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Review

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A SYSTEMATIC REVIEW OF EFFECTIVE BIOAGENT IN CHRONIC WOUNDS: THE MAGGOT BIOTHERAPY PYRAMID

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Abstract

Wound assessment is important in monitoring the effectiveness of treatment in chronic wounds. Therefore, a holistic approach is needed when evaluating effective wound treatment. Most chronic wounds require complementary treatment approaches and conventional treatments in modern medicine. This research was carried out by compiling studies on the past, present, and future of maggot (medical larvae) that cure nonhealing/hard-to-heal wounds; therapeutic larva types, therapy method, healing mechanism, wound healing effect with clinical studies, different usage areas and biological activities of the larvae and the metabolite components in the secretions that provide these activities. In conclusion, medical larvae applied with traditional and complementary medicine techniques to treat nonhealing, difficult-to-heal wounds have a wound-healing effect. However, more research is needed to identify the metabolite components in their secretions that provide their mechanism of action and biological activities.

Keywords: Maggot, Lucilia sericata, chronic wound, wound debridement treatment, bioactive.

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Introduction

The skin is a defense barrier of the organism, protecting the organism against external effects such as environmental microorganisms, chemicals, radiation, and allergens. Some physical and biomechanical factors may adversely affect skin integrity. The inability to prevent passage and accommodation of pathogenic microorganisms biofilm development disrupts the anatomical, functional tissue integrity, which is the main reason for wound formation and wound chronicity. Chronic wounds are those whose healing is delayed due to some underlying problems and do not show normal healing stages. If a wound does not heal within 4-6 weeks despite treatment, it is a chronic wound type. Infections related to chronic wounds are difficult to control. The most important of these difficulties that may be encountered is a risk of disease by tissue damage and tissue death and containing a high amount of bacteria. As a result of the inability to remove excess exudate, wound healing is delayed, so it is necessary to prepare the wound bed and clean death tissue in the wound to ensure wound management. This important stage is possible with the debridement technique, which reduces the risk of infection and speeds up wound healing to remove the dead tissue.[1] Maggot Debridement Therapy (MDT) is the most effective and natural way of debridement performed in necrotic tissue by mechanical and biochemical means. In this method, Maggot therapy applications, ulcerative lesions, burns, various types of malignant tumors, abscesses and osteomyelitis, and traumatic wounds also result in difficult wounds to heal. Low cost, fast, effective, easy, and practical application; It makes the larval treatment more preferred.[2]

Such names as 'Maggot Therapy,' 'Larval Therapy,' 'Therapeutic Myiasis,' 'Biosurgical Debridement,' and 'Biosurgery' are also given to Maggot Debridement Therapy (MDT). Is known that in the 1500s, Maya tribal natives wrapped the infected wound by drying the blood of cattle in the sun, and with this practice, the larvae entered the dead tissues and treated them by feeding on these necrotized tissues, ensuring that the wound was healed within a few days. There is information that a native tribe in Australia cleaned wounds with larvae and continued to relay for generations.[2]According to the first written documents on the treatment of larvae, the French physician Ambroise Pare is cited pioneer in battlefield medicine, especially in the treatment of wounds.[3] In the 1800s, during the civil war, he served as an officer of the surgeon Baron D.J.Larrey used the larvae in wound treatments of Napoleon's used soldiers. They stayed on the battlefield and observed that the larvae attacked only necrotic tissue and accelerated the healing of an infected wound.[4] Dr. W.S Baer conducted the first scientific studies on the clinical application of larvae.[4] After treating two soldiers in the First World War, Baer began working with extensive research on flies. Baer reported that during the 1920s and 1930s, more than 90 patients suffering from osteomyelitis and chronic leg ulcers were treated with the use of larvae. In the 1940s, it declined with the widespread use of antibiotics such as penicillin and sulfamide. At the end of the 1980s, it began to receive attention again due to a rise in antimicrobial resistance rates.[5] Currently, "Maggot Treatment" is accepted worldwide and approved by the national health authorities, and the practice methods are developing daily. At the beginning of the 1990s, more than 8,000 treatments took place in 600 centers in

five years in England alone. Again in the 1990s in the United States and around the world since 1995, Canada, Australia, England, Germany, Switzerland, Sweden, Finland, France, Austria, Denmark, Ukraine, Netherlands, Egypt, Israel, Thailand, and nonrecyclable stubborn larval therapy in the treatment of wounds or injured patients by applying positive results have been achieved.[6] Maggot Therapy in Turkey has been used at the Gulhane Military Medical Academy since 2002.[7] Since 2008; it has been involved in Istanbul University Cerrahpasa Faculty of Medicine, Department of Medical Microbiology.[8] T.R. With the efforts of the Ministry of Health, the 'Regulation on Traditional and Complementary Medicine Practices' was published in the Official Gazette dated 27.10.2014 and numbered 29158, and standards were established for Maggot (Larvae) practices.. MDT is defined as the use of sterilized larvae of the *Lucilia (Phoenicia) sericata* fly, species of fly belonging to the Insecta class, the Diptera order, the Cyclorrhapha suborder, the family Calliphoridae, the Lucilia lineage, in the treatment of infected open wounds. These larvae have the property of not damaging the dermis and subcutaneous tissue of the skin. The elimination products of *Lucilia sericata* larvae are a source of antimicrobial, antibacterial, antibiofilm, and other biological activity in the wound. The purpose of our study; To recognize wound healing maggots, develop maggot therapy, present research on the forms and methods of treatment used today, and emphasize their bioactivity with a multidisciplinary approach.

Materials and Methods

This study is a systematic review. The conditions that should be included in the writing of the Systematic Review research report for the research and the preparation of the study report are based on the relevant checklist protocol (PRISMA).[9] Firstly, Maggot Therapy is used to comprehensively analyze the literature and evidence on the potential activities of Maggot secretion and secretions;

- (‘maggot (OR) larvae’) (AND)
- (‘maggot (OR) larvae’) (AND) (’chronic wounds OR ‘wound debridement’)
- (‘*Lucilia sericata’*) (AND) (‘secretion’)
- (‘*Lucilia sericata* secretion’) (AND) (‘bioactivities)

A four-fold search algorithm was created using Turkish and English terms. PubMed, Science Direct, Scopus, Web of Science, and Cochrane databases were systematically interrogated. Four-layer search algorithms were determined as:

1. MDT development,
2. Therapeutic maggot species used in chronic wounds and chronic wounds,
3. MDT controlled clinical trials and MDT application,
4. According to the need for analysis, active metabolite components and bioactivity in larval secretion.

To better analyze and raise the development of treatment practices with unknowns about Maggot in the process from the history of Maggot therapy to the present, information sources works of literature that have reached twenty years ago to the present day were systematically evaluated. As summarised in Figure 1, priority in the relevant literature; titles and abstracts were then read in full text. A thorough evaluation was carried out by extracting copies of the same articles obtained from unrelated literature and databases.[10] The scale score was determined as 1-9 in full-text comparative randomized control articles. The methodological quality of the research included in this systematic review was evaluated using checklists published by CASP, Critical Appraisal Skills Programme. Accordingly, the quality assessment of randomized controlled trials was carried out with 9-item[11] checklists. Each item included in these lists is evaluated as "yes, no, indefinite, and not applicable." The status determined for each study is given in Table 2. The reference list of included studies was reviewed to obtain related studies that would contribute.

