Probiotics and Hydrogels in Wound Healing: A Synergistic Approach to Advanced Wound Care

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Abstract - ***Chronic wounds remain a significant challenge in modern medicine due to persistent infections, prolonged inflammation, and the rise of antibiotic-resistant bacteria. Traditional wound treatments often fail to address these root causes effectively. Recent advancements highlight the promising role of probiotic-based hydrogels, which combine the moisture-retaining and protective properties of hydrogels with the antimicrobial and immunomodulatory benefits of probiotics. Hydrogels create a conducive environment for wound healing by maintaining moisture, promoting cell migration, and facilitating tissue regeneration. When infused with probiotics, such as Lactobacillus and Bifidobacterium strains, these hydrogels can modulate the wound microbiome, outcompete harmful bacteria, reduce biofilm formation, and stimulate the immune system. This review explores the synergistic mechanisms of action between probiotics and hydrogels, including their ability to deliver therapeutic agents, accelerate wound closure, and promote angiogenesis. Additionally, this article addresses the challenges of formulating probiotic-infused hydrogels and the potential for future clinical applications, including personalized wound care solutions and multifunctional hydrogel systems. By integrating probiotics and hydrogels, this approach offers a novel and effective solution for treating chronic wounds, potentially reducing infection risks and the overuse of antibiotics.***

Keywords - Probiotics, Hydrogels, Wound healing, Skin microbiome, Infection control, Tissue regeneration, Biomedical applications

I. INTRODUCTION

1.1 Overview of Wound Healing and Current Challenges

Wound healing is a vital biological process that is essential for restoring tissue integrity and function after injury. It involves a series of highly regulated stages: haemostasis, inflammation, proliferation, and remodeling. Each stage plays a distinct role in tissue repair:

1. Haemostasis: This initial phase begins immediately after injury, where blood clotting is triggered to prevent excessive blood loss. Platelets aggregate to form a fibrin clot, initiating a cascade of events that lead to tissue repair [12,15] .
2. Inflammation: Following clot formation, immune cells such as neutrophils and macrophages infiltrate the wound site to clear debris and pathogens. This phase is crucial for preventing infection and preparing the wound bed for healing, but if prolonged, it can lead to chronic inflammation, delaying the healing process [12,15].
3. Proliferation: In this phase, fibroblasts migrate into the wound bed and begin synthesizing collagen and other extracellular matrix (ECM) components. Angiogenesis (the formation of new blood vessels) occurs, and epithelial cells migrate to cover the wound, forming new tissue [11,15].
4. Remodelling: During the final phase, collagen is reorganized, and the tissue regains strength and elasticity. Scar tissue forms as the ECM is remodelled, and the wound closes [13,15].

Despite advancements in wound care technologies, healing can be impeded by various factors, particularly infection. Chronic wounds, such as diabetic ulcers, pressure sores, and venous leg ulcers, are prone to delayed healing due to microbial colonization, biofilm formation, and an extended inflammatory phase[3,4]. Infections introduce pathogens that disrupt normal wound closure and can lead to severe complications like sepsis. The rise in antibiotic-resistant bacteria further complicates the treatment of chronic wounds, making it crucial to explore alternative, more effective therapeutic strategies [7,9].

1.2 Hydrogels and Their Role in Wound Healing

Hydrogels are three-dimensional, hydrophilic polymeric networks that possess the ability to absorb and retain large amounts of water while maintaining their structural integrity. Their high-water content and biocompatibility make them ideal materials for use in wound care [3,8,18]. Hydrogels provide a moist wound environment, which is essential for several reasons:

- Cell migration and proliferation: The moist environment created by hydrogels promotes keratinocyte migration and fibroblast activity, which are critical for tissue regeneration [3,8].
- Prevention of scab formation: By keeping the wound hydrated, hydrogels help prevent the formation of scabs, which can slow the healing process [11,18].
- Gas exchange and drainage: Hydrogels allow oxygen to permeate the wound while also absorbing excess wound exudate, maintaining an optimal healing environment [8,10].

Additionally, hydrogels can be functionalized to serve as carriers for therapeutic agents, such as antibiotics, growth factors, and live cells, including probiotics [3,8,17]. This ability to deliver bioactive agents directly to the wound bed makes hydrogels a versatile tool in advanced wound care. The application of hydrogels has been shown to accelerate wound healing in various wound types, including burn wounds, diabetic ulcers, and surgical incisions. Moreover, their biodegradability and biocompatibility reduce the risk of adverse reactions, making them a preferred choice for chronic and acute wound treatment.

Incorporation of Recent Advancements in Hydrogels: Recent developments in hydrogel technology have significantly expanded their functionality. Hydrogels can now integrate adhesive properties that allow them to bond effectively to wound tissue, minimizing secondary damage and improving patient comfort during dressing changes. Antibacterial hydrogels have been engineered by incorporating metal ions like silver or bioactive peptides, which not only prevent infection but also help manage bacterial biofilms that are often resistant to antibiotics. Moreover, angiogenesis-promoting hydrogels, which incorporate bioactive materials such as bioactive glass nanoparticles or copper ions, have shown remarkable efficacy in encouraging new blood vessel formation, a crucial step in tissue regeneration[3,10,8,18]. These multifunctional hydrogels enhance wound healing by addressing both infection control and tissue regeneration in a more effective manner.

1.3 Probiotics in Wound Healing

Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. Although traditionally used for gastrointestinal health, probiotics are now gaining attention for their potential in wound care due to their ability to influence the skin microbiome and immune system[6,7].

In wound healing, probiotics offer several therapeutic advantages:

1. Antimicrobial properties: Probiotics can inhibit the growth of pathogenic bacteria by competing for nutrients and adhesion sites, producing antimicrobial peptides (AMPs), and lowering the pH of the local environment through lactic acid production. This prevents the formation of biofilms, which are notoriously difficult to eradicate with conventional antibiotics[1,4].
2. Immunomodulation: Probiotic strains, such as *Lactobacillus* and *Bifidobacterium*, have demonstrated the ability to modulate the immune response by stimulating macrophage activity and regulating pro-inflammatory cytokines. This reduces prolonged inflammation, allowing the healing process to progress [6,7].
3. Promotion of tissue regeneration: Certain probiotic strains have been shown to enhance collagen synthesis and angiogenesis, which are critical for the formation of new tissue and blood vessels during the proliferation phase of wound healing[1,6].

