Traditional Therapies for Skin Wound Healing

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Significance: The regeneration of healthy and functional skin remains a huge challenge due to its multilayer structure and the presence of different cell types within the extracellular matrix in an organized way. Despite recent advances in wound care products, traditional therapies based on natural origin compounds, such as plant extracts, honey, and larvae, are interesting alternatives. These therapies offer new possibilities for the treatment of skin diseases, enhancing the access to the healthcare, and allowing overcoming some limitations associated to the modern products and therapies, such as the high costs, the long manufacturing times, and the increase in the bacterial resistance. This article gives a general overview about the recent advances in traditional therapies for skin wound healing, focusing on the therapeutic activity, action mechanisms, and clinical trials of the most commonly used natural compounds. New insights in the combination of traditional products with modern treatments and future challenges in the field are also highlighted.

Recent Advances: Natural compounds have been used in skin wound care for many years due to their therapeutic activities, including anti-inflammatory, antimicrobial, and cell-stimulating properties. The clinical efficacy of these compounds has been investigated through *in vitro* and *in vivo* trials using both animal models and humans. Besides the important progress regarding the development of novel extraction methods, purification procedures, quality control assessment, and treatment protocols, the exact mechanisms of action, side effects, and safety of these compounds need further research.

Critical Issues: The repair of skin lesions is one of the most complex biological processes in humans, occurring throughout an orchestrated cascade of overlapping biochemical and cellular events. To stimulate the regeneration process and prevent the wound to fail the healing, traditional therapies and natural products have been used with promising results. Although these products are in general less expensive than the modern treatments, they can be sensitive to the geographic location and season, and exhibit batch-to-batch variation, which can lead to unexpected allergic reactions, side effects, and contradictory clinical results.

Future Directions: The scientific evidence for the use of traditional therapies in wound healing indicates beneficial effects in the treatment of different lesions. However, specific challenges remain unsolved. To extend the efficacy and the usage of natural substances in wound care, multidisciplinary efforts are necessary to prove the safety of these products, investigate their side effects, and develop standard controlled trials. The development of good manufacturing practices and regulatory legislation also assume a pivotal role in order to improve the use of traditional therapies by the clinicians and to promote their integration into the national health system. Current trends move to the development of innovative wound care treatments, combining the use of traditional healing agents and modern products/practices, such as nanofibers containing silver nanoparticles, *Aloe vera* loaded into alginate hydrogels, propolis into dressing films, and hydrogel sheets containing honey.

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SCOPE AND SIGNIFICANCE

SKIN IS A MULTILAYER ORGAN that acts as an interface between the internal organs and the external environment, forming a barrier that prevents the body dehydration and the penetration of external microorganisms.[1] As the skin is permanently exposed to the external atmosphere, it is extremely vulnerable to the appearance of different types of lesions, such as burns, ulcers, and wounds. At the moment of the injury, the human body initiates a complex cascade of biological processes toward the repair and regeneration of the damaged or lost tissue. These processes rely on the interaction between several mediators like extracellular matrix (ECM) molecules, platelets, inflammatory cells, growth factors, cytokines, and chemokines, occurring in a synchronized and integrated manner throughout different phases of hemostasis, inflammation, migration, proliferation, and tissue remodeling.[1,2] To stimulate the healing process, reduce the scar formation, and improve the properties of the new skin, several wound care products and therapies have been developed.[3–16] Wound-healing therapies can be broadly classified into traditional and modern therapies, which have distinct levels of efficacy, clinical acceptance, and side effects. Traditional therapies have been used for many centuries mainly by the rural populations in developing countries. Usually, these therapies involve the use of herbal- and animal-derived compounds, living organisms, silver and traditional dressings.[17,18] On the other hand, modern therapies comprise the use of grafts, modern dressings, bioengineered skin substitutes, and cell/growth factor therapies.[19–22] The concept of *in situ* biomanufacturing is also under investigation for skin regeneration.[1] In general, modern therapies are more expensive than traditional ones, being readily available in the most developed countries.

TRANSLATIONAL RELEVANCE

The increasing interest on the use of traditional therapies for skin wound care has led to a significant increase in the number of scientific research works that investigate the clinical efficacy, safety, and side effects of these therapies. These works allowed the development of novel products and clinical practices that are currently used by the clinicians and surgeons in the treatment of different types of skin injuries. Despite these advances, further efforts are needed toward the approval of traditional therapies and natural healing compounds for clinical use, in order to allow their introduction into the national healthcare systems.

CLINICAL RELEVANCE

Traditional healing agents assume a central role in wound care due to their clinical efficacy, simplicity, and affordability. These therapies represent a cost-effective alternative for the treatment of diverse difficult-healing wounds (*e.g.*, ulcers, burns, and infected wounds) by providing a wide range of therapeutic effects that stimulate the healing process and improve the quality of the new skin. Traditional therapies can also be combined with modern clinical practices, biomaterials, and drugs, allowing the development of innovative therapeutic treatments that address important medical needs, such as minimize the bacterial resistance and reduce the healing time.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Overview of the wound-healing process

Wound healing is a complex process that occurs in almost all tissues after damage, aiming at repairing a lost or injured tissue. The first phase of the healing process, the hemostasis, starts immediately after injury and aims to control the bleeding and to limit the spread of microorganisms within the body. Hemostasis involves several events, such as vascular constriction, platelet aggregation, and fibrin clot formation, with subsequent development of a scab that provides strength, protection, and support to the damaged tissue.[21–23] During this process, platelets release several growth factors, including the transforming growth factor-β (TGF-β), epidermal growth factor (EGF), insulin-like growth factor-1, and platelet-derived growth factor (PDGF), which are responsible for the activation of fibroblasts, endothelial cells, and macrophages in the surrounding environment.[20,24] The inflammatory phase, occurring simultaneously with the hemostasis, is characterized by the release of several proinflammatory cytokines, cationic peptides, proteases, reactive oxygen species, and growth factors, allowing the wound cleaning.[2,20] Growth factors like TGF-β, PDGF, fibroblast growth factor, and EGF play an important role in the communication between cells and their ECM, stimulating cell recruitment, proliferation, morphogenesis, and differentiation.[23,24] After bleeding, the healing process involves the migration and infiltration of inflammatory cells into the wound. At this phase, neutrophils, macrophages, and lymphocytes are responsible for multiple functions, including the promotion of the inflammatory response, inhibition of the penetration of exogenous microorganisms, elimination of microbes, and stimulation of

keratinocytes, fibroblasts, and angiogenesis.[23] Once the bleeding and inflammation are controlled, epithelial cells and fibroblasts migrate to the damaged region, supporting capillary growth, collagen synthesis, and new tissue formation. At this stage, epithelial cells replace dead cells, while fibroblasts are responsible for the production of collagen, fibronectin, hyaluronan, glycosaminoglycans, and proteoglycans, which are the major constituents of the ECM and confer strength to the skin.[2,21,24] A granulation tissue is produced as a result of the growth of capillaries and lymphatic vessels from existing vessels present at the site of injury (neovascularization). Finally, in the maturation or remodeling phase, the new tissue is continuously remodeled until its composition and properties are close to those of the healthy tissue.[23] The ultimate goal of the wound-healing process is the regeneration of the injured skin without scar formation.

Traditional therapies for wound healing

Although the human skin has a natural ability to promote the self-regeneration after damage, this capacity can be compromised under specific conditions, like extensive skin loss, deep burns, chronic wounds, nonhealing ulcers, and diabetes.[20,23] An inappropriate healing process can lead the wound to enter in a chronic state, which increases the risk of infection and affects the patient health and his/her quality of life. Chronic wounds, such as venous ulcers and ischemic wounds, are characterized by the disruption of the normal regeneration process, usually as a result of bacterial colonization, vascular insufficiency, and diabetes, leading to a complicated and delayed healing process.[24,25] Such wounds represent one of the most debilitating, painful, and costly skin conditions, being a critical medical and social problem for both patients and countries. Chronic wounds may also require longer hospitalization times and/or the employment of sophisticated and expensive wound care products (*e.g.*, cellular tissue-engineered skin substitutes and medicated dressings), increasing medical costs. Although several clinical practices have been tested in order to prevent delayed healing and improve the healing process, the treatment options for chronic wounds are still very limited. To address this need, significant efforts have been performed in the research into traditional therapies as alternative clinical treatments for the treatment of these wounds.

Practices and compounds that arise from traditional medicine have been used to create the optimal conditions for the skin regeneration process and to prevent the failure of the healing process, due to their therapeutic activities, availability, affordability, and relative low cost.[26] According to the World Health Organization (WHO), traditional medicine, also referred as "alternative" or "complementary" medicine, underlines on the use of traditional therapies toward the maintenance of health and the prevention, diagnosis, improvement, or treatment of physical and mental illnesses.[26,27] These therapies comprise practices, products, and knowledge from different countries, involving the use of living organisms and natural compounds obtained from a wide range of sources (*e.g.*, animals, plants, fungi, and minerals). Silver-based products and traditional dressings have also been employed in wound care and are commonly used in most public healthcare systems.

