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REVIEW ARTICLE

A Comprehensive Review of Advanced Modern Treatments, Strategies, and Techniques for Accelerating Wound Healing

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Abstract

Skin wounds are common events and may occur in different situations for each person, but it's better to start the skin repairing process as soon as possible. So, the healing happens in the best time with the minimum scar and skin tissue damages. Recent developments in wound management have introduced the advanced therapeutic modalities for repairing the tissue, and each of the approaches contributes to enhancing tissue regeneration. Therefore, according to all developments in methods for increasing the speed of healing, it can be seen in skin wound healing processes too. So, it's necessary to know more about wound healing and different modern treatments and techniques for accelerating wound healing, especially in skin injuries. This review focuses on exploring the wound healing process and different advanced and modern treatments to introduce and review all these items together. It is useful for anyone who needs general data about advanced wound healing techniques.

1. Introduction

In different situations when an injury happens, we have to care for the wound and find a way for accelerating the wound healing in the oral and facial wound healing after traumatic injuries in different situations such as car accidents, knife wounds, lacerations, and surgeries, or wounds heal through hemostasis, inflammation, proliferation, and remodeling phases of wound healing, involving multiple cells and cytokines in the oral and maxillofacial regions. The healing process and all of its stages are explained below for more information and studying with the best data.

2. Stages of Wound Healing

According to all data that have been given by physiologists about the physiological changes that are observed in obesity, it is possible that they directly influence the wound healing process. So, for understanding possible interaction mechanisms, it requires familiarity with the wound healing process under normal physiological conditions. In other words, hemostasis, the first step in wound healing, occurs when endothelial cells are damaged. After hemostasis, the inflammatory phase begins with edema and an influx of inflammatory cells. The inflammatory phase is followed by proliferation and remodeling, the latter of which can last for more than 2 years (Gurtner *et al.*, 2008).

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2.1. Hemostasis

Hemostasis, the first step in wound healing, occurs through endothelial damage and can last from minutes to hours. Immediately after injury, vasoconstriction of the injured vessel occurs, and both the intrinsic and extrinsic coagulation cascades are activated by adjacent platelets and endothelial cells. The blood clot that forms is rich in platelets, collagen, fibronectin, and thrombin. It acts as a scaffold for neutrophils, monocytes, and other invading cells, triggering the release of local vasoconstrictors such as cytokines, growth factors, and serotonin (George Broughton *et al.*, 2006). After hemostasis, the release of histamine induces the migration of inflammatory cells to the injury site, and it is the beginning of the inflammatory phase.

2.2. Inflammatory Phase

The inflammatory phase typically begins shortly after the initial injury and hemostasis phase (Eming *et al.*, 2014), and this phase is important for the wound healing process as it marks the recruitment of the innate immune system. Within the first 48–96 hours after injury, monocytes are recruited from the surrounding tissues and transformed into macrophages (George Broughton *et al.*, 2006), and these activated macrophages are required for the transition from the inflammatory phase to the proliferative phase. In addition, macrophages also release vascular endothelial growth factor, fibroblast growth factor, and TNF-α to stimulate angiogenesis. At this time, the major cell type at this stage is neutrophil. These important cells are recruited to the injury site via IL-8 that was released from platelets during the degranulation process and secrete IL-1, TNF-α, and TGF-β at this time too (George Broughton *et al.*, 2006). After injury in skin, Toll-like receptors are expressed on host cells and activate two distinct signaling pathways: 1. the nuclear factor kappa beta signaling pathway and 2. the mitogen-activated protein kinase signaling pathway. Activation of these signaling pathways is a hallmark of the inflammatory phase (Landén *et al.*, 2016). Once the inflammatory phase subsides, the body enters the proliferative phase. The inflammatory phase can be the reason for healing and other skin issues. So, the healing will begin only when the inflammatory phase ends completely (Guo and DiPietro, 2010).

2.3. Proliferative Phase

During the proliferative phase, the body prioritizes the restoration of the local vascular network and re-epithelialization of the wound surface too (Landén *et al.*, 2016). When the keratinocytes begin to migrate from the edges of the wound bed, and the epithelial stem cells begin to proliferate in response to influenced chemical and mechanical signals from both anti-inflammatory and pro-inflammatory cells, it will lead to fibrosis. During the fibrogenesis, fibroblasts are activated and transform into myofibroblasts and secrete components of the extracellular matrix that promote the wound contraction and contribute to permanent scar formation (Zangooei and Jalili, 2013). These signals lead to the development of granulation tissue, which is primarily composed of collagen III, new blood vessels, and fibroblasts. Fibroblasts are the main cell type in granulation tissue and initiate the re-epithelialization in response to cytokines that were released by macrophages. In addition to IL-6, fibroblasts release keratinocyte growth factors 1 and 2, and these cytokines stimulate local keratinocyte migration, proliferation, and differentiation in the wound bed (George Broughton *et al.*, 2006). Studies have shown that collagen deposition, epithelialization, and angiogenesis are reduced in wounds lacking IL-6 (Lin *et al.*, 2003). The complex of the four stages of wound healing is important to consider, especially how these stages are affected by obesity.

2.4. Remodeling

During the maturation and remodeling phase, the wound reaches its maximum strength as it matures (Bowden *et al.*, 2016).

3. Process of Skin Wound Healing

The complex healing process of skin wounds involves various cellular and molecular processes. Wound healing is highly dependent on reactive oxygen species (ROS), and it is essential for the control of various processes such as inflammation, cell proliferation, angiogenesis, granulation, and extracellular matrix formation. Nevertheless, excess reactive oxygen species (ROS) caused by increased oxidative pressure can lead to delayed or failed wound healing. It is important to understand the function of reactive oxygen species (ROS) and develop biomaterials that efficiently scavenge ROS and improve the skin wound healing process. This study represents a thorough investigation of the role of reactive oxygen species (ROS) in the wound healing process and examines existing knowledge regarding biomaterials that were used for ROS removal. Additionally, this article describes various techniques and substances that can be used to treat skin wounds. The clinical application of improved biomaterials is also highlighted. In fact, it has the potential to bind to reactive oxygen species and promote skin repair. Its goal is to develop new strategies for the effective treatment of cutaneous wounds (Bedard and Krause, 2007; Roy *et al.*, 2006; Soneja *et al.*, 2005).

