TANG HUMANITAS MEDICINE

Review

Immunomodulatory properties of medicinal maggots *Lucilia sericata* in wound healing process

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ABSTRACT

The healing properties of medicinal maggots (larval stage of *Lucilia sericata*) are widely used in the chirurgical debridement of non-healing wounds including diabetic foot ulcers, venous and pressure ulcers, where classical approaches have failed. Several kinds of wounds are prone to complications coming out of a specific wound bed environment. There are multi-resistant bacterial species present, their pathogenic impact is multiplied by their ability to form a biofilm. Moreover, immunological events in chronic wounds differ from those in acute wounds. Non-healing wounds are cycled in the early inflammation phase with increased levels of inflammation attributes like inflammation cytokines and matrix metalloproteinases produced by inflammation phase cells. Application of larval therapy promotes progress in the healing process to the next stages involving tissue granulation and re-epithelisation. Larval debridement is an effective method of cleaning the wound of cell debris, necrotic tissue and bacterial load. This happens in a mechanical and biological manner, but the whole complex mechanism of the maggot healing activity is still not fully elucidated. Centuries of clinical practice brings noticeable proof of the maggots' beneficial effect in wound healing management. This long history led to the investigation of the bioactive components of the larval body and its extracts *in vitro*. We introduce a review which describes the immunomodulation impact of maggot body components on the cellular and molecular levels of the wound healing process.

Keywords wound healing, Lucilia, maggot debridement therapy

INTRODUCTION

Chronic, non-healing wounds are major health care problems worldwide. Recent estimates indicate that 1 to 2% of the population in developing countries will experience chronic skin wounds during their lifetimes. In the United States, annual chronic wound treatment costs in excess of US$25 billion. Nearly 2% of European health budgets is spent on the impaired healing of chronic wounds.

The healing process in chronic wounds is generally prolonged, incomplete and uncoordinated, resulting in poor anatomic and functional outcomes. It is now accepted that the tissue of all chronic wounds is colonized by polymicrobial flora (Bowler and Davies, 1999). At present, there are two main strategies used to prevent and treat clinical infections in non-healing wounds: systemic and topical antibiotics or antiseptics (Kosinsky and Lipsky, 2010). However, in recent decades, there has been an increasing world-wide spread of hospital-acquired pathogens exhibiting resistance against one or more of the clinically applied antibiotics. This serious problem attracted renewed attention to the old-fashioned and almost forgotten therapy of maggot debridement therapy (MDT). The use of medicinal maggots in USA was approved by the Food and Drug Administration as a medical device in 2004 (Steenvoorde et al., 2007). Over the last few years, several comparative clinical trials investigating the efficacy of MDT have been performed (Dumville et al., 2009; Sherman, 2002). In terms of debridement, MDT is more effective than conventional therapies (Sherman, 2002). On the other hand, in terms of wound healing and remodeling, the outcomes of clinical trials are controversial (Zarchi and Jamec, 2012).

MDT has attracted great attention due to its successful application and efficacy in the elimination of multi-drug resistant wound pathogens (Bello et al., 2001; Jaklič et al., 2008).

Chronic wounds

The primary function of the skin is to serve as a protective barrier against the environment. Loss of the integrity of a large area of the skin as a result of injury or illness may lead to major disability or even death (Adam et al., 1999). The primary goals of wound healing are wound closure and functional scar creation.

Cutaneous wound healing is highly coordinated and a complex process. Recent advances in cellular and molecular biology have greatly expanded our understanding of the biologic processes involved in wound repair and tissue regeneration and have led to improvements in wound care (Clark, 1996). Several different cell types and molecules, including growth factors and components of the extracellular matrix (ECM), play crucial roles. Normal wound healing involves a series of four phases: (1) haemostasis, (2) inflammation, (3) migration and proliferation and (4) remodeling (Clark, 1996). These phases include events like bleeding, coagulation and initiation of an acute inflammatory response to the initial injury, regeneration, migration and

