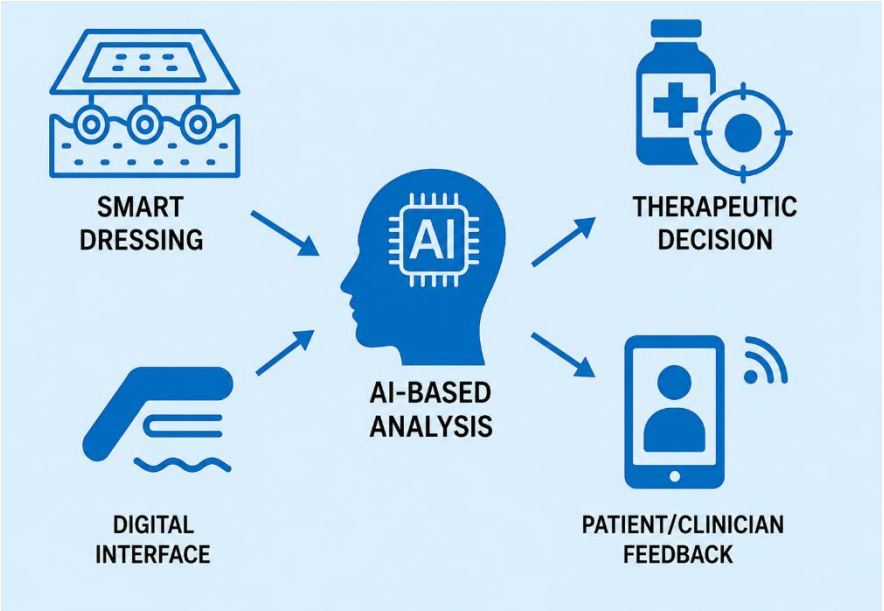


Fig 5.1. Diagram created by the authors for educational and illustrative purposes.

Therefore, the integration of artificial intelligence in wound treatment is no longer a simple technological extension, but a fundamental paradigm shift: from reactive, observation-based medicine to anticipative, personalized and digitally assisted medicine, in which each stage of healing is quantified, optimized and monitored in real time.

5.3. In vitro and in vivo experimental models for the study of the healing process [199–202, 203-208]

The complex understanding of the biological mechanisms that govern wound healing requires the development of rigorous and standardized experimental models, capable of reproducing as faithfully as possible the dynamics of tissue regeneration. These models allow sequential investigation of the healing stages of inflammation, proliferation, cell migration and remodeling under controlled, quantifiable and reproducible conditions. At the same time, they are an essential tool in the testing of

innovative therapies, such as nanomaterials, bioactive biomaterials, cell therapies or controlled-release smart dressings.

In vitro models

In vitro models are the first step in assessing the biological response and biocompatibility of materials intended for clinical application. They provide a controlled environment without the systemic interference of the living organism, allowing precise analysis of cell-matrix interactions and cellular responses to mechanical, chemical or biological stimuli.

The most widely used are models based on human or animal cell cultures:

1. Fibroblasts – involved in collagen synthesis and extracellular matrix restoration;
2. Keratinocytes – epithelial cells that ensure the re-epithelialization of the lesion surface;
3. Endothelial cells – essential for the process of angiogenesis and tissue perfusion.

These cells are grown either in monocultures (for specific studies on a single cell line) or in cocultures or tri-cellular systems, which more closely reproduce the interactions between the epithelium, the connective matrix and the vascular network.

A major step in the evolution of in vitro models is the use of three-dimensional (3D) matrices made of collagen, gelatin, chitosan, fibrin or synthetic materials (PLGA, PEG). These structures recreate the native three-dimensional environment, allowing cells to organize themselves spatially, communicate through paracrine signals, and form architectures similar to those in living tissues.

A commonly used experimental tool is the "wound scratch assay", whereby a standardized wound is created in the cultured cell layer, and its

migration and closure rate is monitored in real time. This method is used to evaluate the effect of growth factors (VEGF, EGF, PDGF), metal nanoparticles or bioactive hydrogels on the cell regeneration process.