Traditional medicine is a common practice in different regions of the world, such as Africa, Asia, and Latin America, contributing to increase the access of population to the healthcare. It is estimated that up 80% of the Asian and African population use traditional medicine therapies for primary healthcare, whereas in China these therapies represent 40% of all healthcare.[26] The use of traditional medicine is also increasing in the most developed countries, being estimated that at least 70% of population in Canada, 42% in United States, 38% in Belgium, and 75% in France use these medicines.[26] Recent data also indicate that in Australia 69% of the total population use traditional medicine, while in New Zealand and Singapore it reaches 30% and 53%, respectively.[27]

Recent developments on novel extraction procedures, purification methods, processing methodologies, and clinical treatments allowed a significant increase in the quality, efficacy, and safety of traditional therapies. However, the use of some therapies is largely supported by wisdom and experience acquired over years, rather than by strong scientific evidence. Nevertheless, in the last few years, several laboratories focused their research activities on the mechanisms behind the therapeutic efficacy of traditional healing compounds, increasing the knowledge about their action mechanisms and biological activities. In the next sections, the most commonly used traditional therapies for skin wound healing are described and the scientific evidence of their use is discussed. According to the origin, these therapies are classified into herbal-derived compounds, animal-derived compounds, living organisms, and silver and traditional dressings (Fig. 1).

Herbal-derived compounds

Herbal-derived compounds are the most commonly used traditional therapies for the treatment

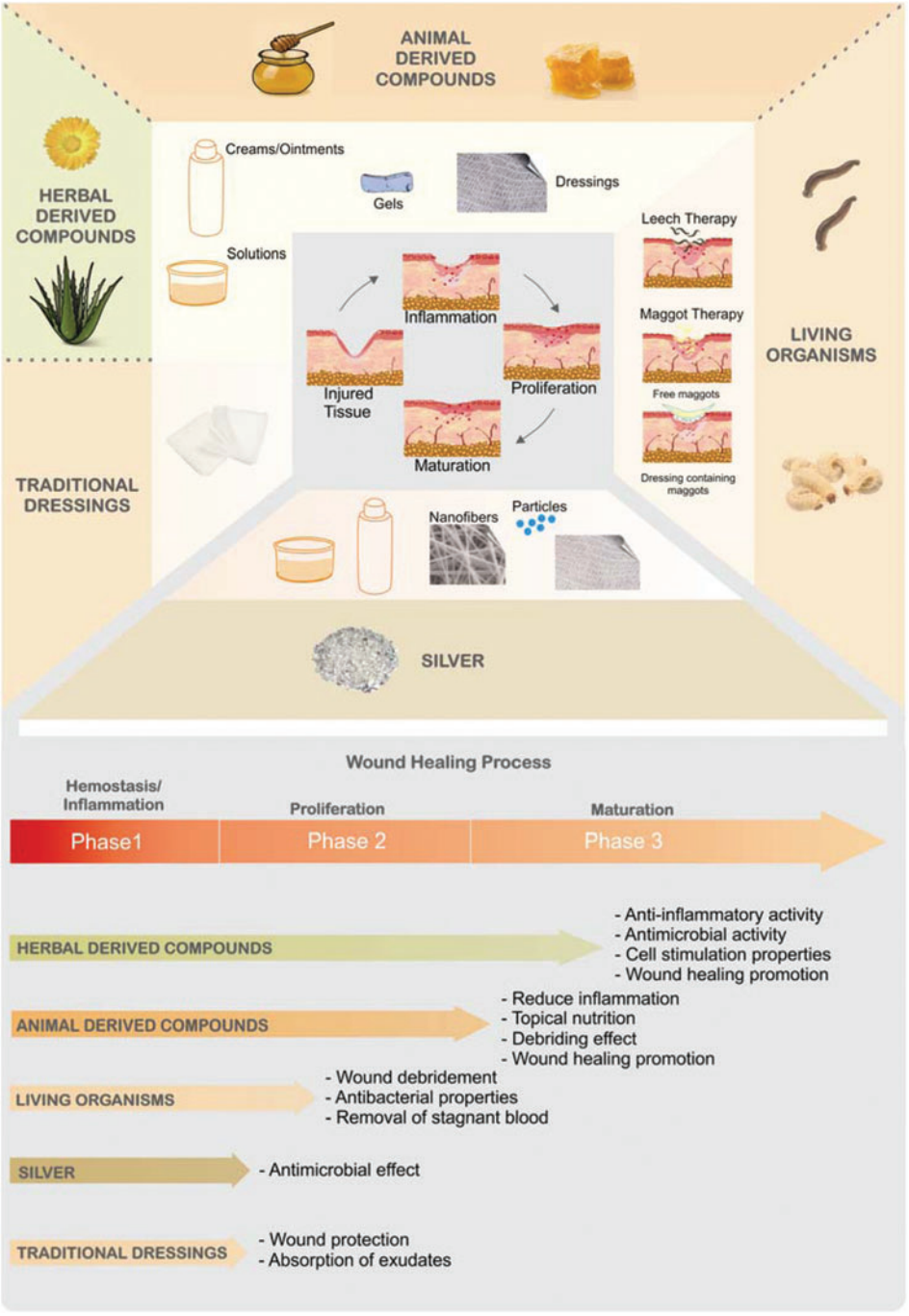

Figure 1. Classification of traditional therapies for skin wound healing. Traditional therapies and compounds are used in different phases of the healing process in a great variety of physical forms, either commercially available or under investigation, stimulating the skin regeneration process. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

of skin lesions. They include the application of herbs, herbal preparations, and finished herbal products, containing biologically active compounds that stimulate the healing process. Today, a great variety of plants, native from different regions of the world, are investigated and used for the treatment of skin lesions.[17,28,29] Herbal-based products are applied as extracts, emulsions, creams, and ointments, being commonly administrated through topical, systemic, and oral routes. Table 1 presents an overview of some plants under investigation for wound-healing applications.[4–6,30–44]

Aloe vera. *Aloe vera* (AV), also known as *Aloe barbadensis* Miller, is the most popular herb in wound healing. AV is a cactus-like plant that be-

Table 1. *Examples of some plants currently investigated for wound-healing applications*

Herb	*Main Constituents*	*Physical Forms and Administration Routes*	*Laboratorial and Clinical Evidence*	*References*
Aloe vera	Soluble sugars, nonstarch polysaccharides, lignin, polysaccharides, glycoproteins, and antiseptic agents	Forms: solutions, creams, mucilage, gels, and dressings Routes: topical and oral	Anti-inflammatory and antimicrobial activities; stimulate cell proliferation, collagen synthesis and angiogenesis; promote wound contraction	4,5,30–32
Hippophae rhamnoides (sea buckthorn)	Flavonoids (*e.g.*, quercetin, isorhamnetin), carotenoids (*e.g.*, α-, β-carotene, lycopene), vitamins (C, E, K), tannins, organic acids, triterpenes, glycerides of palmitic, stearic, oleic acids and, amino acids	Forms: aqueous leaf extract, seed oil Routes: topical and oral	Antioxidant and anti-inflammatory activities; stimulate the healing process; improve wound contraction and epithelialization; increase the hydroxyproline and protein content in the wound	33,34
Angelica sinensis	Essential oils and water-soluble ingredients; ferulic acid is the main active constituent	Forms: ethanol extracts, ferulic acid dissolved in DMSO Routes: n.a. (*in vitro* tests)	Stimulate the proliferation of human skin fibroblasts, the secretion of collagen, and the expression of TGF-β in *in vitro* conditions	35
Catharanthus roseus (*Vinca rosea*)	Contain two major classes of active compounds: alkaloids (*e.g.*, vincamine) and tannins	Forms: leaf ethanol extract Routes: topical	Antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*; increase wound strength, epithelialization, and wound contraction	36
Calendula officinalis (marigold)	Triterpenoids and flavonoids	Forms: gels, aqueous extracts, hexane, and ethanolic extracts dissolved in DMSO Routes: topical	Anti-inflammatory and antibacterial activities; stimulate the proliferation and migration of fibroblasts *in vitro*; stimulate the collagen production and angiogenesis	6,37,38
Sesamum indicum	SM is the main antioxidant constituent, others include sesamolin and sesaminol	Forms: SM (purity > 98%) and SM containing dexamethasone Routes: intraperitoneal and intramuscular routes	Improve the wound tensile strength, wound contraction, and the hydroxyproline levels in both normal and delayed wound models in rats	39
Morinda citrifolia (noni)	Acids, alcohols, phenols, esters, anthraquinones, sterols, flavonoids, triterpenoids, saccharides, carotenoids, esters, ketones, lactones, lignans, and nucleosides	Forms: ethanol extract of plant leaves mixed with water Routes: oral	Improve the hydroxyproline content and reduce both the wound area and the epithelialization time in excision wounds in rats	40,41
Camellia sinensis	Polyphenols, flavonoids, tannins, caffeine, and amino acids	Forms: pure vaseline and ethanolic plant extract (0.6%) ointment Routes: topical	Reduce the healing time and the wound length of incision wounds created in Wistar rats	42,43
Rosmarinus officinalis L. (rosemary)	Most bioactive constituents include terpenoids and polyphenols, such as carnosol, carnosic acid, and rosmarinic acid	Forms: aqueous extract and essential oil Routes: topical and intraperitoneal injection	Reduce the inflammation and improve the wound contraction, re-epithelialization, angiogenesis, and collagen deposition on full-thickness wounds in diabetic mice	44