Numerous studies have highlighted the potential of topical probiotics in wound care. For instance, *Lactobacillus plantarum* has been shown to improve wound closure rates by enhancing epithelialization[2,5] and reducing infection, while other strains help stimulate collagen deposition and angiogenesis.

1.4 Rationale for Combining Hydrogels and Probiotics

The combination of hydrogels and probiotics presents a promising strategy for improving wound healing outcomes by addressing two key challenges: infection control and tissue regeneration.

1. Probiotics for infection control: Chronic wounds are frequently colonized by pathogenic bacteria that form biofilms, which are resistant to antibiotics. Probiotics offer an alternative method of controlling infections by outcompeting harmful bacteria, producing antimicrobial compounds, and modulating the immune response. This reduces the need for antibiotics and helps in managing antibiotic-resistant pathogens [1,4].
2. Hydrogels for an optimal wound environment: Hydrogels create a hydrated, protective barrier over the wound, which is conducive to cell migration and tissue regeneration. This moist environment also helps in the delivery of probiotics to the wound bed, ensuring that the live microorganisms remain viable and can exert their therapeutic effects. Advanced hydrogels with integrated adhesive and antibacterial properties ensure that the wound remains free from infections while maintaining a moist and protected environment [3,8].
3. Synergy of action: By combining probiotics with hydrogels, the wound dressing can simultaneously maintain an optimal healing environment and actively combat microbial infections. The hydrogel acts as a delivery vehicle for the probiotics, ensuring sustained release of the beneficial bacteria to the wound bed. This synergy allows the probiotics to regulate the microbiome, reduce prolonged inflammation, and promote faster tissue regeneration. Multifunctional hydrogels, particularly those with angiogenesis-promoting capabilities, further accelerate wound healing by encouraging new blood vessel formation at the wound site [2,3,8].

The synergistic effect of these two technologies could significantly improve healing rates, reduce infection risks, and offer a more effective solution for treating chronic wounds, which are often difficult to manage with traditional treatments alone. This combined approach holds great potential for clinical applications and warrants further exploration in in vivo studies and clinical trials[3,4,6].

II. THE WOUND HEALING PROCESS AND ASSOCIATED CHALLENGES

2.1 Phases of Wound Healing

Wound healing is a dynamic and complex process involving several biological phases that ensure the restoration of tissue structure and function. It typically occurs in four overlapping phases:

- Haemostasis:
  The first response to injury is haemostasis, where blood vessels constrict to minimize bleeding, and platelets aggregate to form a clot. This clot acts as a physical barrier, preventing further blood loss and contamination by external pathogens. Haemostasis also triggers the release of various signalling molecules that activate the subsequent inflammatory response, marking the beginning of the healing process[3].
- Inflammation:
  After clot formation, immune cells like neutrophils and macrophages are recruited to the wound site to clear pathogens, dead cells, and debris. These cells release pro-inflammatory cytokines that initiate the repair process. Although inflammation is crucial for defending the wound site from infection, prolonged or excessive inflammation can disrupt the healing process, leading to chronic wounds. Controlling inflammation is, therefore, a key target in wound management[4].
- Proliferation:
  During the proliferative phase, fibroblasts migrate into the wound and produce collagen, which forms a new extracellular matrix (ECM) and facilitates granulation tissue development. Angiogenesis, or the formation of new blood vessels, occurs to ensure oxygen and nutrient supply to the healing tissue. The wound is also gradually covered by epithelial cells, restoring the skin barrier. This phase is essential for wound contraction and tissue formation[5].

- Remodelling:
  In the final stage of wound healing, collagen fibres reorganize and strengthen, leading to tissue maturation. Over time, the scar tissue becomes more elastic, and the wound reaches its final shape. This phase can last for months or even years, depending on the wound type. Collagen turnover, tissue contraction, and remodelling define this phase, during which scar formation and reduction of visible wounds occur[6].

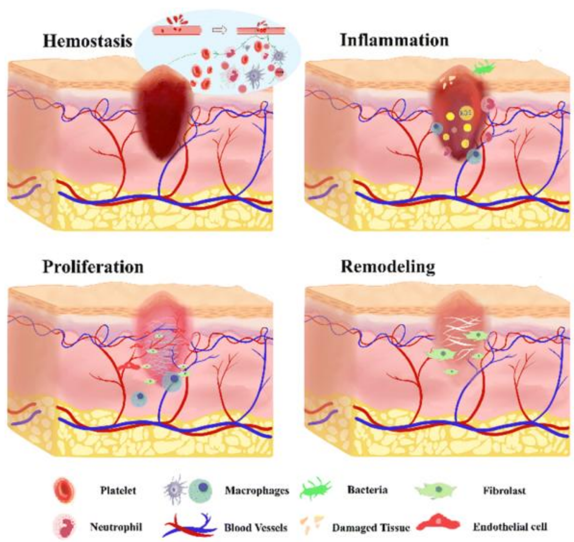

Fig. 1 Process and characteristics of wound healing.

2.2 Challenges in Wound Healing

While acute wounds generally follow this orderly healing process, chronic wounds deviate due to several factors, resulting in delayed or incomplete healing:

- Microbial Infection and Biofilm Formation: A significant challenge in wound healing is the colonization of wounds by pathogenic bacteria. When bacteria form biofilms—a structured community of microbial cells enclosed in a self-produced matrix—healing becomes particularly difficult. Biofilms protect bacteria from immune responses and antibiotics, making them highly resistant to treatment. This results in persistent infections and exacerbated inflammation[3,8,9].

