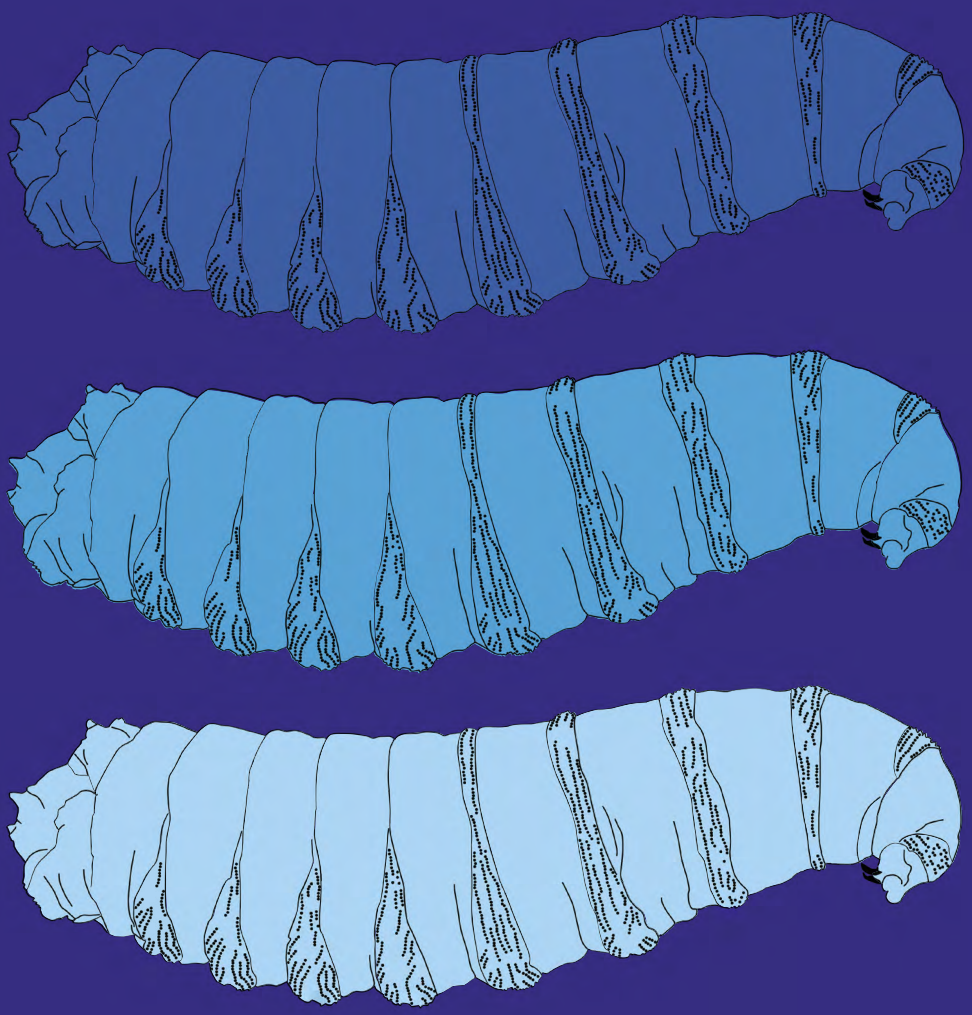
A COMPLETE GUIDE TO MAGGOT THERAPY

Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics



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10. Maggot-assisted Wound Healing

Yamni Nigam and Michael R. Wilson

Unlike any other wound care device or pharmaceutical, medicinal maggots convey multiple therapeutic benefits at the same time when applied to chronic and/or infected wounds. In addition to providing an ideal healing environment through debridement and infection control, maggot excretions and secretions actively promote wound healing through a wide range of specific physiological mechanisms and pathways. After a brief review of early studies into the healing properties of medicinal maggots, and what is known from randomised clinical trials, the chapter explains in detail the biochemical and physiological principles of maggot-mediated wound healing.

Introduction

For years there have been anecdotal reports from clinicians suggesting that wounds treated with maggots have better outcomes and heal faster compared to non-maggot treated wounds. It is only in recent years, however, that these reports have been further investigated. The evidence supporting maggot-induced promotion of healing comes from randomised controlled trials, clinical studies, and scientific laboratory investigations.

A wound typically goes through three major phases on its way to complete healing [1]. These are the phases of inflammation (including haemostasis), proliferation and maturation (or remodelling). The

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inflammatory phase serves to immediately protect the body, and results in the active recruitment of phagocytic and destructive white blood cells (leucocytes) [1]. These cells debride injured and non-viable tissue, and substantially diminish the bioburden of the wound, enabling it to progress to the next phase of healing. Wounds will only progress to healing if the debridement and disinfection processes have been successful [2].

Once the wound has entered the proliferative phase, several physiological events occur. This includes the process of granulation, which involves the laying down of new foundation tissue in the wound base. Specialised cells called fibroblasts migrate inwards from the wound margins and begin to generate and assemble collagen, the major component of wound connective tissue. Fibroblasts are stimulated by chemical activators and messengers, known as cytokines, which are mostly released by macrophages (the dominant leucocyte towards the end of the inflammatory phase). Fibroblasts themselves secrete a variety of cytokines (e.g. platelet-derived growth factor (PDGF) and tissue growth factor beta (TGFb)), allowing other vital cells such as endothelial cells and angiocytes to proliferate and grow new blood vessels [3]. New blood vessels provide much-needed oxygen and nutrients that help to facilitate the growth and proliferation of new tissue filling the wound.