4. Difficult and Poor Wound Healing Formations

Chronic wounds can result in formations where one step of wound healing is impaired. The main characteristics of chronic wounds are a prolonged inflammatory phase, excessive neutrophil infiltration, persistent infection, and high MMP levels. In chronic wounds and ulcers, fibroblasts are phenotypically distinct and have reduced migration capacity compared to those in acute wounds (Brumberg *et al.*, 2021). Dysregulation of pro-inflammatory cytokines such as IL-1β and TNF prolongs the inflammatory phase and delays healing. It has been shown that IL-1β and TNF are increased in chronic wounds, leading to elevated MMP levels and excessive degradation of local ECM, impairing cell migration (Hesketh *et al.*, 2017; Krzyszczyk *et al.*, 2018). MMPs such as collagenase and gelatinase A and B have been demonstrated to be increased in body fluids of chronic wounds compared to acute wounds (Eming *et al.*, 2007). Additionally, insufficient angiogenesis may contribute to chronic wounds due to the lack of pro-angiogenic factors such as members of the VEGF family, caused by proteolysis and subsequent impairment of biological activity in the chronic wound microenvironment (Lauer *et al.*, 2000). Chronic wound development involves several cellular and signaling pathways and several molecular markers downstream of the Wnt signaling pathway. Activation of the β-catenin/c-myc pathway promotes the healing of injured tissues by inhibiting keratinocyte migration, altering their differentiation, and suppressing keratinization (Stojadinovic *et al.*, 2014; Stojadinovic *et al.*, 2005). In this way, EGF receptors are inhibited at the non-healing edges of keratinocytes (Brem *et al.*, 2007). Furthermore, in chronic wounds, TGFβ signaling is suppressed by downregulation of TGFβ receptors and attenuation of Smad signaling (Pastar *et al.*, 2010). Such cell signaling may serve as a target for rapid wound healing in chronic wounds.

5. Current Wound Healing Treatment Methods

- Dressings
- Negative Pressure Therapy
- Surgery
- Hyperbaric Oxygen Therapy

6. Wound Healing Dressing

A variety of cells and growth factors are involved in the wound healing process, and traditional dressings cannot control the release of drugs in response to the healing process. Intelligent wound dressings can selectively release some active ingredients in response to changes in temperature, light, magnetic fields, pH, enzyme levels, and ROS at the wound site. Additionally, the physical properties of the hydrogel's synthetic components and its 3D pore-like structure enable excellent drug loading capacity. Medicated hydrogels, compared with traditional wound dressings, can respond to the local environment by releasing active substances and can regulate different stages of wound healing. Hydrogel acts as a bio-dressing loaded with functional drugs in response to stimulation and can promote the drug release process (Qian *et al.*, 2020).

7. Hydrogels in Wound Healing

Hydrogels are a category of materials with widespread applications in the field of skin regeneration. These are polymeric structures that exist in three-dimensional forms and are formed by physical or chemical cross-linking of hydrophilic polymer chains (Koehler *et al.*, 2018). These can be synthesized using various techniques such as irradiation, freeze-thaw, and chemical processes. Hydrogels are called "reversible" or "physical" gels when their structural integrity is maintained by molecular entanglements or ionic compounds. Hydrogen bond gels are composed of covalent bonds and are called "permanent" or "chemical" gels. These networks can potentially undergo covalent water expansion (illustrated until reaching the 5 state). These networks can reach water expansion equilibrium while maintaining their original structure. They are retained until an equilibrium state is reached while maintaining the original structure. This provides a remarkable ability to absorb exudate from the wound, promote oxygen flow, and maintain an even moisture level in the injured area. So, you can speed up the healing process and increase the moisture levels in the injured area (Van Vlierberghe *et al.*, 2011). These special physical properties enable the fabrication of hydrogels of various sizes and shapes, facilitating complete coverage of irregularly shaped wounds (Bilici *et al.*, 2016). The hydrogel is biodegradable and biocompatible. It serves as a temporary template during the re-epithelialization and remodeling of chronic wounds. Additionally, the hydrogels exhibit sufficient bioadhesion, which is important to ensure sustained stability. This property improves hemostasis and maintains optimal water content within the wound (Arif *et al.*, 2021). Furthermore, hydrogels provide versatile scaffolds that improve their overall effectiveness in promoting wound healing by incorporating various components such as antibacterial and antimicrobial agents, drugs, and other complementary biomolecules. It can be concluded that hydrogel-based materials are most suitable as bandages to cover skin wounds.

8. Functional Hydrogels for Wound Healing

Traditional wound dressings, including gauze and bandages, are commonly used for dry, clean wounds (Brumberg *et al.*, 2021). Hydrogels, compared with traditional wound dressings, due to their porous structure, have great advantages like biodegradability, drug delivery ability, and sustained release ability (Kamoun *et al.*, 2017). Hydrogels can be used to improve wound healing by tuning their properties and developing hydrogels with characteristics that promote adhesive, anti-inflammatory/antioxidant, antibacterial, hemostatic, angiogenesis, and re-epithelialization. So, they can be tailored to the type of traumatic process (Qian *et al.*, 2020).

8.1. Different Functional Hydrogels for Wound Healing

- Adhesive hydrogels
- Anti-inflammatory/antioxidant hydrogels
- Antibacterial hydrogels
- Hemostatic hydrogels
- Pro-angiogenic hydrogels
- Pro-re-epithelialization hydrogels

8.2. Different Temperature-Responsive Hydrogels

- Photosensitive hydrogels
- Magnetically responsive hydrogels
- pH-responsive hydrogels
- ROS-responsive hydrogels
- Multiple responsiveness hydrogels

8.3. Natural and Synthetic Hydrogels

8.3.1. Natural Hydrogels

- Chitosan
- Gelatin
- Hyaluronic Acid
- Alginate

8.3.2. Synthetic Hydrogels

- Polyethylene Glycol (PEG)
- Polyvinyl Alcohol (PVA)
- Polyvinylpyrrolidone (PVP)

8.3.3. Advanced Hydrogels

- Sprayable Hydrogels
- "Smart" Hydrogels

8.4. Alternative Gels for Wound Healing

- Nanogels
- Aerogels
- Cryogels

8.5. Healing-Promoting Cargoes Carried by Hydrogels

Hydrogels play an important role in promoting wound healing because their porous structure and biocompatibility enable the transport of various biologically active cargoes such as cells and exosomes (Arif *et al.*, 2021; Bilici *et al.*, 2016; Koehler *et al.*, 2018; Van Vlierberghe *et al.*, 2011).

8.6. Some of the Healing-Promoting Cargoes Carried by Hydrogels Are

- Nucleic acids
- Cytokines
- Small-molecule drugs
- Stem cells
- Exosomes
- Nanomaterials

9. Impact of ROS on Skin Regeneration and Wound Healing

Wound healing is a natural and biological process that occurs when the skin tissue is damaged and injured. This process involves blood vessel narrowing and thrombus formation, followed by inflammation, cell proliferation, and ultimately wound reformation. In this process, oxygen is essential in almost every step of the wound healing process. This is because of the energy required for various processes involved in wound healing, including biosynthesis, intracellular transport, and cell movement. ATP synthesis, which serves as the main energy source, is optimized in the presence of oxygen. During wound healing, functions such as cell proliferation and extracellular matrix (ECM) formation increase, and as a result, higher energy requirements emerge. Consequently, the need for oxygen also increases to meet the elevated energy demands during wound healing (Tk, 1969). Oxygen plays an important role in the wound healing process, not only by providing energy but also by supporting many important

enzymatic reactions (Dissemond *et al.*, 2015; Lee *et al.*, 2022).

The potential sources of ROS are mitochondria, endoplasmic reticulum (ER), peroxisomes, various oxidases, and phospholipid metabolism. Additionally, the NADPH oxidase (NOX) family can generate ROS using specific enzymes (Bedard and Krause, 2007). Nitrous oxide enzymes reside on cell membranes and help electrons pass through biofilms. This process leads to the reduction of oxygen and the production of superoxide (O2?). In this pathway, superoxide can then undergo chemical reactions to form various reactive oxygen species, including hydrogen peroxide (H2O2), proxy radical (HO2?), and hydroxyl radical.