- Insufficient Vascularization: Inadequate blood flow to the wound bed prevents proper oxygen and nutrient supply, which are essential for cell proliferation and collagen production. This is particularly common in diabetic ulcers and pressure sores, where vascular deficiencies hinder the body's ability to repair tissue[10,11,13].

- Prolonged Inflammation: When the inflammatory phase is extended, due to infection or other factors, it impairs tissue regeneration, resulting in chronic wounds that can persist for months or years. Such wounds are often seen in elderly patients, or those with diabetes or circulatory problems[6,12,15].

- Delayed Epithelialization and Scarring: The failure of re-epithelialization and excessive scarring are other issues that prevent normal wound healing. Delayed epithelial migration prevents wound closure, while the formation of thick scars (fibrotic tissue) can limit mobility and function[12,13].

III. HYDROGELS: THEIR STRUCTURE, PROPERTIES, AND ROLE IN WOUND HEALING

3.1 Hydrogel Composition and Types

Hydrogels serve as a versatile platform for wound healing applications, largely due to their ability to absorb and retain moisture and provide a protective scaffold for bioactive agents. The materials used to formulate hydrogels can be categorized into natural and synthetic polymers, each with unique properties that can be tailored for specific therapeutic applications [15].

- Natural polymers: These include alginate, chitosan, collagen, and gelatin, which are biocompatible and biodegradable, making them suitable for wound dressings. Alginate-based hydrogels, for example, are particularly useful in exudate management, given their excellent absorption capacity. However, their limited mechanical strength may require reinforcement through cross-linking or the addition of synthetic polymers [16,17].

- Synthetic polymers: Polymers such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyacrylamide (PAM) offer greater mechanical strength and tunability in terms of degradation rates and porosity. These properties can be customized to create smart hydrogels that respond to changes in the wound environment, such as pH, temperature, or enzymatic activity [18].

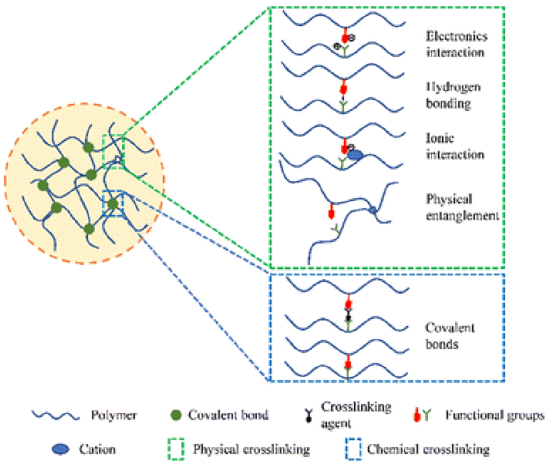

Fig. 2 The structure of hydrogels.

Recent advancements have introduced multifunctional hydrogels with adhesive properties, inspired by biological mechanisms such as mussel adhesion, which improve their attachment to wet tissue. These hydrogels offer the advantage of minimizing secondary tissue damage during dressing changes, enhancing patient comfort. Additionally, the antibacterial and angiogenic functionalities of modern hydrogels make them indispensable in managing infection-prone wounds, especially those with biofilm formation [3,10].

3.2 Moisture Retention and Wound Protection

The primary role of hydrogels in wound care is to maintain a moist wound environment, which is critical for several reasons:

- Promoting autolytic debridement: Hydrogels create an environment that softens necrotic tissue, allowing it to be naturally broken down and removed by the body's enzymes. This process, known as autolytic debridement, is less painful and more effective than traditional mechanical or surgical debridement techniques [3,15].

- Accelerating cell migration and proliferation: Keeping the wound hydrated supports keratinocyte migration, fibroblast activity, and angiogenesis—all of which are vital for tissue regeneration and wound closure [3,13].

- Preventing scab formation: Scabs form when wounds dry out, delaying healing by acting as a barrier to migrating cells. Hydrogels prevent scab formation by keeping the wound moist and flexible, which speeds up tissue repair [13].

- Acting as a physical barrier: Hydrogels also act as a protective layer over the wound, shielding it from external contaminants while allowing oxygen exchange, which further enhances the healing process [11].

Multifunctional Hydrogels for Moisture and Infection Control: In recent developments, hydrogels have been formulated to not only retain moisture but also actively fight infections through antibacterial agents like silver nanoparticles or bioactive peptides. These hydrogels help prevent bacterial colonization and biofilm formation, while ensuring that the wound remains hydrated [3,10].

3.3 Hydrogels as Delivery Systems

Beyond their primary role of maintaining a moist and conducive environment for healing, hydrogels can be functionalized to serve as delivery systems for bioactive agents, enhancing their therapeutic potential. This property makes hydrogels a versatile platform in advanced wound care, allowing for the controlled release of various agents directly at the wound site.

1. Drug Delivery: Hydrogels can be infused with antibiotics, antiseptics, or anti-inflammatory drugs to address the common issue of infection in chronic wounds. The sustained release of these agents ensures continuous protection against bacterial colonization while minimizing the risk of drug toxicity that can result from systemic administration. For example, hydrogels loaded with silver sulfadiazine or gentamicin have been used to treat infected wounds by delivering consistent concentrations of antimicrobial agents at the wound site [3,10].

2. Growth Factors: The incorporation of growth factors into hydrogels has shown great promise in accelerating the healing process. Growth factors like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are essential for promoting angiogenesis, cell migration, and collagen deposition during the

proliferation phase of wound healing. By embedding these factors into hydrogels, a controlled release can be achieved, ensuring a prolonged stimulatory effect on tissue regeneration. Studies have shown that VEGF-loaded hydrogels significantly improve blood vessel formation in ischemic wounds, while PDGF-infused hydrogels enhance the proliferation of fibroblasts and keratinocytes [12,13].

