

Traditional Therapies for Skin Wound Healing

Rúben F. Pereira^{1,2,3} and Paulo J. Bártolo^{1,*}

¹Centre for Rapid and Sustainable Product Development (CDRsp), Polytechnic Institute of Leiria, Marinha Grande, Portugal.

²INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal.

³ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.



Paulo J. Bártolo, PhD

Submitted for publication August 28, 2013.
Accepted in revised form November 21, 2013.

*Correspondence: Centro Empresarial da Marinha Grande, Rua de Portugal—Zona Industrial, Marinha Grande 2430-028, Portugal (e-mail: paulo.bartolo@ipleiria.pt).

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.

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

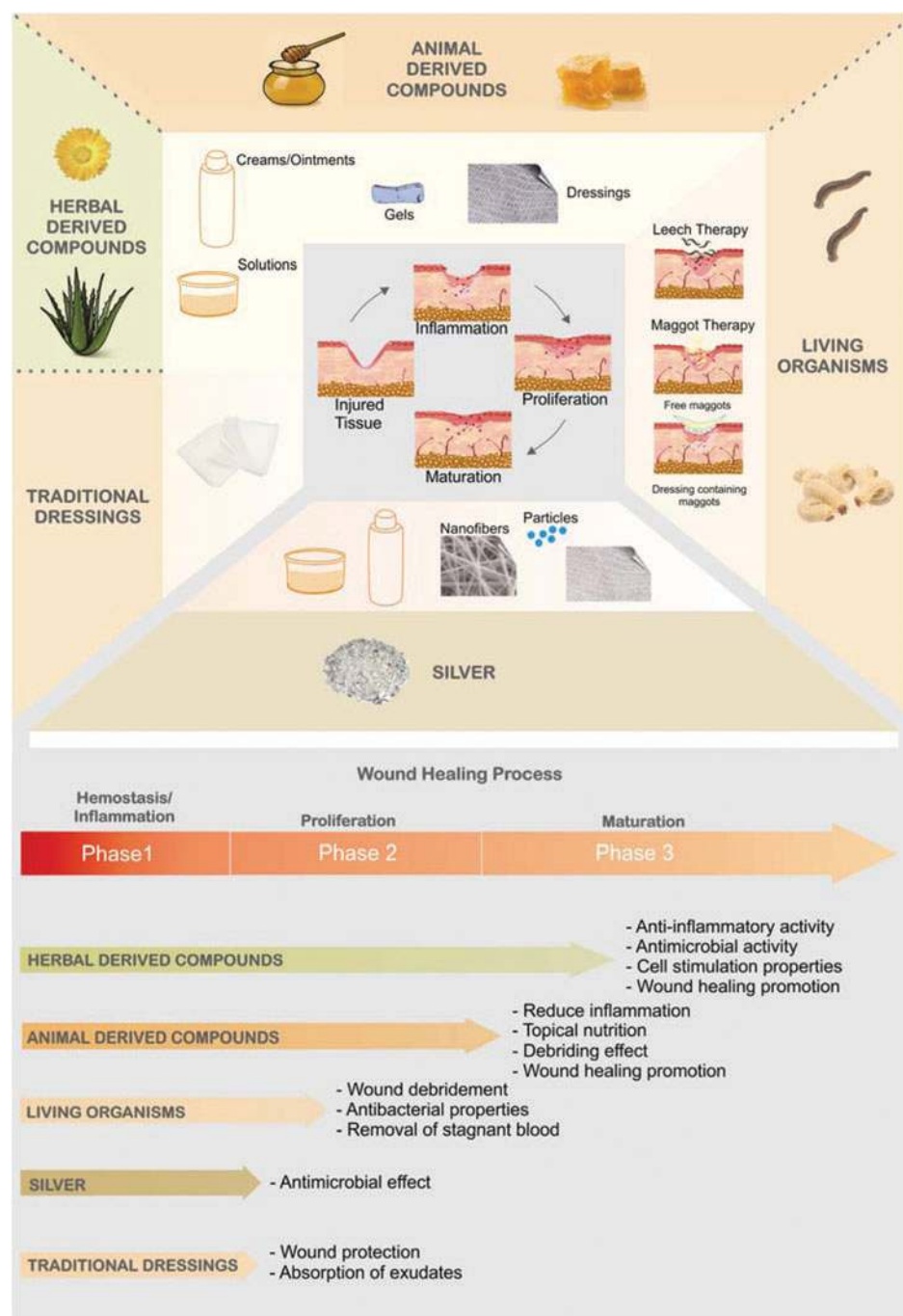


Figure 1. Classification of traditional therapies for skin wound healing. Traditional therapies and compounds are used in different phases of the healing process in a great variety of physical forms, either commercially available or under investigation, stimulating the skin regeneration process. To see 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
<i>Aloe vera</i>	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
<i>Hippophae rhamnoides</i> (sea buckthorn)	Flavonoids (<i>e.g.</i> , quercetin, isorhamnetin), carotenoids (<i>e.g.</i> , 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
<i>Angelica sinensis</i>	Essential oils and water-soluble ingredients; ferulic acid is the main active constituent	Forms: ethanol extracts, ferulic acid dissolved in DMSO Routes: n.a. (<i>in vitro</i> tests)	Stimulate the proliferation of human skin fibroblasts, the secretion of collagen, and the expression of TGF- β in <i>in vitro</i> conditions	35
<i>Catharanthus roseus</i> (<i>Vinca rosea</i>)	Contain two major classes of active compounds: alkaloids (<i>e.g.</i> , vincamine) and tannins	Forms: leaf ethanol extract Routes: topical	Antimicrobial activity against <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> ; increase wound strength, epithelialization, and wound contraction	36
<i>Calendula officinalis</i> (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 <i>in vitro</i> ; stimulate the collagen production and angiogenesis	6,37,38
<i>Sesamum indicum</i>	SM is the main antioxidant constituent, others include sesamol 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
<i>Morinda citrifolia</i> (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
<i>Camellia sinensis</i>	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
<i>Rosmarinus officinalis</i> 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 leaves, 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 sol-

uble sugars, nonstarch polysaccharides, lignin, lipids, vitamins (B₁, B₂, B₆, 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, anti-fungal, 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.