DMSO, dimethyl sulfoxide; SM, sesamol; TGF-β, transforming growth factor-β; n.a., not applicable.

longs to the Liliaceae Family, growing in tropical climates.[45] From the processing of fresh plant leafs, two main products are obtained: (1) a bitter yellow juice, usually known as "*Aloe vera* latex or aloe juice," and (2) a clear mucilaginous gel obtained from the parenchymal tissue, commonly referred as "*Aloe vera* gel or mucilage."[45–47] Aloe juice was approved by the U.S. Food and Drug Administration as a laxative and cathartic agent.[48] AV gel is the most valuable product for the treatment of skin lesions, being composed of a water fraction (99–99.5%) and a solid fraction (0.5–1.0%) containing several biologically active compounds, such as soluble sugars, nonstarch polysaccharides, lignin, lipids, vitamins (B_1, B_2, B_6, and C), enzymes (acid phosphatase, alkaline phosphatase, amylase, and lipase), salicylic acids, proteins, and minerals (sodium, calcium, magnesium, and potassium).[45,47] Several therapeutic activities have been attributed to the AV gel, including anti-inflammatory, antiseptic, and antimicrobial properties. The AV gel also retains the ability to stimulate the fibroblast proliferation, collagen synthesis, and angiogenesis.[30,49,50] Although these properties are mainly due to the synergy established between the plant constituents,[45,47] several authors claim that the

biological activity of polysaccharides (*e.g.*, acemannan, mannose-6-phosphate, pectic acid, galactan, and glucomannan) and glycoproteins (*e.g.*, lectins), present in the leaf pulp, play a major role in the wound-healing process, being responsible for specific properties like anti-inflammatory, antifungal, or cell stimulation.[51,52] The cell-stimulating properties of AV are related to the composition of polysaccharides and the binding ability of mannose to some receptors present in the surface of fibroblasts.[45,48] *In vitro* studies have also showed the anti-inflammatory activity of AV, as well as its ability to stimulate the gap junctional intercellular communication and the proliferation of human type II diabetic skin fibroblast cells.[50,53] AV is commonly applied in skin lesions as oral solutions,[30] topical preparations,[48] creams,[31] mucilage,[5] gels,[32] and dressings.[4]

In vivo trials, using animal models and humans, confirm the positive effects of AV in the wound-healing process by increasing the synthesis and the degree of collagen crosslinking, growth factor expression, proliferation of fibroblasts, blood vessel formation, and wound contraction.[5,30–32,54–56] A randomized controlled clinical trial that investigates the effects of AV gel, thyroid hormone cream, and silver sulfadiazine (SSD) cream on the healing process of sutured incision wounds in rats showed that AV gel significantly increases the fibroblast proliferation, angiogenesis, re-epithelialization, and wound closure. These effects can be due to the improved infiltration of AV within the skin tissue, which stimulates the biological activities involved in the healing throughout the repair process.[5] Khorasani *et al.*[56] conducted a randomized clinical trial to investigate the efficacy of AV cream (0.5% of AV gel powder) in second-degree burn wounds. The study involved 30 patients with similar burn wounds at two different sites in the body (hands or feet). One wound was treated with AV, while the other one was topically treated with SSD for comparison. The patients treated with AV exhibited both significantly faster re-epithelialization rate and shorter mean healing times (15.9 days vs. 18.73 days for SSD). The burn wounds treated with AV also required less time to heal (16 days vs. 19 days) with no evidence of microbial contamination during the healing process.

AV gel has also been combined with natural polymers to produce blend films for wound-healing applications. Our group is developing thin hydrogel films composed of calcium alginate and AV gel (5%, 15%, and 25%) for applications in both exuding and dry wounds.[57] The main goal of this research work is to combine the occlusive and hemostatic properties of calcium alginate gels with the healing properties of AV gel in the form of biocompatible and biodegradable thin films. These films create the optimal conditions for an improved healing process, and simultaneously release the AV compounds directly to the wound site, according to specific release profiles. Experimental results showed that AV has a great influence on the film properties, significantly improving the transparency, hydrophilicity, water absorption, and *in vitro* degradation rate.[58–60] In another work, Inpanya *et al.*[4] developed blended films based on fibroin and AV gel extract for wound-healing applications. The authors showed that the films enhance the *in vitro* attachment and proliferation of skin fibroblasts, while the *in vivo* application of the films in diabetic rat wounds accelerated the healing process (Fig. 2) and promoted the collagen synthesis and organization.

Although the use of both topical and oral AV preparations is considered safe without serious side effects, like toxicity and mortality,[31,61] some adverse reactions have been experienced by the patients. Topical preparations are commonly associated to skin itching, irritation, contact dermatitis, erythema, and photodermatitis, while oral administration can lead to diarrhea and vomiting.[46,47,62] The existing clinical evidence about the therapeutic activities of AV demonstrates its ability to stimulate the healing process. However, a significant number of the available research works are based on poor methodologies involving a small number of studies with few patients. Thus, there is a need for high-level evidence and further large, randomized control trials to support the use of AV-derived products as topical agents or incorporated within dressings for the treatment of skin lesions. The physicochemical properties of AV are highly dependent on the species, climate, region, growing conditions, processing, and storage methods, which can result in significant changes in terms of both chemical constituents and therapeutic properties. To avoid this variability, it is necessary to improve the standardization and the quality control assessment of AV products.

Calendula officinalis. *Calendula officinalis* also known as marigold, is an herb native from the Mediterranean that has been used for skin applications, mainly as wound-healing and anti-inflammatory agent.[36] Its chemical composition includes a great variety of substances, such as phenolic compounds (*e.g.*, flavonoids and coumarins), steroids, terpenoids, carbohydrates, lipids, tocopherols, quinones, carotenes, essential oils, fatty acids, and minerals.[37,63–65] Diverse therapeutic activities have been assigned to the *C.*

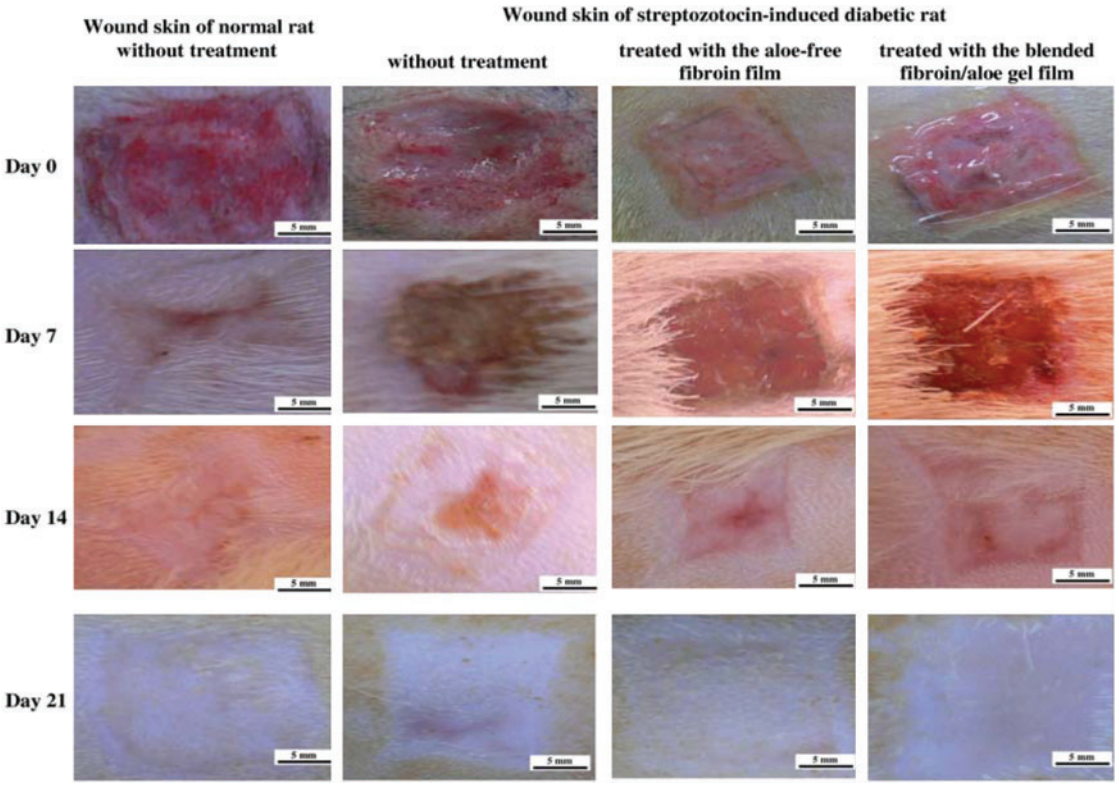

Figure 2. Influence of fibroin/aloe gel film dressings on the wound healing of normal rat and streptozotocin-induced diabetic rat.[4] To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