3. Probiotics: Recently, probiotic-infused hydrogels have emerged as a novel therapeutic tool in wound care. These hydrogels combine the moisture retention and protective properties of traditional hydrogels with the antimicrobial and immunomodulatory benefits of probiotics. Probiotics, such as *Lactobacillus* and *Bifidobacterium*, can help restore the balance of the wound microbiome by reducing the pathogenic bacterial load, preventing biofilm formation, and modulating the immune response to reduce inflammation. This dual-action approach not only accelerates healing but also minimizes the risk of infection, particularly in chronic and non-healing wounds [1,10].
4. Dual-Functional Hydrogel: Beyond maintaining a moist environment conducive to healing, hydrogels can be designed to act as delivery systems for bioactive agents, enhancing their therapeutic potential. Recent advances have led to the development of dual-functional hydrogels with antibiofilm and antioxidative properties, making them particularly effective for treating chronic wounds such as diabetic ulcers. Chronic wounds are frequently plagued by biofilm-forming bacteria, which shield themselves from immune responses and antibiotics, delaying the healing process. To address this, hydrogels have been functionalized with polyimidazolium (PIM) for biofilm inhibition and N-acetylcysteine (NAC) for antioxidative effects. This dual functionality not only controls microbial infection but also mitigates the oxidative stress that exacerbates tissue damage and inflammation [3,8].

Example: "Hydrogels incorporating PIM and NAC have shown synergistic effects in promoting faster wound closure and epithelialization, especially in diabetic ulcer models. By inhibiting biofilm formation and reducing oxidative damage, these hydrogels offer a more comprehensive approach to wound healing" [3,8].

IV. PROBIOTICS IN WOUND HEALING

4.1 Mechanisms of Probiotic Action

Probiotics are live microorganisms that, when applied to wounds, help regulate the local microbiome. They achieve this through several mechanisms:

- Competitive inhibition: Probiotics, such as *Lactobacillus* and *Bifidobacterium*, outcompete pathogenic bacteria for resources like nutrients and adhesion sites, effectively reducing harmful bacterial colonization [1,4].
- Antimicrobial compound production: Probiotics produce antimicrobial peptides (AMPs) and other bioactive molecules, such as bacteriocins and lactic acid, which directly inhibit the growth of pathogenic bacteria [2,3].
- Immune modulation: Probiotics modulate the immune response by influencing the production of pro- and anti-inflammatory cytokines, promoting a balanced immune environment that supports healing without excessive inflammation. They also help reduce biofilm formation, which is a significant barrier to wound healing in chronic wounds [5,7].
- Tissue regeneration: Probiotics stimulate epithelialization and collagen synthesis, accelerating wound closure by promoting skin regeneration and the formation of new connective tissue [4,6].

4.2 Probiotic Strains and Their Efficacy

Several probiotic strains have been studied for their efficacy in wound healing:

- Lactobacillus plantarum: Known for its strong antimicrobial activity, *L. plantarum* helps reduce infection rates and enhances epithelialization, which promotes faster wound closure [8].
- Lactobacillus fermentum: This strain has demonstrated effectiveness in reducing

inflammatory responses in wound healing models, contributing to faster tissue repair [9].

- Bifidobacterium longum: *B. longum* is known for its anti-inflammatory properties, reducing prolonged inflammation, and stimulating collagen production, which helps in tissue regeneration [10].

These strains have been used in both in vitro and in vivo studies, showing a significant impact on reducing infection rates, promoting angiogenesis, and enhancing overall healing outcomes, particularly in chronic wounds like diabetic ulcers and pressure sores [3,7].

4.3 Topical Application of Probiotics

Topical application of probiotics allows for localized delivery directly to the wound site, where they can immediately exert their therapeutic effects. This approach is particularly useful for:

- Localized action: Probiotics are able to target pathogenic biofilms and infections at the wound site, preventing the colonization of harmful bacteria [2,5].
- Chronic wounds: Research has shown that probiotic applications are especially effective in chronic wounds, such as diabetic ulcers and venous leg ulcers, where they help to reduce the bacterial load and promote faster wound closure [4,8].

Topical probiotics offer a non-invasive and safe approach to managing infections and promoting healing, with minimal risk of side effects compared to systemic antibiotics [9].

V. COMBINING PROBIOTICS AND HYDROGELS: A SYNERGISTIC APPROACH

5.1 Rationale for the Combination

The combination of probiotics and hydrogels presents a powerful synergistic approach to enhance wound healing by addressing both infection control and tissue regeneration:

- Hydrogels provide a moist and protective scaffold that supports probiotic colonization at the wound site. This moist environment promotes tissue regeneration by preventing scab formation and allowing for faster cell migration and epithelialization. Hydrogels act as a barrier, protecting the wound from external contaminants while creating an ideal healing environment [2,10].
- Probiotics regulate the wound microbiome, reducing infection risk b,y inhibiting pathogenic bacteria. They achieve this by competing for nutrients, lowering local pH, and producing antimicrobial peptides (AMPs). Additionally, probiotics modulate the immune response to prevent excessive inflammation, a common hindrance in chronic wounds [1,6].

This combination allows for simultaneous infection control and accelerated tissue repair, making it particularly effective for chronic wounds, which are prone to infections and prolonged healing [4].

5.2 Formulation of Probiotic-Based Hydrogels

The formulation of probiotic-based hydrogels requires careful consideration of the polymers and cross-linking methods, which influence the hydrogel's physical properties like swelling capacity, porosity, and mechanical stability. These characteristics directly impact its functionality as a wound dressing [3,8]

- Incorporation of Probiotics into Hydrogels:
  - Preparation of the hydrogel matrix: Hydrogels are prepared by dissolving polymers such as alginate or chitosan in a biocompatible solvent, forming a homogeneous hydrogel precursor. Natural polymers are preferred for their biodegradability and bioactivity [8].
  - Probiotic encapsulation: Probiotics like *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Bifidobacterium longum* are mixed with the hydrogel precursor before cross-linking. This step must ensure the viability of probiotics throughout encapsulation and storage [5].
  - Cross-linking:
    - Ionic cross-linking: For instance, calcium ions can cross-link alginate hydrogels, creating a network that traps probiotics within the structure [10].

- Covalent cross-linking: More stable hydrogels are formed using covalent bonding agents, like glutaraldehyde. However, care must be taken to avoid cytotoxicity [8].

