

# Naturally derived factors and their role in the promotion of angiogenesis for the healing of chronic wounds

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**Abstract** Chronic diseases such as vascular disease and diabetes are witnessing a global increase in prevalence. Such diseases often predispose patients to the development of severe, debilitating, chronic wounds. Angiogenesis, the formation of new capillaries from the pre-existing vascular network, is an essential component of wound healing and aberrant angiogenesis is evident in almost all chronic wounds. Natural products, derived from both plants and animals, provide a significant haven of compounds which have proved to be of great benefit to man and his ailments. Whilst significant advances have been made in the understanding of impaired angiogenesis in a non-healing wound, in the clinical setting, few effective agents exist that can expedite wound healing and closure. The lack of effective healing agents has led to a renewed interest in investigations into natural wound healing resources. In this review, we collate new evidence that details the potential for several natural compounds to promote angiogenesis and wound healing, most predominately via the up regulation of VEGF expression, that warrant urgent further investi-

gation for development into new pro-angiogenic/wound healing therapies.

**Keywords** Angiogenesis · Chronic wounds · Plants · Maggots · Growth factors · VEGF

## Introduction

Angiogenesis is the term given to the formation of new blood vessels from pre-existing vessels. It is a complex process, which relies on the interplay between endothelial cells, fibroblasts, macrophages and the surrounding extracellular matrix (ECM). Macrophages produce growth factors to stimulate angiogenesis [1], and the ECM also provides a reservoir of pro-angiogenic factors which are released during proteolytic degradation of the ECM during endothelial cell migration [2]. Examples of such growth factors can be seen in Table 1. Angiogenesis itself is controlled by a fine balance between these pro-angiogenic compounds and also anti-angiogenic factors (e.g. angiostatin, endostatin and thrombospondin) [3]. If the balance is tipped in favour of angiogenesis it can lead to diseases such as macular degeneration, rheumatoid arthritis, cancer growth and metastases [4]. However, if the balance is tipped towards a reduction in angiogenesis, cardiovascular disease [5], peptic ulcers and impaired wound healing can result [6].

## Wounds and wound chronicity

Wounds occur when the structure of a tissue, most notably the epidermis, is damaged following injury. Once injury has occurred the wound healing process is activated.

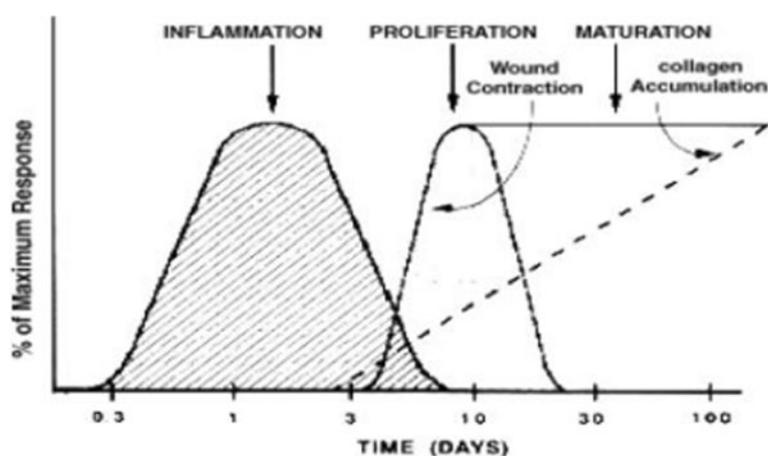
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**Table 1** Some of the major growth factors involved in wound healing [7]

Growth factor family	Members	Cell source	Actions
Transforming growth factor beta	TGF $\beta$ -1, TGF $\beta$ -2	Platelets	Fibroblast chemotaxis and activation
		Fibroblasts Macrophages	ECM deposition $\uparrow$ Collagen synthesis $\uparrow$ TIMP synthesis $\downarrow$ MMP synthesis
Platelet derived growth factor	TGF $\beta$ -3 PDGF-AA, PDGF-BB, VEGF	Platelets	May reduce scarring
		Macrophages Keratinocytes Fibroblasts	Activation of immune cells and fibroblasts ECM deposition $\uparrow$ Collagen synthesis $\uparrow$ TIMP synthesis $\downarrow$ MMP synthesis Angiogenesis
Fibroblast growth factor	Acidic FGF, Basic FGF, KGF	Macrophages Endothelial cells Fibroblasts	Angiogenesis Endothelial cell activation Keratinocyte proliferation and migration ECM deposition
Insulin-like growth factor	IGF-I, IGF-II, Insulin	Liver Skeletal muscle Fibroblasts Macrophages Neutrophils	Keratinocyte proliferation Fibroblast proliferation Endothelial cell activation Angiogenesis Collagen synthesis ECM deposition Cell metabolism
Epidermal growth factor	EGF, HB-EGF, TGF $\alpha$	Keratinocytes	Keratinocyte proliferation and migration
Connective tissue growth factor	Amphiregulin, Betacellulin CTGF	Macrophages	ECM deposition
		Fibroblasts Endothelial cells Epithelial cells	Mediates action of TGF- $\beta$ on collagen synthesis

