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NANOSTRUCTURED MATERIALS USED IN WOUND THERAPY

~ Ph.D. Thesis Summary ~

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1. Introduction

Improper wound healing is a significant medical health issue, leading to increased morbidity, mortality, and healthcare costs. The presence of prolonged and uncontrolled inflammation, recurrent bacterial infections, and poor cell migration and angiogenesis characterizes most chronic wounds. Conventional treatment methods often fail in enhancing the wound healing process, necessitating novel approaches to improve wound outcomes [1].

In this context, nanotechnology introduces novel approaches that aim to accelerate tissue repair and improve therapeutic outcomes, using biocompatible nanomaterials [1,2]. So, their physicochemical properties, including nanoscale size (which provides a high surface area-to-volume ratio), tunable surface properties, and customizable drug loading capabilities, enhance the biological interactions, being advantageous over traditional treatment strategies [1,3]. Moreover, nanomaterials' characteristics represent effective instruments because of their capacity to interact precisely with the biological environment at the molecular and cellular levels [3]. With the help of nanotechnology, multifunctional platforms can be obtained that mimic the extracellular matrix (ECM), thereby promoting cell adhesion, proliferation, and migration, as well as modulating signaling pathways involved in tissue regeneration, while combining antimicrobial activity and prolonged delivery of drugs and bioactive substances [3].

NPs represent an important strategy regarding wound healing because of their properties, such as versatility and ability to interact with biological systems, including controlled drug delivery, antimicrobial properties, and bioactivity [4]. In particular, metal nanoparticles are good candidates for wound dressing components, having the potential to stimulate the wound healing process by stimulating keratinocyte proliferation, leading to optimal re-epithelialization, and due to their potent antimicrobial properties [5,6]. Additionally, their surface can be functionalized and used to selectively bind to specific cell types or tissues involved in wound healing, improving treatment efficiency [4]. In controlled drug delivery systems, polymeric and lipid NPs are used due to their capacity to control the drug release over time, reducing the risks of drug degradation, frequent administration, and prolonged treatment effectiveness [7]. Otherwise, other nanomaterials, such as hydrogels and nanofibers, are used to obtain scaffolds that provide a platform for cell migration and proliferation, mimicking the ECM. They also provide optimal moisture at the wound site, enhance skin hydration, and prevent necrotic tissue from appearing by allowing the body to rid itself of it [4,7].

In this respect, nanotechnology offers the advantage of creating new multifunctional platforms that promote wound healing in the most efficient way possible, thus making them potential next-generation wound therapies. However, there are still disadvantages that limit their use, such as long-term safety, stability, and clinical translation, which remain to be addressed in order to harness their potential in wound management fully [1-4,8].

Due to the urgency and necessity of developing new therapies that overcome the limitations of traditional dressings, which are ineffective in terms of inflammation management, infection occurrence, and inadequate healing, nanotechnology is intervening in the development of new strategies. Thus, this thesis aims to advance the field of modern dressings through the development of composite nanomaterials with antimicrobial and pro-regenerative effects that sustain the controlled release of bioactive compounds. Therefore, this theme reflects an innovative perspective that combines biomaterials science and nanotechnology to develop state-of-the-art dressings with superior healing potential, biocompatibility, and clinical applicability.

2. Skin

2.1. Biological barrier function

The human skin represents the largest organ of the body, playing a key role as a barrier between the external environment and the internal body systems. The skin offers protection against a wide range of external stressors while preventing water and electrolyte loss [9-11]. These roles are sustained by the skin structure, which is composed of three primary layers (Figure 1): the epidermis, the outer layer of the skin, which presents stratified keratinized epithelium, the dermis, located between the epidermis and hypodermis, which presents connective tissue, vasculature, lymphatics, glands, and hair follicles, and the subcutaneous tissue (also known as the hypodermis), made of fat and connective tissue [9,11,12].

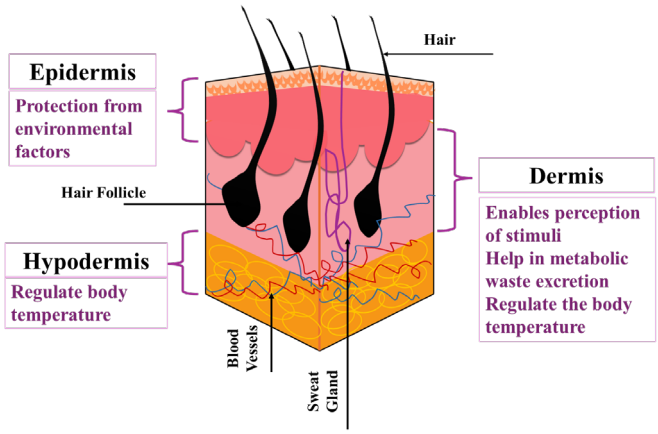


Figure 1. Skin structure and its roles. Created based on the information from [9,11,12].