officinalis and its constituents, including anti-inflammatory, antibacterial, antifungal, antioxidant, and the ability to stimulate angiogenesis.[7,37,63,66] Although the specific compounds responsible for the wound-healing properties of *C. officinalis* remain unknown, it has been reported that triterpenes play an important role in the healing process by stimulating the fibroblast migration and proliferation.[38] Other compounds have also been isolated and characterized, showing anti-inflammatory, antitumor, and antioxidant activities.[65,67,68] *In vivo* trials show that the topical application of *C. officinalis* promotes the healing of acute wounds and burns in rat models by reducing the epithelialization time and increasing the wound contraction, collagen content, and blood vessel formation.[6,37,69] Naeini *et al.*[6] investigated the effect of *C. officinalis* gel (5%, 7%, and 10% of gel concentration) on cutaneous collagen production and hydroxyproline content of wound incisions created in rats. The topical application of the *C. officinalis* gel at 7% significantly improved the collagen production compared with the control and placebo groups. Authors observed that the other gel concentrations were less effective in the stimulation of the healing process, probably due to the low concentration (5% gel) and cytotoxic effects (10% gel). Similar results related to the influence of the concentration dose on the therapeutic effect of aqueous-ethanol extracts of *C. officinalis* in a rat hepatocarcinogenesis model were reported.[70]

Clinical trials have also been conducted to evaluate the therapeutic efficacy of *C. officinalis* in the treatment of ulcers and acute dermatitis during breast cancer irradiation.[7,71–73] A pilot study that involves a total of 32 patients was performed by Binić *et al.*[7] to investigate the effect of herbal treatments in the healing process of noninfected venous leg ulcers. The patients were randomized into two groups: one group (15 patients) was treated with a topical antibiotic as control, while the second group (17 patients) was treated with Plantoderm® ointment (it contains alcohol extracts of *C. officinalis*) and Fitoven® gel (phytotherapy treatment [PT] group). After 7 weeks of treatment, the topical administration of herbal products resulted in a significant difference in the percent decrease of the surface area of the ulcers and a decrease in the bacterial colonization, while in control group no significant difference in the percent decrease of the surface area of the ulcers was observed. A reduction of 42.68% in the surface of the ulcers treated with herbal products was verified, against 35.65% in the control group, which

indicates the positive effects of *C. officinalis* in the wound-healing process. Although the study involved a low number of patients with comparable patient characteristics (sex, age, venous leg ulcer duration, and ankle brachial index) and wound surface area, the predominance of mixed bacterial flora into the ulcers of the control group (73.33% vs. 41.17% in PT group) may influence the healing rate of the wounds.

These research works support the wound-healing activity of *C. officinalis*. However, the mechanisms that underlie the therapeutic activities of *C. officinalis* are poorly understood, which preclude its clinical application. Evidence from animal and human trials is still required to support the clinical use of *C. officinalis* extracts for skin-wound-healing applications. The side effects of *C. officinalis* are also poorly investigated, existing limited scientific evidence in literature. It has been reported that the *in vivo* use of *C. officinalis* extracts at high concentrations produces genotoxic effects in a rat hepatocarcinogenesis model, while clinical trials show either no side effects,[7] or the occurrence of allergic dermatitis in 2.03% of the treated patients.[74]

Animal-derived products

Animal-origin products, like honey and propolis, have been used in wound care since ancient times due to their therapeutic properties. Honey has been applied as a natural bioactive dressing material that fills and covers either superficial or deep wounds, providing a moist environment and topical nutrition. Propolis has also been employed as a result of its antioxidant, anti-inflammatory, and antibacterial properties. Frog skin and its secretions have also been explored in traditional medicine as ointment or temporary dressing that cover the wound, preventing the penetration of pathogens and the dehydration.[18,75]

Honey. Honey is a highly viscous and superconcentrated acidic sugar solution (pH = 4.0) derived from nectar gathered and modified by the honeybee *Apis melífera*. Its chemical composition includes carbohydrates like fructose (40%), glucose (30%), and sucrose (5%); water (20%); amino acids (5%); antioxidants; vitamins; minerals; and enzymes.[17,76] Honey can be collected from different sources, which may result in different chemical compositions and, consequently, various levels of therapeutic activity.[8,77,78] The use of honey as a natural healing agent has been increasing in healthcare, primarily, due to its ability to provide topical nutrition to the wound, reduce inflammation, and absorb the excess of exudate, this way avoiding maceration.[17,75] Several therapeutic activities have been assigned to the honey, including antibacterial, anti-inflammatory, antifungal, and the ability to stimulate angiogenesis, granulation, wound contraction, and epithelialization.[77,79–81] Honey also provides a debriding effect, reduces edema, and deodorizes the wound.[79]

The antibacterial activity is one of the most investigated properties of honey, being attributed to the synergy between several factors, namely, (1) the high sugar concentration, (2) the acidity, (3) the low water content, and (4) the presence of antimicrobial substances like hydrogen peroxide, methylglyoxal, antimicrobial peptide bee defensin-1, flavonoids, and phenolic acids.[18,76,80,81] Several studies demonstrated the bactericidal activity of honey against a broad spectrum of nonresistant and antibiotic-resistant bacteria, as well as its ability to inhibit or even eradicate biofilm formation in both animal models and humans.[80,82–85] *In vitro* studies also showed that honey promotes the angiogenesis in a rat aortic ring assay,[86] and stimulates the proliferation of human keratinocyte cells,[87] which are involved in the healing process and play a pivotal role in re-epithelialization. The effect of honey and its dominant protein major royal jelly protein 1 (MRJP1) on the activation of human keratinocytes was further investigated by Majtan *et al.*,[87] showing that either honey solution or MRJP1 protein induces the proliferation of human keratinocytes. Different effects in terms of cytokine and matrix metalloproteinase (MMP)-9 mRNA expression in primary keratinocytes were observed. Honey upregulates the expression of cytokines and MMP-9 mRNA in primary keratinocytes, while the isolated use of MRJP1 increases the level of tumor necrosis factor-α mRNA expression. However, the beneficial effects of the upregulation of cytokines and MMP-9 mRNA for the wound-healing process are not totally clarified by the authors. They also stated that the wound-healing activity of honey is influenced by additional factors, such as the pH and the release of hydrogen peroxide.

An important concern related to the therapeutic efficacy of honey relies on the progressive dilution of honey when in contact with the wound exudate, which may lead to a significant decrease in the antibacterial effect, increasing the risk of infection.[82] In a recent work, Kwakman *et al.*[88] reported that the addition of a synthetic antimicrobial peptide (bactericidal peptide 2) into a medical-grade honey results in a significant improvement in the bactericidal activity against antibiotic-resistant pathogens. These findings suggest that the de-

velopment of innovative formulations that contain honey and antimicrobial peptides represent a promising alternative to overcome the just-mentioned limitation.

The wound-healing activity of honey-based products (*e.g.*, solutions, gels, and dressings) has been investigated in both laboratorial studies and clinical trials. Laboratorial research works in animal models showed that honey significantly improves the healing rate, reduce the scar formation, and inhibit the bacterial growth in burns and acute wounds.[8,89,90] Recently, Wang *et al.*[8] developed an hydrogel dressing composed of gelatin (20 wt.%), honey (20 wt.%), and chitosan (0.5 wt.%) for the treatment of burn injuries. The dressing exhibits a remarkable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, without inducing adverse skin reactions. After application into second-degree burns created in a rabbit model, the hydrogel dressing promoted a significant increase in the healing process and wound contraction, comparatively to the control group and the group treated with a commercial ointment (MEBO®). The burns treated with the honey dressing were completely healed with intact epidermis after 12 days of treatment, while the other groups needed 14 (MEBO) and 17 days (control) to heal.

Prospective randomized clinical trials show that honey accelerates the healing process in diabetic ulcers, malignant wounds, and burns compared with commercial topical agents and traditional dressings.[9,91–93] In a recent clinical trial, Kamaratos *et al.*[9] investigated the effect of manuka-honey-impregnated dressings on the healing and microbiology of neuropathic diabetic foot ulcers in 63 patients, during 16 weeks. As a control, one group of patients was treated with conventional dressings. Although the ulcers treated with honey exhibited a significant decrease in the average healing time (31 days vs. 43 days for control) and a rapid clearance of bacteria, no significant differences between honey and comparative treatment were observed regarding the percentage of healed ulcers. Other clinical trials also observed similar effects in the treatment of patients with venous ulcers and malignant wounds.[94,95] The clinical efficacy of honey was also tested for the treatment of acute wounds (*e.g.*, burns, lacerations, abrasions, and minor surgical wounds) and compared with commercial products like conventional dressings and SSD.[93,96] Ingle *et al.*[96] performed a prospective, randomized, double-blind controlled trial to investigate the healing properties of honey and IntraSite Gel in patients with lacerations or shallow abrasions. Forty wounds (25 shallow wounds and 15 abrasions or partial-thickness burns) were treated with honey, while 42 wounds (25 shallow wounds and 17 abrasions, donor sites or partial-thickness burns) were treated with the hydrogel. Even though no significant differences in the mean healing time between the wounds treated with honey and hydrogel were found, honey proved to be a safe and cost-effective healing agent.