These ROS play various roles in cellular functions such as differentiation, proliferation, apoptosis, migration, and contraction (Roy *et al.*, 2006; Soneja *et al.*, 2005). The ROS group includes oxygen derivatives such as hydroxyl radical (OH), peroxide, superoxide anion, and hydrogen peroxide (H2O2). During the non-healing phase of a wound, excessive ROS production can cause cell damage, as ROS molecules oxidize adjacent molecules or cellular components. ROS are widely considered to be the main cause of cell damage during the aging process (Beckman and Ames, 1998). Studies have shown that moderate amounts of reactive oxygen species (ROS) help maintain intracellular balance, but increased ROS levels can negatively impact wound healing processes (Zhu *et al.*, 2019). In other words, as long as ROS levels remain constant, cell integrity and its functions are maintained.

However, ROS also have positive effects. Coordinated production of ROS by immune cells is important and effective for various parts of the body, such as host defense and cell signaling (Jones *et al.*, 2018; Koo *et al.*, 2019). During the homeostatic phase, ROS generated by NADPH oxidase in vascular cells can stimulate the expression of chemotactic and adhesion molecules, thereby reducing local blood flow (Hoffmann and Griffiths, 2018). During the inflammatory phase, neutrophils and macrophages produce superoxide and H2O2, which play important roles in killing bacteria and preventing wound infection. ROS also stimulate the release of tumor necrosis factor alpha (TNF-α) and platelet-derived growth factor (PDGF), supporting cell migration (He *et al.*, 2022; Hoffmann and Griffiths, 2018; Khorsandi *et al.*, 2022; Xu *et al.*, 2020).

During the proliferation phase, ROS/REDOX signaling is required to mediate the tissue growth factor-α1 (TGF-α1) signaling pathway and enhance the expression of fibroblast growth factor (FGF). Furthermore, ROS can stimulate processes such as angiogenesis by expressing vascular endothelial growth factor (VEGF), so endothelial cell division and migration can promote angiogenesis. Therefore, maintaining optimal levels of reactive oxygen species (ROS) is important to combat microorganisms and ensure the survival of cells. Increased ROS at the wound site can lead to the development of chronic inflammation. ROS overactivation impairs the function of transcription factors such as activator protein 1 (AP-1), mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-κB), and nuclear factor erythroid 2–related factor 2 (Nrf2) (An *et al.*, 2018; Asai *et al.*, 2018). Nrf2 plays an important role in protecting against increased internal oxidative stress and significantly contributes to wound healing (Koike *et al.*, 2020).

On the other hand, stimulation of NF-κB and AP-1 increases the amount of matrix metalloproteinases (MMPs) in dermal fibroblasts, causing degradation of the extracellular matrix (ECM) and slowing the wound healing process. ROS influence inflammatory responses in diabetic models through the NLR family pyrin domain–containing protein 3 (NLRP3), IL-1β pathway (Cheng *et al.*, 2012), and TNF-α (Lord *et al.*, 2017; Seiwerth *et al.*, 2018). Currently, there is a clear desire to restore ROS levels, improve the impaired physiological state around the injury, and promote the wound healing process. According to these data, considering the problems of excessive accumulation of reactive oxygen species (ROS) near wounds, we provide an overview of commonly used materials for ROS removal. These materials are designed to promote cell growth and migration, accelerating the wound healing process by reducing ROS levels within a certain range.

9.1. ROS-Scavenging Materials

- Carbon matrix materials
- Metal nanoparticles
- Small antioxidant molecules
- Antioxidant enzymes
- Natural materials

9.2. Application of ROS-Scavenging Materials in Wound Healing

- Using the main nanomaterials for the purpose of promoting the wound healing process
- Skin wound therapeutics with application of enzymes and peptides with antioxidant properties
- Use of free radical capture treatments for the wound healing process
- Using cells and cell products for the purpose of wound healing

10. Scaffold Properties for Wound-Healing Treatment

Scaffold design plays a central role in wound healing therapy. Generally, scaffolds can be made from syn-

thetic polymers in combination with extracellular matrix components, primarily collagens. These are associated with angiogenesis and revascularization, inflammation, and oxidative stress, and all these functions depend on functionalization with cells, microvascular fragments, or nanoparticles, and conjugation with additives like growth factors, antibacterial, anti-inflammatory, or antioxidant molecules. All of these can be involved in various applications, including antibacterial, antioxidant, and anti-inflammatory properties in microbial infections (La Monica *et al.*, 2024).

10.1. Scaffold Properties for Wound-Healing Treatment

- Promoting angiogenesis and revascularization
- Counteracting the inflammation phase
- Counteracting oxidative stress
- Counteracting microbial infections
- Scaffolding with antibacterial, antioxidant, and anti-inflammatory properties
- Promoting cell proliferation
- Stimulating ECM regeneration
- Particular case (La Monica *et al.*, 2024)

11. Hydrogels as Dressing for Burn Wounds

Hydrogels exhibit three-dimensional, hydrophilic, insoluble structures that are composed of cross-linked polymer chains and swell in aqueous solutions without collapsing (Yao *et al.*, 2021). Hydrogels can be synthesized from natural or synthetic polymers or a combination of both (Chong *et al.*, 2023). Polymer hydrogels have attracted considerable attention in wound dressing research because of their hydrophilicity, ability to mimic the extracellular matrix (ECM), biocompatibility, and biodegradability (Madaghiele *et al.*, 2014; Pan *et al.*, 2021). Hydrogels act as physical barriers, effectively protecting the wound bed from external contamination while creating an ideal moist environment to promote wound healing (Stoica *et al.*, 2020). Additionally, the inherent high-water content of hydrogels provides cooling and soothing effects, making them particularly suitable for burn dressings (Stoica *et al.*, 2020). Extensive references have discussed the polymer sources, cross-linking methods for hydrogel fabrication, and the different types of hydrogels used for wound healing purposes (Firlar *et al.*, 2022; Pan *et al.*, 2021; Yu *et al.*, 2021).

12. Effects of Hydrogels as Transdermal Drug for Burn Wound Healing

Burns that heal are often complicated by infection. Therefore, traditional hydrogel dressings based on the concept of moist wound healing are not sufficient to promote the wound healing process. Burn wound healing in the presence of infection requires administration of drugs and therapeutics such as antimicrobials, growth factors, bioactive compounds, and stem cells, which have shown promising results in promoting burn wound healing (Rowan *et al.*, 2015; Shu *et al.*, 2021; Stoica *et al.*, 2020; Yao *et al.*, 2021). Because burns can affect a variety of body surfaces (Markiewicz-Gospodarek *et al.*, 2022), transdermal drug delivery through the skin-the largest and most easily accessible organ of the human body-is preferred for local or systemic administration and absorption of various drugs and therapeutics in wound healing. Compared to parenteral routes involving needle-based injections, transdermal drug administration is a noninvasive and painless method that supports self-administration without specialist assistance, thereby improving patient compliance (Zaid Alkilani *et al.*, 2015). Furthermore, transdermal administration ensures local and long-acting drug distribution at the wound site through controlled release and permeation of drugs and therapeutics through the skin, while preventing systemic toxic complications and optimizing bioavailability by bypassing pre-systemic metabolism (Wang *et al.*, 2021).