Through its site and roles, the skin is susceptible to damage, which can disrupt the skin barrier and increase the risk of infection. [9]. In this regard, skin barrier integrity is crucial in preventing skin diseases, as any dysfunction in any component of the barrier can lead to skin conditions. Additionally, the damage to the skin appendages can affect the quality of healing. In this sense, the therapeutic approaches aim to focus on restoring lipid composition, strengthening structural proteins, and regulating the cytokine activity by using topical formulations or microbiota-based interventions [13-16].

2.2. Classification of Skin Injuries

Being in contact with the external environment, the skin is prone to being injured by a wide range of physical, chemical, and biological factors, which can produce various injuries, such as bruises, burns, ulcers, and deep cuts [17]. Thus, classifying wounds plays a key role in precise diagnosis and selecting the appropriate therapy strategy to improve the healing time and prevent the occurrence of potential complications [18]. In this regard, from a clinical perspective, skin injuries are classified as acute or chronic wounds (Table 1) based on their healing time.

Table 1. Key differences between acute and chronic wounds. Created based on the information from [18-22].

Characteristics	Acute wounds	Chronic wounds
Healing time	2-4 weeks	Months, even years, or no healing
Healing process	An organized and appropriate healing	Remain stuck in the inflammation stage
Comorbidity association	There are usually no underlying problems	They are associated with diabetes or vascular diseases, pressure injuries
Infection Risk	Lower	Higher

2.3. Wound Healing Process

Wound healing represents a complex and dynamic biological process that aims to restore the structure and function of injured skin, occurring in four main stages (Figure 2) based on coordinated signals for cell migration, immunity, angiogenesis, and tissue repair [23,24].

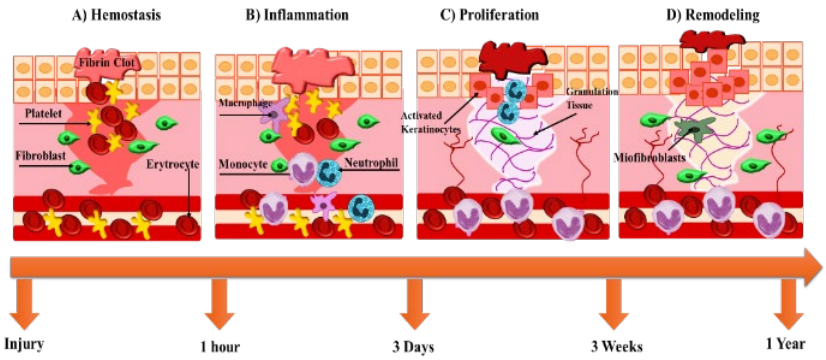


Figure 2. Wound healing stages. Created based on information from [23,25].

In this regard, the wound healing process represents a complex mechanism, regulated by biological processes that involve immune, vascular, epithelial, and neural systems. Thus, any perturbation in the healing process, whether due to systemic diseases, aging, or persistent inflammation, can lead to complications and the occurrence of chronic wounds or pathological scarring, which need more attention and special therapeutic interventions [23,25,26].

2.4. Common Complications and Limiting Factors in Wound Healing

Wound healing is a complex biological process, and a variety of systemic and local factors can impair it. These disruptions can occur at any stage of the healing process [27-29]. Complications that arise during the healing process can be categorized into delayed or non-healing, poor healing, and uncontrolled healing. Thus, non-healing wounds are characterized by the disruption in the normal physiological processes of the repair mechanism., Without appropriate treatment, they become chronic and are associated with biological alterations such as hypoxia, reduced growth factor levels, increased inflammatory cytokines, proteolytic enzyme overactivity (e.g., MMPs), and the accumulation of senescent cells that impair tissue regeneration [17,27,30].

Due to their prolonged healing time and altered immune response, chronic wounds are prone to infection. Thus, the wound microenvironment promotes biofilm formation by polymicrobial communities and resident bacteria, which impair immune clearance and antibiotic penetration [31,32]. Moreover, the colonization of wounds with resistant bacterial pathogens can hamper their management by limiting therapy options and increasing the risk of treatment failure [33]. The extended administration of antibiotics, aiming to treat wound infections, can contribute to mild and severe complications, such as diarrhea, but most importantly, the development of antibiotic resistance, and, in some cases, life-threatening leukopenia. Additionally, the overuse of antibiotics can increase the risk of developing multidrug-resistant (MDR) organisms, which will complicate wound management [33,34]. These infections could progress into life-threatening diseases such as gangrene, sepsis, and limb amputation, especially in older or diabetic patients [10,32,34].

2.5. Socioeconomic Impact of Chronic Wounds

Chronic wounds represent a significant global public health burden, as wound-related medical services pose considerable financial pressure [35,36]. Moreover, the presence of comorbidities (i.e., co-existing health problems) complicates both wound management and the epidemiologic follow-up of chronic conditions [37]. Thus, patients having chronic wounds, in general, experience persistent impaired mobility, pain, social isolation, depression, and

significant lifestyle restrictions, which hurt their psycho-emotional state [36] (Figure 3). Thus, their impact on both patients and health systems underlines the urgent need to enhance prevention strategies, early intervention, and more effective wound management approaches.