Selection Criteria and Selection of Research

The studies that are suitable for this systematic review have been selected according to the following inclusion criteria;

1. **Studying group:** Chronic wound treatment, biological activity in wound healing
2. **Intervention:** Maggot (larval) treatment using the larvae of *Lucilia sericata*
3. **Comparison:** Not using *Lucilia sericata* larvae, hydrogel treatment, traditional treatment
4. **Results:** Wound healing by achieving complete debridement in chronic wounds with maggot therapy with controlled clinical trials, identification of larval secretory metabolite components effective in wound healing
5. **Study design:** Descriptive research, qualitative research, randomized controlled or comparative clinical studies

The exclusion criteria in the study are the method of non-specific, full-text inaccessible, repeated studies of non-experimental studies published in different languages is the work of Turkish and English languages. This literature analysis describes the many functional features of the larvae used in MDT, which emerge from different disciplines, sources of information, and research findings and reveals their qualified nature.

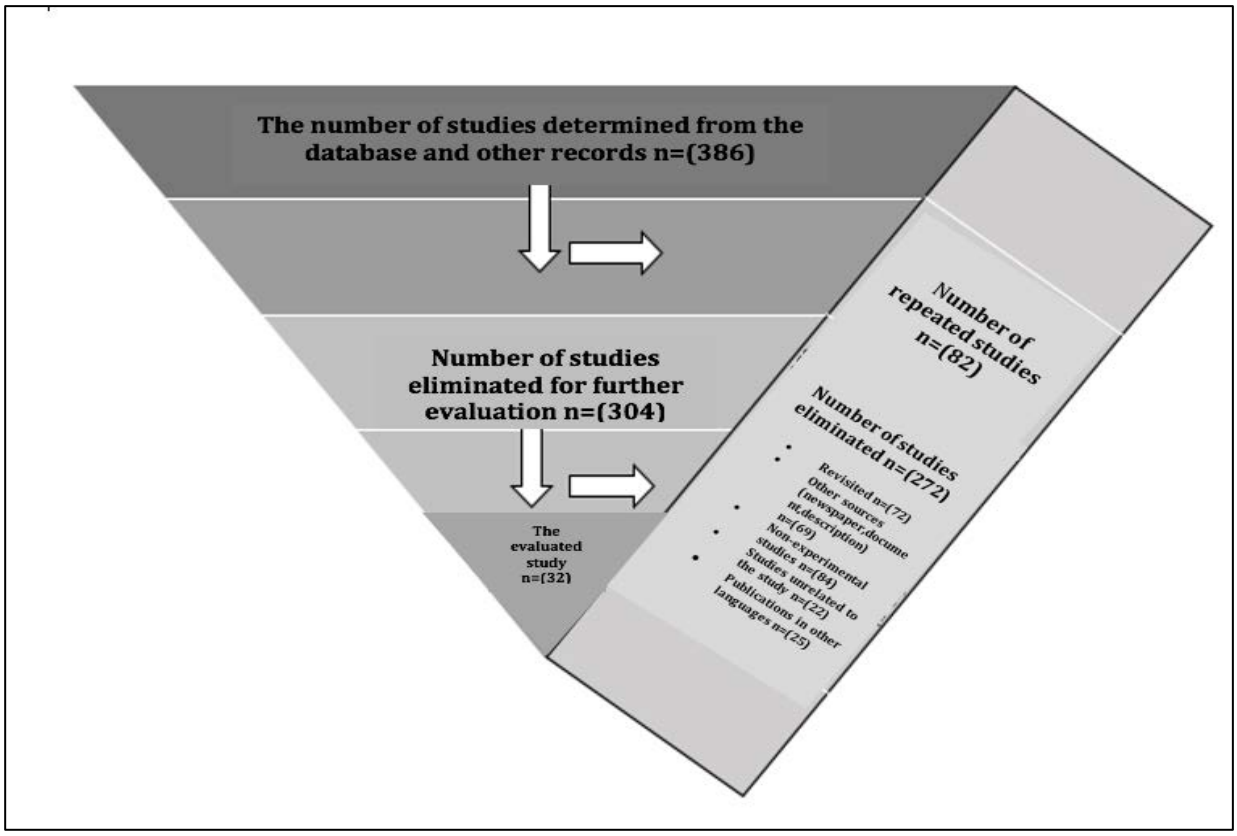

Figure 1: Selected Working Procedure Pyramid

Discussion

Larvae Used in Maggot Therapy

The fly larvae used for Maggot Therapy are usually found in the family Calliphoridae. The main thing in selecting larvae is the ability to feed only from dead tissues without damaging living tissues.[12] It has been noted that wound treatment can also be used for nonhealing skin infections, including eight species of obligate non-parasites from the family Calliphoridae as a worldwide treatment.[13] (Table-1). However, the other six species were not preferred in medical practice except *Lucilia sericata* (widespread preference) and *Lucilia cuprina* (limited choice). It has been observed that different types of larvae can change the effectiveness and reliability of treatment.[13]

Table 1. Calliphoridae species used in larval wound treatment

FAMILY	SPECIES
Calliphoridae	*Calliphora vicina*
	Chrysomya rufifacies
	Lucilia caesar
	Lucilia cuprina
	Lucilia illustris
	Lucilia sericata
	Phormium regina
	Protophormia terraenovae

Lucilia (Fly: Calliphoridae) members are in the group of organisms that are not parasitic in the adult period but cause infection (myiasis) by settling in human and animal tissues during the larval period.[14] In medical entomology, they are included in the group called myiasis flies.[15] Myiasis flies are examined in two groups obligate parasites and non-obligate parasites. The species *Lucilia sericata* is included in the mandatory non-parasitic group.[15] *Lucilia sericata*, first described by Meigen in 1826, was named the greenfly because of its metallic green color. *L.sericata* is preferred because it feeds on superficial necrophage in living tissues. It has been observed that *L.sericata* larvae starve to death when released into living healthy granulation tissue. It has been reported that *L. sericata* is a suitable species for Maggot Therapy due to their necrophage feeding.[16]

Members of this family are remarkable for exhibiting community behavior during reproduction. When the first female begins lay eggs, other females who see this also tend to ovulate, and different species of this family lay eggs on each other's eggs. They are holometabolous and undergo a complete metamorphosis with one egg, three larvae, one prepupa, one pupa, and an adult stage. Figure 2 gives an overview of a related simplified larval developmental biology.[17] Accordingly, the life cycle of *L.sericata* lasts about 16 days at 25ºC. The larvae of Calliphoridae do not resemble the adult at all. They differ in their structure, biology, and ecology.[18]

The maximum amount of nutrients that the larvae need is the third period. Due to the unbelievable feeding speed of the larvae, their growth is also proportionally quite fast. They change skin twice during development; the elasticity of the upper layer of the skin allows them to grow faster.[19] Towards the end of the third period, the larvae enter the navigation phase (post-feeding). The larva finishes feeding and moves away from the nutrient source, looking for a suitable place to pupate. Larva completely empties the digestive tract to pupate, and contractions of longitudinal muscles are observed. There is a rapid water loss from the upper skin until the pupation contraction is over. The adult emerges from the pupal sheath upwards with the help of its feet; when it first appears, it appears as a pupa, but in about one day, it regains its normal appearance and begins to fly.[19]