Hydrogels have desirable properties and characteristics such as biocompatibility, biodegradability, non-toxicity, non-allergenicity, ease of application and removal, and high water content, making them suitable as carriers for transdermal drug delivery (Jacob *et al.*, 2021). The moisturizing effect of hydrogels on the skin improves the penetration of therapeutic agents into the skin, thereby facilitating transdermal drug delivery systems (Kim *et al.*, 2020). Furthermore, drugs and therapeutic agents can be incorporated into the porous and cross-linked polymer networks of hydrogels, allowing controlled release by adjusting the porosity, degree of cross-linking, and swelling behavior of the hydrogels (R Johnson and Wang, 2015). External environmental stimuli such as temperature and pH (Zhu and Chen, 2022) can trigger drug release by changing the chemical properties of hydrogels (Firlar *et al.*, 2022). This versatility expands the application of hydrogels as wound dressings (Saghazadeh *et al.*, 2018; Zhong *et al.*, 2020). Drugs and therapeutics incorporated into hydrogels can be classified as: (a) antibacterial agents (b) antioxidants and anti-inflammatory agents (c) analgesics (d) growth factors.

12.1. Hydrogels as Transdermal Drug Delivery Carriers for Burn Wound Healing

- Antimicrobial agents–incorporated hydrogels
- Antioxidant and anti-inflammatory agents–incorporated hydrogels
- Analgesic drugs–incorporated hydrogels
- Growth factors–incorporated hydrogels

13. Burn Wound Healing and Transdermal Drug Delivery

Hydrogels have shown their potential as transdermal drug delivery vehicles for burn wound healing due to their inherent high-water content, which acts as a natural penetration enhancer (Karande and Mitragotri, 2009). However, the unique characteristics of burn wounds pose challenges to the efficacy of transdermal drug delivery. The layer of hard necrotic tissue that forms over the wound bed is a local complication of burn wounds (Saghazadeh *et al.*, 2018). The presence of scar tissue impedes the effective penetration of drugs and therapeutic agents contained in hydrogels into deeper injured tissues (Cartotto, 2017; Souto *et al.*, 2020; Tiwari, 2012). Additionally, high levels of release in burn wounds may further impact the bioactivity and bioavailability of penetrating drugs and therapeutic agents (R Johnson and Wang, 2015; Whittam *et al.*, 2016; Yao *et al.*, 2021).

As a result, efforts are being made to overcome these barriers and improve the transdermal delivery of drugs and therapeutic agents through scar tissue to deeper wound areas, as well as their bioavailability, by using hydrogels as carriers. Various approaches to improvement have been investigated.

13.1. Strategies for Burn Wound Healing

13.1.1. Nanoparticles (NPs)

- Non-polymeric NPs
- Metal and metal oxide NPs
- Lipid-based NPs
- Polymeric NPs

14. Benefits of Nano-Emulsions in Wound Healing

The emergence of innovative therapeutics in the field of wound healing has highlighted the potential of nanotechnology-based drug delivery systems as a viable approach to promote healing mechanisms at various stages. Nano-emulsions are considered an excellent option due to their diverse properties, leading to a wide range of applications in wound treatment.

The main advantages of using nano-emulsions as drug delivery carriers in the treatment and management of wound healing include high drug loading capacity, improved drug solubility and bioavailability, and relative ease of preparation, scaling, and control. They enable controlled drug release and protection from enzymatic degradation. By lowering surface and interfacial tensions and thereby increasing overall viscosity and ductility of the system, nano-emulsions provide thermodynamic stability for the main system. Nano-emulgels offer several advantages over lipid nanoparticles, micro-emulsions, or liposomes for transdermal or dental delivery due to high drug loading capacity, improved diffusivity, permeability, and reduced skin irritation (Gorain *et al.*, 2022; Lo and Fauzi, 2021; McClements and Jafari, 2018). According to references, manufacturing methods of nano-emulsions for wound treatment are broadly divided into two categories: (a) high-energy approaches (b) low-energy approaches.

High-energy methods related to wound healing are further divided into:

- high-pressure homogenization
- ultrasound therapy
- Low-energy methods include:
- phase inversion
- spontaneous emulsification (Qadir *et al.*, 2016; Solans and Solé, 2012)

15. Conventional Methods in Wound Management

Standards of wound care include debridement, irrigation, infection control, and dressings (Margolis *et al.*, 2011). The goal of debridement is to remove nonviable tissue and expose healthy, well-perfused tissue using surgical or autolytic/enzymatic approaches (Stiehl, 2021). After debridement, the wound can be cleaned with saline or sterile water (Maemoto *et al.*, 2023). Detergents, hydrogen peroxide, and concentrated povidone-iodine solutions are not recommended due to tissue damage and their toxicity (Rai *et al.*, 2023). Treating infections is essential for the wound healing process. Failure to control infection results in poor healing and abscess formation. Both topical and systemic antibiotics are used on infected wounds. While topical agents are commonly used for superficial wound infections, systemic antibiotics are used in patients with deep or systemic infections (Powers *et al.*, 2016).

Different types of dressings have been developed to protect wounds from infection and promote the healing process. For example, dry gauze wound dressings have traditionally been used, but removing them can cause secondary damage. Moisture Retention Dressing (MRD) is a material with a water vapor permeability (MVTR) of less than 35 g/m^2/h, allowing wound healing in a moist environment. There are five basic types of MRD, including films, foams, hydrocolloids, alginates, and hydrogels. Some are sticky or absorbent; in other cases, a secondary dressing must be kept in place (Obagi *et al.*, 2019).

Skin substitutes include various biological, synthetic, or biosynthetic materials that can temporarily or permanently cover open skin wounds successfully. These alternatives are valuable in the treatment of both acute and chronic wounds and are useful for covering defects resulting from burns and other injuries, as well as for reconstructive purposes such as resolving extensive post-burn contractures. Split-thickness autografts are often used in chronic wounds. Efficiency is highly dependent on the quality and quantity of donor skin. Donor site pain and infection also limit this approach (Braza and Fahrenkopf, 2019).

The dermal matrix is the underlying tissue that supports the skin. The bioengineered skin matrix is a type of dressing that mimics normal skin structure and promotes wound healing in patients. It is classified into epidermis, dermis, and complete skin. According to the biological source, the substrate can be autologous (the host of the transplant), allogeneic (another human donor), or xenogeneic (another species).

There are several traditional approaches such as plant extracts and herbal medicines that have been used to promote wound healing. Aloe vera is often used in primary care. It has various biological and pharmacological activities, including antioxidant, anti-inflammatory, immunomodulatory, antibacterial, and skin-protective effects, all of which are important in the healing process (Hekmatpou *et al.*, 2019; R. Kumar *et al.*, 2019). One of the herbal formulae developed by the institute is NF3, an innovative herbal formula containing two herbs, astragalus and astragalus, in a 2:1 (w/w) ratio. This formula improved the healing of diabetic ulcers by promoting angiogenesis and inhibiting inflammation through in vitro, in vivo, and clinical studies (Ko *et al.*, 2014; Tam *et al.*, 2015; Tam *et al.*, 2014).