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proliferation of connective tissue and parenchyma cells, synthesis of extracellular matrix proteins, remodeling of new parenchyma and connective tissue and collagen deposition regulated by cytokines and growth factors (Rivera and Spencer, 2007). Clinically, wounds can be categorized as either acute or chronic – non-healing wounds depending upon the timescale of healing or the tendency to relapse (Yager et al., 2007). Non-healing wounds represent a significant cause of morbidity and mortality for a large amount of the population (Brem et al., 2000). One of the underlying mechanisms responsible for the failure of chronic wounds to heal is an out-of-control inflammatory response that is self-sustaining (Menke et al., 2007). Chronic wounds may result from various causes, including naturopathic, pressure, arterial and venous insufficiencies, burns and vasculitis. The three major types of chronic wounds that are prevalent are venous and arterial ulcers, diabetic ulcers and pressure ulcers (Velnar et al., 2009).

As mentioned above, chronic wounds result in enormous health care expenditures. Also, patients with chronic wounds suffer incalculable deprivation in their private life fields (Bjarnsholt et al., 2008). Diagnosis of chronic wounds limits mobility, and this leads to being limited in work opportunities and making social contacts. In many cases, patients suffering from chronic wounds have to deal with intensive pain (Nelzen et al., 1991).

Chronic wounds are prone to complications. The complications of chronic wounds include functional limitations, infections and malignant transformation. Abscess formation, osteomyelitis, gangrene, and even sepsis all may occur as a result of an infected wound. Chronic wounds in foot ulcers are one of the most common causes of nontraumatic amputation (Chraibi et al., 2004).

The abnormal microbiologic environment of the wound is among the many other factors suggested as the cause of reversed healing (Bowler and Davies, 1999). The abnormal presence of bacteria in wounds prolongs the wound healing inflammatory phase (Diegelmann, 2003). This delays the process in which wounds start to heal. In chronic wounds, the critical factors are the density of microorganisms and spectrum of specific pathogens present in the wound bed (Bowler and Davies, 1999; Mangram et al., 1999). Bendy et al. (1964) reported that healing in deceits ulcers was inhibited if the bacterial load was greater than 106 colony forming units/ml (CFU/ml) of wound fluid. The majority of wounds are polymicrobial, involving both aerobes and anaerobes. The microenvironment of a chronic wound shows a domination of anaerobes. Dowd et al. (2008) performed a broad survey of wounds using a variety of molecular methods and concluded that the bacterial communities in diabetic foot ulcers had a high degree of diversity. The most prevalent populations of bacteria identified in chronic wounds from patients with diabetic ulcers are *Staphylococcus* (especially *S. aureus*), *Peptoniphilus, Pseudomonas, Anaerococcus, Enterococcus, Bacteroides, Veillonella, Finegoldia*, and *Clostridium spp.* (Dowd et al., 2008). According to Bowler and Davies (1999) there is a greater diversity of microorganisms in infected leg ulcers than in non-infected leg ulcers. These observations support the view of Kingston and Seal (1990), who argued that all species associated with a microbial disease should be considered potentially synergistic. This indicates that microbial interactions may induce an enhanced pathogenic effect (Bello et al., 2001).