The administration of honey as a natural healing agent is considered safe, rarely resulting in allergic reactions or adverse effects. However, there are clinical trials that report that the use of honey may result in itching, and the contact between honey and the wound site can be painful for the patient due to its acidic nature.[94,96,97] The scientific evidence about the use of honey in wound healing indicates that its therapeutic properties together with the nonadherent interface with the wound bed promote an increase in the healing rate and elimination of infections. Medical-grade honeys, prior submitted to sterilization processes, usually using gamma radiation, are applied to the lesion site as topical solutions, gels, and dressings, creating a natural coverage that provides a moist environment and topical nutrition, enhancing the skin regeneration. Besides these positive effects, there is a need for further laboratorial studies, and especially controlled clinical trials, focusing on the properties of the regenerated skin and the healing efficacy of honey preparations in different types of wounds. Honey treatment is not necessarily superior to other existing treatments for either acute or chronic wounds, but offers another treatment option with a good relationship between clinical efficiency and manufacturing cost.

Propolis. Propolis, also known as bee glue, is a resinous-like substance collected by the honeybees (*Apis mellifera*) from several tree species. Propolis has been used in folk medicine due to its wide range of biological properties and low toxicity.[17,98] Similarly to other natural-origin substances, propolis has a complex composition, containing resin and balsam (50%), wax (30%), essential and aromatic oils (10%), pollen (5%), and other substances such as organic debris (5%).[98,99] Among these constituents, the most representative are polyphenols like flavonoids (*e.g.*, quercetin, galangin, and chrysin), phenolic acids (*e.g.*, ρ-Coumaric acid, caffeic acid, and ferulic acid), and aromatic compounds, which play an important role in the pharmacological activities of propolis.[98,100,101] A wide range of compounds have been extracted, isolated, and identified from propolis, contributing to elucidate

the actuating mechanisms and the role on its biological activities.[100,102–104] Several therapeutic activities have been claimed, such as the antimicrobial, antioxidative, antiseptic, antiviral, anti-inflammatory, immunomodulatory, and healing properties.[99,101] These properties are sensitive to the chemical composition of propolis, which in turn strongly depends on the tree source, region, climate, or production conditions.[98,100] Kumazawa *et al.*[101] reported significant variations in the antioxidant activity of ethanol extracts of propolis collected from different geographic locations. The authors observed that the antioxidant properties depend on the content of polyphenols, flavonoids, and antioxidative compounds, including kaempferol and phenethyl caffeate.

A large number of laboratorial research works have been performed in order to investigate the biological properties of propolis, in particular, the mechanisms behind the antioxidant,[105] anti-inflammatory,[104] and antibacterial activities.[106] In a recent *in vitro* study, Bufalo *et al.*[104] demonstrated that propolis and one of its constituents, caffeic acid, have a strong anti-inflammatory activity, by inhibiting the production of nitric oxide in macrophages without inducing cytotoxic effects on the cells. The authors suggest that the anti-inflammatory effect can be mediated by the down-regulation of transcription nuclear factor-κB, p38 mitogen-activated protein kinase, and c-jun NH_2-terminal kinase (JNK1/2). Similar results were reported in another study conducted in surgical wounds created in rat models.[107]

The antibacterial activity of propolis has been studied against a broad spectrum of bacteria, including Gram-positive, Gram-negative, yeasts, and antibiotic-resistant bacteria. However, this activity depends on the concentration and is strictly related with the contents of polyphenols and flavonoids.[106,108,109] Although the exact actuating mechanisms remain unknown, it is believed that specific compounds like rutin, quercetin, and naringenin have an important role in the antibacterial activity by improving the permeability of the bacterial membrane and decreasing both the production of adenosine triphosphate (ATP) and the transport mechanisms across the membrane.[108] Propolis also has the ability to establish synergic effects with synthetic antibiotics, leading to an improvement in the antimicrobial effects in both *in vitro*[109,110] and *in vivo*.[111] This synergetic action may contribute to reduce the administration of synthetic drugs and the development of antibiotic-resistant microorganisms, opening promising perspectives for the synthesis of novel drugs.

Recently, the scientific evidence about the healing properties of propolis has increased, although the number of *in vivo* preclinical studies that investigate its healing properties in animal models and humans is limited.[112–116] Animal studies showed the ability of propolis to promote the keratinocyte proliferation, the stimulation of glycosaminoglycan deposition in the wound, and the modification of the chondroitin/dermatan sulfate structure.[112,114] Pessolato *et al.*[113] reported the efficacy of a propolis ointment on the healing process of second-degree burn wounds by promoting wound debridement, stimulating the collagen synthesis, and reducing the wound inflammation. The healing mechanism of propolis remains a controversial issue, though this characteristic is likely due to the synergetic effects between the chemical constituents and its antibacterial and anti-inflammatory activities.

Clinical trials have been conducted to investigate the therapeutic activities of propolis for different skin lesions.[10,117,118] Gregory *et al.*[10] conducted a clinical study to compare the healing effect of propolis cream and SSD in superficial second-degree burns. Despite the limitations of the study, in particular, the low number of patients, the time between treatments, and the absence of data about bacterial colonization, results show a beneficial effect of propolis, leading to a reduced inflammation and an improved healing process. In another clinical trial, the healing efficacy of propolis was tested through the topical administration of a propolis ointment combined with short stretch bandage compression in 28 patients with chronic nonhealing venous leg ulcers. All ulcers treated with propolis were completely healed after 6 weeks of treatment, while in the control group (treated with compression dressings) the healing time was significantly higher (16 weeks).[117]

Evidence suggests a significant increase in the use of propolis in wound care, mainly due to its anti-inflammatory, antioxidant, and healing activities. However, in order to improve the clinical use of propolis, it is necessary to develop novel manufacturing strategies and quality control methods, ensuring an extensive characterization of its chemical constituents and pharmacological properties. It is also critical to investigate the therapeutic levels and the cytotoxic concentrations of propolis products in both *in vitro* and *in vivo* studies in order to guarantee its safety and to identify possible side effects. Although the adverse reactions related to the use of propolis in wounds are poorly documented in the literature, contact dermatitis is referred as the most common side

effect. Allergic contact dermatitis from propolis is due to the presence of allergens, such as 3-methyl-2-butenyl caffeate and phenylethyl caffeate, which are constituents of LB-1, the first allergen identified in propolis. Phenylethyl caffeate leads to strong reactions in propolis-sensitive patients, while benzyl salicylate and benzyl cinnamate, two less-frequent allergens present in propolis, result in very weak-to-moderate reactions.[119]

Living organisms

The interest in the use of living organisms for wound healing has been significantly increasing in last years, providing alternative approaches for skin repair. Maggots have a remarkable antimicrobial activity and ability to stimulate the wound debridement, while leeches are very useful in the treatment of venously congested wounds.

Maggot debridement therapy. The use of fly larvae in wound care, also designated as maggot debridement therapy, larval therapy, or biosurgery, is rapidly growing due to its efficacy, safety, and simplicity. Medicinal maggots are extensively used to promote the debridement of diverse types of wounds through the digestion and removal of devitalized or necrotic tissue. Maggots also have the ability to decompose organic matter and exogenous pathogens, providing wound cleaning and disinfection, which is fundamental for a successful healing process.[18] Currently, maggot therapy is employed in chronic skin wounds that have failed the healing after the application of either conventional or modern treatments.[120] In these cases, sterilized maggots are introduced into the wound with the support of traditional bandages (*e.g.*, gauzes) or modern dressings (*e.g.*, Le Flap™), providing either free or constrained access to the lesion site. In the "free-access mode," maggots are usually suspended in isotonic saline solution and subsequently introduced onto the wound in direct contact with the injured tissue (Fig. 3A).[121] Before the introduction of maggots, a hydrocolloid dressing that contains a hole corresponding to the wound dimensions is applied to the skin surrounding the wound, preventing maggots to escape and protecting the skin from the proteolytic enzymes. A sterile and porous sheet of nylon mesh is also fixed onto the hydrocolloid dressing to cover the maggots, and a gauze pad is used for the drainage of exudate and liquefied necrotic tissue.[120,122] In the "constrained-access mode," maggots are introduced within small nylon bags (*e.g.*, Biobag™) or incorporated within dressings, avoiding the direct contact with the wound (Fig. 3B). These materials act as a barrier between the injured tissue and the larvae, allowing the diffusion of maggot excretions/secretions (ES) to the wound.[123] The bag loaded with maggots is generally covered by a hydrocolloid dressing and/or absorbent bandages. The number of maggots introduced into the wound depends on the maggot properties (*e.g.*, age and size) and patient health (*e.g.*, wound size, and content of necrotic tissue), but an average amount of 5–10 maggots/cm^2 of wound surface area is usually used, remaining in the site during 48–72 h.[120,124–126]