**Fig. 1** Wound healing can be divided into three main stages: inflammation, proliferation and maturation [9]

Although elaborate and complex, the cellular processes involved are well understood comprising of (1) inflammation, (2) proliferation (formation of granulation tissue) and (3) maturation [8] and are shown in Fig. 1.

#### Chronic wounds

Wounds can be classified as acute or chronic. Acute wounds can result from burns, surgery or trauma, while

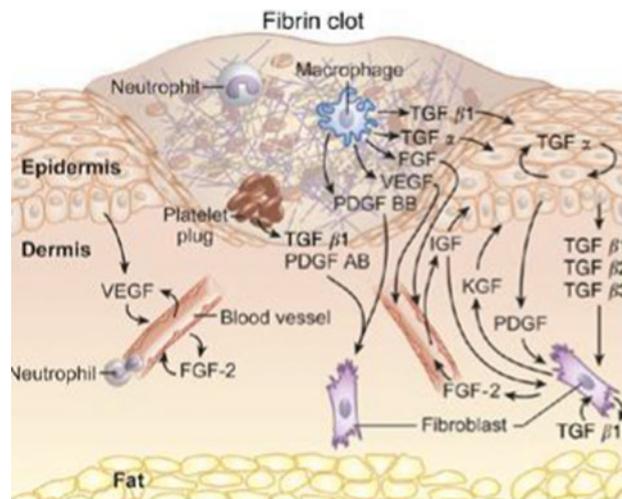
chronic wounds predominately arise due to an underlying pathophysiological problem, such as vascular insufficiency, and include arterial, diabetic, pressure and venous ulcers [1]. Since the global prevalence of chronic diseases such as vascular disease and diabetes is increasing [10], there is also a rise in the number of patients that are now susceptible to the development of severe chronic wounds. Whilst an uncomplicated acute wound will progress physiologically through the three major phases leading to complete healing, chronic wounds are characteristically non-healing. Resulting from a myriad of aetiologies (pressure, venous, arterial and diabetic neuropathies), they are unable to progress rapidly through these phases in an orderly and timely fashion, and often remain static in the inflammatory phase, providing a necrotic, sloughy, and compromised environment for bacterial proliferation. These wounds can quickly become infected and notoriously difficult to manage. Effective wound debridement and disinfection strategies are the first essential step in wound management, before a wound can progress to healing and closure.

### Wound healing

During the normal healing process, the proliferative phase begins around 4 days after tissue injury [1] when new stroma or granulation tissue starts to form. Granulation tissue is comprised of inflammatory cells, fibroblasts and newly formed blood vessels [11]. Angiogenesis is an essential component of wound healing. These newly formed blood vessels transport oxygen and nutrients to aid tissue growth [8] at the damaged site as well as the removal of the by-products of cellular metabolism [11]. Delayed wound healing is a hallmark of chronic wounds [12]. Chronic wounds differ from the acute form due to the failure of the epithelial cells to migrate over the wound bed but instead, proliferate at the wound margins and are unresponsive to growth factor signals [7]. While wound repair depends on a multitude of factors such as age, sex of patient, type of wound, depth and its location [13], aberrant angiogenesis is associated with almost all chronic wounds [14] (Fig. 2).

Wound fluid stimulates endothelial cells to proliferate and migrate [16]. Growth factors are present in the wound bed and in wound fluid [17–19]. However, it is believed that within certain chronic wounds and their fluids, there are lower levels of growth factors present due to their abnormal localisation within fibrin cuffs and co-localisation with plasma proteins that essentially “trap” the growth factors and prevent them functioning [7, 20]. In addition, it has been hypothesised that the high levels of proteinases present could degrade these growth factors, impair their function and in doing so contribute to the chronic wound phenotype [21].