Bacteria grow in various forms. Planktonic bacteria are very common. An alternative form in which bacteria are able to exist is biofilm. Bacterial biofilms grow in a sessile or in an adherent form, which differs from the planktonic-free living form of bacteria. The biofilm is often associated with chronic infections and is resistant to antimicrobial agents (Bello et al., 2001). According to the definition by Donlan et al. (1999), biofilm is a microbial-derived sessile community characterized by cells that are irreversibly attached to a substratum, interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rates and gene transcription. It has been shown that chronic wound pathogenic biofilm is generally polymicrobial with a wide variety of bacteria with different physiological and phenotypic preferences (Costerton et al., 1999). Bacterial biofilms consist of about 80% extracellular polymeric substances (EPS), composed of polysaccharides, proteins and nucleic acids, and about 20% bacterial cells (Costerton and Stewart, 2001). Biofilm is structured by certain species such as *Pseudomonas aeruginosa* and *S. aureus* which are often predominant. The ways in which biofilms are resistant to different antimicrobial agents include an inability of the antimicrobial agent to penetrate the formation of the biofilm, slow the rate of growth of the biofilm bacteria due to nutrient limitations or an adoption of a distinct phenotype of bacteria as a response to growth on a surface (Costerton et al., 1999). The inflammatory reaction of a chronic wound differs markedly from that of an acute healing wound. At the molecular level, non-healing wounds tend to be stuck in a chronically pro-inflammatory cytokine status level, which reverses when the wounds begin to heal (Granick and Gamelli, 2007). The normal function of inflammation in an acute wound is to prepare the wound bed for healing by removing necrotic tissue, debris and bacterial contaminants as well as activating fibroblasts (Velnar et al., 2009). In a non-healing wound, neutrophils are present all the time during the healing process. Persistent recruitment and activation of neutrophils in the wound bed are caused by pressure tissue trauma, bacterial overgrowth, leukocyte trapping or ischemic perfusion injury (Diegelmann, 2003). The results of the continuing up-regulation of the inflammatory cascade lead to abnormal inflammatory cells and cytokine profile of a chronic wound. The large number of activated neutrophils leads to the excessive production of degradative matrix metalloproteinases (MMPs) (Lobmann et al., 2005). Another study where biopsies taken from the edge of chronic venous ulcers revealed that epidermal cells were in a heightened proliferative state, but the epidermal basement membrane lacked type IV basement membrane collagen, which is necessary if the epithelial cells are to attach and migrate. These observations suggested that wound cells were present but did not have an appropriate structure over which to migrate (Schultz, 2007). Attention turned to the role of proteases in wound healing. Proteolytic degradation of the ECM is an essential part of wound repair and remodeling, removal of damaged components, cell migration during wound re-epithelialization and revascularization. Restructuring of the ECM is necessary to allow cells to adhere and form basement membrane. The activities of MMPs are regulated by a family of tissue inhibitors of metalloproteinases (TIMPs), which decrease their activities. Successful wound healing requires a balance between MMP and TIMP levels to control the synthesis and degradation of ECM components (Brem et al., 2007). Also, elevated levels of MMPs of chronic wounds suggest that a highly proteolytic environment impedes healing (Rogers et al., 1995). Vaalamo et al. (1996) in a study on normally healing acute wounds versus chronic wound - venous ulcers found that the inhibitor TIMP-1 was only detectable in acute wounds. Furthermore, the wound fluid of chronic wounds shows an increased level of MMPs. Trengove et al. (1999) found that MMP activity was 30-times higher in chronic wounds compared with that in acute wounds. Bullen et al. (1995) found

that TIMP levels were lower and MMP-9 levels were higher in the chronic wound fluid of pressure ulcer. Besides degrading ECM components like fibronectin, proteases in chronic wound fluids degrade exogenous growth factors important for the following phases of wound healing such as epidermal growth factor (EGF), tissue growth factor 1-α (TGF1-α), or platelet derived growth factor (PDGF) (Ladwig et al., 2002; Trengove et al., 1999). Elevated proteinases levels in chronic wounds are primarily of neutrophil origin, including collagenase (MMP-8), gelatinase (MMP-9), neutrophil elastase, cathepsin G, and urokinase - type plasminogen activator (Yager and Nwomeh, 1999). The over-expression of the MMPs as well as the elevated levels of pro inflammatory cytokines are observed in chronic wounds. Trengove et al. (1999) reported high levels of the inflammatory cytokines IL-1, IL-6, and TNF-α in fluids collected from venous ulcers, and Harris et al. (1995) also found that cytokine levels were generally higher in wound fluids from non-healing ulcers than healing ulcers. This suggest that chronic wounds have elevated levels of pro-inflammatory cytokines, and the molecular environment changes to a less pro-inflammatory cytokine environment as chronic wounds begin to heal (Falabella and Kirsner, 2005).