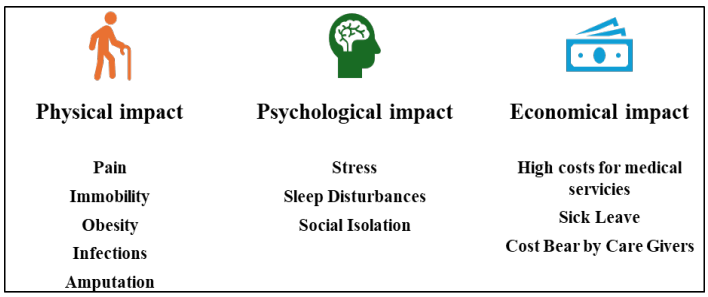


Figure 3. Consequences of Chronic Wounds. Created based on information from [35].

2.6. Limitations of Conventional Dressings

In wound management, the choice of wound dressing represents a key factor that affects the healing time and must be based on the type of wound and its physiological needs. Thus, conventional wound dressings such as gauze, cotton wool, and plasters were generally used as dry dressings [38]. However, these dressings present several limitations regarding wound management. They fail to provide a moist environment at the wound site, need to be changed frequently, resulting in a high risk of maceration, while their adherence to wounds produces mechanical trauma, pain, and more damage to newly formed granulation tissue during removal [38-40]. Regarding the antimicrobial effect, conventional dressings can offer limited protection, having a higher contamination risk compared to advanced dressings [40]. Another limitation of traditional dressings is their inability to actively support angiogenesis, growth factor release, or tissue regeneration [39,41].

Therefore, effective wound treatment requires the use of advanced dressings able to overcome the limitations of conventional options.

3. Modern Wound Dressings

3.1. Functional Requirements for Modern Wound Dressing

To solve the issues of conventional wound dressings, an ideal wound dressing must provide a moist environment, allow gas exchange, maintain an optimal temperature and circulation, minimize wound adherence, relieve pain, be sterile, non-allergenic, non-toxic, biocompatible, biodegradable, and cost-effective [42-44]. Moreover, the wound dressing must properly fit the wound, without adhering to its surface, to protect from microbial contamination, mechanical, and thermal stress [44]. Furthermore, maintaining the microenvironment of the wound, including optimal moisture, limited transepidermal water

loss, and skin microbiome balance, represents a key strategy for supporting normal wound healing [45,46].

Thus, research has focused on developing new dressings using advanced materials and functionalities to optimize wound healing outcomes. Modern wound dressings include hydrocolloids, films, foams, hydrogels, hydrofibers, and alginate dressings, each having advantages and disadvantages that render them suitable for specific wound applications.

Regarding the development of modern dressings, researchers are showing increased interest in integrating bioactive compounds, which can address inflammation, oxidative stress, infections, and promote skin regeneration [47,48]. These kinds of dressings have the potential to accelerate wound closure and promote the wound healing outcome in chronic wounds [49].

3.2. Biomaterials Used in the Development of Modern Dressings

Modern dressings can be fabricated from a variety of materials, which are divided into two categories, namely natural polymeric materials and synthetic polymeric materials. Natural polymers are characterized by properties such as biocompatibility, biodegradability, and favorable tissue regeneration properties, but they have mechanical limitations, low stability, and a high rate of degradation. On the other hand, synthetic polymers are used because of their ability to provide superior mechanical stability, chemical versatility, and therapeutic agent loading capacity. However, they are also limited by their lack of direct cellular interaction, which is why they are often combined with natural polymers to achieve optimal performance.

3.3. Integration of Nanotechnology into Modern Wound Dressings

The integration of nanotechnology into the development of dressings represents one of the most promising strategies that can contribute significantly to improving wound healing by speeding up healing and enhancing treatment. In this respect, some materials possess anti-inflammatory, antibacterial, and proangiogenic properties and can be administered directly or integrated into other structures (e.g., hydrogels, nanofibers), creating nanocomposites [1,7]. Thus, two broad classes of nanomaterials are used in wound dressing designs: (1) those with intrinsic properties that are beneficial for wound healing, and (2) those used as delivery vehicles for therapeutic agents [50].

3.3.1. Intrinsic activity of nanoparticles in wound dressings

Metal-based nanoparticles are widely studied for their potential in modulating the wound-healing process [1,51-53].