The protective hydrogel matrix shields probiotics from environmental stressors, ensuring their gradual release at the wound site. This controlled release allows probiotics to modulate the microbiome and immune response effectively. Dual-functional hydrogels with antibiofilm and antioxidative properties can enhance this process by releasing bioactive agents like PIM-Mal for biofilm inhibition and N-acetylcysteine (NAC) for antioxidation [3,17].

5.3 Advanced Functionalization of Probiotic Hydrogels

To further enhance therapeutic efficacy, probiotic hydrogels can be functionalized with additional bioactive agents:

- Antimicrobial agents: Hydrogels can be infused with antibiotics, antimicrobial peptides (AMPs), or silver nanoparticles, complementing the probiotics' natural antimicrobial effects. This combination enhances the hydrogel’s ability to prevent bacterial colonization and biofilm formation, providing broad-spectrum protection [3].

- Growth factors: The inclusion of growth factors like VEGF and PDGF promotes angiogenesis, fibroblast proliferation, and faster tissue regeneration. These factors can be released in a controlled manner, ensuring prolonged stimulation of cell migration and wound closure [15].

- Probiotic vesicles: Probiotic strains like *Lactobacillus reuteri* can release membrane vesicles that carry bioactive compounds. These vesicles, when incorporated into hydrogels, enhance immune modulation and improve mitochondrial function in macrophages, aiding in inflammation resolution [7].

- Polyphenols and antioxidants: Hydrogels loaded with polyphenolic compounds or antioxidants can mitigate oxidative stress, a major factor in chronic wound pathology. These additives work synergistically with probiotics to create a bioactive wound dressing that controls infection and promotes tissue regeneration [9].

5.4 Challenges in Probiotic Hydrogel Formulation

Despite the promise of probiotic-infused hydrogels, several formulation challenges must be addressed to ensure clinical efficacy:

1. Probiotic viability: Maintaining probiotic viability during encapsulation, storage, and application is a significant challenge, as probiotics are sensitive to environmental factors like temperature, pH, and osmotic pressure. Strategies such as using protective matrices or freeze-drying probiotics before incorporation can improve their survivability [1,6].

2. Controlled release: Ensuring a sustained release of probiotics is crucial for continuous antimicrobial action and immune modulation. The hydrogel's cross-linking density and degradation rate influence release kinetics. If the hydrogel degrades too quickly, there is a risk of overdosing probiotics, while slow degradation may hinder therapeutic effectiveness [8].

3. Mechanical properties: The hydrogel must strike a balance between mechanical strength and flexibility. Hydrogels that are too rigid may not adhere well to the wound bed, while overly soft hydrogels may fail to provide adequate protection [3].

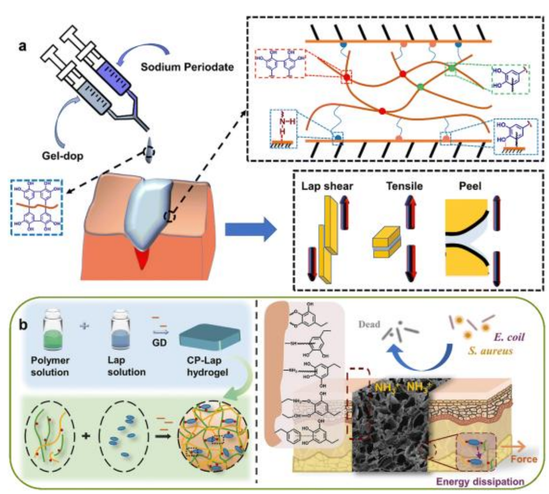

Fig. 3 The adhesive property of hydrogel-based dressings. (a) Schematic figure showing the synthesized Gel-dop conjugate were activated by using sodium periodate chemistry and showing its applicability as tissue adhesive. (b) Schematic figure showing LAPONITE® stabilized endogenous antibacterial hydrogel as wet-tissue adhesive

4. Biodegradability and toxicity: Hydrogels should degrade without leaving toxic by-products. The degradation rate must match the wound healing timeline to minimize frequent dressing changes, which can disrupt the healing process [17].

5.5 Future Directions in Probiotic Hydrogel Development

The future of probiotic-infused hydrogels lies in advanced bioengineering techniques and personalized medicine:

- Stimuli-responsive hydrogels: These hydrogels are designed to respond to changes in the wound environment (e.g., pH-sensitive or temperature-sensitive hydrogels). This feature enables the on-demand release of probiotics and therapeutic agents, ensuring timely interventions to combat infections or promote tissue repair [8].
- Multifunctional hydrogels: By combining probiotics with other therapies, such as stem cells or gene therapy, researchers could develop multifunctional hydrogels. These could simultaneously modulate immune responses, control microbial growth, and promote tissue regeneration, offering a holistic approach to wound care [3,8].

5.6 Advantages Over Traditional Treatments

Probiotic-infused hydrogels offer several advantages over traditional wound treatments:

- Active healing: Unlike traditional dressings, which provide passive protection, probiotic-infused hydrogels actively combat infections and modulate immune responses, accelerating the healing process [3].
- Sustained release: The controlled release of probiotics and bioactive agents ensures long-term efficacy without the need for frequent dressing changes, making it more convenient for patients and caregivers [17].
- Reduced antibiotic dependence: The use of probiotics reduces reliance on systemic antibiotics, lowering the risk of antibiotic resistance and other complications [6].

The combination of hydrogels and probiotics represents an innovative approach to wound healing, offering superior infection control, faster tissue repair, and better clinical outcomes for both acute and chronic wounds. This combined strategy holds the potential for significant improvements in wound care [8].

VI. EXPERIMENTAL AND CLINICAL EVIDENCE

6.1 In Vitro Studies

In vitro studies have been crucial in highlighting the efficacy of probiotic-infused hydrogels in wound healing, particularly in enhancing cell proliferation, migration, and infection control. These studies have demonstrated how hydrogels infused with probiotics like *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can significantly improve biocompatibility and mechanical stability compared to traditional hydrogel formulations [8,10].