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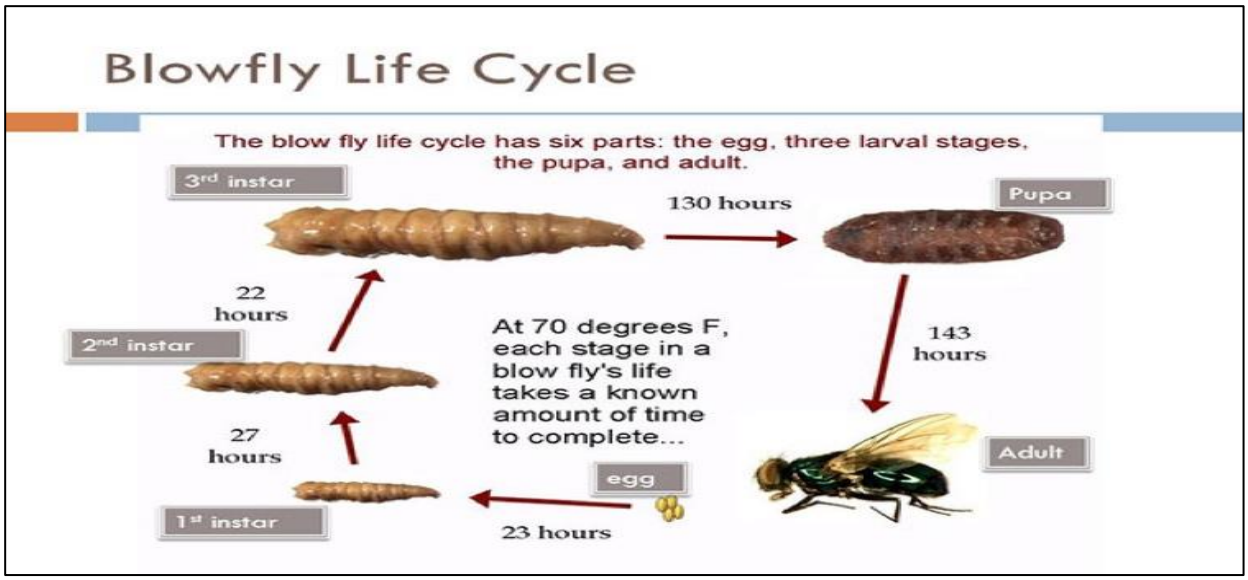

Figure 2. *"Lucilia sericata"* Blowfly Lifecycle
(https://www.nlm.nih.gov/ visibleproofs/galleries/technologies/blowfly.html)

The Mechanism of Action of Maggot Therapy

In deep wounds, blood flow slows down due to insufficient tissue oxygenation. It becomes difficult for antibiotics to enter the area and suppressive immune mediators to work; recovery is delayed. MDT method is preferred when there are situations where a healthy physical, biochemical macro, and microenvironment can not build with medical treatment methods, and progressive tissue loss can not be prevented.[20] The most powerful aspect of Maggot Treatment is the debridement of the wound. Others are the formation of tissue by defecation and granulation. Debridement is removing necrotic tissue and cellular tissue from the wound bed.[21] Full larval debridement requires an average of 2-3 larval cycles lasting 3-5 days.[15] Debridement occurs through two mechanisms. First is mechanical; the larvae themselves break down substrates into small particles with the help of hooks located in the mouth, then they liquefy and lubricate necrotic tissues in the wound with salivary enzyme secretions.[22] Second mechanism is more detailed and complex. In this complex mechanism, various chemical enzymes and substances are secreted. The pH values of larval secretions in the range of 8.6-8.7 provide an available environment for proteolytic enzyme activity such as trypsin and chymotrypsin. In disinfection, larvae secrete antibacterial substances from the intestinal, hemolymph, and salivary glands on the wound and ensure the destruction of bacteria. Another factor that plays an important role in the breakdown of bacteria is the changed pH value of the digestive tract of larvae. Bacteria can also be destroyed in the wound since antibacterial substances effective in the intestines and proteolytic enzymes such as protease lipase collagenase in the digestive system are released during nutrition.[23] With ammonia and calcium carbonate

metabolite components in secretions, it is removed from the acidic environment of the wound pH, and wound alkalinization is carried out. However, therapeutic substances such as allantoin and urease of larvae are supporters that will ensure the integrity of the disinfection stage.[23] The larvae act as a natural mechanical stimulant as they constantly move on the wound. Cytokines such as interferon-γ and interleukin-10 with secretions such as ammonium, ammonium bicarbonate, urea, and allantoin accelerate granulation tissue formation. They stimulate wound healing by secreting growth-promoting factors.[24]

Application of Maggot Therapy

Clinical applications of Maggot Therapy (MT) are usually performed by cage dressing or free larval application. (Figure-3a)[25] The larvae used in MT are disinfected and sterilized green bottle fly *Lucilia sericata* feed on necrotized cells rather than healthy tissues. Sterile larvae left on the wound can circulate freely on the wound bed. For this purpose, before applying the larvae, the wound should be thoroughly washed, and all possible tissue residues that can be removed from the residues of wound care products should be removed. The wound and its surroundings should be carefully cleaned. To prevent the escape of larvae from the wound and protect against secretory digestive enzymes., the upper part of the wound is covered with hydrocolloid dressings surrounding the wound or a sterile piece of thin nylon tulle that acts as a net fixed with non-allergic transparent adhesives. The wound surface is kept in the dressing at a density of 5-10 larvae/cm2 for 1-3 days during the treatment. In recent years, maggot Therapy practices have also been carried out using "Biobag." (Figure-3b)[25] In this method, the maggots are placed in pouches with foam particles inside, consisting of two pieces of tulle made of a special material (polyvinyl alcohol-hydro-sponge) with a thickness of 0.5 mm, similar to tea bags. The mouth of the bag is attached. Gauze or bandage keeps larvae in pouches fixed in the wound.[25,4]

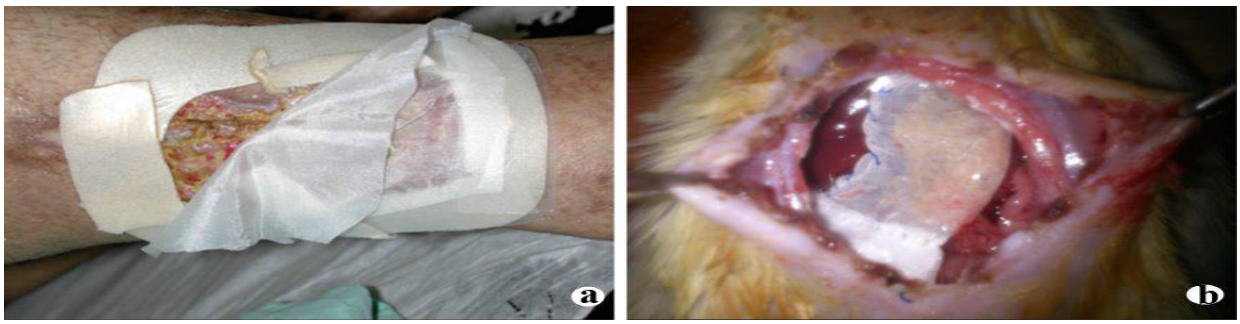

Figure 3. Method of Use of Larvae in MDT a) Cage-Shaped Dressing b) Biobag

In the bio-bag method, mechanical irritation is prevented so larvae cannot act directly on the wound. Accordingly, the decrease of pain sensation, inability of larvae to escape through the sac, and provision of a hygienic environment on the wound bed are beneficial aspects. On the contrary, the disadvantage of this method is that due to the restriction of movement of larvae on the wound, cleaning off dead tissues can not be done adequately. One of the factors that should be considered when applying Maggot Therapy is the number of larvae to be applied to the wound and how long it should be applied.[26] The number of larvae used in the treatment varies according to necrotic tissue, wound width, depth, and type of infecting bacteria. In the medical sector, commercial companies usually sell tubes containing about 300 larvae that have been sterilized. Less than 200 larvae should be used during treatment in low wound depth, while up to 1000 larvae can be applied to wounds with huge depth.[4] Control management is important in treatment. After each application, a sterile physiologic solution should be squeezed out, and larvae should be collected from the wound with pliers and removed. The larvae develop most rapidly between the sixteenth and fortieth hours after decanting from the egg stage. The nutritional needs during this period are about 20-25 mg. Applying 16-hour larvae kept in their substrate to the wound is recommended for effective treatment. (Figure-4) [4] In the application of wound care, it is practical to leave young larvae on the wound for 2-3 days; in application with large maggots, both faster and more effective treatment is performed.