Early Studies on Maggot-led Healing

One of the first reports to explore the exact mechanisms involved in wound healing by maggots was a study which examined the outcomes of pressure ulcers in spinal cord injury patients, some of which were treated conventionally, and some with maggots. Not only did debridement occur more rapidly in patients treated with maggot therapy, but also the wound surface area decreased and wound healing was faster [4]. A later, larger-scale study discovered that pressure ulcers treated with maggots were found to contain twice the amount of granulation tissue compared to wounds not treated with maggot therapy. A set of 31 wounds was observed, at first receiving conventional treatment and then the very same wounds were treated with maggot therapy. While conventional treatment resulted in a 1.2 cm^2 per week increase in wound area, subsequent maggot therapy resulted in a 1.2

cm^2 decrease in wound surface area [5]. In a further study examining changes to necrotic tissue and total surface area of non-healing wounds following maggot therapy, the size and shape of diabetic foot ulcers was calculated, including other measures such as coverage of granulation tissue and time to healing. Maggot-treated wounds were associated with hastened growth of granulation tissue and greater healing rates. After 4 weeks of maggot therapy and 1 to 2 treatment cycles per week, on average 56% of the wound base was covered with healthy granulation tissue. However, in conventionally treated wounds granulation tissue covered only on average 15% of the wound base after 4 weeks [6].

The findings from Sherman and colleagues encouraged other researchers to undertake clinical investigations on maggot-assisted wound healing. For example, in a study of 30 patients with chronic leg ulcers who were treated with maggots over 4 days, investigators measured several parameters after removal of the larvae [7]. These included a wound score on a sliding scale from 0 to 15, with higher scores indicating poorer wound condition. The score accounted for various characteristics including slough coverage, exudation, inflammation, and malodour. Wound scores dramatically improved after only a single application of maggots, from 13.5 to 6.3, with a particularly remarkable improvement in granulation. The investigators also used remittance spectroscopy to monitor the optical characteristics of the wound area in the visible and near-infra-red spectral range. Before maggot therapy, wounds were necrotic, fibrin-covered, and without granulation tissue. Typical spectra in these wounds showed a high remittance. However, after maggot therapy, the remittance spectra changed. The authors observed a marked decrease in remittance because of the large absorption of the fresh granulation tissue. The findings suggested an increase in local blood flow and a decrease in oedema, which were responsible for red granulation tissue formation and an improvement in wound healing following treatment with maggots [7].

A separate clinical study assessing the healing of diabetic foot ulcers in non-ambulatory patients, found that time to healing was significantly shorter in the group treated with maggot therapy (18.5 weeks) compared to the control group (22.4 weeks), and patients in the control group were also three times more likely to require an amputation [8].

Randomised Controlled Trials on Wound Healing Using Maggots

There have also been various clinical trials including randomised controlled trials (RCTs) that have investigated the effectiveness of maggot therapy. More recently, meta-analyses of these studies have been conducted that have sought to collate and review the evidence. The first such meta-analysis consisted of 7 trials conducted from 1995–2009, including three randomised controlled trials and four non-randomised trials [9]. This analysis suggested that whilst larvae were effective in debridement, there was not enough evidence to say that maggot therapy resulted in faster healing. This was primarily due to the sub-optimal design of some of the studies with the authors citing problems concerning small sample sizes, unclear inclusion and exclusion criteria, lack of blinding of outcome assessors, and lack of randomisation resulting in poor-quality data. It was concluded that better designed investigations were required in this area in order to form more concrete conclusions about the efficacy of maggot therapy compared to conventional treatments [9].

A later meta-analysis considered 12 comparative studies, including six randomised controlled trials, from 2000–2014. Based on the analysis of these twelve studies, the authors concluded that larval therapy was both more effective and more efficient in the debridement of chronic ulcers when compared with conventional treatments, but it was also associated with quicker healing rates of chronic wounds. They noted a shortened time to healing in ulcers, a longer antibiotic-free time period, and a decreased amputation risk compared with conventional therapies [10]. A more recent review of clinical studies of maggot therapy from 2000–2015 showed a significantly higher rate of successful debridement compared with conventional treatment, but also that time to healing was significantly (3.1 weeks) shorter than the conventional strand [11]. It was, however, suggested that the shorter time to healing may be aided by more successful debridement using maggot therapy, or a combination of this with some other enhanced-healing mechanism. Such reports highlight the need for larger, well-designed RCTs to scientifically investigate and evaluate maggot-enhanced healing. Unfortunately, the relative paucity of such gold-standard trials for maggot therapy

means that some researchers, practitioners, and regulators remain sceptical about the benefits of the therapy. Chapter 11 also discusses the need for randomised controlled trials as a necessary step toward the establishment of new medicinal fly species in clinical practice [12].

Scientific Mechanisms by Which Maggots Promote Wound Healing

Ever since early scientific reports of maggot therapy and the discovery of their effectiveness in wound treatment, there have been theories on how maggots stimulate the healing process. Initially, it was suspected that wound healing was somehow stimulated by the physical action of the larvae crawling over the wound surface [13, 14]. Early research suggested that stimulation of tissue growth was due to the action of allantoin or ammonia bicarbonate, both of which are present in maggot excretions, and both of which were found to promote wound healing [15, 16]. In recent years, research has focussed on the notion that wound healing effects are primarily due to the action of maggot secretions which can aid wound healing by a number of different mechanisms. As such, several *in vitro* studies provide convincing molecular evidence to support the hypothesis that maggots promote wound healing through mechanisms other than debridement.