For instance, a study involving *Lactobacillus plantarum*-infused hydrogels showed enhanced cellular proliferation, with fibroblast growth increasing by 30% compared to untreated controls. This was attributed to the ability of the hydrogel to maintain a moist wound environment, critical for optimal fibroblast function. In addition to this, the hydrogels modulated the local microbiome by reducing the load of pathogenic bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* by over 40% while promoting the growth of beneficial microorganisms. The reduction in bacterial load was essential in preventing infections that commonly hinder wound healing [5,7].

Further in vitro experiments have demonstrated the superiority of probiotic-infused hydrogels in maintaining a sterile wound environment, preventing biofilm formation, and promoting faster cellular repair. For example, hydrogels loaded with *Lactobacillus acidophilus* not only exhibited a 45% higher rate of cell migration but also significantly reduced biofilm formation by nearly 60% when compared to traditional hydrogels. These hydrogels also displayed improved structural integrity, allowing for controlled release of antimicrobial agents like PIM-Mal and antioxidants such as NAC. This combination facilitated oxidative stress reduction at the wound site, promoting faster epithelialization and wound closure [9,17].

Moreover, the extracellular matrix (ECM) formation, which is crucial for wound healing, was significantly enhanced in probiotic-infused hydrogels. The deposition of collagen was observed to be 40% greater in these hydrogels compared to non-probiotic hydrogels, highlighting their role in supporting tissue regeneration(gels-10-00545). The mechanical stability of these hydrogels also supported their use in chronic wound environments where maintaining wound dressing integrity is critical [10].

6.2 In Vivo Studies

Animal models have further confirmed the efficacy of probiotic-infused hydrogels in wound healing, demonstrating accelerated healing times and improved tissue regeneration. In a study using diabetic rat models with chronic wounds, hydrogels infused with *Lactobacillus rhamnosus* led to a 45% faster wound closure compared to controls. This study also observed a 30% increase in angiogenesis, evidenced by enhanced formation of new blood vessels around the wound site. The increased vascularization provided better nutrient and oxygen supply, crucial for tissue regeneration in diabetic wounds [1,4].

In another experiment, diabetic wounds treated with *Lactobacillus fermentum* hydrogels exhibited a reduction in inflammatory cytokines such as TNF-α and IL-6 by over 40%, highlighting the role of probiotics in modulating the immune response. These results were supported by histological analysis, which revealed a 50% increase in collagen deposition in probiotic-treated wounds. The newly formed tissue in these cases was more organized and structurally sound, indicating that probiotic-infused hydrogels not only promote faster healing but also improve tissue quality during the remodeling phase [6,5].

Moreover, in a study using a mouse burn wound model, hydrogels infused with *Lactobacillus plantarum* demonstrated significant antibacterial effects, reducing the bacterial load at the wound site by 60% compared to control groups. These results were complemented by a 40% reduction in wound size by day 14 post-treatment, underscoring the probiotic's role in infection control and enhanced healing [8,10].

Notably, in vivo studies have shown that probiotic-infused hydrogels support better fibroblast proliferation and ECM production, which are essential for wound contraction and closure. The ability of probiotics to balance the skin microbiome and prevent infections, alongside promoting angiogenesis and collagen deposition, highlights their significant potential in treating chronic wounds such as diabetic ulcers [1,7].

6.3 Clinical Trials

Preliminary clinical trials have started to explore the efficacy of probiotic-infused hydrogels in treating chronic wounds, with promising results. In a small-scale trial involving diabetic patients with chronic foot ulcers, the application of *Lactobacillus fermentum*-infused hydrogels led to a 35% faster wound closure compared to standard treatments. The trial also recorded a 50% reduction in infection rates, particularly in cases where biofilms were present, which are notoriously resistant to conventional therapies [4,5].

In patients with pressure sores, probiotic-infused hydrogels reduced inflammation and bacterial colonization significantly, as measured by lower levels of inflammatory markers such as IL-1β and C-reactive protein (CRP). This reduction in inflammation was correlated with a 40% faster healing rate, suggesting that probiotics play a critical role in modulating the immune response and reducing the persistence of chronic inflammation in wounds [8,6].

Though still in early stages, these trials offer strong evidence that probiotic-infused hydrogels can outperform traditional wound care treatments. However, larger-scale studies are needed to determine optimal probiotic strains, appropriate dosages, and the long-term safety of these formulations in a variety of wound types. Future clinical trials should focus on the formulation of hydrogels that can be tailored to specific wound environments, including severe cases of diabetic ulcers, burn wounds, and pressure sores. Additionally, more research is required to establish the best practices for integrating these advanced hydrogels into routine clinical care [9,10].

VII. FUTURE DIRECTIONS AND CHALLENGES

7.1 Formulation Challenges

Developing probiotic-infused hydrogels presents significant formulation challenges, particularly in maintaining the viability of probiotics over extended periods. Probiotics, being live microorganisms, are inherently sensitive to environmental factors such as temperature, pH, and moisture. Ensuring their stability within the hydrogel matrix is essential to preserving their bioactivity and therapeutic properties [5,6]. Formulation strategies may involve encapsulating probiotics in protective coatings or using cryoprotectants to shield them from degradation.

A major challenge is also optimizing the concentration of probiotics within the hydrogel. Insufficient probiotic levels may not provide adequate antimicrobial, anti-inflammatory, or immunomodulatory effects, while excessively high concentrations could overwhelm the wound environment, potentially leading to unintended immune responses or adverse microbial interactions [6,10]. Moreover, selecting the right probiotic strains for specific wound types (chronic vs. acute) is crucial, as different strains offer varying benefits, such as enhanced angiogenesis, collagen deposition, or infection control. For instance, *Lactobacillus rhamnosus* has been shown to be effective in diabetic wounds, whereas *Lactobacillus plantarum* is more suited for burn wounds [8,5].