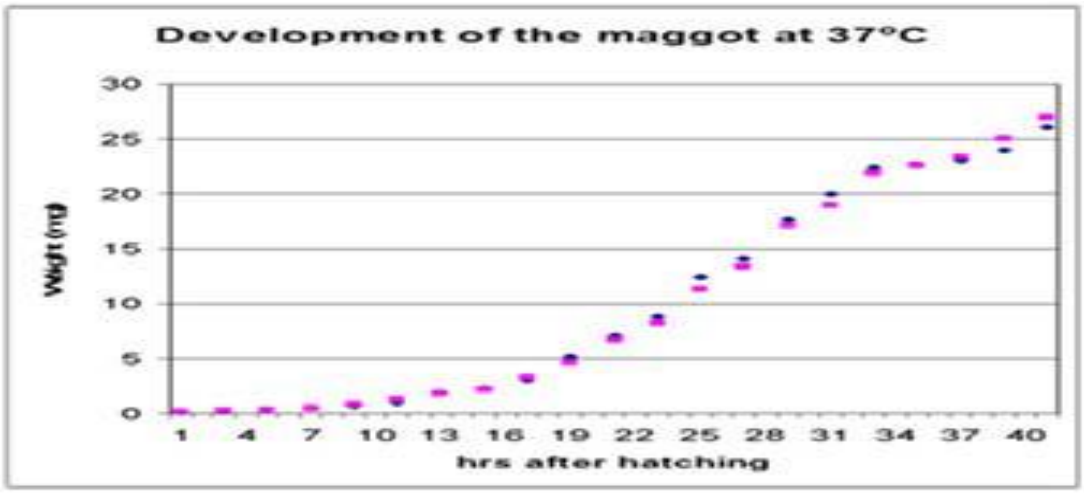

Figure 4. The change in the average body weight of maggots after their first hatching at 37 °C

The most effective function of Maggot Therapy in wound healing is debridement. This function is performed by maggots entering every place of necrotic tissue and separating living tissue from tissue destruction sites (Figure-5).[27] Wound monitoring is important for rapid progression of treatment; during control, the condition

of the wound, necrosis, drainage, inflammatory discharge, bad smell, and the bleeding should be monitored and recorded if the patient has pain.[4]

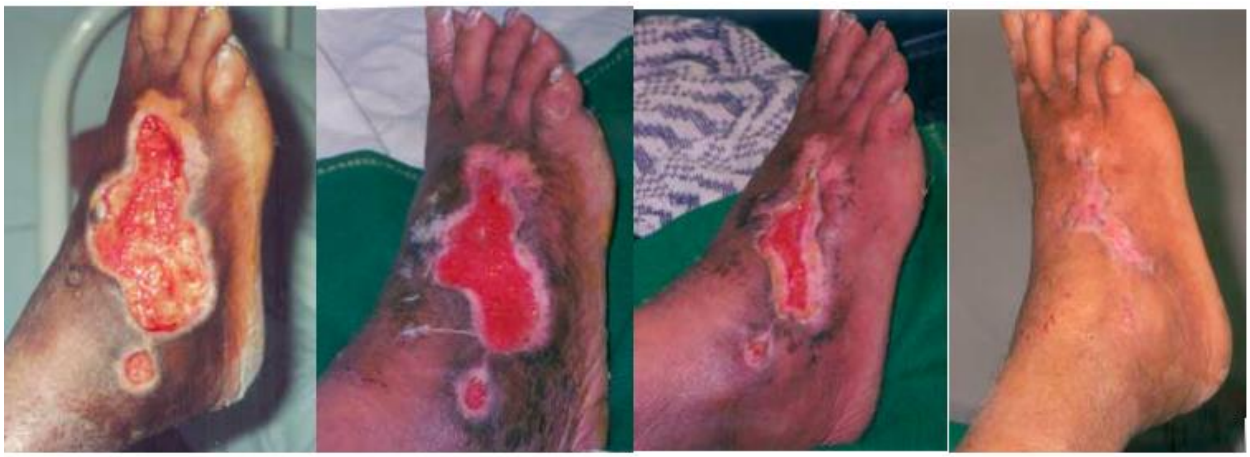

Figure 5. Two diabetic foot ulcers: one before MDT (a) and two after (b), four (c) and eight (d) weeks of MDT

Maggot Therapy can be used to treat ulcers due to conditions such as wounds that do not heal due to trauma, burns, bone marrow inflammation, mastoiditis, Burger's disease, necrotic tumors, and crusted or not completely healed wounds.[28] Psychological and aesthetic anxiety in patients is more prominent than possible surgical side effects. Pain is the most common complaint reported by 6-40% of patients during treatment. In Maggot Therapy, pain is related to the length of stay of the dressing on the wound. As this period increases, pain increases in sensation. The pain, which is mild when applied for the first time, increases gradually due to proteolytic enzymes secreted by larvae stimulating the nerve endings within 24 hours, growth, hardening of the skin, and larvae trying to escape from the wound within 48-72 hours.[29] It raises the patient's body temperature; if ammonium salts produced by larvae are not sufficiently absorbed by dressing, it increases its body temperature. The danger of septicemia can also arise if non-sterile larvae are used in therapy.[30] Systemic antibiotics are applied to prevent septicemia. Nevertheless, this can lead to blockage of the gill slits, through which the larvae supply oxygen, so antibiotics should not be used in ointment form.[28]

Clinical Studies

The first prospective randomized controlled trial was presented by Ronald Sherman, a key figure in the Maggot Therapy revival. In this study, the effect of Maggot Therapy on the treatment of pressure ulcers in patients with spinal cord injuries was investigated, and wound bed surface area and the healing speed and effect rates were observed. The result of treatment is that the larvae used do not cause any side effects in most necrotic wounds; they are debrided faster than all other medical treatment methods.[31] In a different controlled study conducted by Sherman's group, it is the treatment of chronic bilateral plantar foot ulcers that do not respond to

conventional treatments applied to all types but have positive results after using Maggot Therapy.[32] In Maggot treatment applied to 43 patients in pressure ulcer treatment in 2002; it was observed that wounds were completely healed with full debridement compared to patients who received conventional medicine; in another randomized study conducted in 2003 in which 14 patients with venous leg ulcers were treated with Maggot Therapy or hydrogel were followed up; it was stated that after only one application, larvae completely cleared ulcer of necrotic cells and provided effective debridement in all patients.[33-34]

Markevich and his team conducted another study of 140 patients with chronic wounds, diabetic foot neuropathy, medical treatment, and worm. Treatment had the wound and was followed ten days after treatment with maggot wound closure is found to be twice as effective.[35] In a randomized controlled study by Dumville and friends, which examined the comparison of hydrogel therapy and Maggot application, although MDT provided faster debridement than hydrogel therapy in neurotic leg ulcer cases, no difference was observed in bacterial load and healing rates between the two patient groups.[36] In different prospective controlled studies on foot ulcer disease in 2012, 723 patients who underwent Maggot Therapy were treated as outpatients. In 357 (82.1%) hospitalized patients, maggot treatment was performed with frequent controls, and complete debridement was achieved. In addition to supporting and creating evidence, these studies; reported that complex diabetic wounds were treated with Maggot Therapy in 17 of 23 patients, including last-stage renal disease, diabetes, heart disease, and identical conditions.[37] In Table 2, clinical studies in which Maggot Therapy was effective in the debridement, cleaning, and removal of infection of various wounds that did not heal with conventional treatments are discussed.