Silver nanoparticles

Silver nanoparticles (AgNPs) are among the most commonly NPs used in wound management due to their enhanced efficiency, precision, and therapeutic outcomes, particularly due to their antimicrobial and regenerative properties [1,54]. However, their exact mechanism of action is not fully understood, but several antimicrobial mechanisms have been proposed. In this regard, the continuous release of silver ions (Ag^+) provides an effect against multiple bacterial targets simultaneously. Thus, after the AgNPs application on the wound surface, these NPs tend to attach to bacterial membranes, where they act by disrupting their structure and function [51,55]. It was observed that AgNPs have a high antimicrobial effect against both Gram-negative and Gram-positive bacteria, including multidrug-resistant strains, and against fungi such as *Aspergillus niger* and *Candida albicans* [56,57]. Furthermore, AgNPs are well-known for their ability to enhance the efficacy of conventional antibiotics through synergistic effects, reducing the required antibiotic dose and helping to overcome resistance [57,58]. However, it should be noted that although they have a modulating and antimicrobial effect, there is still the possibility that they may be cytotoxic to fibroblasts and keratinocytes that repair the site of infection. In high concentrations, AgNPs induce cell death and inhibit the proliferation not only of bacteria but also of cells involved in the healing process (e.g., keratinocytes), slowing down their growth, metabolism, and potential for migration and proliferation [59]. Moreover, there are still concerns regarding cytotoxicity and aggregation of AgNPs, which limit their usage in clinical practice. By overcoming their limitations, AgNPs can offer renewed hope for patients with hard-to-treat wounds [1,53].

Gold nanoparticles

Gold nanoparticles (AuNPs) represent valuable candidates used in wound management due to their biocompatibility, chemical stability, and ease of surface functionalization [60,61]. Compared to AgNPs, their direct antimicrobial effect is limited, but they present antioxidant, anti-inflammatory, and regenerative mechanisms that offer significant benefits in the wound healing process, by inducing VEGF expression and potentially functioning as antioxidants [60,61]. Complementarily, AuNPs present potential antioxidative effects, which contribute to the neutralization of free radicals. [51,62]. Due to their surface capacity to be modified, AuNPs can be functionalized with antibiotics (e.g., vancomycin), improving the antibacterial properties and their effects on multidrug-resistant strains like *E. coli* and *S. aureus* [53]. These NPs do not excel in terms of a strong antimicrobial effect, but they do have the ability to modulate healing processes and serve as drug delivery platforms [51,53,60].

Zinc and zinc oxide nanoparticles

Zinc and zinc oxide nanoparticles (ZnNPs and ZnONPs, respectively) are well known for their antimicrobial, anti-inflammatory, and regenerative potential regarding wound regeneration. Their small size allows them to adhere to bacterial membranes, penetrate membranes, disrupt them, and produce ROS [1,63,64]. Compared to other antimicrobial agents, ZnONPs were reported to exhibit a moderate to strong antimicrobial activity against both Gram-negative and Gram-positive bacteria [65,66]. It has been demonstrated that ZnONPs promote collagen deposition, tissue remodeling, and angiogenesis, presenting a high biocompatibility and low cytotoxicity, particularly when optimally sized [67]. Overall, ZnONPs can be considered as potential candidates for use in the treatment of wounds, due to their balanced combination of antimicrobial protection, oxidative modulation, and regenerative stimulation.

Copper and copper oxide nanoparticles

Copper nanoparticles (CuNPs) have been considered in the treatment of wounds due to their antimicrobial, anti-inflammatory, and regenerative properties. Their antimicrobial effect is characterized by the Cu^{2+} ions released, which disrupt bacteria's membranes, interfere with enzymatic activity, and ROS production, resulting in lipid peroxidation and DNA degradation. In this context, CuNPs prevent biofilm formation and provide an increased efficacy against drug-resistant bacteria such as *Escherichia coli* and *Staphylococcus aureus* [1,3,51]. Furthermore, CuNPs accelerate new blood vessel formation by stimulating the production of VEGF hypoxia-inducible factor (HIF-1α) [51,68]. However, these NPs can be toxic at high concentrations, being unstable and prone to rapid oxidation, producing free radical that induces cell death and lipid peroxidation [69]. Despite their limitations, CuNPs and CuONPs remain promising candidates in wound healing therapy.

Antioxidant and epithelializing nanoparticles

ROS play a key role in promoting cell signaling to modulate chemotaxis, cell growth and migration, angiogenesis, stem cell fate, and ECM formation, and protecting wounds from pathogens at low levels [70-72]. However, the increased concentration of ROS can produce DNA damage and a persistent inflammatory state that results in improper healing [70-72]. Due to oxidative stress production, there is a need for an antioxidant mechanism to improve the delayed healing, particularly in chronic wounds. In this regard, antioxidant compounds, classified as enzymatic (e.g., catalase, superoxide dismutase) and non-enzymatic (e.g., flavonoids, polyphenols), are being used as ROS scavengers with a role in stabilizing or neutralizing harmful species [73].

Thus, a strategy for ROS levels modulation is represented by the biomaterials functionalized with antioxidants, by providing materials such as scaffolds, drug delivery systems that sustain a prolonged and controlled antioxidant release, while ensuring an optimal wound environment that enhances tissue regeneration [73,74]. In wound repair, antioxidants such as carotenoids and polyphenols, found in fruits and vegetables, showed a significant effect in scavenging free radicals, in protecting lipid membranes, and improving collagen synthesis, promoting faster tissue regeneration [75-78].