Lately, a renewed attention has focused on the use of maggot therapy in modern wound care due to the therapeutic effects of medicinal maggots: (1) efficacy to provide the wound debridement,[127] (2) capacity to inhibit or even eradicate the biofilm formation,[128] (3) antimicrobial activity,[129] and (4) ability to stimulate the healing process.[12]

The wound debridement ability is attributed to the powerful proteolytic enzymes (*e.g.*, collagenase, trypsin-like, and chymotrypsin-like enzymes) secreted by the maggots. These enzymes liquefy and dissolve the necrotic tissue, solubilize fibrin clots, and degrade ECM molecules (*e.g.*, fibronectin, laminin, and acid-solubilized collagens I and III), facilitating the digestion by the larvae and stimu-

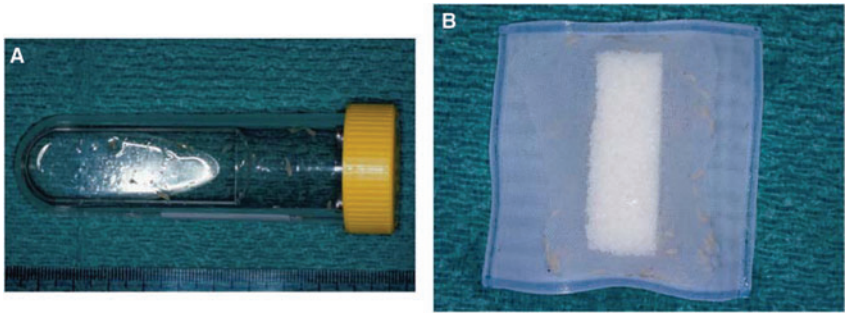

Figure 3. **(A)** Free maggots suspended in isotonic saline solution before application onto the wound. **(B)** Biobag that contains maggots inside and a sponge to prevent the net to collapse.[121] To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

lating the healing.[130,131] Maggots also play an important role in the elimination of bacteria and other pathogens from the wound, including antibiotic-resistant bacteria, such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus*.[129,132] An *in vivo* study showed that maggot therapy is efficient in the treatment of patients with bacteria-infected wounds, but this effect is most pronounced in wounds that contain Gram-positive bacteria.[133] The actuating mechanisms behind the antimicrobial activity of larvae are not yet completely understood, though laboratorial and clinical evidence point out that bacterial ingestion and digestion, the high levels of wound exudate, the secretion of natural bactericidal agents (*e.g.*, lucifensin), and the alkalinity of the wounds play a crucial role in the inhibition/elimination of biofilm formation and bacterial growth.[120,121,128,134,135] Recent works investigated the synergetic effects between maggot ES and commercial antibiotics on the viability of bacteria and biofilm breakdown.[136,137] These works reveal that maggot ES act synergistically with some antibiotics without affecting their therapeutic activity, allowing the effective biofilm breakdown with consequent elimination of derived bacteria. Proposed underlying mechanisms suggest that maggot ES increase the permeability of the cell wall, which facilitates the action of antibiotics.[137] The use of maggot therapy is also associated to the stimulation of the healing process by increasing tissue oxygenation, fibroblast proliferation,[120,138] angiogenesis,[139] and the formation of granulation tissue.[12] These effects are mainly attributed to the maggot ES and its constituents (*e.g.*, serine proteinases), rather than the isolated removal of dead/necrotic tissue. However, the debridement activity of maggots is fundamental for the healing process as it degrades and removes ECM molecules and necrotic tissue, which are important barriers to a successful regeneration process.[130] Wang *et al.*[140] showed the ability of maggot ES to effectively stimulate the migration of microvascular endothelial cells through the activation of the enzyme V-akt murine thymoma viral oncogene homolog 1 during the wound healing, which is crucial in the angiogenesis. Similarly, van der Plas *et al.*[141,142] showed the capacity of maggot ES to inhibit proinflammatory responses of human monocytes and neutrophils without alterations in the antimicrobial properties. Horobin *et al.*[138] developed a three-dimensional (3D) *in vitro* assay to study the influence of maggot ES in the fibroblast migration and morphology. They found that fibroblast cells embedded within collagen gels in the presence of maggot ES exhibited spread morphologies with longer cytoplasmic extensions and matrix organization, revealing the cell-stimulation activity of maggots in 3D environments. Laboratorial studies have also identified several biologically active constituents in the ES products that play a crucial role in diverse phases of the wound-healing process. Bexfield *et al.*[139] identified amino-acid-like compounds (*e.g.*, histidine, valinol, and 3-guanidinopropionic acid) from larvae ES and demonstrated their ability to stimulate the growth of human endothelial cells. These findings suggest that these amino acids might play an important role in the angiogenesis.

Nonetheless, laboratorial and clinical studies demonstrated the safety and efficacy of maggots in wound care; therapies that involve the introduction of living organisms onto the wound have some important limitations, including (1) reluctance of the patients to the sensing caused by the movement of the larvae into the wound, (2) pain and discomfort, (3) escaping maggots, and (4) relatively short life-cycle stage of larvae.[11,124,127,133] To improve patient acceptance, reducing the discomfort, and minimizing the risk of escaping maggots, modern dressings that contain either living larvae or maggot secretions have been designed and tested.[11,143–145] In these systems, maggots are usually enclosed between thin permeable membranes, restricting their access to the lesion site. During the treatment, maggot secretions diffuse through the membrane to the injured site, promoting the wound debridement and stimulating the healing process. Smith *et al.*[11] developed a poly(vinyl alcohol)-based hydrogel wound dressing that contains *Lucilia sericata* larvae ES products and investigated its ability to modulate the behavior of fibroblasts and epithelial cells (Fig. 4). The presence of high concentrations of maggot secretions in the culture media increases the rate of wound closure in fibroblast monolayer cultures by stimulating cell migration. On the other hand, the release of maggot secretions from the hydrogel dressing into 3T3 fibroblasts and HaCaT (keratinocytes) model wound promotes a significant increase in the wound closure rate after 12 h of incubation, suggesting beneficial effects of maggot secretions in the wound-healing process.

Prospective controlled trials supported the safety and efficacy of maggot therapy for the treatment of diverse wounds, including leg ulcers,[126,127] diabetic ulcers,[12,125] pressure ulcers,[122,125] venous ulcers,[146] and diabetic wounds.[147] Two clinical trials report that maggot therapy is effective in the debridement of the wound, but it does not produce significant differences in terms of the healing rate.[127,146] However, there are clinical trials that report the ability of maggot therapy to provide antimicrobial

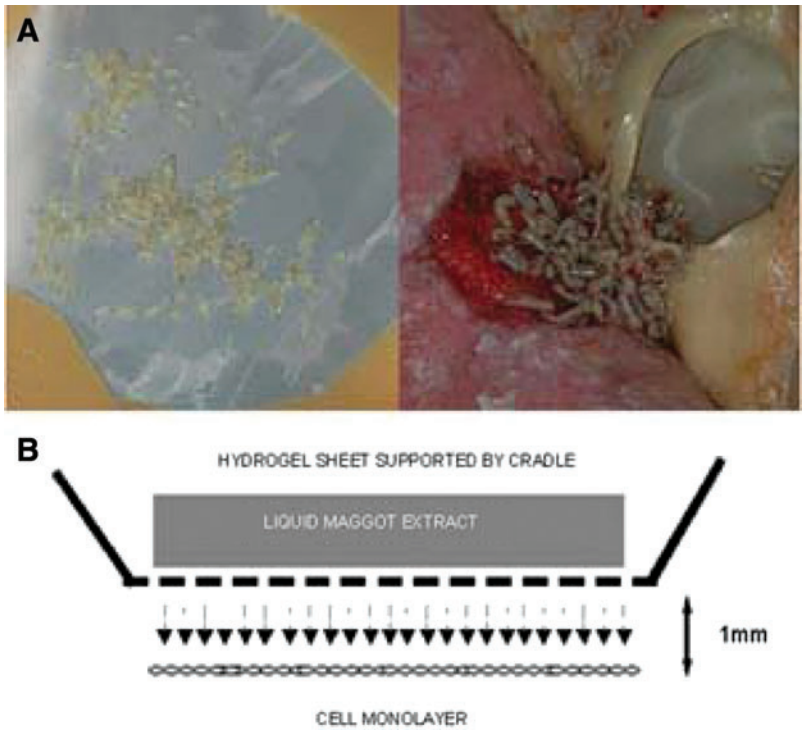

Figure 4. **(A)** Maggots before the application into a chronic wound, and maggots in direct contact with the wound at the end of the treatment, during the removal. **(B)** The experimental scheme used to test the effect of the delivery of maggot extract from a hydrogel wound dressing onto model wounds in monolayer cell culture.[11] To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

activity and to stimulate the granulation tissue formation and the wound-healing process.[12,147] Dumville *et al.*[127] conducted a randomized controlled trial that involves 267 patients with venous or mixed venous and arterial leg ulcers, to investigate the clinical efficacy of maggot therapy compared with hydrogels. In this study, 94 patients received loose larvae treatment, 86 patients were treated with bagged larvae, and 87 patients received the hydrogel treatment. Although maggot therapy significantly reduced the debridement time of the wounds, no significant changes were observed between the groups regarding the healing rate (236 days for maggot groups and 245 days for hydrogel group) and the reduction in the bacterial load. Contradictory results regarding the effect of maggots on the healing rate were reported by Sherman,[12] in a clinical trial that involves 18 patients with 20 nonhealing diabetic foot and leg ulcers. The wounds were treated with maggot therapy (six wounds), conventional therapy (six wounds), and conventional therapy followed by maggot therapy (eight wounds). Maggot therapy was more effective in the wound debridement than conventional therapy, leading to an increase in both the formation of granulation tissue and the healing rate of the ulcers.