Opletalová and friends[38] statistically significant faster debridement of exfoliation with MT treatment but reported that this was only in the first week of MT. Mudge and friends [39] demonstrated that MDT debrides the wound more quickly with the difference in the number of wounds completely debrided in the MT group than Hydrogel treatment. In another study organized by Wang and friends, a significant difference between the MDT and hydrogel group was not found in rates of MRSA decimation capacity and the bacterial load reduction in traditional treatment and MT treatment applied to diabetic foot and leg ulcer patients for 60 days.[40]

Different Uses of Larvae

In medicine, Maggot Therapy is mostly used to treat necrotic, suppurative, watery, gangrenous wounds that are difficult to heal. Chronically infected wounds such as ulcers, burns, and osteoarthritis, as seen in Figure-6.[41] In treatment, larvae are classically applied as a last resort, usually after long-term systemic antibiotic administration and if successful recovery has not been achieved despite medical intervention.

Table 2. The summary of the included clinical trials

Authors/Year	Type of wound	Number of participants	Intervention and Control	Follow	Result	Quality score
Sherman (2002)	Pressure ulcers	MDT: 43 Control :49	MDT and traditional treatment	17-19 weeks	Debridement, Granulation tissue, Complete recovery of surface area,	Yes 5/9 No 2/9
					Adverse impact	Indefinite 2/9
Sherman (2003)	Diabetic foot and leg ulcers	MDT: 14 Control: 14	MDT and traditional treatment	Eight weeks	Debridement, Granulation tissue, Complete recovery of surface area,	Yes 5/9 No 2/9
					Adverse impact	Indefinite 2/9
Dumville et al. (2009)	Venous or mixed ulcers	MDT:180 Control:87	MDT and hydrogel therapy	6-12 weeks	Complete debridement, The recovery period, Bacterial growth, Adverse events	Yes 7/9 No 2/9
Mudge et al. (2014)	Venous or complex leg ulcers	MDT:46 Control:42	MDT and hydrogel therapy	28-35 days	Debridement, Wound surface area Bacterial growth, Adverse events	Yes 8/9 No 1/9
Markevich et al. (2000)	Diabetic neuropathic foot	MDT:70 Control:70	MDT and traditional therapy	Ten days	Complete recovery and debridement	Yes 8/9 No 1/9
Opletalova et al. (2012)	Venous leg ulcers	MDT: 51 Control: 54	MDT and traditional therapy	30 days	Complete debridement, Bacterial growth, Recovery period, Adverse events	Yes 8/9 No 1/9
Wang et al. (2010)	Diabetic foot and leg ulcers	MDT: 53 Control:53	MDT and traditional therapy	60 days	Debridement of granulation tissue, Bacterial growth, Adverse events	Yes 7/9 No 2/9

CASP, Critical Appraisal Skills Programme. 1 Did the trial address a clearly focused issue? 2 Was the assignment of patients to treatments randomized? 3. Were all of the patients who entered the trial properly accounted for at its conclusion? 4 Were patients, health workers and study personnel 'blind' to treatment? 5 Were the groups similar at the start of the trial? 6 Aside from the experimental intervention, were the groups treated equally? 7 Can the results be applied to the local population or in your context? 8 Were all clinically important outcomes considered? 9 Are the benefits worth the harms and costs?

If we talk about the use of larval therapy in veterinary, it is not as widespread as its use in medicine, but it is used, albeit limited. Infected wounds of 2 dogs, four cats, one rabbit, and 13 horses, seven lames and six dying, were started to be treated with Maggot Therapy by American veterinarians; animals survived amputation and death except for only one horse. There were no complications other than pain during the treatment process. After the study, it was understood that Maggot Therapy is also effective and safe for some serious hoof and leg wounds in horses.[41]

The interesting aspect of therapeutic agent larvae is that it has an important place in forensic entomology. The science of "Forensic Entomology" or "Biocriminal Entomology" can be defined as information about insects' biology, behavior, adaptation abilities, and ecology in forensic research using their life cycles. Carrion-feeding

(necrophage) flies are mediators that play an important role in the decay phase of corpses. This connection between insects and carcasses and their use in criminal investigations is important in forensic entomology. Insects are living species that detect and reach corpses as soon as possible. They lay eggs or larvae on the corpse in openings such as the face, inside the mouth, between lips and teeth, eye cavity, ear hole, nostrils, and wound surface area. They can be fed more comfortably and protected easily.[42] *L. sericata* is typically known by studies as a fly with a bad smell that is released when a corpse decays and explodes after death. Hence, it is important species from a Forensic Entomological point of view. The time of death was estimated according to the larvae or eggs arriving at the corpse in a certain order, the order of arrival of insects left on the corpse and the lengths and numbers of larvae emerging from them, and the number of respiratory slits in the stigma by Post Mortem Interval (PMI) calculation. In the last years, Entomotoxicological analysis studies using larvae have had an important place in providing information about the time after death, especially larval stages of insects found at the crime scene, and providing important evidence about poisoning or drug consumption by the victim.[43]

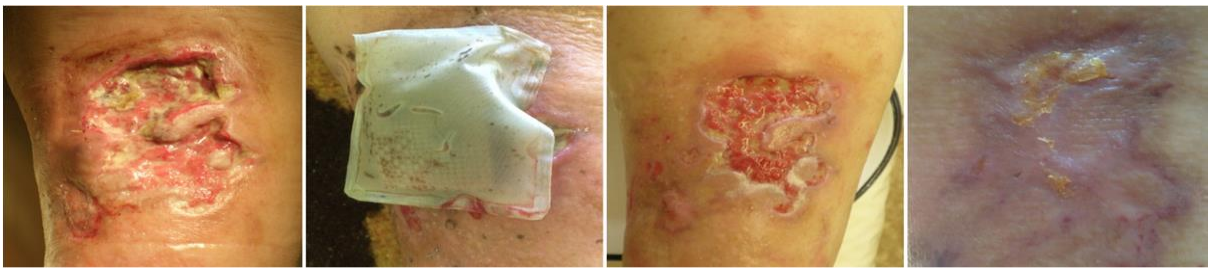

Figure 6. The successful outcome of the patient after treatment with *Lucilia sericata* larvae

Biological Activity of Larval Secretions

The secretion of *Lucilia sericata* larvae contains allantoin, cysteine, sulfhydryl radicals, glutathione, ammonia, calcium carbonate, and growth-stimulating factor. Additionally, they have many digestive enzymes (Table-3) while feeding on the wound.[44] With the latest in vitro studies, it has been determined that secretion (secretions) of larvae contain at least two substances with antibacterial properties. They are hydrophobic substances with a molecular weight of 3-10 kDa and hydrophilic substances of <1 kDa with peptide-like structures.[45]

Since the discovery of larvae, scientific studies in the process and today's larval development and the available function of larvae in the application of Maggot Therapy in wounds have been discussed in the simplified pyramid. The stage in each layer, specific to the potential impact of the larvae, is the hierarchy of the sublayers (Table-7). Regarding the activation step in effect, the fascinating power in the secretions obtained from the whole body fluid of the larvae is an antibacterial effect, which was first described in the 1930s. The mechanism of action is the ingestion of bacteria by larvae, the direct killing of bacteria in the digestive tract, and wound alkalinization. In addition, its debridement activities have also been reported to reduce bacterial load on the

wound with its numerous antibacterial effects. It has been noted that some substances in secretions of sterile *Lucilia sericata* larvae have a significant antimicrobial effect. Simmons was the first to perform an antibacterial analysis of larvae against various microorganisms (*Staphylococcus, Streptococcus, Proteus, Clostridium*). It was found that the secretory extract can kill bacteria in 5-10 minutes, and the bactericidal effect of the larvae was revealed.

Table 3. The secretory enzyme content and activities of *Lucilia sericata* larvae.