Overall, antioxidant-based biomaterials and nanoparticles improve wound healing by reducing oxidative stress and promoting tissue regeneration. Their use in wound dressings represents a significant development in addressing ROS-related wound complications.

3.3.2. Smart Delivery Systems

Liposomes

Since nanotechnology is used to make nanostructured systems that help wounds heal, researchers have come up with lipid-based nanocarriers for controlled and targeted drug delivery to manage these conditions. Among lipid-based nanosystems, liposomes can be ideal candidates for smart delivery, having the ability to encapsulate both hydrophilic compounds and lipophilic molecules [79-81]. Moreover, due to their increased biocompatibility and similarity with the biological environment, they have improved integration with skin tissue, providing an enhanced therapeutic effect with reduced toxicity risk [82,83].

Liposomes can be classified as unilamellar or multilamellar, their lamellarity and sizes further influencing their half-life as a drug delivery system, but also the drug-loading capacity [84]. Their structure consists mainly of phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin) that are stabilized with cholesterol to maintain membrane rigidity, reduce permeability, and thus facilitate control of the release of active substances [84,85].

When it comes to wound healing, liposomes act not only as passive systems but also as bioactive agents capable of modulating the inflammatory response and proliferation for tissue repair. Thus, they can maintain optimal wound moisture and be used to deliver growth factors, antioxidants, and substances with powerful antimicrobial effects directly to the damaged tissue, promoting accelerated re-epithelialization and angiogenesis [86-88]. Moreover, liposomes are promising platforms in complex wounds such as diabetic ulcers, burns, and chronic infections, because of their ability to penetrate tissue layers and synergize with encapsulated drugs, improving healing outcomes [87].

In this regard, liposomes' mechanisms of action can be realized through several pathways. Thus, they can be adsorbed to cell membranes through electrostatic interactions or

ligand-receptor recognition, transported through endocytosis, and merge directly with cell membranes for rapid intracellular administration, or exchange lipids with target cells to slowly release therapeutic medicines [85]. Moreover, liposomes' flexibility promotes both passive targeting, by accumulating at the wound site due to vascular permeability, and active targeting by surface functionalization with bioactive molecules such as peptides, antibodies, or other ligands to direct liposomes to specific cells or tissues [89,90].

However, liposomes' therapeutic versatility and efficiency are limited by their unfavorable long-term stability, caused by phospholipid oxidation or hydrolysis, and the possible leakage of encapsulated drugs. In this regard, lyophilization, spray drying, cholesterol inclusion, and surface modification with polysaccharides or antioxidants can be considered as strategies to improve their stability and storage over time [81]. Moreover, their industrial production is limited by the high costs involved, and the process needs to overcome optimization barriers before reaching large-scale production [91].

Stimuli-Responsive Polymers

Wound healing is a multi-phase process influenced by microenvironmental factors such as pH, ROS, glucose level, and enzymatic activity. Stimuli-responsive systems were designed as an advanced therapeutic platform, capable of responding and adapting to the wound microenvironment, thereby improving drug delivery, enabling real-time monitoring, and enhancing healing results. Stimuli-responsive systems are classified as hydrogels, nanofibers, and functional films that respond dynamically to endogenous or exogenous cues, reducing systemic toxicity and enhancing treatment precision [36,92,93]. Overall, stimulus-responsive strategies are evolving from simple controlled release platforms to intelligent systems capable not only of administering treatment according to local conditions, but also of providing real-time feedback. The integration of multiple functionalities is currently of interest due to its significant potential for clinical translation, although there are still limitations that need to be overcome.

3.4. Commercially available wound dressings

Although research into developing new therapeutic opportunities for wound treatment continues, there are already examples of dressings used in clinical applications and available on the market. However, the integration of nanotechnology can lead to the optimization of their performance. The use of nanomaterials allows for the improvement of antimicrobial properties, increased absorption capacity, controlled release of therapeutic agents, and even real-time monitoring of the healing process. Thus, there is a trend toward smart dressings, capable not only of passively protecting and treating the wound, but also of actively intervening in the healing process through bioactive properties and customized functions.

3.5. Emerging Trends in Wound Therapy and Regenerative Medicine

Due to the need for improvement and the need to develop new therapeutic strategies for wound healing, dressings have evolved considerably. Starting with passive dressings, the platform evolves into a multifunctional, biointeractive, and intelligent system that promotes real-time monitoring, targeted therapy, and environmental responsiveness, thereby addressing the limitations of conventional wound dressings [94]. In this respect, dressings that incorporate biosensors and use polymeric materials can be designed to monitor wound biomarkers while promoting bioactive compounds delivery [49]. Thus, integrating sensing, data transmission, and therapeutic capabilities supports personalized treatment strategies. Monitoring parameters such as oxygenation, temperature, pH, humidity, bacterial activity, pressure, electrical impedance, and ECM can lead to remote monitoring of wounds, as well as changes that require immediate intervention, significantly reducing complications [47,95,96]. Moreover, introducing smart responsive polymers in designing advanced wound dressings can help in the design of sensor-triggered platforms for the release of bioactive compounds. For example, these dressings can detect infection-related changes and release the antimicrobial substances, via clinician-triggered systems, that activate drug release through microcontrollers [47].