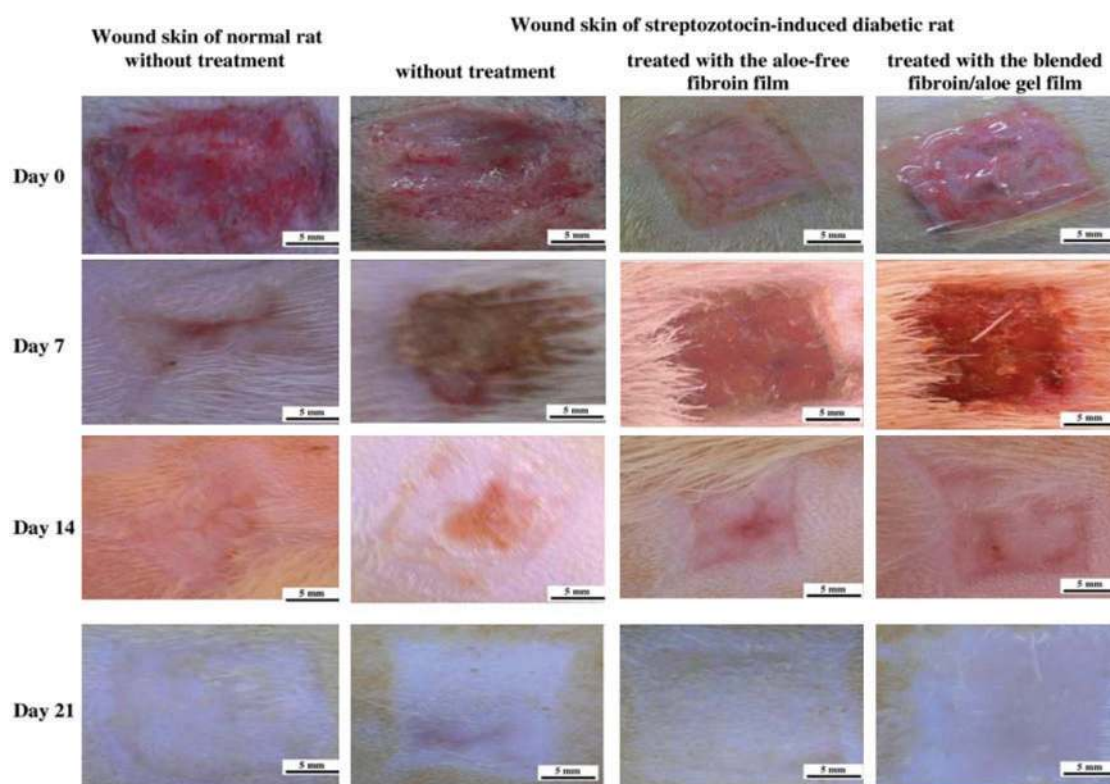


Figure 2. Influence of fibroin/aloe gel film dressings on the wound healing of normal rat and streptozotocin-induced diabetic rat.⁴ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

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 Planoderm[®] 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 super-concentrated acidic sugar solution (pH=4.0) derived from nectar gathered and modified by the honeybee *Apis mellifera*. 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 shal-

low 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.*, *p*-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- κ B, p38 mitogen-activated protein kinase, and c-jun NH₂-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 FlapTM), 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.*, BiobagTM) 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/cm² 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-

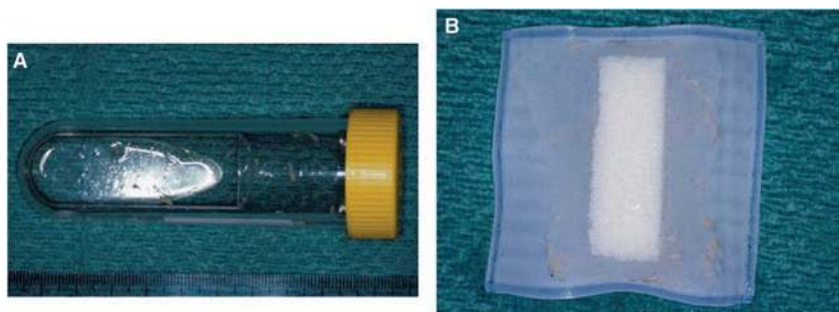


Figure 3. (A) Free maggots suspended in isotonic saline solution before application onto the wound. (B) Biobag that contains maggots inside and a sponge to prevent the net to collapse.¹²¹ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

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 cytoplas-

mic 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

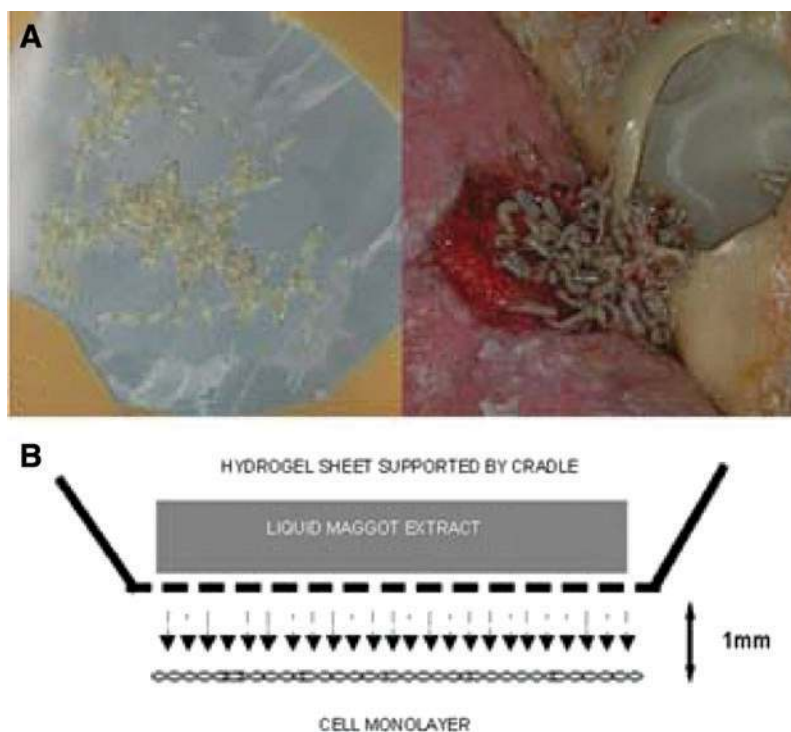


Figure 4. (A) Maggots before the application into a chronic wound, and maggots in direct contact with the wound at the end of the treatment, during the removal. (B) The experimental scheme used to test the effect of the delivery of maggot extract from a hydrogel wound dressing onto model wounds in monolayer 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 glaucoma.^{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, trypsin 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 Burd¹⁶³ 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