There is a concerted worldwide effort to discover compounds that can aid chronic wound healing in the hope of saving patients from severe complications and amputations



**Fig. 2** Normal, physiological healing depicting the complex interplay between cells, and growth factors in the ECM of the wound bed [15]

while at the same time reducing the cost to health-care institutions [22]. It is not surprising that the topical application of growth factors to promote chronic wound healing is of interest. For example, Becaplermin, a recombinant human derived growth factor, is the only growth factor approved for the treatment of diabetic foot ulcers [23]. Meta-analysis of four multi-centred randomised control trials showed a once daily application of 100 µg/g becaplermin (in conjunction with a regimen of good ulcer care) significantly increased the prevalence of complete wound healing and significantly shortened the time taken for wounds to completely heal compared to a placebo control group (regimen of good ulcer care plus placebo gel) [24]. However, while further research into the addition of exogenous growth factors have been encouraging *in vitro*, the extrapolation into the clinical setting has not been met with such promising results [23]. This might explain the renewed interest in natural resources used years ago to help treat chronic wounds. The medical use of natural products is not a new concept and several well established therapies stem from them. For example the drug morphine is derived from the opium poppy and the anti-cancer drugs taxol and docetaxel are isolated from the bark of the Pacific yew tree and the European yew tree respectively.

In this article we will explore some of the more novel natural resources which have been shown to promote angiogenesis and therefore may have a therapeutic benefit in the treatment of non-healing, chronic wounds.

### The role of plant extracts in the stimulation of angiogenesis

Research into the use of plants to aid wound healing has highlighted several species with pro-angiogenic properties,

species include *Aloe vera* [25], *Panax ginseng* [26] and *Cinnamomum cassia* [27] (reviewed comprehensively by Majewska et al. [29] and Fan et al. respectively [28]). Many, if not all of these plant extracts have been analysed as single compounds. However, a study carried out by Gupta et al. [30], developed a poly herbal formulation (PHF) comprising of leaf extracts from the species *Hippophae rhamnoides*, *Aloe vera* and, *Curcuma longa*, which had previously been reported to have wound healing properties [31–36]. PHF or vehicle control (sterile water) were applied to wounds on both normal and diabetic rats and wound surface area measured to determine wound contraction. PHF activity was also assessed using the in vitro chorioallantoic membrane (CAM) assay. Results showed that PHF-treated wounds contracted faster than vehicle treated wounds for both normal and diabetic rats, while western blot analysis performed on granulation tissue treated with PHF revealed an increased expression of VEGF compared to vehicle control. Further analysis also revealed PHF increased angiogenesis in the CAM assay suggesting PHF potentially aids wound healing through the stimulation of angiogenesis via increasing VEGF expression [30].

#### *Chromolaena odorata* (Eupolin)

Another plant reported to have pro-angiogenic properties is *Chromolaena odorata* (Eupolin). Eupolin is an herbaceous perennial that originates from South and Central America [37]. It has been shown to have antimicrobial [38] and anti-inflammatory [39] properties as well as acting as an antioxidant [40] and has been used in the treatment of soft tissue wounds, burn wounds and skin infections [41]. Aqueous extracts of Eupolin have been shown to enhance fibroblast and endothelial cell proliferation in vitro [42]. Although the specific mode of action was not elucidated (e.g. through the enhanced expression of specific growth factors), the effect of endothelial cell proliferation may account for the wound healing benefits seen with this treatment.

#### *Picrorhiza kurrooa* (Picroliv)

Another product, Picroliv, which is derived from the roots of *Picrorhiza kurrooa* (a small herb that grows in the Himalayas) [43] has also been investigated with regards to wound healing. Singh et al. [44], investigated the effects of picroliv using an ex vivo rat aorta ring model to determine its effect on angiogenesis and its ability to heal full thickness cutaneous wounds. Picroliv was shown to increase angiogenesis (compared to untreated controls) in the rat aortic ring assay by enhancing endothelial cell capillary formation. Picroliv-treated wounds also showed a

greater number of endothelial cells and a significantly greater degree of endothelial cell migration ( $p < 0.05$ ), 7 days post wounding, when compared to untreated controls. Finally, immunohistochemical staining for VEGF showed picroliv-treated wounds had a higher expression of VEGF than untreated wounds 4 days post wounding. The authors findings suggest that picroliv may contribute to the wound healing process by enhancing angiogenesis through increased VEGF expression.