16. Modern Approaches in Wound Healing

In modern treatments, the use of stem cells is becoming a promising candidate. They are characterized by high self-renewal ability and the ability to differentiate into different lineages. In wound healing, the limitations of traditional approaches such as contamination and tissue stimulation can be overcome by promoting tissue regeneration.

16.1. Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) were first isolated from bone marrow in the 1970s as mesoderm-derived progenitor cells (Naji *et al.*, 2019). They are able to differentiate into mesenchymal lineages including osteoblasts, chondrocytes, myocytes, and adipocytes, but are unable to differentiate into hematopoietic stem cells. MSCs can be isolated from various tissues such as bone marrow, adipose tissue, peripheral blood, umbilical cord, Wharton's jelly, and dental pulp (P. Kumar *et al.*, 2019).

According to a report published by the International Society for Cell Therapy (ISCT), when cultured in medium containing 10 μL under standard conditions (37 °C, 5% CO2, atmospheric O2 concentration ∼20%) in a humidified incubator (with obtained bovine serum), minimum inclusion criteria to define MSCs include: (a) ability to adhere to plastic surfaces under standard culture conditions (b) presence of surface markers such as CD73, CD105, CD90, and absence of markers such as CD14, CD19, CD34, CD45 (c) ability to differentiate into osteoblasts, chondrocytes, and adipocytes in vitro under specific medium conditions (Dominici *et al.*, 2006) MSCs exhibit therapeutic effects through immunomodulatory, anti-inflammatory, pro-angiogenic, antioxidant, and anti-apoptotic activities (Mishra *et al.*, 2020). These effects are expressed through paracrine activity rather than direct cell differentiation. The secretome or conditioned medium (CM) is a complex product secreted by MSCs. It consists of soluble proteins (mainly growth factors, cytokines, and chemokines) and extracellular vesicles (EVs) in which proteins, lipids, and genetic material are encapsulated and transported. Although many skin substitutes are available on the market, stem cells are considered a better option for wound healing. They have been shown to promote healing of various types of wounds, including acute wounds, chronic wounds, and burns. Living cells are used that can actively interact with the wound environment and promote healing through the secretion of growth factors and other signaling molecules. This approach has proven effective in treating chronic wounds that do not respond to conventional treatments.

In addition, cell-free products such as growth factors, cytokines, and extracellular vesicles can be used to stimulate the body's natural healing mechanisms and functions without the need for living cells (Mazini *et al.*, 2020). In contrast, many commercially available skin substitutes are often made from synthetic materials that lack the biological complexity and versatility of living cells.

16.2. Advantages of MSC-Based Cell-Free Therapy

Although using cell-based products to promote wound healing is not new, there are still hurdles to overcome. Most commercially available cell-based skin graft substitutes such as Apligraf®, Recell®, PolyActive®, and OrCel® are expensive (Oualla-Bachiri *et al.*, 2020). Storage and transportation require special and strict conditions. In addition, potential risks such as tumorigenicity, rejection, and infection complicate frequent use. Delivering living cells to the wound site is also a challenge. Injecting cells through a needle damages cell membranes and viability (Wahlberg *et al.*, 2018). After injection, excess apoptotic or necrotic cells can trigger a local immune response and cause secondary damage. Therefore, different cell-free approaches are required. Numerous preclinical studies have demonstrated the therapeutic efficacy of MSC-derived cell-free products (MSC-CM). Various animal models have been used to study their effectiveness and potential. Compared to cell-based products, MSC-CMs can be more easily manufactured, packaged, stored, and transported. This allows for large-scale production. Cell-free products are treated as drugs, making quality control easier. Donor and recipient matching is not required, and immune rejection can be avoided. Furthermore, they can reduce the possibility of embolization, tumorigenicity, and infection transmission (Abbasi-Malati *et al.*, 2018). Therefore, unlike cell-based products, MSC-CM can be considered a ready-to-use pharmacological agent. Generally, isolated cell-free products can be stored for 6 to 7 months at temperatures between -20 °C and -80 °C (Torizal *et al.*, 2022).

16.3. Continuous Oxygen Therapy

In wounds, continuous delivery of 98–100% oxygen to the wound bed at a rate of 3–15 ml/h requires patients to wear a small wearable device attached to the wound 24 hours a day for a predetermined number of days. Of the 49 included studies, continuous oxygen therapy was examined to treat different wound types, with the most common being diabetic foot ulcers (DFU) (n=10), followed by venous leg ulcers (n=10), surgical wounds (n=1), and other wounds (n=1). Four studies used Natrox, four used TransCu O2, two used unspecified transdermal continuous oxygen therapy, one used Epiflo, one used micro-oxygenation devices, and in one case, oxygen-perfused negative pressure was used. Nine of the 13 studies were conducted as RCTs, while two RCTs (Niederauer *et al.*, 2015, 2017) were interim results of a larger RCT using TransCu O2 in DFU (Niederauer *et al.*, 2018). This review presents only the final RCT results to prevent double counting. After 12 weeks, improved wound healing was observed in DFUs. A significantly higher percentage of DFUs healed in the treatment group compared to placebo (32.4% vs. 16.7%, p=0.033). The time to 50 μU was significantly reduced in patients receiving CDO therapy (mean 18.4 days vs. 28.9 days, p=0.001) (Niederauer *et al.*, 2018). Yu *et al.* (2016) conducted an RCT in 20 patients and showed that the use of Natrox resulted in a 100% healing rate of grade II diabetic foot ulcers in the treatment group compared to 0% in the control group. A randomized clinical trial by Driver *et al.* (2013) using Epiflo in 17 patients with diabetic foot ulcers concluded a statistically significant difference in mean wound size reduction of 87% (55.7%–100%) in the treatment group compared to 46% (15%–99%) in the control group (p<0.05) (Driver *et al.*, 2017). Driver also conducted an RCT evaluating transcutaneous continuous oxygen therapy in 130 DFU patients. After 12 weeks, 54% of wounds in the treatment group were completely healed compared to 49% in the control group, although the results were not statistically significant (p=0.4167). A randomized controlled trial of 145 DFU patients using Natrox (Serena *et al.*, 2021) reported complete wound closure at 12 weeks in 44.4% of the TOT group compared to 28.1% in the standard treatment group (p=0.044). In a recent RCT by Al-Jalodi *et al.* (2022), 28.1% of patients in the SOC group healed in 12 weeks compared to 44.4% in the SOC plus TOT group (p=0.044). Furthermore, 85% of patients in the TOT group remained healed after 1 year compared to 60% in the control group. He and 24 colleagues conducted a clinical trial using a micro-oxygenation device in DFU. The mean wound healing time was significantly reduced when standard care was combined with continuous oxygen therapy (p<0.05), with a 10% reduction in scar length after 4 weeks (88.8% in the intervention group vs. 28.5% in the control group, p=0.049). In an RCT by Wang and Yu, the use of negative pressure showed statistically significant results in reducing wound site area and depth (all p<0.01), and granulation tissue increased in more hospitalized patients (p<0.01). A prospective comparative study in 27 cases showed that transcutaneous continuous oxygen therapy for venous leg ulcers promoted wound closure (p<0.001).