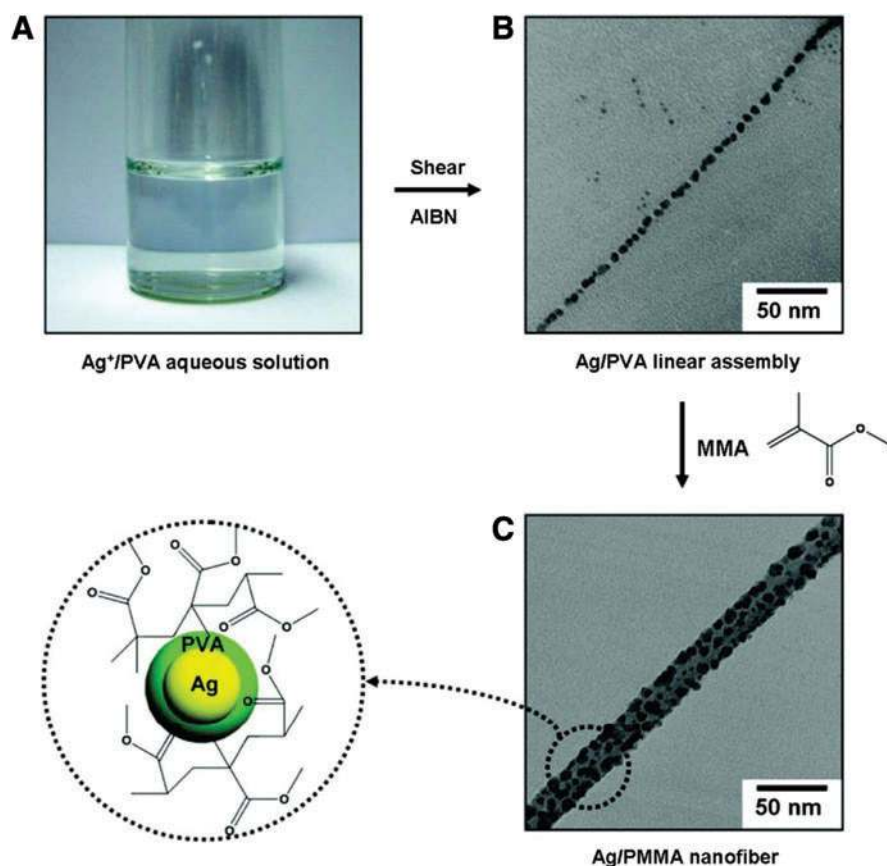


Figure 5. Processing steps in the fabrication of PMMA nanofibers that contain silver nanoparticles through radical-mediated dispersion polymerization. Macroscopic image of Ag^+ /PVA aqueous solution (**A**) and transmission electron microscopy images of Ag/PVA linear assembly (**B**) and Ag/PMMA nanofiber (**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.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The authors would like to thank the support of the Portuguese Foundation for Science and Technology through the Strategic Project PEST-OE/EME/UI4044/2011. R.F.P. is grateful for the financial support from Portuguese Foundation for Science and Technology by the Grant SFRH/BD/91151/2012.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have no other affiliations or financial involvement in commercial associations that might create conflict of interest with the article. No ghostwriters were used in the preparation of this article.

ABOUT THE AUTHORS

Rúben F. Pereira is a researcher at the Center for Rapid and Sustainable Product Development and a PhD student at the University of Porto. His research focuses on the development and use of biomaterials, traditional healing agents, and *in situ* biofabrication strategies for skin tissue regeneration. He is author and coauthor of several articles in scientific journals, book chapters, and patents. **Paulo J. Bárto** is a Professor of Ad-

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.

vanced Manufacturing Processes at the Polytechnic Institute of Leiria, director of the Center for Rapid and Sustainable Product Development (a Center of Excellence in Mechanical Engineering of the Portuguese Foundation for Science and Technology), adjunct professor at Queensland University of Technology (Australia), visiting professor at Nanyang University (Singapore), professor of the “Catedra UNESCO” of Biomaterials at the University of Habana (Cuba), member of CIRP (the International Academy of Production Engineering), Portuguese representative at GARPA (the Global Alliance of Rapid Prototyping Associations), and member of the Direction Board of the International Society of Biomanufacturing. He is also editor-in-chief of *Virtual and Physical Prototyping Journal* published by Taylor & Francis, and member of the Editorial Board of several journals like the *Biofabrication Journal*, the *Rapid Prototyping Journal*, the *International Journal of Precision Engineering and Manufacturing*, the *Journal of Biomaterials and Tissue Engineering*, the *ISRN Tissue Engineering*, and the *International Journal on Mechatronics and Manufacturing Systems*.