#### *Centella asiatica* (Gotu Kola)

*Centella asiatica* (Gotu Kola) is a perennial herbaceous plant found throughout India and has been used to treat high blood pressure, promote longevity and renew nerves cells [45]. The wound healing properties of Gotu Kola have also been documented [46–48] and are attributed to an isolated compound called asiaticoside. Reports of enhanced wound healing by asiaticoside have shown this extract to increase antioxidant levels in wounds [49] as well as stimulating collagen [50] and fibroblast proliferation [51, 52]. A study has shown asiaticoside can also promote wound healing via increasing angiogenesis [22]. The CAM assay was utilised and cellulose disks containing 20–80 µg of asiaticoside were applied to the membranes. New vessel formation was observed over a 72 h period. All concentrations produced an angiogenic response, with the greatest response observed at 40 µg. A recent study conducted by Kimura et al. [53], reported that a topical application of asiaticoside increased the levels of interleukin 1β, monocyte chemoattractant protein-1 (MCP-1) and VEGF in burn wound exudates. Furthermore, they concluded that enhanced wound healing was through the promotion of angiogenesis via increased MCP-1 expression stimulating VEGF expression.

#### *Blechnum orientale* (Blechnaceae)

A perennial fern native to Malaysia, *Blechnum orientale* (Blechnaceae) has also been shown to promote wound healing via its proangiogenic effects. Lai et al. [54], have shown that wounded Sprague–Dawley rats treated with a 2 % topical application of a water extract from *Blechnum orientale*, had significantly higher wound contraction compared to controls ( $p < 0.001$ ) and higher collagen synthesis. Upon histopathological examination of excised tissue, a greater number of new capillaries were observed in the treated group in comparison to control subjects.

#### *Allium sativum* (garlic)

The health benefits of garlic have been known for a long time and there are a plethora of in vitro studies showing it

to have antifungal, antimicrobial, antioxidant and anti-cancer properties as well as a beneficial effect on lowering blood pressure and cholesterol. One study has reported wound healing properties using aged garlic extract (AGE). Dorsal skin excision wounds on 1 week old chicks were topically exposed to different concentrations of AGE for 6 days. Chicks treated with the highest concentration of AGE (15 %) had significant wound closure compared to the vehicle control group ( $p < 0.001$ ). Histological examination revealed a dose-dependent and significant increase in angiogenesis ( $p < 0.001$ ) between AGE treated groups and control groups [54]. The authors concluded that further research is needed to elucidate the specific mechanisms through which AGE could be exerting its pro-angiogenic effects.

#### *Nicotiana alata* (nicotine)

Nicotine is found in cigarette smoke and plays a role in the development of cardiovascular diseases, respiratory diseases, cancers [55] as well as impairment of wound healing [56–58]. It is an alkaloid naturally present in *Nicotiana alata* tobacco plant [59] and it can also be found in lower concentrations in the tomato, potato and green pepper plants [60]. Nicotine exerts its effects by acting on nicotinic acetylcholine receptors (nAChR) [61]. However, endothelial cells also express nAChR and several studies have shown that via nAChR, nicotine stimulates endothelial cell proliferation [61, 62]. Nicotine has also been shown to increase the number of endothelial progenitor cells (EPCs) in the bone marrow and spleen of mice; increase the recruitment of EPCs to sites of angiogenesis, resulting in an increased angiogenesis in ischemic tissue [63]. A study conducted by Jacobi et al. [64], hypothesised that nicotine, via its effect on endothelial cells, could accelerate wound healing. Using normal and diabetic mouse models, skin wounds were created and treated over a 1 week period with either phosphate buffered saline (PBS) or nicotine ( $10^{-8}$  or  $10^{-9}$  M). A positive control, using basic fibroblast growth factor (bFGF) was also used. Wound healing in the normal animal models was unaffected by nicotine but in the diabetic animals, wound closure was significantly accelerated when compared to PBS control. The degree to which nicotine closed the wound in the diabetic mice was comparable to bFGF. Nicotine also significantly increased neovascularisation in the diabetic mice, and induced capillary-like sprouting in vascular explants. Another study by Morimoto et al. [65], also used the murine excisional wound model and applied PBS, bFGF and nicotine at various concentrations ( $10^{-10}$ – $10^{-1}$  M). As in the Jacobi study, Morimoto et al. showed that the rate of wound closure was increased in bFGF and nicotine treated mice compared to controls. However, at