In vitro models also allow for the analysis of the expression of genes and proteins involved in healing (MMP-8, TGF-β, IL-6, COL1A1), as well as molecular signaling through pathways such as MAPK, PI3K/AKT, and NF-κB, providing valuable data on how different factors influence cellular response.

In vivo models

In vivo models represent an essential step in translational research, allowing the integrated observation of the healing process within the living organism, where cellular, immunological, vascular and nervous components interact simultaneously.

The most used animal models are:

1. mice and rats, due to their low cost, short regeneration time and the possibility of genetic manipulation;
2. rabbits and pigs, which offer a skin model closer to the human one, in terms of dermis thickness and vascular density.

Frequently used experimental models include:

1. excisional wounds – created by the controlled removal of a skin fragment, useful for the evaluation of dressings and biomaterials;
2. standardized thermal burns – for studying inflammatory processes and deep epithelial regeneration;
3. Induced diabetic ulcers – obtained by administering streptozotocin or aloxane, which reproduce the microvascular and neuropathic complications of diabetes.

These models provide complex information on immune response, angiogenesis, collagen deposition and scar formation, being essential for

testing the effectiveness of advanced dressings, PRP therapies, mesenchymal stem cells or low-intensity laser treatments.

In addition, in vivo models allow the analysis of systemic biocompatibility and toxicity of materials, by monitoring biological markers (CRP, leukocytes, liver enzymes, creatinine) and the histological evolution of the lesion tissue.

Ex vivo models and organ-on-chip systems

In recent years, research has shifted towards ex vivo models and "organ-on-chip" microplatforms, which combine the advantages of cellular systems with artificial microcirculation. These miniature devices integrate microfluidic channels that replicate blood flow, oxygen gradient, and nutrient transport, providing a faithful simulation of the physiological environment.

The "skin-on-chip" model, for example, uses layers of keratinocytes and fibroblasts grown on semipermeable membranes, crossed by a microfluidic flow that mimics capillary perfusion. This technology enables dynamic testing of drugs, biomaterials, and nanotherapies in a predictive system that reduces the need for animal testing.

Ex vivo models (tissue fragments harvested from human or animal donors) are used to analyze the local response to treatment and the interaction between material and biological tissue, while preserving the natural architecture of the skin.

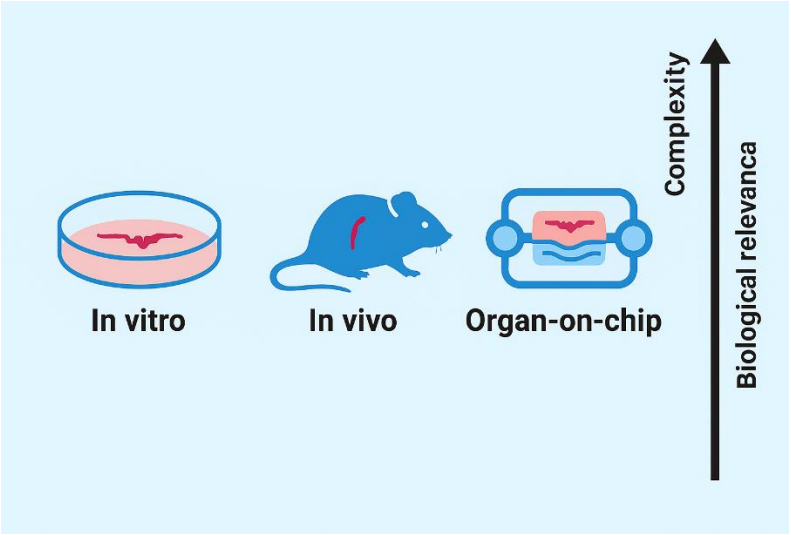


Fig 5.2. Comparison of the levels of complexity of experimental models used to study the wound healing process: from in vitro cell cultures to in vivo animal models and advanced "organ-on-chip" platforms. (original illustration)

Importance and future directions

Experimental models, regardless of their level of complexity, form the basis of applied biomedical research in the field of wound surgery. They allow a mechanistic understanding of healing phenomena and facilitate the rapid translation of laboratory results to clinical applications.