REFERENCES

- Pereira RF, Barrias CC, Granja PL, and Bartolo PJ: Advanced biofabrication strategies for skin regeneration and repair. *Nanomedicine* 2013; 8: 603.
- Zahedi P, Rezaeian I, Ranaei-Siadat S-O, Jafari S-H, and Supaphol P: A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polym Adv Technol* 2010; 21: 77.
- Hart CE, Loewen-Rodriguez A, and Lessem J: Dermagraft: use in the treatment of chronic wounds. *Adv Wound Care* 2012; 1: 138.
- Inpanya P, Faikrua A, Ounaron A, Sittichokechaiwut A, and Viyoch J: Effects of the blended fibroin/aloe gel film on wound healing in streptozotocin-induced diabetic rats. *Biomed Mater* 2012; 7: 035008.
- Tarameshloo M, Norouzian M, Zarein-Dolab S, Dadpay M, Mohsenifar J, and Gazor R: Aloe vera gel and thyroid hormone cream may improve wound healing in Wistar rats. *Anat Cell Biol* 2012; 45: 170.
- Naeini AT, Miri R, Shafiei N, Tabandeh MR, Oryan A, and Nazifi S: Effects of topical application of *Calendula officinalis* gel on collagen and hydroxyproline content of skin in rats. *Comp Clin Pathol* 2012; 21: 253.
- Binić I, Janković A, Janković D, Janković I, and Vrucinić Z: Evaluation of healing and antimicrobial effects of herbal therapy on venous leg ulcer: pilot study. *Phytother Res* 2010; 24: 277.
- Wang T, Zhu X-K, Xue X-T, and Wu D-Y: Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. *Carbohydr Polym* 2012; 88: 75.
- Kamaratos AV, Tzirogiannis KN, Iraklianos SA, Panoutsopoulos GI, Kanellos IE, and Melidonis AI: Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. *Int Wound J* 2012 Sep 18 [Epub ahead of print]; DOI: 10.1111/j.1742-481x.2012.01082.x.
- Gregory SR, Piccolo N, Piccolo MT, Piccolo MS, and Heggors JP: Comparison of propolis skin cream to silver sulfadiazine: a naturopathic alternative to antibiotics in treatment of minor burns. *J Altern Complement Med* 2002; 8: 77.
- Smith AG, Powis RA, Pritchard DI, and Britland ST: Greenbottle (*Lucilia sericata*) larval secretions delivered from a prototype hydrogel wound dressing accelerate the closure of model wounds. *Biotechnol Prog* 2006; 22: 1690.
- Sherman RA: Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. *Diabetes Care* 2003; 26: 446.
- Burd A, Kwok CH, Hung SC, Chan HS, Gu H, Lam WK, and Huang L: A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. *Wound Repair Regen* 2007; 15: 94.
- Choi JS, Leong KW, and Yoo HS: In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). *Biomaterials* 2008; 29: 587.
- Ma K, Liao S, He L, Lu J, Ramakrishna S, and Chan CK: Effects of nanofiber/stem cell composite on wound healing in acute full-thickness skin wounds. *Tissue Eng Part A* 2011; 17: 1413.
- Sriwiriyanont P, Lynch KA, Maier EA, Hahn JM, Supp DM, and Boyce ST: Morphogenesis of chimeric hair follicles in engineered skin substitutes with human keratinocytes and murine dermal papilla cells. *Exp Dermatol* 2012; 21: 783.
- Vujanovic S and Vujanovic J: Bioresources in the pharmacotherapy and healing of burns: a mini-review. *Burns* 2013; 39: 1031.
- Bodeker GC, Ryan TJ, and Ong CK: Traditional approaches to wound healing. *Clin Dermatol* 1999; 17: 93.
- Rustad KC and Gurtner GC: Mesenchymal stem cells home to sites of injury and inflammation. *Adv Wound Care* 2012; 1: 147.
- Groeber F, Holeiter M, Hampel M, Hinderer S, and Schenke-Layland K: Skin tissue engineering—in vivo and in vitro applications. *Adv Drug Deliv Rev* 2011; 63: 352.
- Boateng JS, Matthews KH, Stevens HNE, and Eccleston GM: Wound healing dressings and drug delivery systems: a review. *J Pharm Sci* 2008; 97: 2892.
- Gurtner GC, Werner S, Barrandon Y, and Longaker MT: Wound repair and regeneration. *Nature* 2008; 453: 314.
- Guo S and DiPietro LA: Factors affecting wound healing. *J Dent Res* 2010; 89: 219.
- Enoch S and Leaper DJ: Basic science of wound healing. *Surgery* 2007; 26: 31.
- van der Plas MJ, Dambrot C, Dogterom-Ballering HC, Kruithof S, van Dissel JT, and Nibbering PH: Combinations of maggot excretions/secretions and antibiotics are effective against *Staphylococcus aureus* biofilms and the bacteria derived there from. *J Antimicrob Chemother* 2010; 65: 917.
- WHO: Traditional Medicine Strategy 2002–2005. World Health Organization, 2002.
- WHO: The Regional Strategy for Traditional Medicine in the Western Pacific (2011–2020). World Health Organization, 2012.
- Sharma Y, Jeyabalan G, and Singh R: Potential wound healing agents from medicinal plants: a review. *Pharmacologia* 2013; 4: 349.
- Ayyanar M and Ignacimuthu S: Herbal medicines for wound healing among tribal people in Southern India: ethnobotanical and scientific evidences. *Int J Appl Res Nat Prod* 2009; 2: 29.
- Atiba A, Nishimura M, Kakinuma S, Hiraoka T, Goryo M, Shimada Y, Ueno H, and Uzuka Y: Aloe vera oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor- β and fibroblast growth factor production. *Am J Surg* 2011; 201: 809.
- Eshghi F, Hosseinimehr SJ, Rahmani N, Khardemloo M, Norozi MS, and Hojati O: Effects of Aloe vera cream on posthemorrhoidectomy pain and wound healing: results of a randomized, blind, placebo-control study. *J Altern Complement Med* 2010; 16: 647.
- Takzare N, Hosseini MJ, Hasanazadeh G, Mortazavi H, Takzare A, and Habibi P: Influence of Aloe vera gel on dermal wound healing process in rat. *Toxicol Mech Methods* 2009; 19: 73.
- Upadhyay NK, Kumar R, Mandotra SK, Meena RN, Siddiqui MS, Sawhney RC, and Gupta A: Safety and healing efficacy of Sea buckthorn (*Hippophae rhamnoides* L.) seed oil on burn wounds in rats. *Food Chem Toxicol* 2009; 47: 1146.
- Gupta A, Kumar R, Pal K, Singh V, Banerjee PK, and Sawhney RC: Influence of sea buckthorn (*Hippophae rhamnoides* L.) flavone on dermal wound healing in rats. *Mol Cell Biochem* 2006; 290: 193.
- Hsiao C-Y, Hung C-Y, Tsai T-H, and Chak K-F: A study of the wound healing mechanism of a traditional chinese medicine, *Angelica sinensis*, using a proteomic approach. *Evid Based Complement Alternat Med* 2012; 2012: Article ID 467531.
- Nayak BS and Pereira LMP: *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement Alternat Med* 2006; 6: 41.
- Parente LML, Júnior RSL, Tresvenzol LMF, Vinaud MC, de Paula JR, and Paulo NM: Wound healing and anti-inflammatory effect in animal models of *Calendula officinalis* L. growing in Brazil. *Evid Based Complement Alternat Med* 2012; 2012: 375671.
- Fronza M, Heinzmann B, Hamburger M, Laufer S, and Merfort I: Determination of the wound healing effect of *Calendula* extracts using the scratch assay with 3T3 fibroblasts. *J Ethnopharmacol* 2009; 10: 126.
- Shenoy RR, Sudheendra AT, Nayak PG, Paul P, Kutty NG, and Rao CM: Normal and delayed wound healing is improved by sesamol, an active constituent of *Sesamum indicum* (L.) in albino rats. *J Ethnopharmacol* 2011; 133: 608.
- Singh DR: *Morinda citrifolia* L. (Noni): a review of the scientific validation for its nutritional and therapeutic properties. *J Diabetes Endocrinol* 2012; 3: 77.
- Nayak BS, Sandiford S, and Maxwell A: Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf.