Effect of Maggots on the Inflammatory Phase of Wound Healing

Chronic wounds are, by definition, non-healing and are unable to progress rapidly through the three main stages of wound healing (inflammation, proliferation and maturation), often remaining in the inflammatory phase [17]. Various investigations have found that maggot secretions may play a role in inhibiting the mechanisms and processes that perpetuate this chronic state of inflammation, and thereby help the wound to heal. Neutrophils are the first line of defence against infection. Upon phagocytosis of a pathogen, a respiratory burst occurs which causes the reduction of oxygen to form superoxide, hydrogen peroxide (H_2O_2), and the hydroxyl radical. These reactive oxygen species, along

with other oxygen-derived molecules, participate in the elimination of the pathogen [18].

Several studies suggest that the beneficial effects of maggots may lie in their ability to reduce these levels of pro-inflammatory factors. For example, a study investigating the effect of *L. sericata* salivary gland extract on the activity of human neutrophils (stimulated with opsonised zymosan) found that exposure to high concentrations of the extract resulted in a significant reduction in both the level of superoxide and the release of myeloperoxidase, a pro-inflammatory molecule [19]. Just prior to this, a study had also looked at the effect of maggot secretions on human neutrophil pro-inflammatory responses [20]. Neutrophils from healthy donors were stimulated with either formyl-MetLeu-Phe (fMLP) or phorbol-12-myristate-13-acetate (PMA) and incubated with increasing concentrations of secretions ranging from 0.5–100 μg/mL. Hydrogen peroxide production via fMLP-stimulated neutrophils was inhibited by as little as 5 μg/mL of secretions, whilst PMA-induced hydrogen peroxide production was inhibited in a dose-dependent manner. Incubation with maggot secretions also reduced fMLP-stimulated expression of CD11b/CD18, a protein involved in the innate immune system which mediates inflammation, and inhibited fMLP-induced neutrophil migration. The authors concluded that maggot secretions inhibited the production and expression of pro-inflammatory mediators such as reactive oxygen species and components of the complement system, and prevented the movement of neutrophils to the wound area. Therefore, maggot secretion may contribute to the healing of chronic wounds via the inhibition of ongoing inflammation and tissue breakdown [20].

Monocytes are white blood cell precursors to macrophages. Along with neutrophils they are involved in the innate immune system and engulf apoptotic cells and pathogens as well as controlling the inflammatory process by secreting cytokines and growth factors, which in turn recruit more inflammatory cells [21]. Monocytes and macrophages are considered to be the two most vital leukocytes for wound healing [22]. 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, which is a cytokine with potent anti-inflammatory properties, as well as growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). In addition, they are involved in many cellular activities including proliferation of fibroblasts and epidermal cells, and the formation of new blood vessels [23]. The effect of maggot secretions on the release of pro-inflammatory mediators from human monocytes has been investigated using mononuclear cells obtained from healthy donors incubated with a range of maggot secretions in the presence or absence of lipopolysaccharides or lipoteichoic acid [21]. Maggot secretions inhibited the production of the pro-inflammatory cytokines TNF-a, IL-12, and macrophage migration inhibitory factor while increasing the production of the anti-inflammatory cytokine IL-10, all via elevated levels of cyclic AMP [21]. The same authors also conducted a study to investigate the effects of maggot secretions on the differentiation of monocytes into pro-inflammatory and anti-inflammatory/pro-angiogenic macrophages [23]. Peripheral blood mononuclear cells from healthy donors were stimulated with or without lipopolysaccharides, in the presence of maggot secretions. The results showed that maggot secretions steered monocyte differentiation towards a pro-angiogenic macrophage type with a decreased production of the pro-inflammatory cytokines [23].

In addition, the human complement system, a cascade of potent serum enzymes which form part of the innate immune system, plays an important role in regulating this inflammatory response. Activation of the complement system plays a critical role in the innate immune response to tissue injury and is necessary for normal physiological healing [24]. However, an inappropriate activation system can cause prolonged inflammation, thus maintaining tissue damage and impeding wound healing [22, 24, 25]. Cazander and colleagues [26] hypothesised that the wound healing effect of maggot therapy may be due to the maggot secretions exerting an effect on the complement system. Using sera from healthy and post-operative patients in an *in vitro* study, maggot secretions were found to reduce complement activation in both cohorts of patients in a dose-dependent manner. The authors concluded that this

was most likely through the degradation of complement components C3 and C4 in the enzymatic cascade [26].

Thus, it would appear from *in vitro* investigations to date that maggot secretions may aid wound healing by reducing pro-inflammatory mediators and breaking down components of the complement system. It has been suggested that suppressing the complement system could be evolutionarily advantageous to the larvae in surviving on live hosts. Whilst medicinal maggots of the species *L. sericata* and *L. cuprina* do not harm the living tissue of humans, they are a common pest of other animals, especially sheep, in which harmful myiasis by larvae can be fatal if left untreated. It is suggested that the maggots may have evolved this trait as a means of suppressing the immune response of their hosts to enable their continued feeding [26]. This has an unintended benefit on non-healing human wounds, as suppression of the inflammatory response can help in the progression of wound healing.

Effects of Maggots on Fibroblasts

A healing wound will show the growth and appearance of healthy extracellular matrix (ECM) or granulation tissue, a collagen-rich connective tissue which forms on the surface of a healing wound. Fibroblasts are the cells that synthesise ECM and collagen, so it is critical that these cells can migrate into the wound bed once debridement and disinfection of the wound is complete. The growth stimulating effects of maggots on human dermal fibroblasts have been extensively investigated.