The clinical use of maggot therapy is considered safe with no significant side effects or allergic reactions for the patients. The most common adverse reactions include pain and discomfort associated to the escaping maggots, which are easily solved through the administration of analgesics and the immobilization of maggots within dressings.[120,122–124,126] Contra-indications for maggot therapy include open wounds in the abdominal cavity, septic arthritis, and pyoderma gangrenosum in patients with immunosuppressive therapy.[123]

The use of maggot therapy for wound-healing applications significantly increased in recent years, allowing the treatment of diverse types of skin wounds. Clinical trials showed that maggot therapy accelerates wound debridement and promotes a bactericidal effect, but no consistent trials demonstrate its efficacy regarding the healing process. Thus, further studies are required to clarify the effect of maggot therapy in the wound healing and to define standardized clinical practices. Standardization is a critical issue in maggot therapy, since there are many factors (*e.g.*, maggot source and production, composition of maggot secretions, and treatment protocols) that affect the therapeutic activities of maggots. Multidisciplinary efforts from different research groups

will assume a major role in the development of more standardized procedures of maggot therapy, proving and highlighting the therapeutic properties and the action mechanisms of maggots.[148] New research works should also be conducted to evaluate the clinical effectiveness of maggot therapy combined with other treatments either traditional (*e.g.*, plant extracts) or modern (*e.g.*, tissue-engineered skin substitutes), which should be more effective in the promotion of the healing process. In this field, it is expected that maggots will assume a prominent position as natural debridement agents for the treatment of nonhealing wounds, playing a crucial role in the wound-bed preparation. However, other agents with high healing-stimulation properties should be subsequently applied in order to reduce the healing time and to improve the properties of the new skin.

Leech therapy. Leech therapy or hirudotherapy is an alternative therapeutic treatment for diverse skin disorders that involves the administration of medicinal leeches (*Hirudo medicinalis*) into the injured site. Hirudotherapy has been used in plastic and reconstructive surgery since the ancient times to promote the healing of a wide range of lesions, including venously congested tissues, free flaps, pedicled flaps, replanted tissues, and glucoma.[149–151] The action mechanism that underlies the medicinal leeches relies on the secretion of a complex mixture of compounds (*e.g.*, vasodilators, anticoagulants, anesthetics, and analgesics) with relevant biological and pharmacological properties from the salivary glands into the lesion site, locally stimulating the healing process. The main constituent of leech saliva is hirudin, which is a potent natural anticoagulant that inhibits the blood coagulation through the binding to thrombin, allowing the ingestion of blood by the leeches. Hirudin also acts as a bacteriostatic and bactericidal agent.[149,152] Other compounds with relevant biologically active properties (*e.g.*, antibacterial, anti-inflammatory, vasodilation, and analgesic) include calin, destabilase, hirustatin, bdellins, hyaluronidase, tryptase inhibitor, eglins, factor Xa inhibitor, acetylcholine, and histamine like.[149] Leech therapy has been extensively employed in wound healing to remove stagnant blood from wounds after reconstruction or plastic surgery, due to the ability of leeches to absorb blood through either puncture the skin or bite, and to release therapeutic compounds (*e.g.*, hirudin) directly into the lesion.[152] During the application, leeches absorb the stagnant blood and restore the normal blood flow, oxygenation, and nutrient supply to the affected area, reducing the venous pressure and promoting the healing process.[149] In a recent systematic review, Whitaker *et al.*[153] evaluated the current scientific evidence regarding the use of medicinal leeches in plastic and reconstructive surgery for the treatment of diverse skin conditions. From the 277 patients treated, the overall success rate of leech therapy was 77.98%, which indicates the clinical efficacy of leech therapy. Among these patients, 49.75% required blood transfusions due to the continuous blood loss, 79.05% received antibiotics, 54.29% received concomitant anticoagulant therapy, and few patients received antispasmodics. The incidence of complications was reported in 21.8% of patients with infection to be the most common one. This literature survey indicates that leech therapy can be used as an alternative therapeutic treatment for wound healing. However, there are some important limitations pointed out by the authors that can influence the overall success rate, including the lack of information about the flap size and the administration of antibiotics, as well as the variable number of leeches and time interval between leech applications. Although the current scientific evidence for leech therapy in wound healing (treatment of soft tissue hematomas, penile replantation, tissue flap reconstructions, soft tissue injury, and surgical replantation) is mainly composed of case studies and case reports that involve a low number of patients,[154] there are randomized controlled trials that investigate the efficacy of leech therapy in patients with osteoarthritis, revealing promising results in terms of pain reduction and enhancement of the joint function.[150,155] Possible side effects of leech therapy include bacterial infections, bleeding, local itching, allergies, and anemia.[149,152,155] Local infections with *Aeromonas* species (*Aeromonas hydrophila*) are one major complication of hirudotherapy being well-documented in literature. *A. hydrophila* is a gram-negative rod that lives symbiotically in the intestinal flora of the leech, producing proteolytic enzymes for the leech digestion of the vertebrate blood. These bacteria are introduced into the wounds during the leech attachment, leading to an infection incidence rate in a range of 2.4–20%.[154] Even though *A. hydrophila* is resistant to penicillin and first-generation cephalosporins due to the production of beta-lactamase, prophylactic antibiotic therapy can be used to prevent local infections during the leech therapy.[154,156]

Currently, there is a need for long-term controlled randomized trials that investigate the clinical efficacy of leech therapy in different wound

types. Further studies that focus on the number of leeches to be used, administration period, time intervals between applications, and cost-benefit ratio are also required to support the clinical practice and establish standardized treatment protocols.

Silver and traditional dressings

Silver is a broad-spectrum antimicrobial agent that is commonly used in the treatment of skin lesions, in particular, wounds and burns. Silver is one of the most commonly applied antimicrobial agents in wound care, being available as the active ingredient of diverse products, such as solutions (*e.g.*, silver nitrate), creams (*e.g.*, SSD), gauze dressings (*e.g.*, Urgotul® SSD), foams (PolyMem® Silver), and dressings (*e.g.*, Acticoat™). Among the great variety of silver-based products, SSD is one of the most used, being considered the gold standard for the topical treatment of burns.[157,158] Several laboratorial studies have shown the excellent antimicrobial properties of silver-based products against a wide range of microorganisms, including Gram-negative, Gram-positive, and antibiotic-resistant bacteria.[159–161] These studies suggest that the mechanisms by which silver in ionic form (Ag^+) interferes with the normal metabolism of bacteria involve the accumulation of silver ions inside the cells and their binding with negatively charged components in proteins and nucleic acids, which leads to the protein denaturation and structural modifications in the cell walls/membranes.[13,157,161,162] Besides the relatively safety and potent bactericidal effect of silver, its use is strongly limited by the cytotoxic effects in mammalian cells.[13,163,164] Poon and Burd[163] showed that silver from either a silver nitrate solution or a commercial dressing is highly toxic for keratinocytes and fibroblasts in monolayer culture in a dose-dependent manner. Lately, AshaRani *et al.*[164] reported similar results about the cytotoxic effects of starch-coated silver nanoparticles in normal human lung fibroblast cells and human glioblastoma cells. The authors suggest that the actuating mechanism involves the disruption of the mitochondrial respiratory chain with consequent production of reactive oxygen species and the interruption of ATP synthesis, leading to the DNA damage. These studies revealed that the cytotoxic effects of silver in mammalian cells depend on the concentration of silver ions, which varies according to the solubility of silver salts, the release medium, or the dressing type.[13,162]

An additional concern about the use of silver is related with the delay on the wound-healing process. Burd *et al.*[13] conducted a series of *in vitro* and *in vivo* studies to evaluate the effects of five commercial silver-based dressings on the wound-healing rate. *In vitro* results showed that in all dressings, silver leads to a significant delay in the re-epithelialization in an epidermal cell proliferation model. On the other hand, *in vivo* results in a mouse excisional wound model revealed a delay in the wound healing or an inhibition of the wound epithelialization after the application of some dressings. To overcome these important limitations, alternative formulations that contain silver ions have been developed and tested, like silver loaded within hydrogel dressings,[165] nanoparticles,[166] and nanofibers containing silver nanoparticles (Fig. 5).[161] In this field, it is critical the development of smart materials capable to deliver low concentrations of silver ions into the wound bed, avoiding toxic concentrations that might inhibit the healing process, and ultimately lead to the wound entering in a chronic state. These materials should also deliver an adequate amount of silver in order to produce a powerful antibacterial activity.