Enzymes	Ingredients	Specific activity (µ mol Min^{-1} Mg^{-1})	Km (MM)	Vmax (µ mol Min^{-1})	Molecular weight
General proteases	Kazin	0.688	-	-	-
Collagenase	Collagen	0	-	-	-
Trypsin	BApNA	0.010	0.2	0.5	26±2,9
Leucine aminopeptidase	LNA	0.043	0.1	2.7	280±37
Carboxypeptidase A	HPA	0.034	0.02	2.7	40±9.4
Carboxypeptidase B	HA	0.015	0.08	1.5	42±5.9
Elastase	Elastin Congo red	0.002	-	-	-
Carboxypeptidase B	Chymotrypsin	GPA	0	-	-

On the other hand, a study analyzed that 104 bacteriae were completely broken down; it has been attended the ability of larval secretions alone to accelerate wound healing. In addition to indicating the potential for lytic activity against *Pseudomonas and Candida*, it has also been reported that larvae completely degrade MRSA found in infected wounds and ulcers.[46] Since then, many findings in many studies have led to the need to investigate further antibacterial activity in larval secretions against both Grams (+) and Gram (-) bacteria.

When examined in terms of bioactivity of medical larvae, the main factors and mechanisms were systematized as in Table-5.[47] On the other hand, there is a strong activity of larval elimination products and body fluids against *the P.aeruginosa.* They showed various peptides and genetic rearrangements of *L.sericata*, especially against this species, and noted the synergistic effects of these peptides. It is highly effective against *Staphylococcus aureus* and *Escherichia coli* by applying various antibacterial activity analyses for the secretions obtained from sterile larvae.

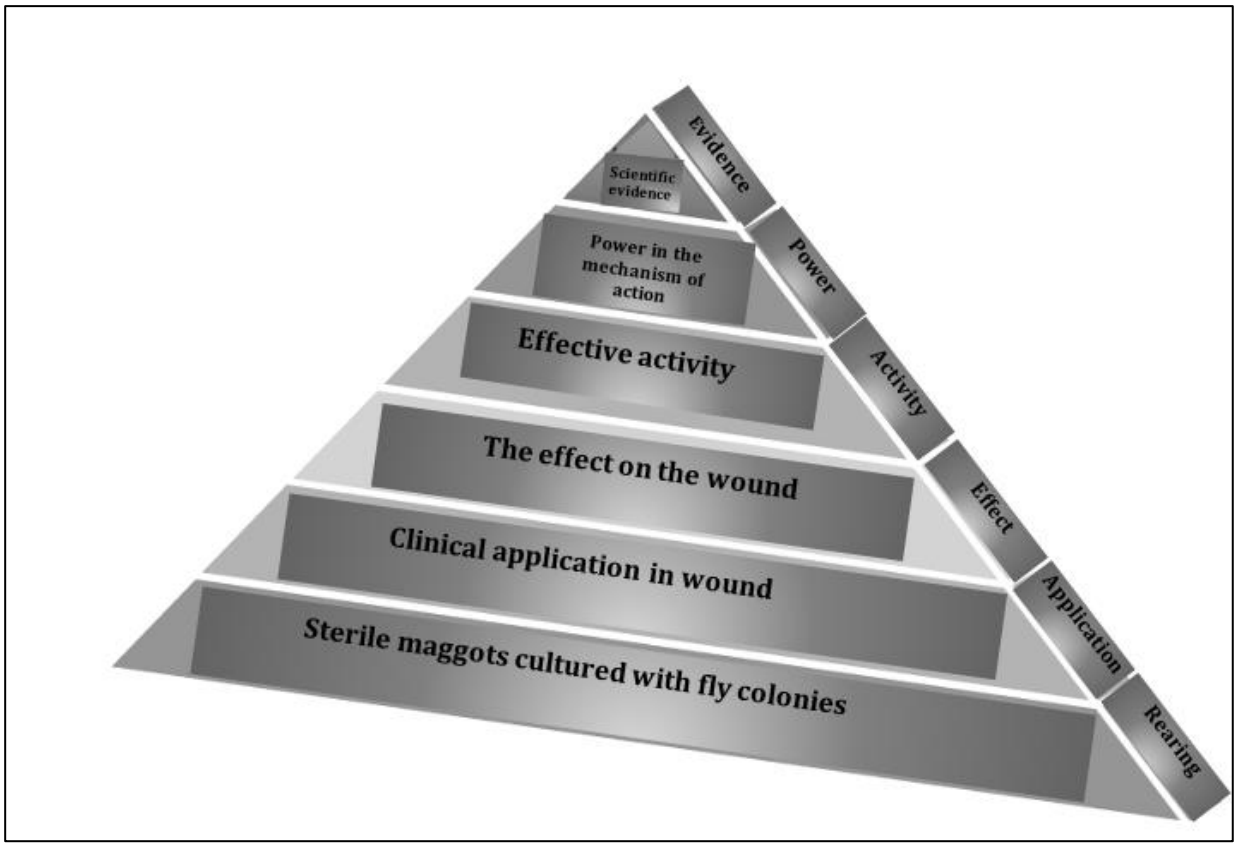

Figure 7. The Simplified Pyramid for Maggot Therapy

In contrast, its antibacterial effect is weak against *P. aeruginosa* and *S.aureus*.[48] Larval secretions are mediated by at least two different molecules, chymotrypsin, and DNAase, which can prevent bacterial biofilm formation and degrade resident biofilms. It has been revealed that it is necessary to study the antimicrobial molecules in larval secretions in more detail. By isolating several antimicrobial molecules from the *L. sericata*, secretory components' structural characterization and antimicrobial activity were revealed. Of these molecules, Lucifensin 1, Lucifensin 2, MAMP, and Seraticin 1 have been identified as potential antimicrobial molecules.[49] The molecular structure of Seraticin 1 is investigated so that it can respond to chemical synthesis. The mode of action, the minimum inhibitory concentrations, and molecular targets' determination are also being analyzed. (Nigam Y. and others, unpublished) Therefore, the presence of antibacterial molecules contained in *L.sericata* secretions has now become universally accepted.[50]

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Table 5. Overview of the bioactivity of maggots

Bioactivity	Component	Molecular weight	Case	Source	Mechanism
Antibacterial	Ammonia, calcium carbonate	0.5–10 kDa	Undefined	Whole-body extract	Increasing the pH value
	Ammonium carbonate	0.5–10 kDa		Whole-body extract	Increasing the pH value
	Allantoin	0.5–3 kDa		Whole-body extract	Stimulation of granulation
	Lucifensin I	4,11 Da	Strains of *Staphylococcus Streptococcus*	Intestinal salivary glands, body fat, hemolymph, ES	Regulating ion channels or transmembrane pores
	Lucifensin II	4,127Da	*Staphylococcus aureus, Pseudomonas aeruginosa*	Whole-body extract	----
	Lucilin	Undefined	Multidrug resistance Gram-negative bacterium	Excretion-Secretion (ES)	----
	MAP	45.1 kDa	Standard and antibiotic-resistant strains of *Staphylococcus aureus*	Excretion-Secretion (ES)	Increasing membrane permeability
	Alfa-metoksifenil	Undefined	Undefined	ES	
	C10H16N6O9 -Seraticin	<500 Da	MRSA, *Staphylococcus spp., Bacillus spp., E. coli, Pseudomonas spp., Proteus spp., Enterococcus spp., Serratia spp., Candida spp.,*	Whole-body extract	Melting of the bacterial membrane
	Alloferon I & II	Undefined	*Enterobacter spp.*		
	pHydroxyphenylacetic acid	152 Da	*Micrococcus luteus, Pseudomonas aeruginosa*	Whole-body essence	Changing the membrane potential of bacteria by the flow of K + in the cell membrane
	p-Hydroxybenzoic acid	138 Da		Whole-body essence	
	octahydro-dipyrrolo[1,2-a;1',2'-d] pyrazine-5,10-dione	194 Da Undefined	*M. luteus, P. aeruginosa*		
	Proline diketopiperazine	Undefined	*Micrococcus luteus, Pseudomonas aeruginosa*	Whole-body essence	
Antibiofilm	Chymotrypsin 1, MEP	25 kDa, 45kDa	*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruainosa*	Excretion-Secretion (ES)	Biofilm formation in protein
	DNAase				
Antifungal	Lucimysin	8.2 kDa	Ascomycota, Basidiomycota, Zygomycota, Candida albicans, Phytophthora parasitica	Excretion-Secretion (ES)	Formation of a metal complex at a particular receptor
Anti-inflammatory	Excretion-Secretion (ES)	56 kDa	Macrophage		MIP-1β, RANTES, PDGF-BB reduction
Immunomodulator	BLIP	Undefined	T lymphocytes	Excretion-Secretion (ES)	IFN-γ, IL-4, IL-10, IL-13, and CD25 reduction, TNF-α and TGF-β increasing editing
Antileishmania	Excretion-Secretion (ES) and Hemolymph	<10 Da	*Leishmania tropica* Promastigote and intracellular amastigotes	ES and Hemolymph	The effect of cytotoxicity on promastigote and intracellular amastigotes
Proangiogenesis	Unsaturated fatty acid	Undefined	Microvascular endothelial cell	Whole-body essence	Increase migration, activating the AKT1 signaling pathway
	Histidine	Undefined	Human umbilical vein endothelial cell		Increasing immigration
Procoagulant	Jonah-like protein	Undefined	Human plasma		Reduce clotting time
Antitumor	ω-6 PUFA	Undefined	A-549 lung cancer cells		Activating the p38MAPK signaling pathway
Antiatherosclerosis	Excretion-Secretion (ES)	Undefined	Murine serum		Increase HDL
Antiviral	Excretion-Secretion (ES)	Undefined	Cytotoxicity of Vero cells	Petroleum ether extract	Herpes Simplex Virus (HSV-1) potential