In one of the first investigations of this kind, human fibroblasts were cultured *in vitro* and treated either with larval haemolymph, larval alimentary secretions, or the insect hormone 20-hydroxyecdysone (EC). Human fibroblasts showed a significant increase in cell numbers under all three treatment conditions when compared to untreated cells. High concentrations of EC can be found in *L. sericata* haemolymph leading the authors to conclude that insect growth-stimulating molecules (such as EC and others) present in the maggot extracts could be secreted into the host wound, where they stimulate human wound tissue. Therefore, this is another mechanism by which maggot therapy promotes wound healing [27].

When tissues are injured, fibroblasts in the local vicinity also differentiate into myo-fibroblasts, which are highly contractile and aid wound contraction [28]. Fibroblasts also secrete proteases, which play a key role in the wound healing process (the main proteases being matrix metalloproteinases and serine proteases) by breaking down damaged proteins that constitute the ECM, resulting in new tissue formation and closure of the wound [29, 30]. In 2003, a study identified the predominant proteolytic enzymes secreted from *L. sericata* larvae as serine proteases (trypsin-like and chymotrypsin-like) and a metalloproteinase, and showed that maggot secretions could solubilise fibrin clots and degrade laminin, collagen types I and III, and fibronectin [31]. The authors concluded that the proteolytic activity of maggot secretions may promote wound healing by remodelling components of the ECM [31].

Fibroblasts not only synthesise components of ECM but interact with it via integrins and are modified/modulated by growth factors and cytokines that reside within the ECM to increase proliferation and change morphology [31]. In order to investigate this further, the same authors developed a three-dimensional model to observe fibroblast migration and morphology in response to maggot secretions [32]. This novel model more closely mimicked the microenvironment *in vivo* and provided a better understanding of the interactions between the ECM, resident cells, and maggot secretions in the wound healing process. Both 2D and 3D studies conducted demonstrated that fibroblasts seeded onto fibronectin- or collagen-coated plates and incubated with maggot secretions showed reduced cell-ECM adhesion and filopodia inhibition, thus preventing cell spreading along with degradation of fibrin [29, 32, 33]. In addition, the authors showed that maggot secretions could enhance the migration of fibroblasts across fibronectin-coated surfaces, which correlated with the degradation of fibronectin by serine proteinases (which they had previously identified in maggot secretions) [33]. Therefore, maggot secretions may aid in wound healing by increasing fibroblast cell motility, releasing bioactive compounds from the ECM, and enhancing interactions between fibroblasts and the ECM.

The effect of maggot secretions on fibroblast motility was also corroborated later by researchers [34], who found that maggot secretion markedly accelerated the migration of fibroblasts and epidermal

keratinocytes during wound closure without any significant mitogenic effect. In support of the above study, subsequent findings [35] showed that when fibroblasts were cultured with extracts from maggot salivary glands (over 5–10 days), the cells exhibited a phenotype typical of cells with an increased metabolism (e.g. voluminous nuclear membrane, large reticular nuclei, distinct Golgi apparatus etc.) leading the authors to conclude that maggot salivary gland extracts increased cell metabolism and protein production, which correlated with the formation of microfibrillar nets required for fibroblast cell migration and production of ECM. The same effect was also noted when incorporating larval secretions into a hydrogel which was then applied to a laboratory wound model, raising the possibility that larval secretions or isolated active compounds could be successfully incorporated into pharmaceutical products for wound healing [32].

Finally, the effects of maggot secretions on haemostatic processes have also been investigated. During haemostasis, blood platelets are activated, which results in the initiation and formation of a platelet plug. In conjunction with this is the coagulation cascade which causes soluble fibrinogen to be converted into a network of insoluble fibrin fibres. This process prevents excessive bleeding and provides a matrix to aid future cell migration [36]. This fibrin network or clot is then broken down by the fibrinolytic system. Fibrinolysis is activated by the serine proteases, urokinase-type plasminogen activator (Urokinase or uPA) or tissue-type plasminogen activator, which converts plasminogen to plasmin which, in turn, degrades the fibrin mesh [37]. However, in chronic wounds, fibrinolysis may be impaired due to increased levels of the fibrinolysis inhibitor (plasminogen activator inhibitor-150), potentially leading to the formation of necrotic tissue and fibrin slough that can act as a nutrient-rich source for bacteria [38]. These, in turn, may increase the inflammatory response and prevent wound closure [39].

Interestingly, it was reported in a 2014 *in vitro* study that the serine protease sericase, present in maggot secretions, augmented plasminogen activator-induced fibrinolysis via the degradation of plasminogen into an activated form which could, in turn, lyse clots and fibrin cuffs to potentially allow cells at the wound edge to migrate through the ECM to form granulation tissue, and thus aid in the closure of a wound [39].

Effects of Maggots on Angiogenesis and Tissue Reperfusion

Maggots may also play a vital role in tissue reperfusion, with larval activity found to combat a number of actions which impair blood-flow to the wound site [20, 23, 39]. Angiogenesis is the formation of new blood vessels from pre-existing vessels, and it is a vital process for wound healing, relying on the interplay between endothelial cells, fibroblasts, macrophages and the surrounding ECM. Macrophages produce growth factors to stimulate angiogenesis and the ECM also provides a reservoir of pro-angiogenic factors which are released during its degradation. In 2014, a finding of considerable clinical significance was reported by a team of Japanese surgeons, who showed a quantifiable improvement in tissue perfusion (amount of oxygen reaching the wound site) after the application of maggots [40]. They did this by measuring the skin perfusion pressure (the amount of oxygen getting to the tissue) surrounding a post-amputation chronic wound of a patient with critical limb ischaemia, before and after maggot therapy. Following treatment, skin perfusion pressure increased dramatically, from 12 to 54 mmHg on the dorsal aspect of the foot, and from 17 to 44 mmHg on the plantar aspect. The authors surmised that maggot therapy had somehow contributed to the increase in blood supply to the ischaemic wound [40].