- Evid Based Complement Alternat Med 2009; 6: 351.
42. Sharangi AB: Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)—a review. Food Res Int 2009; 42: 529.
43. Asadi SY, Parsaei P, Karimi M, Ezzati S, Zamiri A, Mohammadizadeh F, and Rafieian-Kopaei M: Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat. Int J Surg 2013; 11: 332.
44. Abu-Al-Basal MA: Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. J Ethnopharmacol 2010; 131: 443.
45. Boudreau MD and Beland FA: An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera*. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2006; 24: 103.
46. Dat AD, Poon F, Pham KB, and Doust J: *Aloe vera* for treating acute and chronic wounds. Cochrane Database Syst Rev 2012; 2: Art. No. CD008762.
47. Hamman JH: Composition and applications of *Aloe vera* leaf gel. Molecules 2008; 13: 1599.
48. Jia Y, Zhao G, and Jia J: Preliminary evaluation: the effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. J Ethnopharmacol 2008; 120: 181.
49. Pellizzoni M, Ruzickova G, Kalhotka L, and Lucini L: Antimicrobial activity of different *Aloe barbadensis* Mill. and *Aloe arborescens* Mill. leaf fractions. J Med Plants Res 2012; 6: 1975.
50. Vijayalakshmi D, Dhandapani R, Jayaveni S, Jithendra PS, Rose C, and Mandal AB: In vitro anti-inflammatory activity of *Aloe vera* by down regulation of MMP-9 in peripheral blood mononuclear cells. J Ethnopharmacol 2012; 141: 542.
51. Das S, Mishra B, Gill K, Ashraf MS, Singh AK, Sinha M, Sharma S, Xess I, Dalal K, Singh TP, and Dey S: Isolation and characterization of novel protein with anti-fungal and anti-inflammatory properties from *Aloe vera* leaf gel. Int J Biol Macromol 2011; 48: 38.
52. Choi SW, Son BW, Son YS, Park YI, Lee SK, and Chung MH: The wound-healing effect of a glycoprotein fraction isolated from *Aloe vera*. Br J Dermatol 2001; 145: 535.
53. Abdullah KM, Abdullah A, Johnson ML, Bilski JJ, Petry K, Redmer DA, Reynolds LP, and Grazul-Bilska AT: Effects of *Aloe vera* on gap junctional intercellular communication and proliferation of human diabetic and nondiabetic skin fibroblasts. J Altern Complement Med 2003; 9: 711.
54. Chithra P, Sajithlal GB, and Chandrakasan G: Influence of *Aloe vera* on the healing of dermal wounds in diabetic rats. J Ethnopharmacol 1998; 59: 195.
55. Hosseinimehr SJ, Khorasani G, Azadbakht M, Zamani P, Ghasemi M, and Ahmadi A: Effect of aloe cream versus silver sulfadiazine for healing burn wounds in rats. Acta Dermatovenol Croat 2010; 18: 2.
56. Khorasani G, Hosseinimehr SJ, Azadbakht M, Zamani A, and Mahdavi MR: *Aloe* versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. Surg Today 2009; 39: 587.
57. Pereira R, Carvalho A, Vaz DC, Gil MH, Mendes A, and Bartolo PJ: Development of novel alginate based hydrogel films for wound healing applications. Int J Biol Macromol 2013; 52: 221.
58. Pereira R, Carvalho A, Gil MH, Mendes A, and Bartolo PJ: Influence of *Aloe vera* on water absorption and enzymatic in vitro degradation of alginate hydrogel films. Carbohydr Polym 2013; 98: 311.
59. Pereira R, Tojeira A, Vaz D, Mendes A, and Bártoło P: Preparation and characterization of films based on alginate and *Aloe vera*. Int J Polym Anal Charact 2011; 16: 449.
60. Pereira RF and Bartolo PJ: Degradation behavior of biopolymer-based membranes for skin tissue regeneration. Procedia Eng 2013; 59: 258.
61. Langmead L, Feakins RM, Goldthorpe S, Holt H, Tsironi E, De Silva A, Jewell DP, and Rampton DS: Randomized, double-blind, placebo-controlled trial of oral *Aloe vera* gel for active ulcerative colitis. Aliment Pharmacol Ther 2004; 7: 739.
62. Foster M, Hunter D, and Samman S: Evaluation of the nutritional and metabolic effects of *Aloe vera*. In: *Herbal Medicine, Biomolecular and Clinical Aspects*, 2nd edition, edited by Benzie IFF and Wachtel-Galor S. Boca Raton, FL: CRC Press, 2011, pp. 37–54.
63. Muley BP, Khadabadi SS, and Banarase NB: Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): a review. Tropic J Pharmaceut Res 2009; 8: 455.
64. Basch E, Bent S, Foppa I, Haskmi S, Kroll D, Mele M, Szapary P, Ulbricht C, Vora M, and Yong S: Marigold (*Calendula officinalis* L.): an evidence-based systematic review by the Natural Standard Research Collaboration. J Herb Pharmacother 2006; 6: 135.
65. Butnariu M and Coradini CZ: Evaluation of biologically active compounds from *Calendula officinalis* flowers using spectrophotometry. Chem Cent J 2012; 6: 35.
66. Preethi KC, Kuttan G, and Kuttan R: Antioxidant potential of an extract of *Calendula officinalis* flowers in vitro and in vivo. Pharm Biol 2006; 44: 691.
67. Ukiya M, Akihisa T, Yasukawa K, Tokuda H, Suzuki T, and Kimura Y: Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. J Nat Prod 2006; 69: 1692.
68. Hamburger M, Adler S, Baumann D, Förg A, and Weinreich B: Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*). Fitoterapia 2003; 74: 328.
69. Chandran PK and Kuttan R: Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. J Clin Biochem Nutr 2008; 43: 58.
70. Barajas-Farias LM, Pérez-Carreón JL, Arce-Popoca E, Fattel-Fazenda S, Alemán-Lazarini L, Hernández-García S, Salcido-Neyoy M, Cruz-Jiménez FG, Camacho J, and Villa-Treviño S: A dual and opposite effect of *Calendula officinalis* flower extract: chemoprotector and promoter in a rat hepatocarcinogenesis model. Planta Med 2006; 72: 217.
71. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, and Montbarbon X: Phase III randomized trial of *Calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. J Clin Oncol 2004; 22: 1447.
72. Hadfield RA, Vlahovic TC, and Khan MT: The use of marigold therapy for podiatric skin conditions. Foot Ankle J 2008; 1: 1.
73. Duran V, Matic M, Jovanović M, Mimica N, Gajinović Z, Poljacki M, and Boza P: Results of the clinical examination of an ointment with marigold (*Calendula officinalis*) extract in the treatment of venous leg ulcers. Int J Tissue React 2005; 27: 101.
74. Reider N, Komericki P, Hausen BM, Fritsch P, and Aberer W: The seamy side of natural medicines: contact sensitization to arnica (*Arnica montana* L.) and marigold (*Calendula officinalis* L.). Contact Dermatitis 2001; 45: 269.
75. Mashreghi M, Rezazade Bazaz M, Mahdavi Shahri N, Asoodeh A, Mashreghi M, Behnam Rassouli M, and Golmohammadzadeh S: Topical effects of frog "*Rana ridibunda*" skin secretions on wound healing and reduction of wound microbial load. J Ethnopharmacol 2013; 145: 793.
76. Sato T and Miyata G: The nutraceutical benefit, part III: honey. Nutrition 2000; 16: 468.
77. Kwakman PHS and Zaat SAJ: Antibacterial components of honey. IUBMB Life 2012; 64: 48.
78. Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, and Kirpal-Kaur B: The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement Alternat Med 2009; 9: 34.
79. Molan PC: Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2001; 2: 13.
80. Kwakman PH, te Velde AA, de Boer L, Speijer D, Vandenbroucke-Grauls CM, and Zaat SA: How honey kills bacteria. FASEB J 2010; 24: 2576.