To summarize, all chronic wounds begin as acute wounds but fail to progress through the normal healing process and become locked in an extended inflammatory phase. In this phase, there are increased levels of proteases such as MMPs, elastase, plasmin, and thrombin, leading to the deterioration of the structure of the provisional matrix and an inability of the wound cells to proliferate and migrate (Granick and Gamelli, 2007). As the ECM is constantly degraded, the tissue perceives that there is still injury and maintains the inflammatory cascade which continues to draw in neutrophils, macrophages and other phagocytic cells. The massive influx of neutrophils release cytokines, reactive oxygen species (ROS), and inflammatory mediators, which injure host tissue in a continuous cycle (Tarnuzzer and Schultz, 1996).

Maggots of *L. sericata* and their immunomodulatory effects

The mechanisms underlying the healing properties of maggot therapy have been examined since the initiation of maggot therapy as a medical treatment option. The molecular and cellular mechanisms of the maggots' healing effect are still not definitely elucidated nor are the substances produced by maggots which are responsible for the stimulation of the healing process of chronic wounds.

Maggot E/S and neutrophils

Neutrophils are phagocytic cells that are a component of the innate immune system and which play a primary role in the defence against bacterial infection. Following binding to the vascular endothelium at sites of inflammation, they extravasate and migrate down concentration gradients of chemotactic cytokines/chemokines such as IL-8, IL-1 and the complement component C5a. Neutrophils are activated as a consequence of interactions with chemotactic agents and complement components, and of the phagocytosis of opsonized bacteria. Neutrophil degranulation and cell death result in the loss of proteolytic enzymes and ROS to the wound tissue, and, if neutrophil infiltration is prolonged, it can contribute to a delay in healing. Although ROS are thought to possess certain beneficial antimicrobial properties, prolonged exposure to elevated levels of ROS causes cell damage and may inhibit the healing of both acute and chronic wounds (Gordillo and Sen, 2003).

Examination of maggot E/S effects on neutrophil activity and viability revealed that E/S contains heat labile components which are able to dose-dependently inhibit the release of elastase and hydrogen peroxide production in activated neutrophils. Elastase degrades the ECM and delays epithelial repair mechanisms. This happens without negatively affecting neutrophil viability. The most efficient concentration of maggot E/S-inhibiting neutrophil product is 50 mg/ml. Maggot E/S did not affect the phagocytosis and intracellular killing of *Candida albicans* by neutrophils. There was also a dose-dependent inhibition of activated neutrophil migration observed. The concentration of 0.5 mg/ml of maggot E/S inhibited neutrophil migration towards N-formyl-methionine-leucine-phenylalanine (fMLP), which is a strong chemoattractant of bacterial origin. E/S dose-dependently reduced the fMLP-stimulated expression of neutrophil adhesion CD11b/CD18 (Integrins involved in attachment to endothelial cells and transendothelial migration into blood vessels to facilitate access to a wound bed by neutrophils.). These findings suggest that maggot E/S is able to modulate neutrophil adhesion to endothelial cells. Maggot E/S dose-dependently induced a rise in intracellular cAMP concentration, suggesting that cAMP-dependent mechanisms may be involved in the E/S-mediated inhibition of neutrophil pro-inflammatory responses (Van der Plas et al., 2007).

Pečivová et al. (2008) have studied the effect of salivary glands' extract (SGE) of maggots on opsonized zymosan-stimulated whole blood chemiluminescence, superoxide generation and MPO (myeloperoxidase) release from human neutrophils. Crude maggots SGE had no significant effect either on superoxide generation and MPO release from isolated unstimulated human neutrophils or on the activity of the isolated enzymes. However, the crude extract from maggot salivary glands in the highest concentration (0.5 mg/ml) significantly decreased opsonized zymosan-stimulated blood chemiluminescence, superoxide generation and MPO release. This study concluded that *L. sericata* maggots SGE dose-dependently decreased neutrophil respiratory bursts and degranulation.

Finally, it can be concluded that maggot E/S inhibits multiple neutrophil pro-inflammatory responses, including chemotaxis, degranulation, respiratory burst and integrin expression, without affecting antimicrobial activity, which may protect against prolonged inflammation and silence the chronic inflammation.