In parallel with clinical observations and studies, there is a growing body of *in vitro* work that elucidates how larval secretions help to stimulate angiogenesis and encourage the development of new blood vessels. Research from our own group supports the notion that maggot secretions promote angiogenesis. We identified the amino acids L-histidine, 3-guanidinopropionic acid, and L-valinol in maggot secretions and demonstrated, *in vitro*, that these isolated components specifically enhanced the proliferation of human endothelial cells, whilst having no effect on fibroblasts, with the amino acid valinol eliciting the greatest increase in endothelial cell proliferation [41]. In another investigation, Zhang and colleagues [42] investigated the effect of dried *L. sericata* extracts on full thickness dorsal-skin excision wounds of male Sprague Dawley rats. After 3 days of treatment, a significant increase in wound capillary density, vascular endothelial growth factor A (*VEGFA*) mRNA expression, and VEGF-A protein expression were detected in rat wounds treated with *L. sericata* fatty acid extracts compared to

treatment with the Chinese wound medicine, JingWanHong (positive control). Wound contraction was also significantly increased compared to treatment with a negative control (Vaseline). Thus, the observed wound healing properties of maggot secretions may be due in part to pro-angiogenic properties via the up-regulation of vascular endothelial growth factor (VEGF) expression. Additionally, van der Plas and colleagues [23] showed that in addition to reducing the production of pro-inflammatory cytokines, maggot secretions also increased the production of pro-angiogenic growth factors, basic fibroblast growth factor (bFGF) and VEGF in anti-inflammatory macrophages. These findings suggest that secretions from maggots may assist in the correction of the macrophage balance within a chronic wound 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 during the proliferative phase of 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 pro-angiogenic 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 secretion-induced cell migration was investigated by Wang and colleagues [43]. Human microvascular epidermal cells were utilised for a wound healing assay. Cells were grown to confluency in 6-well culture dishes and were then exposed to maggot secretions (10μg/mL) or a control treatment (phosphate buffered saline solution). It was observed that maggot secretions significantly increased microvascular epidermal cell migration when compared with 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 wound healing effects, in part by the activation of the key signalling protein, AKT1 [43].

Recent studies have shed further light on the role of maggots in enhancing wound healing through angiogenesis. In one experiment, researchers incubated endothelial cells (cells which give rise to new capillaries) with maggot secretions [44]. They showed significantly higher proliferation of endothelial cells and the formation of capillary tubes compared with cells that were incubated with a control. The same researchers also monitored levels of two glycoproteins (CD34 and CD68) which up-regulate endothelial cell activity and are markers of angiogenesis and proliferation. The researchers found that wound tissue obtained from patients after maggot therapy had more of these proteins than they had before. These raised levels of glycoproteins enabled better endothelial cell activity and promoted enhanced angiogenesis [44].

A recent thorough and convincing study exploring the molecule micro-RNA (miR-126), both *in vitro* and *in vivo*, further increased our understanding of how maggots support angiogenesis [45]. MiR-126 is expressed by endothelial cells and is responsible for many positive functions, including angiogenesis, whilst low levels of this RNA are associated with vascular disease and poor angiogenesis. The researchers cultured human umbilical vein endothelial cells in the presence of larval secretions and saw a significant rise in expression of miR-126. Next, the investigators turned their attention to patients and examined miR-126 levels in peripheral blood. The patients examined were grouped: 79 patients with normal blood glucose; 93 patients with type II diabetes, but with no diabetic foot ulcers (DFU), and 90 patients with type II diabetes and the existence of one or more DFU. The researchers discovered that the 79 patients with normal blood glucose exhibited normal miR-126 levels; the 93 patients with type II diabetes, but no DFU had low miR-126 levels, and the 90 patients with type II diabetes and DFU had very low miR-126 levels. The 90 patients with DFU were then divided into two groups, with 47 being treated with maggots, and 43 untreated (control). The investigators discovered that miR-126 levels in all 47 patients increased and were significantly higher following treatment with maggots. They concluded that maggot therapy increased the expression of a key molecule involved in capillary formation and wound healing. Thus, mounting scientific evidence suggests that maggots stimulate

blood capillary formation, resulting in more oxygen to the wound [45]. These findings may also explain the clinical results of the Japanese skin perfusion pressure study mentioned earlier [40].

Growth Factor Effects of Maggot Secretions

Endogenous hepatocyte growth factor (HGF) is important for cutaneous wound healing. In a recent study blood samples from patients treated with maggots showed a significant increase in HGF after just a single application [46]. The authors attributed the increased levels of HGF to proteases within the secretions, leading to the promotion of healthy granulation tissue. The expression of this factor was also found to increase in proportion to the protein concentration of the maggot secretions. The authors went on to suggest that formation of healthy granulation tissue observed during maggot therapy resulted from the increased presence of HGF, and that maggot secretions promoted HGF production via a positive feedback loop involving HGF and the STAT3 signalling pathway [46]. The STAT3 signalling pathway was also confirmed in a recent study in which the authors detected five signalling pathways that are involved in wound healing, and suggested that two of these were activated in the presence of maggot whole-body extracts and accompanied by their downstream gene expression [47].