By isolating larval medical proteins, a new antifungal peptide called lusimycin was identified from larval secretions, and its functional characterization with its clones was determined. However, in vivo studies have shown that it prevents the cutaneous development of secretions. Its antiparasitic effect has been reported.[51] Platelets and neutrophils, and monocytes/macrophages are among the first cells to work, contributing to an unending inflammatory phase that can interfere with wound healing beyond their benefits to the young wound and prevent the progression of inflammatory reactions in the wound. Biological activity of maggot secretions in different studies; physical effects on the wound and secrete bioactive molecules with immunomodulatory function, proangiogenic activity, antitumor activity and antiatherosclerosis effect, antiviral effect with scientific information are described.[52]

Maggot therapy is known for its long history, from tribal medicine to today's conventional medicine, and there is solid clinical and biomedical evidence that it is effective.[53] Ideas and studies on larval rearing and supply alternatives in production are insufficient. However, comparatively controlled studies in academia will prove the effectiveness of larval treatment, information gaps in treatment management, potential biases in the treatment process, and psychological and aesthetic concerns.

The development of biological therapy and *Lucilia sericata* larvae are gaining new supporters among clinicians in medicine every year. Still, although the availability of the method is rising, the unit price is increasing due to the difficulties of growing larvae production.[54] In terms of the supply of larvae production and traditional academic studies, widening the website networks of medical larval producer companies, doing the current production, product, service, and distribution practices in functioning health services also provide industrial information.

L.sericata larvae are effective in treating larvae, and their use is widespread. The limited use of *L.cuprina* larvae shows as many positive effects as *L.sericata* larvae. However, it has been reported that only *L. sericata* species larvae were included in the case-cohort studies compiled for MDT treatment. In contrast, *L. cuprina* species that reported positive results in treatment were not included in the evaluation.[55,57] Therefore, it is useful in academic studies to present evidence on the effectiveness of treatment of *L. cuprina* larva species used for MDT. Its overall integrity and safety applicability are similar to that of the *L. sericata* species. For this reason, wider literature on Calliphoridae flies and developmental biology and physiology will contribute more.

In the production of medical larvae, the focus should be on changing growing methods and optimum standardization development parameters with minimum development times to achieve uniformity of larval culture continuity between laboratory colonies. The development of sterilization methodologies for larvae is important. There is no scientific literature on the conventional growing and genetic development of *L. sericata* or *L. cuprina* fly strains and laboratory populations to improve medical performance. The last study

investigates whether genetic modification can enhance the therapeutic gains of medical larvae.[56] It hopes to use Recombinant DNA techniques for these fly strains used in larval treatment to create cDNA libraries. On the other hand, human growth factors have been shown to encourage wound healing. Much research continues in which genetic engineering techniques are used to produce transgenic larvae capable of secreting human growth factors such as human platelet-derived growth factor (PDGF-BB).[56]

In the evaluation of MDT with systematic reviews, its successful role in antibiofilm effect against Gram-positive and Gram-negative bacterial strains, including *S. aureus, P. aeruginosa,* methicillin-resistant *S. aureus,* and other drug-resistant pathogens, have been highlighted. In some studies, it has been reported that MDT does not have a direct antimicrobial effect. In contrast, further clinical studies have confirmed the decrease in bacterial load after larval use;[57,58] the mechanisms of action in these activities are similar to different types of larvae used in Maggot Treatment, or is there a difference in degree? These questions are intriguing. More large-scale short-term studies are needed for specific results.

Currently, treatment methods used for maggot therapy may be replaced by patented drugs obtained due to secretion isolation of larvae in the future. To further determine the advantages of maggot therapy and to reveal the medical utility of species other than *L. sericata*, a large number and large-scale research and clinical studies are needed.[28] Scientists agree that interdisciplinary practices will contribute "synergically and positively to the treatment process" in chronic wound treatment. The biochemical mechanisms underlying curative properties of larval therapy have been studied since larval treatment was considered a medical treatment option. The findings on the molecular and cellular mechanisms of the curative effect of maggots are still unclear. Molecules from the secretions of larvae are valuable substances responsible for stimulating the remedial process of chronic wounds.[59] It is a matter of wonder whether any potential for bioactivity identified in the larvae is due to a symbiotic bacterium present in the maggot's body or due to the maggots themselves.[60] Using recombinant DNA techniques seems to be a more promising strategy. Data describing the molecules that provide bioactivity accelerate scientific discovery and help identify source proteases and antibacterial peptides to positive effects of larval therapy with the possibility of using different techniques. Additionally, it will be possible to prepare such peptides and proteins using recombinant techniques to test activity by in vitro experiments. This attitude will enable us to understand better how the larvae can remove necrotic tissue from the wound, effectively eliminate various pathogens and improve the curative process. Active recombinant peptides and proteins developed from larvae can be used as new biotherapeutic agents in the future treatment process of larvae transitioning to biosurgery.

CONCLUSIONS

MDT was an effective treatment when indigenous tribes first discovered it centuries ago. With the rise of drug-resistant pathogens and the incidence of atherosclerosis with the diabetes epidemic, MDT has re-emerged as a significantly useful therapy. MDT is a fast, easy-to-manage, safe, and cost-effective tool for wound care; It emerges as an effective method of multidisciplinary approaches in treatment. MDT is a dynamic concept, and the Maggot Therapy Pyramid provides a structured approach to therapy promotion and an answer to the curiosities in assessing and managing chronic wounds. The fascinating powers of *Lucilia sericata* and other medically useful species larvae used for debridement treatment support MDT in a generally private demonstrable effect on every step of the therapy pyramid from floor to ceiling. Interventions for treating chronic wounds are debridement, antimicrobial, antibacterial action, and degradation of the biofilm. The existence of antimicrobial, antibacterial, and other effects presented in our study is clear. It is thought that the metabolite components in the larval body fluids play a role in these effects. These metabolite components may also have a role in interventions that need further studies. In order to reveal the true mechanism of action of MDT, advanced functional component definitions should be made and supported by controlled studies.