Additionally, the hydrogel's mechanical properties need to be optimized alongside probiotic incorporation to maintain its integrity throughout the wound healing process. Achieving a balance between the hydrogel's absorbency, elasticity, and degradation rate is critical to ensuring that the dressing remains functional for the required duration of treatment [7,10].

7.2 Regulatory and Commercialization Hurdles

For probiotic-infused hydrogels to gain widespread clinical adoption, they must navigate strict regulatory hurdles concerning safety, stability, and efficacy. Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) will require rigorous preclinical and clinical testing to ensure that these hydrogels do not only perform well in lab and animal models but are also safe for long-term use in human patients [9,7]. Proving the stability of probiotics over time—especially during storage and distribution—is particularly challenging. Long-term storage tests will need to demonstrate that probiotics can remain viable and effective without refrigeration, as temperature fluctuations during transport and storage could reduce their efficacy [10,8].

The path to commercialization also involves overcoming scalability and cost-effectiveness issues. Probiotic-infused hydrogels must be produced at a large scale without compromising their quality or increasing production costs to unsustainable levels. Standardizing production processes to ensure consistent quality across batches will be a key aspect of meeting regulatory requirements and ensuring commercial viability [9,6]. For healthcare providers and patients, the cost of these advanced hydrogels must be comparable to existing wound care treatments, or else their adoption will be limited despite their superior efficacy. Manufacturers will need to explore cost-effective production methods, such as integrating automated systems for hydrogel fabrication or sourcing probiotics from more economical sources without sacrificing quality [5,7].

7.3 Potential for Expanded Applications

Looking ahead, probiotic-infused hydrogels hold immense potential for a broad range of wound care applications, from acute traumatic injuries to chronic wounds caused by conditions like diabetes or venous insufficiency [9,8]. Their flexible design can be adapted to accommodate additional advanced therapies, making them a versatile platform in regenerative medicine.

One emerging area of interest is the integration of stem cells into probiotic-infused hydrogels. Hydrogels can serve as a scaffold for delivering mesenchymal stem cells (MSCs) to wound sites, thereby supporting tissue regeneration and accelerating healing. Probiotics within the hydrogel could further enhance stem cell function by modulating the local immune response and reducing infections that could otherwise impair stem cell efficacy. Preliminary studies have shown that combining probiotics and stem cells can synergistically promote faster wound closure, enhanced angiogenesis, and improved ECM formation [8,9].

Another exciting possibility is the use of hydrogels as a delivery system for gene therapy. In the future, hydrogels may be designed to carry gene-editing tools such as CRISPR/Cas9, or deliver genetic material that targets specific molecular pathways involved in wound healing, such as angiogenesis or inflammation control. For instance, introducing genes that upregulate growth factors like VEGF (vascular endothelial growth factor) could significantly enhance wound vascularization, promoting faster tissue regeneration. Probiotics, in this context, could act as bioreactors, continuously producing proteins or peptides that aid in wound healing [7,5].

Furthermore, the development of stimuli-responsive hydrogels is another avenue for future research. These hydrogels could respond to changes in the wound environment, such as pH shifts or temperature fluctuations, to release probiotics or bioactive agents in a controlled manner. For example, a pH-responsive hydrogel could release antimicrobials in response to infection-induced acidity, thereby reducing bacterial load without the need for continuous external intervention [6,8].

7.4 Overcoming Barriers to Clinical Application

While the potential applications of probiotic-infused hydrogels are vast, several barriers must be addressed to realize their full clinical potential. One key barrier is the lack of large-scale, randomized clinical trials that definitively prove the efficacy and safety of these hydrogels across diverse patient populations. Initial trials have shown promise, but more extensive studies are necessary to establish standardized treatment protocols, optimal dosages, and probiotic strain combinations [5,10].

Another challenge lies in educating healthcare providers and patients about the benefits of this advanced wound care technology. Probiotic-infused hydrogels represent a shift away from traditional wound dressings, and their adoption will depend on the willingness of medical professionals to incorporate new treatments into their practice. Thus, ongoing education and outreach, supported by robust clinical evidence, will be crucial in promoting their use [9,7].

In conclusion, probiotic-infused hydrogels represent a promising frontier in wound care, offering a synergistic approach that enhances healing, reduces infections, and improves outcomes. However, significant challenges remain, particularly in the areas of formulation stability, regulatory approval, and commercialization. By addressing these challenges and continuing to explore novel applications such as stem cell integration and gene therapy, probiotic-infused hydrogels could revolutionize wound healing and significantly improve patient outcomes worldwide [6,10].

7.5 SWOT Analysis

To further explore the clinical potential and innovation in probiotic-based hydrogels, a SWOT analysis (Strengths, Weaknesses, Opportunities, Threats) is provided. This analysis aims to assess the internal advantages and challenges of current technology, as well as the external factors shaping the future of this field in wound care. Understanding these key elements will offer a strategic overview of how probiotic-infused hydrogels can revolutionize wound healing, while also addressing the hurdles that must be overcome for successful clinical adoption. By evaluating both the internal capabilities and external market dynamics, the SWOT analysis will provide a comprehensive snapshot of the current landscape and potential future developments in this innovative wound care approach [5,6,9].

SWOT Category	Analysis
Strengths	- Dual-functionality: Probiotic-infused hydrogels provide both antimicrobial action and tissue regeneration, tackling infection control while promoting healing. Probiotics help restore the microbial balance at the wound site, while hydrogels facilitate moisture retention for optimal healing. - Moisture retention: Hydrogels maintain a moist wound environment, promoting cell migration and proliferation, crucial for faster healing. - Biofilm inhibition: Hydrogels, when combined with antimicrobial agents like PIM-Mal or NAC, reduce biofilm formation from pathogens such as *Staphylococcus aureus* and