the highest concentration, nicotine had no effect on wound closure. This effect was also seen with regards to new capillary formation. The combination of bFGF and nicotine, at  $10^{-4}$  M, produced a significant increase in capillary formation compared to controls but at  $10^{-7}$  M nicotine, only a few capillaries were observed. The greatest effect in capillary formation was seen when bFGF and nicotine ( $10^{-4}$  M) were used in combination. Thus the authors concluded that low concentrations of topically applied nicotine accelerates angiogenesis and promotes wound healing, but at higher concentrations, the beneficial effect of nicotine is abrogated.

#### *Pouteria lucuma* (lucuma)

Fruit extracts have also been shown to promote angiogenesis. One example comes from the fruit of *Pouteria lucuma*, a native fruit of Peru, which is rich in fatty acids. Rojo et al. [66], used transgenic zebrafish larvae as a model of wound healing. The transgenic larvae contained a promoter for the expression of green fluorescent protein (GFP) in endothelial cells. Following removal of the tail primordia, larvae were treated with lucuma nut oil (LNO) ( $10$ – $100$   $\mu\text{g}/\text{mL}$ ) for 24 h. Tail primordial re-growth was measured using fluorescence microscopy. Tail regeneration was significantly faster in the larvae treated with  $100$   $\mu\text{g}/\text{mL}$  LNO compared to vehicle control (water) and a greater number of GFP-positive endothelial cells were also observed in the LNO treated larvae. The authors observed that LNO enhanced wound healing by increasing endothelial cell sprouting (angiogenesis), thus improving vascularisation in the wounded area.

#### Grape seed extract

Chronic wounds fail to progress through the normal stages of wound healing. It is reported that these non-healing wounds remain stagnant in the inflammatory stage. The inflammatory stage is the mechanism during which phagocytic cells destroy invading pathogens and clear cellular debris. A by-product of this inflammatory response is the production of reactive oxygen species (ROS) [67] which are released from the neutrophils and macrophages. In normal wound healing ROS are believed to induce cytokine and growth factor expression, but greatly increased ROS levels negate this beneficial effect in chronic wounds [68]. Grape seed proanthocyanidin extract (GSPE) is reported to have anti-oxidant properties [69, 70]. Proanthocyanidins are better known as condensed tannins [71] which have been shown to play a role in wound healing [72, 73]. An in vitro study conducted by Khanna et al. [74], showed that keratinocytes pre-treated with GSPE

(2.5–10 µg/mL) for 24 h, followed by incubation with hydrogen peroxide (250 µM) had an increased VEGF mRNA and protein expression in comparison to untreated control cells. This suggests that the GSPE treatment may aid wound healing by sensitising cells to oxidant induced VEGF, which is a known growth factor for promoting angiogenesis. However, while this conclusion seems logical and the outcome promising, further works needs to be undertaken which specifically looks at GSPE treatment of endothelial cells in order to specifically determine its effect on angiogenesis.

## Polyphenols

Polyphenols, and in particular flavonoids, (compounds found in fruits, vegetables and certain beverages), are a rich source of antioxidants and as a result, they are believed to have a protective role against diseases such as cancer, cardiovascular disease and diabetes [75]. Research findings also suggest that the health benefits of polyphenols are not just attributable to their antioxidant properties but also their ability to inhibit angiogenesis [76–78]. However, recent studies have shown that polyphenols can also promote angiogenesis, Lin et al. [79] published data on the effects of ferulic acid, a polyphenol found in sweet corn, rice bran and tomatoes. Human umbilical vein endothelial cells (HUVECs) were treated with various concentrations of ferulic acid ( $10^{-7}$ – $10^{-4}$  M) for 18 h. At the higher concentrations ( $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$  M) ferulic acid induced significant endothelial cell migration through an artificial basement membrane, and capillary tube formation (vessel sprouting). Ferulic acid was also assessed using the CAM assay. At all concentrations ferulic acid was shown to induce new vessel formation while  $10^{-5}$  M ferulic acid significantly increased the expression of VEGF and PDGF in endothelial cells compared to vehicle control cells. Tulio et al. [80], have also shown that polyphenol extracts from strawberries, wild blueberries and cranberries also induce significant cell migration of HUVECs, in a wound assay, and capillary tube formation compared to the vehicle control. The authors hypothesised that the berry extracts enhanced angiogenesis via altering the redox status of the endothelial cells via activation of the PI3 Kinase/AKT pathway.