Maggot E/S and monocytes/macrophages

Following neutrophil apoptosis, approximately three days after injury, macrophages immigrate to the wound site. Macrophages belong to the very important group of immune cells involved in wound healing, which exhibit different immunological functions in the skin, including phagocytosis and antigen presentation. Tissue macrophages are cells derived from peripheral blood monocytes. In injured tissue, monocytes migrate through the vessel wall; they release enzymes that fragment ECM proteins, creating space for monocytes to migrate to the wound bed. Macrophages can be activated either classically (by lipopolysaccharide (LPS), INF-γ) or alternatively (by IL-4, IL-13). LPS-stimulated macrophages are capable of synthesizing and secreting inflammatory mediators, including TNF-α, nitric oxide (NO) and IL-6. IL-4-activated macrophages play an important role in wound healing and angiogenesis (Gordon, 2003).

In addition to the abovementioned properties, macrophages produce many other cytokines and growth factors that stimulate new capillary growth, collagen synthesis and fibrosis (Mirza and Dipietro, 2003).

Maggot E/S exerted similar effects on monocytes to those observed with neutrophils. Van der Plas and co-workers (2009a) demonstrated that stimulated monocyte migration was reduced by incubation with maggot E/S using a concentration from 35

to 70µg/ml with no lethal effect on cell viability. Antimicrobial properties like phagocytosis and the intracellular killing of bacteria (*S. aureus*) were unaffected by the E/S treatment.

The expression of CD11b (receptor involved in cell adhesion and phagocytosis) was up-regulated in the maggot E/S presence. One hour of incubation with maggot E/S decreased the production of pro-inflammatory cytokines TNF-α, IL-12p40 and MIF–migration inhibitory factor. Maggot E/S also inhibited the production of macrophage inflammatory protein (MIP) by LPS and lipoteichoic acid (LTA) stimulated monocytes. Furthermore, maggot E/S stimulated the production of the anti-inflammatory cytokine IL-10 in macrophages with an effective concentration of 35µg/ml. A similar effect on monocyte cytokine secretion was observed when maggot E/S was applied before or after LPS exposure. This suggests that maggot E/S is capable of interfering with an ongoing inflammatory response (Van der Plas et al., 2009a).

The pre-treatment of monocytes with an inhibitor of the cAMP dependent protein kinase A-activation reduced the anti-inflammatory effects of maggot E/S on LPS-stimulated cytokine production. This confirms that maggot E/S acts through a cAMP-dependent mechanism (Van der Plas et al., 2009a).

Once monocytes infiltrate into the infected wound bed, they differentiate into either pro-inflammatory or anti-inflammatory and pro-angiogenic macrophages under the influence of cytokines and growth factors present in the wound (Buc, 2001). The numbers of pro-inflammatory and anti-inflammatory macrophages are unbalanced in a chronic wound (Koh and Dipietro, 2011).

Van der Plas et al. (2009b) incubated monocytes for six days in the presence of maggot E/S and a granulocyte macrophage-colony stimulating factor (GM-CSF) in order to develop the pro-inflammatory macrophages (MØ-1) and another monocyte group incubated with maggot E/S and macrophage-colony stimulating factor (M-CSF), the factor able to differentiate anti-inflammatory/pro-angiogenic macrophages (MØ-2). The pro-inflammatory macrophages (MØ-1) are fried egg-shaped macrophages with the intensive secretion of pro-inflammatory cytokine IL-12 and low secretion of anti-inflammatory cytokine IL-10 in response to LPS. Otherwise, anti-inflammatory and pro-angiogenic macrophages (MØ-2) are characterized by a stretched, spindle-like morphology, the expression of CD163, and the low secretion of IL-12 pro-inflammatory cytokine and decreased secretion of anti-inflammatory cytokine IL-10 production in response to LPS. Pro-inflammatory macrophages secrete cytokines and chemokines responsible for recruiting and activating immune cells such as neutrophils, monocytes and macrophages involved in the elimination of infectious agents (Park and Barbul, 2004). In addition, these cytokines lead to the expression of co-stimulatory molecules on macrophages essential for T-cell activation. When the infection is cleared, the balance shifts from pro-inflammatory macrophages to macrophages with anti-inflammatory/pro-angiogenic cytokines and growth factor activities. These cells are involved in the clearance of apoptotic cells, neovascularisation and fibroblast and epidermal cell proliferation (Xu et al., 2006).