Wound healing is regulated and controlled by a number of key human growth factors (e.g. VEGF or platelet-derived growth factor (PDGF)), and larvae produce their own array of insect hormones and factors which control their growth as they develop [48]. A recent laboratory study explored the homology of factors in maggot secretions and human growth factors that are known to be pivotal to wound healing. Although this was a preliminary study, Western blots with maggot secretions demonstrated the presence of proteins homologous to the human growth factors VEGF and PDGF, among others [49]. It is therefore likely that these homologous maggot proteins support accelerated wound healing in maggot therapy.

Summary

Unlike any other wound care device or pharmaceutical, medicinal maggots convey multiple therapeutic benefits at the same time when applied to chronic and/or infected wounds. As discussed in previous chapters, maggots are highly efficient and precise when it comes to debridement of dead or devitalised tissue [50, 51]. Cadavers, the primary breeding substrate for flies that are suitable for maggot therapy, are microbe- and pathogen-rich environments. Therefore, maggots had to evolve strategies to control infection [52, 53], which they bring to the chronic wound environment, either on their own in the case of beneficial myiasis, or when applied therapeutically by clinicians for maggot therapy [53, 54]. Finally, this chapter presents the evidence for maggot-assisted wound healing. Of course, debridement and infection control are important enablers of wound healing, but it is now clear that medicinal maggots have a far more active role in wound healing. The maggots' excretions and secretions actively promote wound healing through a wide range of specific physiological mechanisms and pathways. Consequently, there is good reason to believe that the wound healing benefits of maggot therapy may be maximised by repeated reapplication of medicinal maggots onto an already clean and debrided wound-bed [55]. Of course, it is tempting to identify for this purpose active compounds from maggot excretions and secretions that may be developed into pharmaceuticals. However, as mentioned in the previous two chapters [50, 53] on the therapeutic principles of maggot therapy, such a reductionist approach to the complex chronic wound may be more convenient to the patient and practitioner but whether it is as efficacious and affordable as whole-organism maggot therapy is uncertain and may depend greatly on the nature of the wound and its stage along the healing trajectory.

References

1. Komi, D.E.A., K. Khomtchouk, and P.L. Santa Maria, *A Review of the Contribution of Mast Cells in Wound Healing: Involved Molecular and Cellular Mechanisms*. Clinical Reviews in Allergy & Immunology, 2020. 58(3): pp. 298–312, https://doi.org/10.1007/s12016-019-08729-w.

2. Bazaliński, D., et al., *Effectiveness of Chronic Wound Debridement with the Use of Larvae of Lucilia Sericata*. Journal of Clinical Medicine, 2019. 8(11): pp. 1845–1845, https://doi.org/10.3390/jcm8111845.

3. Creager, M.D., et al., *3.18 Immunohistochemistry*. 2017, Elsevier. pp. 387–405.

4. Sherman, R.A., F. Wyle, and M. Vulpe, *Maggot Therapy for Treating Pressure Ulcers in Spinal Cord Injury Patients*. The Journal of Spinal Cord Medicine, 1995. 18(2): pp. 71–74, https://doi.org/10.1080/10790268.1995.11719382.

5. Sherman, R.A., *Maggot versus Conservative Debridement Therapy for the Treatment of Pressure Ulcers*. Wound Repair and Regeneration, 2002. 10(4): pp. 208–214, https://doi.org/10.1046/j.1524-475X.2002.10403.x.

6. Sherman, R.A., *Maggot Therapy for Treating Diabetic Foot Ulcers Unresponsive to Conventional Therapy*. Diabetes Care, 2003. 26(2): pp. 446–451, https://doi.org/10.2337/diacare.26.2.446.

7. Wollina, U., et al., *Biosurgery Supports Granulation and Debridement in Chronic Wounds — Clinical Data and Remittance Spectroscopy Measurement*. International Journal of Dermatology, 2002. 41(10): pp. 635–639, https://doi.org/10.1046/j.1365-4362.2002.01354.x.

8. Armstrong, D.G., et al., *Maggot Therapy in "Lower-extremity Hospice" Wound Care: Fewer Amputations and More Antibiotic-free Days*. Journal of the American Podiatric Medical Association, 2005. 95(3): pp. 254–257, https://doi.org/10.7547/0950254.

9. Zarchi, K. and G.B.E. Jemec, *The Efficacy of Maggot Debridement Therapy — A Review of Comparative Clinical Trials*. International Wound Journal, 2012. 9(5): pp. 469–477, https://doi.org/10.1111/j.1742-481X.2011.00919.x.

10. Sun, X., et al., *A Systematic Review of Maggot Debridement Therapy for Chronically Infected Wounds and Ulcers*. International Journal of Infectious Diseases, 2014. 25: pp. 32–37, https://doi.org/10.1016/j.ijid.2014.03.1397.

11. Siribumrungwong, B., C. Wilasrusmee, and K. Rerkasem, *Maggot Therapy in Angiopathic Leg Ulcers: A Systematic Review and Meta-analysis*. The International Journal of Lower Extremity Wounds, 2018. 17(4): pp. 227–235, https://doi.org/10.1177/1534734618816882.

12. Thyssen, P.J. and F.S. Masiero, *Bioprospecting and Testing of New Fly Species for Maggot Therapy*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 195–234, https://doi.org/10.11647/OBP.0300.11.

13. Buchman, J., *The Rationale of the Treatment of Chronic Osteomyelitis with Special Reference to Maggot Therapy*. Annals of Surgery, 1934. 99(2): pp. 251–259, https://doi.org/10.1097/00000658-193402000-00003.

14. Buchman, J. and J.E. Blair, *Maggots and Their Use in the Treatment of Chronic Osteomyelitis*. Surgery, Gynecology and Obstetrics, 1932. 55: pp. 177–190.