16.4. Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy was used in seven studies and administered at high pressures of 5 to 50 mbar. The most commonly used device was TWO2 (n=5), which delivered oxygen via a chamber. Two other studies used unspecified topical pressurized oxygen therapy. In an RCT by Frykberg *et al.*, 29 of 73 patients with DFU were treated using the TWO2 HyperBox. The closure rate at 12 weeks was 41.7% in the treatment group compared to 13.5% in the sham group (p=0.010). In an RCT by Azimian *et al.*, unspecified hyperbaric transcutaneous oxygen therapy was applied using humidified oxygen at high pressure (10 L/min) for 20

minutes, three times over 12 days. Treatment of 100 grade II–IV pressure ulcers resulted in complete closure in 16/50 ulcers in the treatment group compared to 1/50 in the control group ($p<0.001$). A prospective controlled study of DFU in 31 patients showed a statistically significant difference in complete epithelialization between treatment and control groups (82.4% vs. 45.5%, $p=0.04$). Two comparative TWO2 studies on venous leg ulcers demonstrated a statistically significant reduction in MRSA infection rates. In one study, 80% of ulcers healed completely in 12 weeks in the TWO2 group compared to 35% in the compression bandage group ($p<0.0001$). Pressure Wound Therapy (NPWT) using conventional NPWT and native NPWT with additional local barometric oxygen therapy showed that patients receiving native NPWT with topical oxygen achieved the highest average reduction in wound area and depth and formed more sterile cultures of infected wounds. A matched group study by Yellin *et al.* concluded that the control group (no TWO2) had a 9 times higher risk of hospitalization and 5 times higher risk of amputation than the TWO2 group.

16.5. Oxygen Dressings

Two studies used oxygen dressings: Oxyzyme and OxyBand. Oxyzyme is an oxygen-enriched hydrogel dressing containing glucose oxidase to generate hydrogen peroxide. Upon contact with the wound surface, hydrogen peroxide is converted to water and dissolved oxygen by serum catalase. OxyBand is a multi-layered dressing pre-loaded with pure oxygen that is continuously delivered to the wound bed. It acts as a reservoir that releases oxygen. Although the Oxyzyme/Iodozyme RCT by Moffatt *et al.* in 40 cases showed no statistically significant difference in 12-week cure rates compared to standard treatment (49.1% control vs. 44.7% treated), the frequency of dressing changes and average cost per patient were reduced in the treatment group. A prospective, randomized, controlled, open-label, single-center study by Lairet *et al.* in 17 patients with foot ulcers showed that OxyBand reduced the mean time to complete healing (9.3 ± 1.7 days vs. 12.4 ± 2.7 days in the Xeroform group, $p<0.001$). Pain scores on postoperative day 4 and day 12 were also significantly lower (both $p<0.05$).

16.6. Oxygen Transfer

Oxygen transfer refers to the delivery of oxygen gas to the wound surface by spray. Five studies investigated the effectiveness of hemoglobin sprays. Four evaluated Granulox (Infirst Healthcare), and one evaluated Granulox plus a 10% aqueous solution containing carbonylated hemoglobin. Each study examined hemoglobin spray on different wound types: foot ulcers, venous leg ulcers, scabies wounds, diabetic foot ulcers, and chronic wounds. Jonker *et al.*'s RCT in 42 cases investigated twice-weekly use of Granulox for foot ulcers but found no significant improvement after 12 weeks. The control group achieved a higher cure rate (8/14 vs. 4/15, $p=0.14$). The mean reduction in ulcer size was 100% in the control group compared to 48% in the treatment group ($p=0.21$). A retrospective cohort study of 45 cases showed that Granulox improved cure rates at 26 weeks (90% treated vs. 38% control, $p<0.001$). The median time to complete wound healing was 11.4 weeks in the treatment group compared to 6.6 weeks in the control group.

17. Wound Healing Techniques

17.1. High Energy Methods

High-energy approaches use mechanical force to provide large destructive energies. Droplet size depends on the equipment, preparation parameters such as temperature and time, and sample properties. High-energy methods are complex and energy-intensive. This makes them expensive and unsuitable for heat-sensitive products (Jasmina *et al.*, 2017). These methods fall into the categories of high-pressure homogenization and ultrasound. This technique is widely used to develop nanoemulsions using different forces, namely pressure. The system can generate particle sizes of approximately 1 nm, as well as intense turbulence, cavitation, and hydraulic shear. A high-pressure piston/homogenizer is used to force two liquids consisting of a surfactant and a co-surfactant into a small orifice at intense pressure (500–5000 psi), forming an interface between water and oil and transforming it. The extent to which the formed droplets are broken into smaller droplets depends on the force applied. The development of nanoemulsions by high-pressure homogenization methods is not impulsive in nature, as it requires force input in the form of chemical or mechanical energy. Furthermore, the preparation may become unstable if the system temperature increases during processing (Jaiswal *et al.*, 2015; Jasmina *et al.*, 2017). This technique has wide applications in wound treatment using herbal active ingredients such as betulin-enriched extract and oil palm leaf (Elaeis guineensis Jacq). Emulsification using this technique significantly reduces droplet size and can impact targeted drug delivery. Optimal drug loading and polydispersity index were also observed (Vater *et al.*, 2022; Zain *et al.*, 2021). Ultrasound nanoemulsions can be easily produced using ultrasound technology. Sound waves are used to move molecules and later break up oil droplets through local turbulence. Typically, a probe, metal horn, generator (which generates radio waves), and piezoelectric transducer are used to generate nanoemulsions via ultrasound (Zain *et al.*, 2021). In con-

trast to high-pressure homogenization methods, ultrasound requires less energy, is easy to use, and is cost-effective. Energy is delivered using ultrasound probes called sonotrodes. These contain a piezoelectric crystal that expands and contracts in response to AC energy. When the tip of an ultrasonic device contacts a liquid, mechanical vibrations and cavitation occur. Despite its power, this approach is still limited to laboratory research and pilot-scale production due to wave effects around molecules that can affect large-scale manufacturing (Che Marzuki *et al.*, 2019; Gharibzahedi and Jafari, 2018; Jaiswal *et al.*, 2015). Using this method, various synthetic nanoemulsions (e.g., clindamycin) and herbal ingredients (e.g., eugenol) were prepared and found effective for wound healing with optimal viscosity and high skin compatibility. Transmission electron microscopy analysis also showed that the formed nanoemulsion was spherical and had reduced droplet size (Abdellatif *et al.*, 2021; Ahmad *et al.*, 2018).