However, even though the dressing provides precise real-time physiological monitoring while actively promoting wound repair, particularly in cold environments, further investigation should be conducted to optimize and obtain a next-generation wearable medical device with enhanced properties, which promote wound healing and its monitoring [95].

Although there has been promising progress in this direction, enabling multiparametric detection and wireless communication, the use of these devices in clinical applications remains experimental. High production costs, incomplete integration of detection and actuation components, excessively slow response times, and biological limitations such as wound complexity, microbial infections, lack of standardized guidelines, and large-scale validation hinder their applicability [47,95,97,98].

4. Importance of the proposed theme

As evidenced by recent literature findings, chronic and infected wounds are a growing global healthcare burden caused by their prolonged healing times, high risk of complications, and limited responsiveness to conventional therapeutic approaches. Even though there is continuous progress in wound care, most commercially available dressings act as passive

barriers, lacking the multifunctional properties required to address the complex pathophysiology of chronic wounds.

These clinical challenges impose the need to develop advanced, multifunctional therapeutic platforms capable of simultaneously controlling infection, modulating inflammation, maintaining optimal hydration, and stimulating the natural regenerative processes of the skin.

The proposed research theme addresses this necessity by introducing several innovative hydrogel-based wound dressings, integrating nanostructured materials, bioactive compounds, and green synthesis strategies into sustainable and biocompatible polymeric matrices. The novelty of these experimental studies comes from the various synergistic combinations of plant-mediated silver nanoparticles, β-carotene nanocarriers, microfluidically synthesized zinc oxide nanoparticles, hyaluronic acid-loaded liposomes, and phytochemical-rich natural extracts within alginate and PVA-based hydrogels.

These distinct but complementary strategies were proposed as a means to create next-generation multifunctional wound dressings capable of providing enhanced antimicrobial, antioxidant, and regenerative effects while ensuring structural integrity and cytocompatibility.

Thus, this doctoral research aimed to develop multifunctional hydrogel-based wound dressings by combining nanostructured materials and bioactive compounds to improve chronic wound management through enhanced antimicrobial, antioxidant, and regenerative properties. Aligning with recent advances in nanotechnology-assisted wound healing and sustainable biomedical materials, the thesis pursued the following specific objectives:

(i) Design and fabrication of multifunctional hydrogel dressings by incorporating various nanostructured systems, including green-synthesized silver nanoparticles, β-carotene-loaded polymeric nanocarriers, zinc oxide nanoparticles obtained via microfluidic synthesis, hyaluronic acid-loaded liposomes, and plant-derived bioactive extracts.

(ii) Comprehensive physicochemical characterization of the synthesized nanoparticles and composite hydrogels to establish correlations between structural, morphological, and chemical properties and their expected biological performance.

(iii) Evaluation of the biological performance of the developed dressings through antimicrobial, antibiofilm, swelling, degradation, and cytocompatibility assays, aiming to achieve optimal infection control, moisture balance, and tissue regeneration in chronic wound environments.

5. Personal contributions

Considering the growing clinical challenges associated with chronic and infected wounds, the experimental part of this doctoral research was dedicated to the development of innovative hydrogel-based wound dressings by integrating nanostructured materials and bioactive compounds into multifunctional therapeutic platforms.

The main goal implied the design of advanced dressing systems that can overcome the inherent limitations of conventional treatments, such as poor infection control, insufficient moisture retention, and limited stimulation of tissue regeneration. To achieve this, three original experimental studies were conducted, each exploring a distinct nanotechnological strategy and employing different combinations of polymers, nanoparticles, and natural bioactive agents.

The overarching aim was to engineer next-generation wound care materials with improved antimicrobial activity, antioxidant protection, and regenerative potential, while ensuring biocompatibility and sustained therapeutic performance. Collectively, the results of these studies demonstrate the feasibility and effectiveness of nanotechnology-assisted hydrogel dressings in accelerating the healing process, preventing microbial biofilm formation, and supporting extracellular matrix remodeling, thus contributing to the advancement of modern wound management strategies.

The published papers are included *in extenso* in the next subsections, describing in detail the personal contributions brought to the field of innovative nanostructured formulations for wound therapy.