Laboratorial studies in animal models reported successful results regarding the regeneration of skin wounds after treatment with silver-containing materials.[158,160,166] In a recent study, crosslinked alginate fibers loaded with silver nanoparticles significantly increased the number of fibroblasts in cell culture, and reduced the infiltration of neutrophils and macrophages in an *in vivo* incisional wound model, which indicates a decrease in the inflammatory response. Ag nanoparticles or fibers loaded with Ag nanoparticles also promoted a fast wound healing with increased epidermal thickness, stressing the benefits of incorporating silver within biomaterials.[158] Possible mechanisms that underlie the wound-healing activity of silver are suggested to be related with the stimulation of keratinocyte proliferation and migration, fibroblast differentiation, and modulation of cytokine production.[166]

A large number of clinical trials demonstrated the efficacy of silver-based products to promote the wound-healing process in patients with venous and pressure ulcers,[165,167] burns,[168,169] and traumatic wounds.[170] These works indicate that silver-containing dressings are effective for the treatment of diverse skin injuries, allowing the stimulation of the healing process, pain reduction, and easy removal with reduced trauma. Side effects of silver-containing products, in particular, SSD, are related to the possibility of local maceration, cell cytotoxic effects, and bacterial resistance.[5,168] Additional adverse reactions include hepatic toxicity, renal toxicity, and leukopenia.[56]

Traditional dressings like gauzes, cotton wool, and natural or synthetic bandages are the most

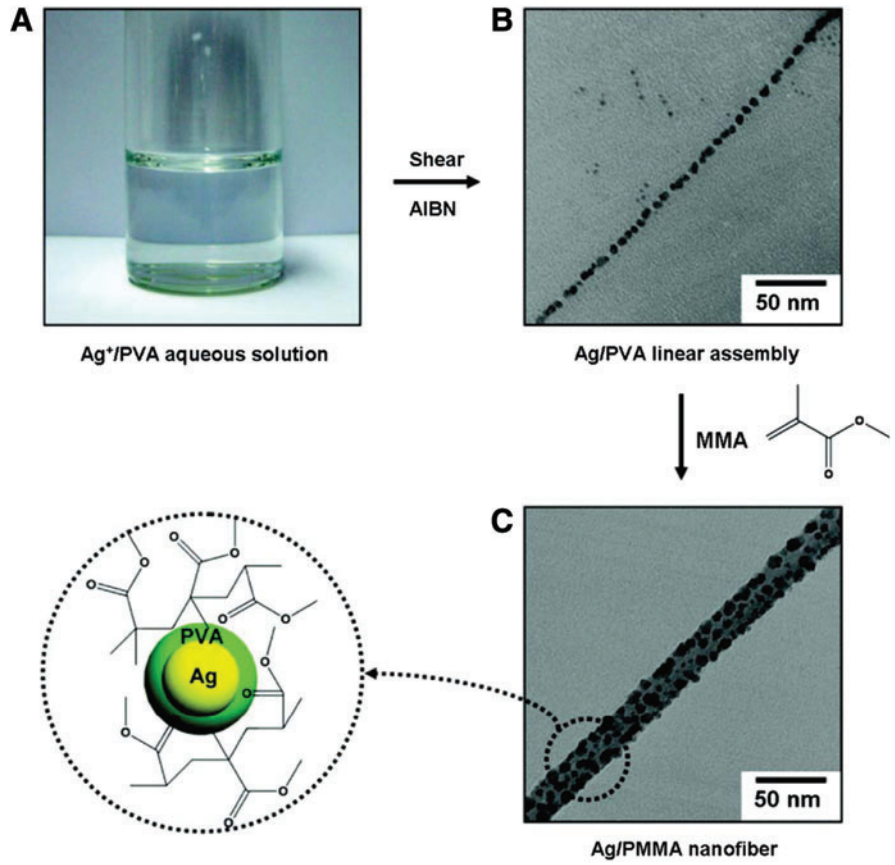

Figure 5. Processing steps in the fabrication of PMMA nanofibers that contain silver nanoparticles through radical-mediated dispersion polymerization. Macroscopic image of Ag^{+}/PVA aqueous solution **(A)** and transmission electron microscopy images of Ag/PVA linear assembly **(B)** and Ag/PMMA nanofiber **(C)**.[161] AIBN, 2,2-Azobis(isobutyronitrile); MMA, methyl methacrylate; PMMA, poly(methyl methacrylate); PVA, poly(vinyl alcohol). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

commonly used products in wound care applications.[1,21] When applied to the wound, these products absorb high volumes of exudate, which may lead to the drying of the wound bed, and ultimately result in cell death and inhibition of the healing process. Additionally, traditional dressings are not able to provide a moist wound environment and may also adhere to the wound bed, which can cause trauma and removal of new epidermis.[170] As a result of these limitations, traditional dressings are commonly applied as secondary dressings or combined with other products such as hydrocolloid and alginate dressings, protecting the wound from the entrance of pathogens and absorbing exudates.

SUMMARY AND FUTURE DIRECTIONS

The increase in the life expectancy and aging population is improving the stress under the healthcare system of each country, which ultimately can restrict the access of populations to primary healthcare. National and international authorities (*e.g.*, WHO) have been establishing general guidelines and priorities concerning to the improvement in the safety and quality of traditional medicines/therapies as a way to promote their use, rationalize the medical costs, and extend the access to the healthcare. Despite the tremendous potential of traditional therapies in terms of wound care benefits and socioeconomic impact, several issues related with the policy, efficacy, quality, safety, manufacturing practices, and rational use need to be addressed in a near future. These issues are of outstanding relevance to improve the safety use of traditional therapies, as well as to fully or partially integrate them into the national health systems. Although clinical trials have proved the efficacy of certain therapies in skin wound healing, some of these studies involve individual case reports or a low number of patients with no control or even any comparison between groups, which limits the scientific evidence. Recent studies are addressing these limitations by the in-

clusion of randomized controlled clinical trials, ensuring the safety of the natural compounds used and providing an adequate follow-up for patients. It is expected that natural compounds will assume a pivotal role in the healthcare, as they are a valuable source of therapeutic substances not only for direct applications as topical wound-healing agents, but also for the development of new classes of drugs with specific activities for each phase of the wound-healing process. This requires the development of specific research methodologies to validate and ensure the efficacy and safety of these products.

Traditional therapies have a wide range of therapeutic properties and, consequently, found different clinical applications, but they cannot permanently substitute the use of high-effective drugs, advanced practices, and innovative cellular therapies. Thus, recent trends are moving to the development of specialized healthcare treatments that involve the combined use of traditional medicine and modern practices/products.

TAKE-HOME MESSAGES

Basic science advances

- Traditional therapies based on herbal- and animal-derived compounds, living organisms, and silver and traditional dressings play an important role in all phases of the wound-healing process, allowing the treatment of a wide range of skin lesions.
- Recent advances on the understanding of the therapeutic effects of traditional healing agents provide new opportunities for the use of each therapy/product according to the specific needs of the wound type and/or the wound-healing phase.

Clinical science advances

- Several traditional therapies have shown the ability to stimulate the healing process and to reduce the scar formation in preclinical and clinical studies, by promoting a wide range of therapeutic effects, such as wound debridement, antimicrobial, cell stimulation, angiogenesis, or wound contraction.
- Recent progress regarding the processing methodologies, characterization techniques, and testing assays allowed a better comprehension regarding the mechanisms behind the therapeutic activities of traditional therapies.

Relevance to clinical care

- Traditional therapies are a cost-effective alternative to stimulate the healing of difficult-healing wounds, which is relevant for the clinicians and surgeons.
- Traditional healing agents can be combined with either natural or synthetic biomaterials and processed in a wide range of physical forms, including nanofibers and gels, toward the development of more effective wound care treatments.

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Abbreviations and Acronyms

AIBN = 2,2-azobis(isobutyronitrile)
ATP = adenosine triphosphate
AV = *Aloe vera*
DMSO = dimethyl sulfoxide
ECM = extracellular matrix
EGF = epidermal growth factor
ES = excretions/secretions
MMA = methyl methacrylate
MMP = matrix metalloproteinase
MRJP1 = major royal jelly protein 1
PDGF = platelet-derived growth factor
PMMA = poly(methyl methacrylate)
PVA = poly(vinyl alcohol)
SM = sesamol
SSD = silver sulfadiazine
TGF-β = transforming growth factor-β
WHO = World Health Organization