81. Davis SC and Perez R: Cosmeceuticals and natural products: wound healing. *Clin Dermatol* 2009; 27: 502.
82. Boekema BKHL, Pool L, and Ulrich MMW: The effect of a honey based gel and silver sulphadiazine on bacterial infections of in vitro burn wounds. *Burns* 2013; 39: 754.
83. Nassar HM, Li M, and Gregory RL: Effect of honey on *Streptococcus mutans* growth and biofilm formation. *Appl Environ Microbiol* 2012; 78: 536.
84. Alandejani T, Marsan J, Ferris W, Slinger R, and Chan F: Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngol Head Neck Surg* 2009; 141: 114.
85. Maddocks SE, Lopez MS, Rowlands RS, and Cooper RA: Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology* 2012; 158: 781.
86. Rossiter K, Cooper AJ, Voegeli D, and Lwaleed BA: Honey promotes angiogenic activity in the rat aortic ring assay. *J Wound Care* 2010; 19: 440.
87. Majtan J, Kumar P, Majtan T, Walls AF, and Klaudiny J: Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. *Exp Dermatol* 2010; 19: e73.
88. Kwakman PH, de Boer L, Ruyter-Spira CP, Creemers-Molenaar T, Helsper JP, Vandenbroucke-Grauls CM, Zaat SA, and te Velde AA: Medical-grade honey enriched with antimicrobial peptides has enhanced activity against antibiotic-resistant pathogens. *Eur J Clin Microbiol Infect Dis* 2011; 30: 251.
89. Tan MK, Adli DSH, Tumiran MA, Abdulla MA, and Yusoff KM: The efficacy of gelam honey dressing towards excisional wound healing. *Evid Based Complement Alternat Med* 2012; 2012: Article ID 805932.
90. Khoo YT, Halim AS, Singh KK, and Mohamad NA: Wound contraction effects and antibacterial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydro-fibre. *BMC Complement Alternat Med* 2010; 10: 48.
91. Malik KI, Malik MAN, and Aslam A: Honey compared with silver sulphadiazine in partial-thickness burns. *Int Wound J* 2010; 7: 413.
92. Robson V, Dodd S, and Thomas S: Standardized antibacterial honey (Medihoney) with standard therapy in wound care: randomized clinical trial. *J Adv Nurs* 2009; 65: 565.
93. Jull AB, Walker N, and Deshpande S: Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 2013; 2: CD005083.
94. Jull A, Walker N, Parag V, Molan P, and Rodgers A: Randomized clinical trial of honey-impregnated dressings for venous leg ulcers. *Br J Surg* 2008; 95: 175.
95. Lund-Nielsen B, Adamsen L, Kolmos HJ, Rørth M, Tolver A, and Gotttrup F: The effect of honey-coated bandages compared with silver-coated bandages on treatment of malignant wounds-a randomized study. *Wound Repair Regen* 2011; 19: 664.
96. Ingle R, Levin J, and Polinder K: Wound healing with honey - a randomised controlled trial. *S Afr Med J* 2006; 96: 831.
97. Sharp A: Beneficial effects of honey dressings in wound management. *Nurs Stand* 2009; 24: 66.
98. Toreti VC, Sato HH, Pastore GM, and Park YK: Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evid Based Complement Alternat Med* 2013; 2013: 697390.
99. Burdock GA: Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem Toxicol* 1998; 36: 347.
100. Pellati F, Prencipe FP, Bertelli D, and Benvenuti S: An efficient chemical analysis of phenolic acids and flavonoids in raw propolis by microwave-assisted extraction combined with high-performance liquid chromatography using the fused-core technology. *J Pharm Biomed Anal* 2013; 81-82: 126.
101. Kumazawa S, Hamasaka T, and Nakayama T: Antioxidant activity of propolis of various geographic origins. *Food Chem* 2004; 84: 329.
102. Athikomkulchai S, Awale S, Ruangrunsi N, Ruchirawat S, and Kadota S: Chemical constituents of Thai propolis. *Fitoterapia* 2013; 88C: 96.
103. Tylkowski B, Trusheva B, Bankova V, Giamberini M, Peev G, and Nikolova A: Extraction of biologically active compounds from propolis and concentration of extract by nanofiltration. *J Membr Sci* 2010; 348: 124.
104. Bufalo MC, Ferreira I, Costa G, Francisco V, Liberal J, Cruz MT, Lopes MC, Batista MT, and Sforcin JM: Propolis and its constituent caffeic acid suppress LPS-stimulated pro-inflammatory response by blocking NF- κ B and MAPK activation in macrophages. *J Ethnopharmacol* 2013; [Epub ahead of print]; DOI: 10.1016/j.jep.2013.06.004.
105. Moreira L, Dias LG, Pereira JA, and Estevinho L: Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food Chem Toxicol* 2008; 46: 3482.
106. Dias LG, Pereira AP, and Estevinho LM: Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. *Food Chem Toxicol* 2012; 50: 4246.
107. Barroso PR, Lopes-Rocha R, Pereira EM, Marinho SA, de Miranda JL, Lima NL, and Verli FD: Effect of propolis on mast cells in wound healing. *Inflammopharmacology* 2012; 20: 289.
108. Silva JC, Rodrigues S, Feás X, and Estevinho LM: Antimicrobial activity, phenolic profile and role in the inflammation of propolis. *Food Chem Toxicol* 2012; 50: 1790.
109. Stepanović S, Antić N, Dakić I, and Švabić-Vlahović M: In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microbiol Res* 2003; 158: 353.
110. Orsi RO, Fernandes A, Bankova V, and Sforcin JM: The effects of Brazilian and Bulgarian propolis in vitro against *Salmonella Typhi* and their synergism with antibiotics acting on the ribosome. *Nat Prod Res* 2012; 26: 430.
111. Oksuz H, Duran N, Tamer C, Cetin M, and Silici S: Effect of propolis in the treatment of experimental *Staphylococcus aureus* keratitis in rabbits. *Ophthalmic Res* 2005; 37: 328.
112. Sehn E, Hernandez L, Franco SL, Gonçalves CC, and Baesso ML: Dynamics of reepithelialisation and penetration rate of a bee propolis formulation during cutaneous wounds healing. *Anal Chim Acta* 2009; 635: 115.
113. Pessolato AG, Martins Ddos S, Ambrósio CE, Mançanares CA, and de Carvalho AF: Propolis and amnion reepithelialise second-degree burns in rats. *Burns* 2011; 37: 1192.
114. Olczyk P, Komosińska-Vashev K, Winsz-Szczotka K, Stojko J, Klimek K, and Kozma EM: Propolis induces chondroitin/dermatan sulphate and hyaluronic acid accumulation in the skin of burned wound. *Evid Based Complement Alternat Med* 2013; 2013: 290675.
115. McLennan SV, Bonner J, Milne S, Lo L, Charlton A, Kurup S, Jia J, Yue DK, and Twigg SM: The anti-inflammatory agent propolis improves wound healing in a rodent model of experimental diabetes. *Wound Repair Regen* 2008; 16: 706.
116. de Almeida EB, Cordeiro Cardoso J, Karla de Lima A, de Oliveira NL, de Pontes-Filho NT, Oliveira Lima S, Leal Souza IC, and de Albuquerque-Júnior RL: The incorporation of Brazilian propolis into collagen-based dressing films improves dermal burn healing. *J Ethnopharmacol* 2013; 147: 419.
117. Kucharzewski M, Kózka M, and Urbanek T: Topical treatment of nonhealing venous leg ulcer with propolis ointment. *Evid Based Complement Alternat Med* 2013; 2013: Article ID 254017.
118. Kucharzewski M, Kubacka S, Urbanek T, Wilemska-Kucharzewska K, and Morawiec T: Stanscheller: the forerunner of clinical studies on using propolis for poor and chronic nonhealing wounds. *Evid Based Complement Alternat Med* 2013; 2013: Article ID 456859.
119. Walgrave SE, Warshaw EM, and Glesne LA: Allergic contact dermatitis from propolis. *Dermatitis* 2005; 16: 209.
120. Sherman RA, Hall MJR, and Thomas S: Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annu Rev Entomol* 2000; 45: 55.
121. Blake FA, Abromeit N, Bubenheim M, Li L, and Schmelzle R: The biosurgical wound debridement: experimental investigation of efficiency