5.1. Cinnamon-Mediated Silver Nanoparticles and Beta-Carotene Nanocarriers in Alginate Dressings for Wound Healing Applications

The first study, published in *Gels*, explored the development of alginate-based hydrogels co-loaded with silver nanoparticles synthesized using cinnamon essential oil, polymeric β-carotene nanoparticles, and *Centella asiatica* extract. The silver nanoparticles were obtained using a green synthesis approach employing D-glucose as a reducing agent, resulting in a crystallite size of approximately 4.88 nm, a physical diameter of 17.21 ± 0.42 nm as determined by SEM, and a hydrodynamic diameter of 162.26 nm according to DLS measurements. Their high colloidal stability, indicated by a zeta potential of −38.49 mV, facilitated uniform dispersion within the hydrogel matrix. In parallel, β-carotene nanoparticles encapsulated in a PVP carrier exhibited an average size of 49.94 ± 1.68 nm, a hydrodynamic

diameter of 768.43 nm, and a zeta potential of −10.54 mV, ensuring controlled release and antioxidant protection. Physicochemical analyses confirmed the successful integration of all components into the alginate matrix, resulting in a porous architecture that promoted moisture retention and oxygen diffusion. The developed hydrogels exhibited high swelling ratios and maintained structural integrity with controlled degradation over 24 hours, ensuring sustained release of bioactive compounds. Antimicrobial evaluations demonstrated strong inhibition of both *Staphylococcus aureus* and *Escherichia coli* within the first 24 hours, preventing early-stage biofilm formation. Moreover, biocompatibility assays performed on human keratinocytes revealed cell viability above 100% in samples containing β-carotene and *Centella asiatica*, confirming the antioxidant-mediated reduction of silver-induced cytotoxicity. This work demonstrated the successful design of a multifunctional alginate-based dressing with enhanced antibacterial activity, antioxidant capacity, and cytocompatibility, representing a promising strategy for chronic wound management.

5.2. Alginate/PVA Hydrogel Incorporating HA-Liposomes and Aronia-Derived Silver Nanoparticles for Advanced Wound Management

The second study, published in the *International Journal of Molecular Sciences*, reported the fabrication of an innovative bilayer hydrogel dressing designed to spatially separate regenerative and antimicrobial functions, thereby enhancing therapeutic performance while maintaining high biocompatibility. The upper layer, based on polyvinyl alcohol (PVA), was enriched with hyaluronic acid-loaded DPPC liposomes, while the lower alginate layer incorporated silver nanoparticles synthesized via a green approach using *Aronia melanocarpa* extract. The DPPC-based liposomes without and with hyaluronic acid exhibited an average particle size of approximately 81 nm and 78 nm, a hydrodynamic diameter of 401.93 nm and 1608.63 nm, and a zeta potential of -4.06 mV and -12.43 mV, respectively, indicating excellent stability and efficient encapsulation of hyaluronic acid. The Aronia-derived silver nanoparticles had a crystallite size of 30.59 nm, a hydrodynamic diameter of approximately 213 nm, and a zeta potential of around −21 mV, with their polyphenol-rich capping layer providing enhanced cytocompatibility while preserving antimicrobial potency. The bilayer architecture provided an optimized platform for synergistic healing effects: the upper PVA layer facilitated keratinocyte proliferation, hydration, and extracellular matrix remodeling, while the lower alginate layer delivered sustained antimicrobial action. The hydrogels displayed a favorable swelling ratio, ensuring maintenance of a moist wound environment, and a controlled degradation profile, supporting long-term dressing stability. All of the dual-

functional bilayer systems exhibited good cytocompatibility, with HaCaT cell viability going beyond 130% after 24 hours of incubation. This bilayer system successfully combined regenerative stimulation and antimicrobial protection within a single dressing, presenting an innovative, sustainable, and highly effective approach to chronic wound therapy.

5.3. Alginate-Based Wound Dressings Embedded With Microfluidic-Obtained Zinc Oxide Nanoparticles

The third study, submitted to the *U.P.B. Scientific Bulletin*, focused on the development of alginate-based hydrogels incorporating zinc oxide nanoparticles synthesized using an innovative microfluidic approach. The cross-shaped microfluidic platform allowed precise control over reaction conditions, producing highly uniform ZnO nanoparticles with superior physicochemical properties compared to conventional synthesis techniques. Among the two formulations obtained, the optimized ZnO_30-15 sample displayed a crystallite size of approximately 29.13 nm, spherical morphology with homogeneous distribution as revealed by SEM, and a hydrodynamic diameter of around 73 nm. These nanoparticles were incorporated into alginate hydrogels, either alone or in combination with *Mimosa pudica* powder, resulting in three formulations: control alginate hydrogel, ZnO-loaded hydrogel, and ZnO-Mimosa hydrogel. FTIR and SEM analyses confirmed the successful integration of both ZnO nanoparticles and Mimosa extract into the hydrogel matrix, which displayed a porous structure supporting exudate absorption and gaseous exchange. The swelling capacity varied among the formulations, yet all developed materials exceeded 100% swelling rates. The highest absorption capacity was obtained for the control hydrogel (i.e. 274%), followed by ZnO-loaded hydrogels (i.e., 151%), and Mimosa-enriched systems (i.e., 131%) highlighting the influence of nanomaterials and plant-derived compounds in modulating hydration properties. Antimicrobial activity was significantly enhanced by modifying alginate matrices, as ZnO-containing hydrogels achieved a significant reduction in CFU values for *S. aureus* and *E. coli*, while the Mimosa-enriched variant demonstrated the widest inhibition zones for tested bacterial strains due to synergistic phytochemical activity. These results demonstrate that microfluidic synthesis provides a reliable and environmentally friendly method for tailoring ZnO nanoparticle properties and, when combined with bioactive plant extracts, enables the development of multifunctional wound dressings with potent antimicrobial, anti-inflammatory, and regenerative properties.