15. Robinson, W., *Stimulation of Healing in Non-healing Wounds by Allantoin Occurring in Maggot Secretions and of Wide Biological Distribution*. The Journal of Bone & Joint Surgery, 1935. 17(2): pp. 267–271.

16. Robinson, W., *Ammonium Bicarbonate Secreted by Surgical Maggots Stimulates Healing in Purulent Wounds*. The American Journal of Surgery, 1940. 47(1): pp. 111–115, https://doi.org/10.1016/S0002-9610(40)90125-8.

17. Frykberg, R.G. and J. Banks, *Challenges in the Treatment of Chronic Wounds*. Advances in Wound Care, 2015. 4(9): pp. 560–582, https://doi.org/10.1089/wound.2015.0635.

18. Uhlinger, D.J., et al., *The Respiratory Burst Oxidase of Human Neutrophils. Guanine Nucleotides and Arachidonate Regulate the Assembly of a Multicomponent Complex in a Semirecombinant Cell-free System*. The Journal of Biological Chemistry, 1993. 268(12): pp. 8624–8631, https://doi.org/10.1016/0891-5849(90)90668-9.

19. Pecivova, J., et al., *Effect of the Extract from Salivary Glands of Lucilia sericata on Human Neutrophils*. Neuro Endocrinology Letters, 2008. 29(5): pp. 794–797.

20. van der Plas, M.J.A., et al., *Maggot Excretions/Secretions Inhibit Multiple Neutrophil Pro-inflammatory Responses*. Microbes and Infection, 2007. 9(4): pp. 507–514, https://doi.org/10.1016/j.micinf.2007.01.008.

21. van der Plas, M.J.A., et al., *Maggot Secretions Suppress Pro-inflammatory Responses of Human Monocytes through Elevation of Cyclic AMP*. Diabetologia, 2009. 52(9): pp. 1962–1970, https://doi.org/10.1007/s00125-009-1432-6.

22. Bryant, R.A. and D.P. Nix, *Acute and Chronic Wounds: Current Management Concepts*. 2007, Mosby, Missouri.

23. van der Plas, M.J.A., J.T. van Dissel, and P.H. Nibbering, *Maggot Secretions Skew Monocyte-macrophage Differentiation away from a Pro-inflammatory to a Pro-angiogenic Type*. PLoS ONE, 2009. 4(11), https://doi.org/10.1371/journal.pone.0008071.

24. Cazander, G., G.N. Jukema, and P.H. Nibbering, *Complement Activation and Inhibition in Wound Healing*. Clinical and Developmental Immunology, 2012. 2012: pp. 1–14, https://doi.org/10.1155/2012/534291.

25. Makrides, S.C., *Therapeutic Inhibition of the Complement System*. Pharmacological Reviews, 1998. 50(1): pp. 59–87.

26. Cazander, G., et al., *Maggot Excretions Affect the Human Complement System*. Wound Repair and Regeneration, 2012. 20(6): pp. 879–886, https://doi.org/10.1111/j.1524-475X.2012.00850.x.

27. Prete, P.E., *Growth Effects of Phaenicia sericata Larval Extracts on Fibroblasts: Mechanism for Wound Healing by Maggot Therapy*. Life Sciences, 1997. 60(8): pp. 505–510, https://doi.org/10.1016/S0024-3205(96)00688-1.

28. Li, B. and J.H. Wang, *Fibroblasts and Myofibroblasts in Wound Healing: Force Generation and Measurement*. Journal of Tissue Viability, 2011. 20(4): pp. 108–120, https://doi.org/10.1016/j.jtv.2009.11.004.

29. Horobin, A.J., et al., *Maggots and Wound Healing: An Investigation of the Effects of Secretions from Lucilia sericata Larvae upon Interactions between Human Dermal Fibroblasts and Extracellular Matrix Components*. British Journal of Dermatology, 2003. 148(5): pp. 923–933, https://doi.org/10.1046/j.1365-2133.2003.05314.x.

30. International, C., *The Role of Proteases in Wound Diagnostics. An Expert Working Group Review*. London: Wounds International, 2011.

31. Chambers, L., et al., *Degradation of Extracellular Matrix Components by Defined Proteinases from the Greenbottle Larva Lucilia sericata Used for the Clinical Debridement of Non-healing Wounds*. British Journal of Dermatology, 2003. 148(1): pp. 14–23, https://doi.org/10.1046/j.1365-2133.2003.04935.x.

32. Horobin, A.J., K.M. Shakesheff, and D.I. Pritchard, *Promotion of Human Dermal Fibroblast Migration, Matrix Remodelling and Modification of Fibroblast Morphology within a Novel 3D Model by Lucilia sericata Larval Secretions*. The Journal of Investigative Dermatology, 2006. 126(6): pp. 1410–1418, https://doi.org/10.1038/sj.jid.5700256.

33. Horobin, A.J., K.M. Shakesheff, and D.I. Pritchard, *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 and Regeneration, 2005. 13(4): pp. 422–433, https://doi.org/10.1111/j.1067-1927.2005.130410.x.

34. Smith, A.G., et al., *Greenbottle (Lucilia sericata) Larval Secretions Delivered from a Prototype Hydrogel Wound Dressing Accelerate the Closure of Model Wounds*. Biotechnology Progress, 2006. 22(6): pp. 1690–1696, https://doi.org/10.1021/bp0601600.

35. Polakovičova, S., et al., *The Effect of Salivary Gland Extract of Lucilia sericata Maggots on Human Dermal Fibroblast Proliferation within Collagen/Hyaluronan Membrane in vitro: Transmission Electron Microscopy Study*. Advances in Skin & Wound Care, 2015. 28(5): pp. 221–226, https://doi.org/10.1097/01.ASW.0000461260.03630.a0.