A study carried out by Negrão et al. [81], investigated the polyphenol xanthohumol (XN) and its metabolites isoxanthohumol (IXN) and phytoestrogen 8-prenylnaringenin (8PN), which are found in Hops used in beer production. The authors carried out both in vitro (HUVECs and human aortic smooth muscle cells) and in vivo (mouse matrigel plug and rat wound-healing assays) analysis. XN and IXN inhibited in vitro cell migration, invasion,

proliferation and capillary tube formation, whereas 8PN stimulated them. The in vivo assays confirmed that XN and IXN reduced blood vessel density but 8PN increased their formation. Thus it would appear that not all polyphenols and/or their metabolites may exert pro-angiogenic effects and further research on polyphenols is essential before their role in angiogenesis and wound healing can be unequivocally confirmed.

Approximately 25 % of all medication prescribed worldwide are obtained from plants. The World Health Organisation lists 252 drugs as essential for human well being. Of these, 11 % are exclusively of plant origin, and a significant number are synthetic derivatives of natural precursors [82]. While plants are generally accepted as a valuable source to obtain natural products for medicinal use, insects too are increasingly being recognised as an important source.

## The role of insect derived compounds in the stimulation of angiogenesis

Ant venom has been trialled for the treatment of arthritis [83], bee venom is being investigated for its potential therapeutic benefits in treating arthritis [84–86] and cancer [87–89]. Spider and scorpion venom are also being considered as alternative cancer treatments [90–94]. However, the most well-studied of all insects for their medicinal use is the humble maggot [95].

### The medicinal maggot

The medicinal maggot is a larval stage of the green-bottle fly, *Lucilia sericata*. Under careful clinical supervision, newly hatched larvae are placed on a chosen wound within a dressing, or foam bag, and begin to feed on necrotic and infected tissue. After 3–7 days, the larvae reach the end of their final larval stage prior to pupation, and can be removed from the wound. Depending on the nature and size of the wound, another round of first-instar maggots can be applied if needed. Modern day maggot therapy functions as a controlled, therapeutic myiasis (the invasion or infestation of living tissue by immature stages of flies). In recent years, clinical grade medicinal maggots have been used increasingly to treat and heal a variety of different festering wounds [96–105].

### Maggots and wound healing

Maggots are clinically reputed to have a 3-fold action: debridement, disinfection and healing, that is; they rapidly rid a wound of its dead necrotic tissue, whilst leaving healthy tissue intact; they appear to have a remarkable

capacity to clear rampant wound infections; and they have been shown to kick-start healing in stubborn, recalcitrant wounds [106, 107].

Recent *ex vivo* research has shown that maggots interact with the process of cellular wound healing. Several studies have shown that maggot secretions can induce changes in cell morphologies that stimulate fibroblast migration, and due to an observed degradation of fibronectin, this motogenesis is most likely to be promoted by serine proteinases from the larval secretions which may alter surface cell adhesion [106–110]. More recently, the science behind maggot therapy has been focussed on investigating its ability to promote angiogenesis in a chronic wound.

#### Pro-angiogenic factors within maggot secretions

Research from our own group has identified three pro-angiogenic compounds within maggot secretions, the amino acids L-histidine, 3-guanidinopropionic acid and L-valinol. We have demonstrated that these isolated components specifically enhance the proliferation of human endothelial cells, whilst having no similar effect on fibroblasts. In particular, valinol produced the most notable effect on endothelial cell proliferation [111].

Dried larvae of *L. sericata* are used as traditional Chinese medicine known as WuGuChong for the treatment of furuncles and carbuncles [112]. To investigate the effect of dried *L. sericata* extracts on wound healing Zhang et al. [112], applied dried extracts of *L. sericata* larvae to full thickness dorsal skin excision wounds of male Sprague–Dawley rats. After 3 days of treatment a significant increase in wound capillary density, VEGFA mRNA expression and VEGFA protein expression were detected in rat wounds treated with *L. sericata* fatty acid extracts when compared with the positive control (the Chinese wound medicine, JingWanHong). Wound contraction was also significantly increased by *L. sericata* fatty acids compared with a Vaseline negative control. Thus, the authors conclude that the observed wound healing properties of *L. Sericata* are due to its pro-angiogenic properties via the up-regulation of VEGF expression.