6. General Conclusions

Chronic and infected wounds represent a significant medical challenge. They are associated with prolonged healing times, susceptibility to microbial infections and biofilm formation, and limited responsiveness to conventional treatments. Despite the availability of numerous wound dressings, most existing solutions act as passive barriers and fail to provide the multifunctional therapeutic support required to promote tissue regeneration, maintain an optimal healing environment, and prevent infections.

In this context, the present doctoral research introduces innovative strategies for designing hydrogel-based wound dressings by integrating nanostructured materials, bioactive compounds, and green synthesis approaches into sustainable and biocompatible therapeutic platforms. The novelty of this work lies in the multimodal integration of functional components—including plant-mediated silver nanoparticles, β-carotene nanocarriers, microfluidically synthesized zinc oxide nanoparticles, hyaluronic acid-loaded liposomes, and natural phytochemical extracts—within polymeric matrices based on alginate and polyvinyl alcohol. By employing distinct but complementary experimental approaches, this thesis contributes to the development of next-generation multifunctional wound dressings that combine broad-spectrum antimicrobial activity, antioxidant protection, and regenerative potential while ensuring structural stability and cytocompatibility. Collectively, these advances position the work at the intersection of nanotechnology, biomaterials engineering, and regenerative medicine, addressing critical limitations in current wound care and offering new perspectives for personalized, effective therapeutic solutions.

In this respect, three experimental studies were conducted, each employing distinct nanotechnological strategies, yet united by the common objective of designing next-generation dressings with enhanced antimicrobial, antioxidant, and regenerative properties. The findings demonstrate the feasibility of combining natural compounds, polymeric systems, and nanomaterials into stable, biocompatible hydrogels with significant therapeutic potential.

Taken together, these three studies demonstrate the potential of nanostructured hydrogel-based wound dressings to address the multifaceted challenges of chronic wound management by integrating diverse bioactive agents and synthesis strategies. Across the investigations, the developed dressings exhibited desirable swelling capacities, strong antimicrobial potential against both Gram-positive and Gram-negative pathogens, controlled degradation rates extending over several days, and high keratinocyte viability, indicating excellent cytocompatibility. The integration of green-synthesized silver nanoparticles, β-carotene nanocarriers, microfluidically obtained zinc oxide nanoparticles, hyaluronic acid-

loaded liposomes, and plant-derived bioactive extracts such as *Centella asiatica*, M*imosa pudica*, and *Aronia melanocarpa* resulted in multifunctional systems capable of providing antimicrobial defense, antioxidant protection, and regenerative support. These findings advance the field of nanotechnology-driven wound care and offer valuable insights into the design of next-generation hydrogel-based platforms tailored for chronic and infected wounds. Through the combined application of nanomaterials and naturally derived therapeutics, this thesis lays the foundation for the development of innovative, personalized wound management strategies with strong translational potential in clinical practice.

Therefore, this thesis fulfilled its initially set objectives: (i) designing and fabricating multifunctional dressings by incorporating various nanostructured systems, (ii) comprehensively characterizing the synthesized nanoparticles and composite hydrogels from a physicochemical perspective, and (iii) evaluating the biological performance of the developed dressings through antimicrobial, antibiofilm, swelling, degradation, and cytocompatibility assays.

In this manner, the present doctoral thesis expands knowledge in the field of advanced wound management strategies, contributing to the literature with several innovative formulations that have shown promising results and potential for translation to clinical settings. The importance of the obtained experimental findings is also confirmed by their publication in ISI-indexed journals with an impact factor.

Published Papers

1. **Țăin, A.E.;** Bîrcă, A.C.; Nedelcu, M.S.; Holban, A.M.; Niculescu, A.-G.; Grumezescu, A.M.; Hudiță, A. Cinnamon-Mediated Silver Nanoparticles and Beta-Carotene Nanocarriers in Alginate Dressings for Wound Healing Applications. *Gels* **2025**, *11*, 738. https://doi.org/10.3390/gels11090738.
2. **Țăin, A.-E.;** Bîrcă, A.C.; Naulea, A.M.I.; Niculescu, A.-G.; Grumezescu, A.M.; Croitoru, G.-A. Alginate/PVA Hydrogel Incorporating HA-Liposomes and Aronia-Derived Silver Nanoparticles for Advanced Wound Management. *Int. J. Mol. Sci.* **2025**, *26*, 9203. https://doi.org/10.3390/ijms26189203.
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