- and practicability. *Wound Repair Regen* 2007; 15: 756.
122. Sherman RA: Maggot versus conservative debridement therapy for the treatment of pressure ulcers. *Wound Repair Regen* 2002; 10: 208.
 123. Cazander G, Gottrup F, and Jukema GN: Maggot therapy for wound healing: clinical relevance, mechanisms of action and future prospects. *J Wound Technol* 2009; 5: 18.
 124. Sherman RA: Maggot therapy takes us back to the future of wound care: new and improved maggot therapy for the 21st century. *J Diabetes Sci Technol* 2009; 3: 336.
 125. Wang SY, Wang JN, Lv DC, Diao YP, and Zhang Z: Clinical research on the bio-debridement effect of maggot therapy for treatment of chronically infected lesions. *Orthop Surg* 2010; 2: 201.
 126. Gilead L, Mumcuoglu KY, and Ingber A: The use of maggot debridement therapy in the treatment of chronic wounds in hospitalised and ambulatory patients. *J Wound Care* 2012; 21: 78.
 127. Dumville JC, Worthy G, Bland JM, Cullum N, Dowson C, Iglesias C, Mitchell JL, Nelson EA, Soares MO, Torgerson DJ, and VenUS II team: Larval therapy for leg ulcers (VenUS II): randomised controlled trial. *BMJ* 2009; 338: b773.
 128. Cazander G, van Veen KE, Bouwman LH, Bernards AT, and Jukema GN: The influence of maggot excretions on PAO1 biofilm formation on different biomaterials. *Clin Orthop Relat Res* 2009; 467: 536.
 129. Margolin L and Gialanella P: Assessment of the antimicrobial properties of maggots. *Int Wound J* 2010; 7: 202.
 130. Chambers L, Woodrow S, Brown AP, Harris PD, Phillips D, Hall M, Church JC, and Pritchard DI: Degradation of extracellular matrix components by defined proteinases from the greenbottle larva *Lucilia sericata* used for the clinical debridement of non-healing wounds. *Br J Dermatol* 2003; 148: 14.
 131. Horobin AJ, Shakesheff KM, and Pritchard DI: Maggots and wound healing: an investigation of the effects of secretions from *Lucilia sericata* larvae upon the migration of human dermal fibroblasts over a fibronectin-coated surface. *Wound Repair Regen* 2005; 13: 422.
 132. Bexfield A, Nigam Y, Thomas S, and Ratcliffe NA: Detection and partial characterisation of two antibacterial factors from the excretions/secretions of the medicinal maggot *Lucilia sericata* and their activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Microbes Infect* 2004; 6: 1297.
 133. Steenvoorde P and Jukema GN: The antimicrobial activity of maggots: in-vivo results. *J Tissue Viability* 2004; 14: 97.
 134. Cazander G, van de Veerdonk MC, Vandenbroucke-Grauls CM, Schreurs MW, and Jukema GN: Maggot excretions inhibit biofilm formation on biomaterials. *Clin Orthop Relat Res* 2010; 468: 2789.
 135. Valachová I, Bohová J, Pálošová Z, Takáč P, Kozánek M, and Majtán J: Expression of lucifensin in *Lucilia sericata* medicinal maggots in infected environments. *Cell Tissue Res* 2013; 353: 165.
 136. van der Plas MJ, Dambrot C, Dogterom-Balling HC, Kruithof S, van Dissel JT, and Nibbering PH: Combinations of maggot excretions/secretions and antibiotics are effective against *Staphylococcus aureus* biofilms and the bacteria derived therefrom. *J Antimicrob Chemother* 2010; 65: 917.
 137. Cazander G, Pawiroredjo JS, Vandenbroucke-Grauls CM, Schreurs MW, and Jukema GN: Synergism between maggot excretions and antibiotics. *Wound Repair Regen* 2010; 18: 637.
 138. Horobin AJ, Shakesheff KM, and Pritchard DI: Promotion of human dermal fibroblast migration, matrix remodelling and modification of fibroblast morphology within a novel 3D model by *Lucilia sericata* larval secretions. *J Invest Dermatol* 2006; 126: 1410.
 139. Bexfield A, Bond AE, Morgan C, Wagstaff J, Newton RP, Ratcliffe NA, Dudley E, and Nigam Y: Amino acid derivatives from *Lucilia sericata* excretions/secretions may contribute to the beneficial effects of maggot therapy via increased angiogenesis. *Br J Dermatol* 2010; 162: 554.
 140. Wang S-y, Wang K, Xin Y, and Lv D-C: Maggot excretions/secretions induces human microvascular endothelial cell migration through AKT1. *Mol Biol Rep* 2010; 37: 2719.
 141. van der Plas MJ, Baldry M, van Dissel JT, Jukema GN, and Nibbering PH: Maggot secretions suppress pro-inflammatory responses of human monocytes through elevation of cyclic AMP. *Diabetologia* 2009; 52: 1962.
 142. van der Plas MJ, van der Does AM, Baldry M, Dogterom-Balling HC, van Gulpen C, van Dissel JT, Nibbering PH, and Jukema GN: Maggot excretions/secretions inhibit multiple neutrophil pro-inflammatory responses. *Microbes Infect* 2007; 9: 507.
 143. Grassberger M, Fleischmann W: The biobag - a new device for the application of medicinal maggots. *Dermatology* 2002; 204: 306.
 144. Lodge A, Jones M, and Thomas S: Maggots 'n' chips: a novel approach to the treatment of diabetic ulcers. *Br J Community Nurs* 2006; 11: 23.
 145. Felder JM, 3rd, Hechenbleikner E, Jordan M, and Jeng J: Increasing the options for management of large and complex chronic wounds with a scalable, closed-system dressing for maggot therapy. *J Burn Care Res* 2012; 33: e169.
 146. Opletalová K, Blaizot X, Mourgeon B, Chêne Y, Creveuil C, Combemale P, Laplaud AL, Sohyer-Lebreuilly I, and Domp Martin A: Maggot therapy for wound debridement: a randomized multicenter trial. *Arch Dermatol* 2012; 148: 432.
 147. Marineau ML, Herrington MT, Swenor KM, and Eron LJ: Maggot debridement therapy in the treatment of complex diabetic wounds. *Hawaii Med J* 2011; 70: 121.
 148. Cazander G, Pritchard DI, Nigam Y, Jung W, and Nibbering PH: Multiple actions of *Lucilia sericata* larvae in hard-to-heal wounds. *BioEssays* 2013; 35: 1083.
 149. Singh AP: Medicinal leech therapy (hirudotherapy): a brief overview. *Complement Ther Clin Pract* 2010; 16: 213.
 150. Michalsen A, Lüttke R, Cesur O, Afra D, Musial F, Baecker M, Fink M, and Dobos GJ: Effectiveness of leech therapy in women with symptomatic arthrosis of the first carpometacarpal joint: a randomized controlled trial. *Pain* 2008; 137: 452.
 151. Gröbe A, Michalsen A, Hanken H, Schmelzle R, Heiland M, and Blessmann M: Leech therapy in reconstructive maxillofacial surgery. *J Oral Maxillofac Surg* 2012; 70: 221.
 152. Elyassi AR, Terres J, and Rowshan HH: Medicinal leech therapy on head and neck patients: a review of literature and proposed protocol. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013; 116: e167.
 153. Whitaker IS, Oboumarzouk O, Rozen WM, Naderi N, Balasubramanian SP, Azzopardi EA, and Kon M: The efficacy of medicinal leeches in plastic and reconstructive surgery: a systematic review of 277 reported clinical cases. *Microsurgery* 2012; 32: 240.
 154. Porshinsky BS, Saha S, Grossman MD, Beery LI PR, and Stawicki SP: Clinical uses of the medicinal leech: a practical review. *J Postgrad Med* 2011; 57: 65.
 155. Michalsen A, Klotz S, Lüttke R, Moebus S, Spahn G, and Dobos GJ: Effectiveness of leech therapy in osteoarthritis of the knee: a randomized, controlled trial. *Ann Intern Med* 2003; 139: 724.
 156. Schnabl SM, Kunz C, Unglaub F, Polykandriotis E, Horch RE, and Dragu A: Acute postoperative infection with *Aeromonas hydrophila* after using medical leeches for treatment of venous congestion. *Arch Orthop Trauma Surg* 2010; 130: 1323.
 157. Rai M, Yadav A, and Gade A: Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 2009; 27: 76.
 158. Neibert K, Gopishetty V, Grigoryev A, Tokarev I, Al-Hajaj N, Vorstenbosch J, Philip A, Minko S, and Maysinger D: Wound-healing with mechanically robust and biodegradable hydrogel fibers loaded with silver nanoparticles. *Adv Healthcare Mater* 2012; 1: 621.
 159. Percival SL, Thomas J, Linton S, Okel T, Corum L, and Slone W: The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds. *Int Wound J* 2012; 9: 488.
 160. Lin YH, Lin JH, Wang SH, Ko TH, and Tseng GC: Evaluation of silver-containing activated carbon fiber for wound healing study: in vitro and in