#### Maggot secretions and pro-angiogenic macrophage activity

Monocytes and macrophages are considered to be two predominantly vital leukocytes for wound healing [113]. Once monocytes infiltrate an area of inflamed tissue, they differentiate into either pro-inflammatory or anti-inflammatory/pro-angiogenic macrophages under the influence of cytokines and growth factors present in the wound. In a chronic wound, the balance between pro-inflammatory and anti-inflammatory macrophages is upset, in favour of pro-

inflammatory cells. Anti-inflammatory macrophages produce high levels of IL 10, bFGF and VEGF and are involved in many cellular activities including proliferation of fibroblasts and epidermal cells, and neovascularisation [114].

A recent study was carried out to investigate the effects of maggot secretions on the differentiation of monocytes into pro-inflammatory and anti-inflammatory/pro-angiogenic macrophages [114]. Peripheral blood mononuclear cells from healthy donors were stimulated with or without Lipopolysaccharides, in the presence of maggot secretions. The results showed that monocytes differentiated towards macrophages with a decreased production of the pro-inflammatory cytokines and pro-inflammatory macrophages tended towards an anti-inflammatory macrophage morphology. In addition to reducing the production of pro-inflammatory cytokines, maggot secretions also increased the production of pro-angiogenic growth factors bFGF and VEGF in anti-inflammatory macrophages [114]. These findings suggest that secretions from maggots may assist in the correction of the macrophage balance within a chronic wound and in doing so, aid wound healing via the promotion of angiogenic growth factors.

The migration of resident epidermal keratinocytes and dermal cells, including fibroblasts and dermal microvascular cells, from the wound margins into the wound bed is a crucial step in wound healing. The most widely studied protein kinase pathways known to regulate cell migration during wound healing are the PI3K:AKT1 and MEK1/2:ERK1/2 pathways. PI3K is activated by many proangiogenic factors, including VEGF and bFGF. Activation of PI3K in turn recruits and activates AKT1, which then alters the activity or abundance of specific transcription factors for cell migration and viability. The involvement of both pathways in maggot secretions-induced cell migration was investigated by Wang et al. [115]. Human microvascular epidermal cells were utilised for a wound healing assay. Cells were grown to confluency in six-well culture dishes. Cells were then exposed to maggot secretions (10 µg/mL) or vehicle control (PBS). It was observed that maggot secretions significantly increased microvascular epidermal cell migration when compared to the control group. To assess the effect of maggot secretions on the PI3K:AKT1 and MEK1/2:ERK1/2 pathways specific inhibitors for the protein kinases, AKT1 and ERK1/2 were added to the culture medium. The inhibitor to PI3K:AKT1 partially blocked maggot secretion enhanced cell migration, however there was no change in cell migration following treatment with the MEK1/2:ERK1/2 inhibitor. Protein analysis confirmed that maggot secretions activated AKT1 but no ERK1/2. Thus it was concluded that maggot secretions could be exerting their angiogenic, and hence wound healing effects, in part by the activation of the key signalling protein, Akt1.

## Conclusion

Non-healing chronic wounds cause significant discomfort and distress to patients worldwide. Currently, there is a rapid, global increase in physiological disorders such as diabetes and cardiovascular disease, conditions which underlie and pre-dispose the development of chronic wounds. Whilst significant advances have been made in the understanding of the cellular and biochemical changes that occur in a non-healing wound bed, few effective wound-healing agents have emerged that are capable of converting an indolent chronic wound into an actively healing one.

This review summarises current published investigations on natural resources which exhibit pro-angiogenic and wound healing properties. We have explored and highlighted the potential reservoir of these natural products, both plant and insect derived, which show significant promise, and could be utilised for the discovery of new pro-angiogenic/wound healing therapies. It is, of course, essential that these compounds, or synthetic derivatives, show efficacy in the clinical setting, but the urgent necessity to manage severe, debilitating chronic wounds and improve the lives of moribund patients, demands that these compounds are quickly and vigorously investigated further to determine their clinical potential as new therapeutic regimens or topical treatments.

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