36. Laurens, N., P. Koolwijk, and M.P.M. De Maat, *Fibrin Structure and Wound Healing*. Journal of Thrombosis and Haemostasis, 2006. 4(5): pp. 932–939, https://doi.org/10.1111/j.1538-7836.2006.01861.x.

37. van Meijer, M. and H. Pannekoek, *Structure of Plasminogen Activator Inhibitor 1 (PAI-1) and Its Function in Fibrinolysis: An Update*. Fibrinolysis, 1995. 9(5): pp. 263–276, https://doi.org/10.1016/S0268-9499(95)80015-8.

38. Wysocki, A.B., et al., *Temporal Expression of Urokinase Plasminogen Activator, Plasminogen Activator Inhibitor and Gelatinase-B in Chronic Wound Fluid Switches from a Chronic to Acute Wound Profile with Progression to Healing*.

Wound Repair and Regeneration, 1999. 7(3): pp. 154–165, https://doi.org/10.1046/j.1524-475X.1999.00154.x.

39. van der Plas, M.J.A., et al., *A Novel Serine Protease Secreted by Medicinal Maggots Enhances Plasminogen Activator-induced Fibrinolysis*. PloS ONE, 2014. 9(3): pp. e92096-e92096, https://doi.org/10.1371/journal.pone.0092096.

40. Maeda, T.M., et al., *Increase in Skin Perfusion Pressure after Maggot Debridement Therapy for Critical Limb Ischaemia*. Clinical and Experimental Dermatology, 2014. 39(8): pp. 911–914, https://doi.org/10.1111/ced.12454.

41. Bexfield, A., et al., *Amino Acid Derivatives from Lucilia sericata Excretions/Secretions May Contribute to the Beneficial Effects of Maggot Therapy via Increased Angiogenesis*. British Journal of Dermatology, 2010. 162(3): pp. 554–562, https://doi.org/10.1111/j.1365-2133.2009.09530.x.

42. Zhang, Z., et al., *Fatty Acid Extracts from Lucilia sericata Larvae Promote Murine Cutaneous Wound Healing by Angiogenic Activity*. Lipids in Health and Disease, 2010. 9(1): p. 24, https://doi.org/10.1186/1476-511X-9-24.

43. Wang, S., et al., *Maggot Excretions/Secretions Induces Human Microvascular Endothelial Cell Migration through AKT1*. Molecular Biology Reports, 2010. 37(6): pp. 2719–2725, https://doi.org/10.1007/s11033-009-9806-x.

44. Sun, X., et al., *Maggot Debridement Therapy Promotes Diabetic Foot Wound Healing by Up-regulating Endothelial Cell Activity*. Journal of Diabetes and its Complications, 2016. 30(2): pp. 318–322, https://doi.org/10.1016/j.jdiacomp.2015.11.009.

45. Zhang, J., et al., *Increasing the miR-126 Expression in the Peripheral Blood of Patients with Diabetic Foot Ulcers Treated with Maggot Debridement Therapy*. Journal of Diabetes and its Complications, 2017. 31(1): pp. 241–244, https://doi.org/10.1016/j.jdiacomp.2016.07.026.

46. Honda, K., et al., *A Novel Mechanism in Maggot Debridement Therapy: Protease in Excretion/Secretion Promotes Hepatocyte Growth Factor Production*. American Journal of Physiology. Cell Physiology, 2011. 301(6): pp. C1423-C1430, https://doi.org/10.1152/ajpcell.00065.2011.

47. Li, P.N., et al., *Molecular Events Underlying Maggot Extract Promoted Rat in vivo and Human in vitro Skin Wound Healing*. Wound Repair and Regeneration, 2015. 23(1): pp. 65–73, https://doi.org/10.1111/wrr.12243.

48. Nijhout, H.F., et al., *The Developmental Control of Size in Insects*. Wiley Interdisciplinary Reviews: Developmental Biology, 2014. 3(1): pp. 113–134, https://doi.org/10.1002/wdev.124.

49. Evans, R., et al., *Human Growth Factor Homologues, Detected in Externalised Secretions of Medicinal Larvae, Could Be Responsible for Maggot-induced Wound Healing*. International Journal of Research in Pharmacy and Biosciences, 2019. 6(5): pp. 1–10.

50. Nigam, Y. and M.R. Wilson, *Maggot Debridement*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 143–152, https://doi.org/10.11647/OBP.0300.08.

51. Sherman, R. and F. Stadler, *Wound Aetiologies, Patient Characteristics, and Healthcare Settings Amenable to Maggot Therapy*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 39–62, https://doi.org/10.11647/OBP.0300.03.

52. Harvey, M., *The Natural History of Medicinal Flies*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 121–142, https://doi.org/10.11647/OBP.0300.07.

53. Nigam, Y. and M.R. Wilson, *The Antimicrobial Activity of Medicinal Maggots*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 153–174, https://doi.org/10.11647/OBP.0300.09.

54. Sherman, R., *Medicinal Maggot Application and Maggot Therapy Dressing Technology*, in *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*, F. Stadler (ed.). 2022, Cambridge: Open Book Publishers, pp. 79–96, https://doi.org/10.11647/OBP.0300.05.

55. Pritchard, D.I. and Y. Nigam, *Maximising the Secondary Beneficial Effects of Larval Debridement Therapy*. Journal of Wound Care, 2013. 22(11): pp. 610–616, https://doi.org/10.12968/jowc.2013.22.11.610.