The current trend is to develop hybrid models, which integrate cell cultures, artificial microcirculation and artificial intelligence components for the automatic analysis of experimental data. At the same time, the focus shifts to reducing the use of laboratory animals, by adopting ethical and predictive platforms, in line with the 3R principles (Replace, Reduce, Refine).

In the future, the combination of biotechnology, microengineering and computational modeling will allow the creation of experimental

systems almost identical to human tissues, opening up new possibilities for the safe and effective testing of personalized regenerative therapies.

5.4. Standardization of clinical protocols and guidelines in wound surgery [203,204]

A fundamental direction for the sustainable development of wound surgery is the need to standardize clinical protocols, diagnostic criteria and methods for assessing the progress of healing.

Currently, the lack of uniformity in the definition of wound types, in the classification of severity and in the selection of adjuvant treatments causes significant variations in the results and in the interpretation of clinical data. To address these differences, international organizations such as the European Wound Management Association (EWMA), the World Union of Wound Healing Societies (WUWHS) and EPUAP have proposed evidence-based guidelines that establish common principles of assessment and intervention.

Standardization involves:

1. clear definition of healing stages and associated biological markers;
2. uniform criteria for the application of modern therapies (laser, VAC, PRP, oxygenation, stem cells);
3. objective scoring systems for assessing clinical and histological outcomes;
4. protocols for digital monitoring of the evolution of wounds, integrated into electronic health records.

The implementation of these guidelines contributes to increasing the quality of care, reducing therapeutic errors and standardizing the results reported in clinical trials, thus facilitating scientific progress and international collaboration.

5.5. Development directions for interdisciplinary research

Wound healing is a complex process, at the intersection of biology, engineering, physics and clinical sciences. Recent advances demonstrate that maximum therapeutic efficiency can only be achieved through interdisciplinary integration of knowledge combining advanced biomaterials, cell therapies, nanotechnologies, artificial intelligence analysis and tissue engineering.

The research of the future will be characterized by:

1. development of integrated experimental platforms, which unite imaging, histological and molecular data in a predictive digital model;
2. collaboration between clinicians, biotechnologists, physicists and computer scientists, to design personalized therapies and smart monitoring systems;
3. application of the concept of digitally assisted regenerative medicine, in which biological processes are quantified and optimized by algorithms;
4. creation of interoperable international registries and databases to validate new treatments on a global scale.

Through the interdisciplinary approach, wound surgery becomes not only a branch of reconstructive medicine, but a science of integrated regeneration, in which technology, biology and artificial intelligence collaborate for the complete restoration of tissue functionality and aesthetics.

Modern perspective and research directions in wound surgery

Modern research direction	Clinical applications	Major advantages	Challenges and limitations
Nanotechnologies and controlled release systems	Nanocomposite dressings, antimicrobial nanoparticles, controlled release of growth factors and antibiotics	Precise infection control, prolonged release of active substances, acceleration of healing	Possible toxic effects, variable biocompatibility, high production costs
Artificial Intelligence in Image Analysis	Automatic wound image analysis, healing prediction, digital monitoring and telemedicine	Rapid diagnosis, personalization of treatment, reduction of evaluation errors	Requires extensive databases, algorithmic standardization, and clinical validation
In vitro and in vivo experimental models	Testing of biomaterials, regenerative therapies and nanotechnologies on cell and animal cultures	Reproduction of real biological processes, evaluation of safety and efficacy before clinical application	Differences from human response, ethical limitations, and high costs

Standardization of clinical protocols and guidelines	Development of EWMA, WUWHS guides, uniform diagnostic and treatment criteria	Standardization of treatments, comparability of studies, increasing the quality of the medical act	Slow deployment, variations between centers, lack of complete digital infrastructure
Integrated interdisciplinary research	Collaborations between clinicians, biotechnologists, physicists, computer scientists, development of international registries	Accelerated innovation, personalized therapies, digital optimization of the healing process	Requires extensive coordination, sustained funding and institutional interoperability

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