The presence of maggot E/S resulted in monocyte differentiation to anti-inflammatory macrophages with a decreased production of the pro-inflammatory cytokines, TNF-α, IL-12p40 and MIF in a dose-dependent manner following stimulation with LPS. Pro-inflammatory macrophages tended towards the anti-inflammatory macrophage morphology in the presence of maggot E/S, but maggot E/S did not induce CD163 expression. According to these results, maggot E/S performed its anti-inflammatory effects on differentiating cells and could not convert the type of matured macrophages (Van der Plas et al., 2009b).

The anti-inflammatory macrophages suppress inflammation directly or indirectly and are important for neovascularisation, cell proliferation and ECM synthesis. The pro-angiogenic activities of anti-inflammatory macrophages through the production of vessel growth factor (VEGF) and basic fibroblast growth factor (bFGF) may induce the formation of new blood vessels and granulation tissue required for progression to the next wound healing phase. These cells also play a major role in matrix synthesis through the secretion of basement membrane components, such as collagen (Bryant and Nix, 2007).

Maggot E/S-derived compounds and fibroblasts and endothelial cells

The migration of epidermal keratinocytes and dermal cells, like fibroblasts and dermal microvascular cells, from the wound margins into the wound bed is crucial for 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 recruits and activates AKT1 which regulates the activities or abundances of specific transcription factors for cell migration and viability (Gentilini et al., 2007, Jiang et al., 2006).

The participation of both pathways in E/S-induced cell migration was investigated with the use of specific inhibitors for the protein kinases, AKT1 and ERK1/2 (Wang et al., 2009). Maggot E/S significantly induced the migration of human microvascular endothelial cells (HMEC1) and increased wound healing by 30% in a scratch test compared with negative controls. E/S concentration of 0.1–10 µg/ml had no effect on HMEC1 cell viability or proliferation. Examination of the maggot E/S effect with a concentration 10 µg/ml on total and phosphorylated cellular AKT1 and ERK1/2 levels using western blot analysis revealed that maggot E/S time-dependently activated AKT1, reaching maximum phosphorylation after 10 min, followed by a decline to basal levels by 60 min. The total AKT1 and ERK1/2 levels were not affected. The inclusion of a specific AKT1 inhibitor completely inhibited E/S-induced AKT1 phosphorylation. Using the inhibitor in a HMEC1 migration assay resulted in only a 50% reduction in migration, suggesting that other signaling pathways may be involved. E/S had no effect on the activation of ERK1/2 (Wang et al., 2009).

Pro-angiogenic compounds were detected within maggot E/S including the amino acids L-histidine, 3-guanidinopropionic acid (GPA) and L-valinol (Bexfield et al., 2009). These three identified compounds stimulated the proliferation of human endothelial cells but had no similar effect on fibroblasts. Valinol showed the most notable effect on endothelial cell proliferation, increasing cell density by 25% after exposure to concentrations present in the E/S fraction less than 500Da for 48 hours. GPA and histidine also significantly increased cell density, achieving a 25% increase after a 72-hour incubation period. Valinol showed at the high concentrations a non-specific toxic effect. The amino acid constitution of maggot E/S may contribute to accelerated wound healing during maggot therapy. GPA is a creatine analogue, a class of compounds which has many biological activities, for example, anti-inflammatory effects, which correspond with the clinical observations associated with maggot therapy (Bexfield et al., 2009).