- vivo. *J Biomed Mater Res B Appl Biomater* 2012; 100: 2288.
161. Kong H and Jang J: Antibacterial properties of novel poly(methyl methacrylate) nanofiber containing silver nanoparticles. *Langmuir* 2008; 24: 2051.
 162. Walker M and Parsons D: The biological fate of silver ions following the use of silver-containing wound care products—a review. *Int Wound J* 2012; [Epub ahead of print]; DOI: 10.1111/j.1742-481x.2012.01115.x.
 163. Poon VK and Burd A: In vitro cytotoxicity of silver: implication for clinical wound care. *Burns* 2004; 30: 140.
 164. AshaRani PV, Low Kah Mun G, Hande MP, and Valiyaveetil S: Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 2009; 3: 279.
 165. Beele H, Meuleneire F, Nahuys M, and Percival SL: A prospective randomised open label study to evaluate the potential of a new silver alginate/carboxymethylcellulose antimicrobial wound dressing to promote wound healing. *Int Wound J* 2010; 7: 262.
 166. Liu X, Lee PY, Ho CM, Lui VC, Chen Y, Che CM, Tam PK, and Wong KK: Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. *ChemMedChem* 2010; 5: 468.
 167. Miller CN, Newall N, Kapp SE, Lewin G, Karimi L, Carville K, Gliddon T, and Santamaria NM: A randomized-controlled trial comparing cadex-omer iodine and nanocrystalline silver on the healing of leg ulcers. *Wound Repair Regen* 2010; 18: 359.
 168. Muangman P, Pundee C, Opananon S, and Muangman S: A prospective, randomized trial of silver containing hydrofiber dressing versus 1% silver sulfadiazine for the treatment of partial thickness burns. *Int Wound J* 2010; 7: 271.
 169. Opananon S, Muangman P, and Namviriyachote N: Clinical effectiveness of alginate silver dressing in outpatient management of partial-thickness burns. *Int Wound J* 2010; 7: 467.
 170. Jurczak F, Dugré T, Johnstone A, Offori T, Vujovic Z, Hollander D, and on behalf of the AQUACEL Ag Surgical/Trauma Wound Study

Group: Randomised clinical trial of hydrofiber dressing with silver versus povidone-iodine gauze in the management of open surgical and traumatic wounds. *Int Wound J* 2007; 4: 66.

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