17.2. Low Energy Methods

Low-energy techniques exploit the properties of surfactant, oil, and water systems and do not require special equipment. Due to their low cost and ease of implementation, these techniques have led to increased research into nanoemulsions for development and broad applications. Low-energy emulsification techniques are energy-efficient as they exploit the chemical energy inherent in the system and produce nanoemulsions with only gentle stirring. These methods can be categorized into phase inversion and natural emulsification techniques. Phase inversion and phase penetration techniques create nanoemulsions on the surface by changing the spontaneous curvature of the surfactant. In this technology, the system temperature is changed using non-ionic surfactants. At high temperatures, oil-in-water (O/W) emulsions transform into water-in-oil (W/O) emulsions. Phase inversion emulsification requires a critical surfactant concentration. Increasing temperature can dehydrate the polymer chains of the surfactant and increase its lipophilicity. At low temperatures, a large positive spontaneous curvature is formed. A sufficient phase transition can be generated either by changing the composition at a constant temperature or by changing the temperature at a stable composition. Additionally, in some cases, generating droplet sizes in the micron range using phase inversion requires high energy input, which can be achieved using high shear agitation or an ultrasonic generator. This method is widely used in wound dressings to deliver active ingredients such as phenytoin and chlorhexidine. Formulations prepared using this technique were found to be stable and highly monodisperse. Enhanced drug entrapment efficiency was observed with no evidence of precipitation. Nanoemulsions produced using this method showed improved wound healing (Teo *et al.*, 2017). Natural emulsification, also called self-emulsification, requires no external energy input. Droplet formation occurs by contacting two immiscible liquids that are not in equilibrium. One of the greatest advantages of this technique is that nanoemulsions can be prepared without special equipment and developed effectively at room temperature. However, the presence of solvent and low formation of the oil phase are two major drawbacks of the self-emulsification technique. This method has been widely used to develop nanoemulsions formulated with herbal active ingredients for wound healing. After sonication for 30 minutes, a stable and transparent nanoemulsion was obtained. Droplet size analysis showed a large surface area, which improved absorption through skin texture. Quantitative analysis of nanoemulsions in the UV and visible wavelength range showed that absorption decreased with increasing sonication time (Bonferoni *et al.*, 2018; Shanmugapriya *et al.*, 2018; Sugumar *et al.*, 2014).

17.3. Advanced Therapies for Wound Healing in Preclinical and Clinical Studies

17.3.1. Advanced Wound Therapies in Preclinical Trials

In conditions such as acute wounds (e.g., surgical and traumatic wounds), dressings control bleeding, absorb exudate, and effectively close wounds to promote the healing process. Recent advances in wound dressings for acute wounds have focused on hemostasis, absorption of wound exudate, and firm wound closure for infection control. For example, a highly adhesive wound dressing composed of alginate and poly(N-isopropylacrylamide) contracted the wound in mouse splint models due to its thermoresponsiveness and high toughness, accelerating wound contraction. Recent attempts to combine adhesive hydrogels with surgical mesh-such as poly(N-isopropylacrylamide)/chitosan hydrogel and polyethylene terephthalate surgical mesh-demonstrated strong adhesion, flexibility, permeability, and strength. These are targeted for chronic wounds, where advanced dressings aim to address the disorganized inflammatory stage, replace skin tissue, and protect against infection. For diabetic wounds, recent focus has been on stimulating the healing process by inducing acute inflammation. Preemptive administration of a mast cell stabilizer and release of the neuropeptide substance P both resulted in severe post-injury inflammation, improved wound re-epithelialization, and accelerated wound healing in diabetic mice (McClements and Jafari, 2018; Whittam et al., 2016). Additionally, removing tissue-damaging pro-inflammatory factors improved tissue regeneration and healing in diabetic mice. In several diabetic mouse models, reactive oxygen species and de-

creased matrix metalloproteinase 9 (MMP9) activity-both continuously released by immune cells—promoted progression to the proliferative phase and enhanced wound healing. Sustained-release hydrogels of deferoxamine, an iron (II) scavenger that inhibits the conversion of hydrogen peroxide to highly toxic hydroxyl radicals, and hydrogels that release small molecules such as MMP9 inhibitors and MMP9-silencing RNA, improved re-epithelialization and promoted wound healing in diabetic mice (Gorain et al., 2022; Lo and Fauzi, 2021; Solans and Solé, 2012). A sustained-release formulation of PPCN hydrogel with stromal cell-derived factor-1 also promoted wound healing in diabetic mice. Dressings that remove pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 through electrostatic interactions demonstrated improved wound closure and reduced chronic inflammation in db/db mice. Hydrogels decorated with the heparin-binding domain of laminin, with or without encapsulated VEGF and PDGF, showed enhanced wound healing after topical application in db/db mice. Thermo-responsive hydrogels modified with conjugated laminin-derived peptide A5G81 promoted migration of keratinocytes and dermal fibroblasts, enhancing wound healing in splinted wounds in db/db mice. A peptide derivative of heat shock protein 90α applied to porcine burn wounds reduced cell apoptosis and inflammation using topical carboxymethyl cellulose hydrogels, resulting in re-epithelialization in this large animal model. Infections with potentially fatal consequences often occur in both acute and chronic wounds. Various anti-infective dressings have shown promising results in preclinical studies. Polymer hydrogels composed of poly (acrylic acid) and poly(acrylamide) loaded with antimicrobial silver/graphene particles exhibited high swelling rates and promoted wound healing in excised rat wounds. New hemostatic, absorbent, antimicrobial wound dressings based on novel mechanobiological strategies have shown promise in animal models of surgical wound healing. Two recently reported agarose and alginate hydrogel systems demonstrated high drug loading and sustained release of three antibiotics, along with good wound closure and positive effects on burn injuries in a pig model (Braza and Fahrenkopf, 2019; Obagi et al., 2019). A suspension of multilayered poly-L-lactic acid nanosheets has been reported to have high barrier function, adhering firmly to burn wounds without adhesive and suppressing Pseudomonas aeruginosa infection in mice for at least 80 days. For wound infections, several therapeutic conductive dressings have been developed to sense infection-related parameters such as pH and temperature and release antibiotics when necessary (R. Kumar et al., 2019; Tam et al., 2015; Tam et al., 2014). One hydrogel system, based on a carbon/poly-aniline working electrode, could measure wound pH and release cefazolin, promoting wound healing in an excision mouse model. Another system used electrical stimulation to provide predictive cues and improve wound healing in diabetic mice. Recently, various wound dressings have been developed that integrate electronics for electrical stimulation, wound monitoring (e.g., pH and temperature), and on-demand drug delivery (Dominici et al., 2006; P. Kumar et al., 2019; Naji et al., 2019). Additionally, several antimicrobial peptides have shown promise in preclinical wound models (Mazini et al., 2020; Mishra et al., 2020; Oualla-Bachiri et al., 2020). Antimicrobial peptide-releasing DNA hydrogels, whose retention mechanism relies on ionic interactions between negatively charged DNA and cationic antimicrobial peptides, reduced S. aureus burden in ex vivo porcine skin explants and promoted wound healing in mice.