Furthermore, it has been reported that fatty acids from homogenised maggots were shown to increase angiogenesis via the stimulation of VEGF protein expression. VEGF has

pro-angiogenic activities via the PI3K pathway. Fatty acids extracted from dried *L. sericata* stimulated angiogenesis and increased the rate of wound healing in rat models via the increased transcription and translation of vascular endothelial growth factor A (VEGFA). VEGFA is a mitogen for endothelial cells and a stimulator of angiogenesis (Lieu et al., 2011). After three days of treatment by fatty acid extracts from dried maggots, a significant increase in capillary density and in levels of VEGFA mRNA and protein expression were observed in acute rat wounds treated with fatty acid extracts compared with a positive control, the Chinese wound medicine JingWanHong. Wound contraction was also significantly increased by *L. sericata* fatty acids compared with a vaseline negative control, but not more than wounds treated with JingWanHong (Zhang et al., 2010).

GC/MS analysis of the above mentioned extract revealed the presence of 10 classes of fatty acids in *L. sericata* maggots: 60% of were monounsaturated, 21% saturated and 19% polyunsaturated. Fatty acids, such as arachidonic acid, are mediators of cell proliferation, angiogenesis and ECM synthesis. Unsaturated fatty acids may also scavenge damaging ROS produced by inflammatory cells during the early phases of the healing process. At low concentrations, ROS can promote angiogenesis by the induction of VEGFA expression in keratinocytes and macrophages as well as stimulate the collagen production. On the other hand, excessive ROS level can induce apoptosis in wound repair cells (Zhang et al., 2010).

Prete (1997) has investigated the growth stimulating effects of the alimentary secretions and haemolymph of *L. sericata* maggots on human fibroblast tissue. In this study, the author compared alimentary secretions and haemolymph to EGF, recombinant IL-6 and the insect moulting hormone, 20-hydroxyecdysone (EC). In comparable concentrations, haemolymph, alimentary secretions and EC all stimulated the proliferation of fibroblasts, but at only 12% of that of EGF. Alimentary secretions significantly increased the growth of fibroblasts in cultures stimulated by IL-6. The additional fibroblast growth was demonstrated by maggot extracts in the presence of stimulatory concentrations of EGF. Therefore, it was suggested that haemolymph and alimentary secretions stimulated this growth by a different mechanism to that in the presence of EGF (Prete, 1997).

Maggots heal by affecting the chronic inflammatory response through the attenuation of neutrophils, monocytes and macrophages, while the pro-angiogenic activity of E/S actively stimulates the formation of granulation tissue. The wound healing capacity of maggot secretions has many aspects, and further research will reveal more interactions with human cells (Nigam, 2010).

CONCLUSION

The last decade has seen an increase in the interest of insect sources of bioactive substances. This review has shown that the body of the medicinal maggot *L. sericata* is a potential source of immunomodulation factors promoting the healing activity in chronic wounds. Maggots produce a wide spectrum of compounds which affect cells acting in immune response during the cutaneous healing process. They are able to inhibit cycling pro-inflammatory responses by decreasing chemotaxis and the production of multiple pro-inflammatory factors like ROS, proteinases (MMPs, elastases), pro-inflammatory cytokines and integrin expression in neutrophils and macrophages.

Contributions to the following healing phases include the stimulation of processes like the migration of epidermal keratinocytes and dermal cells, fibroblasts and dermal microvascular cells, from the wound margins into the wound bed, production of growth factors (EGF, VEGFA, bFGF) and anti-inflammatory cytokines (IL-10, IL-6 in an anti-inflammatory manner). Beneficial is also the stimulation of angiogenesis represented by the increases in capillary density and granulation tissue.

The immunomodulation effects and mechanisms of extracts from *L. sericata* larvae, used in maggot therapy, have not been fully elucidated yet, so we have limited knowledge about particular compounds and molecules responsible for the stimulation of healing process of chronic wounds. Nevertheless, the extract's clinical significance in the progression of healing is great. Therefore, this warrants further research in the field of identification of substances with immunomodulation properties and to define their roles in the healing process should be very beneficial.

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CONFLICT OF INTEREST

The authors have no conflicting financial interests.

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