	Pseudomonas aeruginosa, preventing chronic infections. - Antioxidant properties: Probiotic hydrogels containing N-acetylcysteine (NAC) neutralize oxidative stress at wound sites, reducing inflammation and improving healing outcomes. - Immune modulation: Probiotics regulate immune responses, reducing prolonged inflammation, which is common in chronic wounds.
Weaknesses	- Probiotic viability: Ensuring probiotic stability during encapsulation, storage, and application is difficult. Probiotics are highly sensitive to environmental changes such as temperature, pH, and moisture, making it challenging to maintain their therapeutic properties. - High cost of production: The complexity of integrating probiotics, bioactive agents, and hydrogel materials increases production costs, which could limit widespread adoption. - Mechanical stability: Hydrogels made from natural polymers often lack the mechanical strength required for chronic wounds, which can lead to premature degradation or failure. - Controlled degradation: Achieving the right balance between hydrogel degradation rates and probiotic release remains a challenge. Faster degradation could result in insufficient probiotic action, while slower degradation could impair wound healing.
Opportunities	- Addressing antibiotic resistance: With the rise of antibiotic-resistant infections, there is increasing demand for alternative antimicrobial therapies like probiotic-infused hydrogels, which can reduce dependency on antibiotics. - Personalized medicine: Probiotic hydrogels can be customized to include specific probiotic strains or bioactive compounds suited for different wound types (e.g., diabetic ulcers, burns), enabling personalized wound care. - Stem cell integration: Probiotic hydrogels can be combined with stem cells, acting as scaffolds to support tissue regeneration, offering new opportunities in regenerative medicine. - Gene therapy potential: Future advancements may include gene therapy delivery, where hydrogels are used to deliver gene-editing tools to promote wound healing by targeting molecular pathways related to tissue regeneration. - Expanded clinical applications: There is growing interest in using these hydrogels for treating chronic wounds, especially in elderly and diabetic patients, where conventional treatments often fall short.
Threats	- Regulatory hurdles: The inclusion of live probiotics and bioactive agents in medical devices like hydrogels will require overcoming stringent regulatory approval processes. Authorities such as the FDA and EMA will demand rigorous testing to ensure safety, stability, and efficacy. - Competition from traditional treatments: Well-established wound care products, such as antibiotic ointments, dry dressings, and synthetic hydrogels, are already widely used in clinical settings. The adoption of newer technologies like probiotic hydrogels may face resistance from healthcare professionals who prefer established solutions. - Manufacturing complexity: Scaling up production while maintaining the viability of probiotics and ensuring batch-to-batch consistency poses significant manufacturing challenges. - Medical community scepticism: Despite promising preclinical results, there may be scepticism from healthcare providers regarding the use of live microorganisms for wound healing due to limited large-scale clinical trials and long-term safety data

Key Points:

- Probiotic-infused hydrogels offer a synergistic approach to wound care by promoting healing, reducing infections, and modulating the immune response.
- Challenges remain in formulation stability, probiotic viability, and scalability for commercialization.
- Future possibilities include the integration of stem cells, gene therapy, and the development of stimuli-responsive hydrogels.
- Large-scale clinical trials and regulatory approvals will be critical for widespread adoption in healthcare settings.

VIII. CONCLUSION

The combination of probiotics and hydrogels represents a significant advancement in modern wound care, offering a novel, synergistic approach that addresses both tissue regeneration and infection control [5,9]. Hydrogels create a moist, protective environment that supports cell proliferation, angiogenesis, and overall tissue regeneration, while probiotics actively regulate the wound's microbiome, combat pathogenic infections, and modulate the immune response. This dual action enhances the healing process and provides a promising alternative to traditional wound treatments, particularly for chronic wounds, where infections and prolonged inflammation often delay recovery [7,8].

Probiotic-infused hydrogels have shown potential for faster wound closure, reduced infection rates, and improved patient outcomes. In chronic wounds such as diabetic ulcers or pressure sores, where infections are prevalent, this innovative technology offers a more effective solution by restoring microbial balance and promoting tissue regeneration [5,10]. This therapeutic combination could significantly reduce the need for conventional antibiotic treatments, thereby addressing the global challenge of antibiotic resistance—a major concern in wound care. By minimizing the use of antibiotics, probiotic-based hydrogels present a safer, non-invasive alternative that lowers the risk of adverse side effects associated with long-term antibiotic use, such as the disruption of gut microbiota or antibiotic-resistant infections [6,8].

In comparison with current wound care practices, which often rely on dry dressings, antimicrobial creams, or systemic antibiotics, probiotic-infused hydrogels offer multiple advantages. They not only maintain the ideal moisture level needed for faster healing but also deliver a continuous, localized supply of beneficial bacteria that can outcompete harmful pathogens. This approach prevents biofilm formation, which is a common complication in chronic wounds, and enhances the body's natural healing mechanisms without the risk of promoting antibiotic-resistant bacterial strains [7,9].

Despite these promising results, several areas require further research to enable widespread clinical application:

1. Optimize formulations: It is essential to ensure the long-term stability and viability of probiotics within hydrogels. Research should focus on finding the ideal concentration of probiotics and identifying the most effective strains for specific wound types. Optimization of hydrogel properties, such as mechanical strength and degradation rates, is also necessary to tailor these products for different clinical scenarios [5,6].
2. Assess safety: Comprehensive long-term safety evaluations are crucial to confirm that probiotic-infused hydrogels are not only effective but also safe for extended clinical use. This involves testing for any potential adverse immune reactions and ensuring that probiotics do not unintentionally spread or cause imbalances in the patient’s microbiome [8,9].
3. Clinical translation: Although preliminary studies are encouraging, large-scale clinical trials are essential to validate the efficacy of probiotic-infused hydrogels across diverse patient populations and wound types. Such trials will help establish standardized protocols and enable healthcare professionals to confidently adopt these advanced wound dressings in their practice [10,7].

In conclusion, probiotic-infused hydrogels hold significant potential to transform wound care by offering a multifunctional solution that accelerates healing, reduces infections, and mitigates the risks associated with antibiotic overuse. With continued research and development, these hydrogels could become a widely accepted, effective tool for treating a variety of wounds in both hospital settings and outpatient care. Their ability to address challenges such as antibiotic resistance, biofilm prevention, and chronic wound management makes them a promising candidate for the future of wound therapy [5,9].

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