17.3.2. Advanced Wound Therapies in the Clinical Pipeline

Several advanced wound treatments are currently in clinical trials. A search of clinical trial databases was conducted to identify the most frequently studied interventions targeting wound management, anti-infectives, and biologics. This section summarizes ongoing clinical trials across various wound types. Advanced anti-scarring and pro-healing therapies for surgical wounds are in development. OLX101 is a cell-permeable asymmetric interfering RNA that targets connective tissue growth factor (CTGF) to counteract hypertrophic scarring (OliX Therapeutics). Unlike liposomal or nanoparticle delivery systems, OLX101 is a small interfering RNA that enters cells spontaneously without complex delivery mechanisms. It is currently being developed as an intradermal injectable for hypertrophic and keloid scars. New peptide formulations are also under investigation to promote wound closure and reduce scarring. One example is SLI-F06 (Scarless Laboratories), a fibro-modulin (FMOD)-based amino acid peptide sequence that stimulates fibroblast and endothelial cell migration, myofibroblast differentiation, and contraction. In preclinical studies, intradermal administration of FMOD in a pig wound model reduced scar size, increased tensile strength, and improved dermal collagen organization.

18. Additional Peptides Currently in Development

Additional peptides in development include a Connexin43 (Cx43) mimetic peptide (Granexin). Cx43 is commonly found in the epidermis and dermis, and chronic wound studies have shown its presence at wound edges. Both Cx43 and peptidomimetics of its carboxyl terminus have improved wound closure rates and reduced scar formation. Granexin is being studied in venous leg ulcers, diabetic foot ulcers, and surgical

wounds in phase 1 and phase 2 trials. For soft tissue defects and refractory wounds, several stem cell, exosome, and peptide therapies are in clinical trials. ADSC-SVF-002 (AdiSave) is an autologous fat-derived stem cell therapy injected subcutaneously into soft tissue defects and abnormally healing wounds, with or without unprocessed autologous fat. A single-arm, open-label, single-center safety study is ongoing to evaluate its safety in subjects with soft tissue defects. Unlike cell-based therapies, platelet-derived exosomes are being tested for advanced wound healing (Plexoval, ExoPharm Limited). Autologous exosomes are delivered by local injection over six weeks to study safety, wound closure, and scarring. Advances in surgical wound healing without biologics are also being investigated. Portable NPWT devices such as PICO (Smith and Nephew) achieved non-inferiority in a phase 4 multicenter randomized controlled trial, showing superior wound healing progression over a one-week treatment period. An ongoing RCT is evaluating infection incidence over 1–3 months for surgical site infection prevention after cardiac surgery using extracorporeal circulation, compared with disposable hydrocolloid dressings. Hyperthermia or non-contact normothermia (38°C dressings) have shown promising results in preliminary studies and clinical trials for pressure ulcers and venous stasis ulcers (Driver et al., 2013; Yu et al., 2016). It was hypothesized that warming agents applied to semipermeable dressings (Active Wound Therapy) would increase blood flow and immunogenicity, enhancing healing (Driver et al., 2017; Serena et al., 2021). However, recent studies using similar technologies such as infrared therapy have shown mixed results (Al-Jalodi et al., 2022; Driver et al., 2013), and modern clinical implementation remains limited.

Several diabetic foot ulcer treatments are under investigation. An FDA-approved eye gel containing the beta-adrenergic antagonist timolol is being tested in a phase 3 trial, as beta-blockers have shown angiogenesis and tissue repair benefits in vitro and in vivo. Topical bacteriophage dispersion is in phase 1/2a trials, aiming to reduce infections by targeting Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii. A topical dispersion of genetically engineered Lactococcus lactis bacteria (AuP1602-C) is also in phase 1/2a trials. These bacteria express three proteins: fibroblast growth factor 2, interleukin 4, and macrophage colony-stimulating factor 1. As an alternative to surgical debridement, a protease-containing wound solution is being investigated for outpatient use in a phase 2 study. In an RCT, topical application of ON101 cream-containing two plant extracts that polarize macrophages to the M2 phenotype-was associated with absorbable wound dressing and showed improved healing after 16 weeks. Various biologic dressings based on autografts, allografts, phages, bacteria, and drug-releasing systems for diabetic foot ulcers are under clinical investigation. Next-generation debridement therapy for burns is in clinical trials. NexoBrid (KMW-1) is a topical drug composed of proteolytic enzymes isolated from pineapple stem (Bromelain). These proteins include at least four cysteine proteinases that hydrolyze and solubilize heat-denatured proteins in scabs (Vater et al., 2022; Zain et al., 2021). NexoBrid allows selective and rapid removal of dead or damaged tissue within 4 hours. In a phase 3 trial, 89% of patients had complete scab removal without serious adverse events. Several cell-based therapies are under investigation to improve burn wound healing. StrataGraft (Mallinckrodt) is a bi-layer cellularized scaffold containing keratinocytes and dermal fibroblasts, applied topically to recruit endogenous skin cells. Other technologies include Epicel (Genzyme Corp) and Engineering Skin Substitute (ESS) (Amarantus Bioscience Holdings), which are tissue-engineered skin constructs made from the patient's epithelial cells and collagen-containing fibroblasts. In preclinical studies, ESS created a functional skin barrier. A completed clinical trial evaluated its use in treating severe burns (up to 95% body surface area) in pediatric patients. A phase 2 study is underway to compare ESS with autografted mesh gap skin for treating life-threatening burns. Finally, SkinMed (BioDan) is based on autologous fibroblasts and keratinocytes obtained from a single biopsy and implanted into clotted human plasma as a 3D skin scaffold.

19. Conclusion

The main research focus is on skin wound substitutes to replace invasive autografts. This approach offers a new option for severe burns or other deep injuries where autografts and allografts are currently the standard of care treatments. Recently, three-dimensional (3D) bioprinting has received significant attention in this field by combining scanning and printing approaches to create a personalized system that allows complete coverage of wounds in three dimensions for skin replacement. A portable 3D scanning and bioprinting system is capable of printing autologous fibroblasts (dermis) and keratinocyte cell layers (epidermis) from collagen and thrombin cross-linked fibrinogen. It is designed for successful vascularization and re-epithelialization and demonstrated improvement in mouse wound models (Albanna *et al.*, 2019). Bioprinted gelatin–alginate hydrogel containing mesenchymal stem cells and serving as an angiogenic nitric oxide source promoted re-epithelialization and wound closure in murine burn wounds (Wu *et al.*, 2021). The large mesh size of the gel used as bio-ink can cause burst release of the drug, so the hydrogel was cross-linked during the printing process to sustain drug release. A 3D-printed photo-crosslinked hydrogel composed of

chitosan–methacrylate, the antibiotic levofloxacin, and the analgesic lidocaine demonstrated sustained drug release and accelerated wound closure over 3 days in rat burn wounds (Teoh *et al.*, 2022).

Overall, experimental dressings for acute and chronic wounds with immunomodulatory, anti-infective, skin replacement, and sealing properties have shown promise in animal models of wound healing. These proof-of-concept studies illustrate the growth of the preclinical pipeline and highlight the potential for studying key properties in the pathophysiology and clinical pathology of acute and chronic wounds. According to all these data, advanced modern activities for wound healing are important because they can accelerate the healing process and prevent wound progression. This article can serve as a useful reference for recent studies on advanced modern treatments for wound healing in various conditions.

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