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References

1. Steenvoorde P, Jukema GN. The antimicrobial activity of maggots: in-vivo results. J Tissue Viability, 2004;14(3):97-101.
2. Wolff H, Hansson C. Larval therapy - an effective method of ulcer debridement. Clin Exp Dermatol 2003;28(2):134- 47.
3. Pechter E.A, Sherman R.A. Maggot Therapy: The Surgical Metamorphosis. Plastic Recon. Surgery, Oct. 1983;72(4):567-70
4. Mumcuoğlu K, Özkan AT. Supuratif Kronik Yaraların Maggot Debridman Tedavisi, Turkiye Parazitol Derg., 2009;33(4):307-15.
5. Whitaker IS, Twine C, Whitaker MJ, Welch M, Brown CS et al. Larval therapy from antiquity to the present day: mechanisms of action, clinical applications and future potential. Postgrad Med J, 2007; 83: 409-413.
6. Wayman J, Nirojogi V, Walker A, Sowinski A, Walker MA. The cost-effectiveness of larval therapy in venous ulcers. J Tissue Viability 2000;10(3):91-4.
7. Tanyuksel M, Araz E, Dundar K, et al. Maggot debridement therapy in treating chronic wounds in a military hospital set up in Turkey. Dermatology, 2005;210:115-8.
8. Tekin S, Polat E, Arslan A, et al. Larva debridman tedavisi: Klinik uygulamada ilk sonuçlar. II. Ulusal Yara Bakımı Kongresi.29 Kasım-01 Aralık, İstanbul. p.96. 2007.
9. Karaçam Z. Sistematik derleme metodolojisi: sistematik derleme hazırlamak için bir rehber. Dokuz Eylül Üniversitesi Hemşirelik Yüksekokulu Elektron. Derg 2013;6(1):26–33.
10. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009 Jul 21;6(7):e1000097 (doi: 10.1371/journal.pmed.1000097).
11. CAPS. Critical Appraisal Skills Programme (Randomised Controlled Trial) Checklist. [Internet]. https://casp-uk.net/casp-tools-checklists/(Accessed: 3 February 2022).
12. Krajacic A. Consider Using Maggots. Today's Surgical Nurse, May-Jun. 1998;20(3):28-32.
13. Kingu H. J, Kuria S.K, Villet M.H. Cutaneous myiasis: Is *Lucilia cuprina* safe and acceptable for maggot debridement therapy, J. Cosm. Dermatol. Sci. Appl. 2012;2:79-82.
14. Unat E.K, Yücel A, Altaş K, Samastı M. İnsanın ökaryonlu parazitleri ve bunlarla oluşan hastalıkları, İstanbul Cerrahpaşa Tıp Fakültesi Vakfı Yayınları 1995;15:57-140.
15. Sherman R.A. Maggot therapy takes us back to the future of wound care: new and improved maggot therapy for the 21st century, J. Diabetes Sci Technol.2009;3:336–44.
16. Paul AG, Ahmad NW, Lee HL, et al. Maggot debridement therapy with *Lucilia cuprina*: a comparison with conventional debridement in diabetic foot ulcers. Int Wound J, 2009;6:39–46.

17. Blowfly Life Cycle Cleveland Museum of Natural History (reproduced with permission) [Internet] https://www.nlm.nih.gov/ visibleproofs/galleries/technologies/blowfly.html (Accessed: 3 February 2022).
18. Demirsoy A. Genel Zoocoğrafya ve Türkiye Zoocoğrafyası, Hayvan Coğrafyası, 2001; s.670.
19. Greenberg B, Kunıch J.C. Entomology and the Law: Flies as Forensic Indicators. First Ed., Cambridge University Press. Chapter 2. 2002.
20. Mumcuoglu KY, Clinical applications for maggots in wound care. Am J Clin Dermatol, 2001;2(4):219-27.
21. Moya-López J, Costela-Ruiz V, García-Recio E, Sherman RA, De Luna-Bertos E. Advantages of Maggot Debridement Therapy for Chronic Wounds: A Bibliographic Review. Adv Skin Wound Care. 2020 Oct;33(10):515-25 (doi: 10.1097/01.ASW.0000695776.26946.68. PMID: 32941225).
22. Jarczyk G, Jackowski M, Szpila K, Bozek G, Kapelaty S. Use of *Lucilia sericata* blowfly maggots in treating diabetic feet threatened with amputation. Acta Angiologica 2008;14(2)42–55.
23. Lerch K, Linde HJ, Lehn N, et al. Bacteria ingestion by blowfly larvae: an in vitro study. Dermatology; 2003;207(4):362.
24. Mumcuoglu KY, Ingber A, Gilead L, et al. Maggot therapy for the treatment of intractable wounds. *Int J Dermatol.* 1999;38(8):623-7 (doi:10.1046/j.1365-4362.1999.00770.x).
25. Özalp AH. *Deneysel Nekrotizan Pankreatitte Maggot Terapi (Larva Debritman Tedavisi),* Uzmanlık Tezi, Konya: Necmettin Erbakan Universitesi Meram Tıp Fakultesi Genel Cerrahi Anabilim Dalı, 2013.
26. Fleischmann W, Thoener B. Biobag - a live wound dressing containing maggots. Fifth International Conference on Biotherapy, Wurzburg, Germany, June 29-July 1, p. 8. 2000.
27. Tantawi T, Gohar Y, Kotb M, Beshara F, El-Naggar M. Clinical and microbiological efficacy of MDT in the treatment of diabetic foot ulcers. J Wound Care. 2007;16(9):379–83.
28. Biotherapy - History, Principles and Practice. In: Martin Grassberger, Ronald A. Sherman, Olga S. Gileva, Christopher M.H. Kim, Mumcuoglu KY, eds2013: https://link.springer.com/book/10.1007/978-94-007-6585-6?noAccess=true.
29. Bazalinski D, Wiech P, Szymanska P, Muster M, Kozka M. Application of Lucilia sericata Larvae in Debridement of Pressure Wounds in Outpatient Settings. *J Vis Exp.* 2021(178) (doi:10.3791/62590).
30. Nigam Y, Bexfield A, Thomas S, Ratcliffe NA. Maggot Therapy: The Science and Implication for CAM Part I – History and Bacterial Resistance. Evid-Based Compl Alt, 2006;3(2):223–7.
31. Sherman RA, Wyle F, Vulpe M. Maggot therapy for treatng pressure ulcers n spnal cord injury patents. J Spnal Cord Med. 1995;18(2):71–4.
32. Stoddard SR, Sherman RA, Mason BE, Pelsang DJ, Sherman RM. Maggot debridement therapy. An alternative treatment for nonhealing ulcers. Journal of the American Podiatric Medical Association. 1995;218–21.
33. Sherman R.A. Maggot versus conservative debridement therapy for the treatment of pressure ulcers. WoundRepair Regen. 2002;10:208–14.

34. Sherman, R.A. Maggot Therapy for treating diabetic foot ulcers unresponsive to conventional therapy.Diabetes Care, 2003;26:446–51.
35. Markevich Y, McLeod-Roberts J, Mousley M, Melloy E. Maggot therapy for diabetic neuropathic foot wounds: a randomised study. In: 36th Annual Meeting of the European Association for the Study of Diabetes. 2000.
36. Mohd Zubir MZ, Holloway S, Mohd Noor N. Maggot Therapy in Wound Healing: A Systematic Review. Int J Environ Res Public Health. 2020 Aug 21;17(17):6103 (doi: 10.3390/ijerph17176103).
37. Marineau ML, Herrington MT, Swenor KM, Eron LJ. Maggot debridement therapy in the treatment of complex diabetic wounds. 2011, Hawaii Med J. 2009;70(6):121–4.
38. Opletalová K, Blaizot X, Mourgeon B, et al. Maggot therapy for wound debridement: A randomized multicentertrial. Arch. Derm. 2012;148:432–8.
39. Mudge E, Price P, Walkley N, Harding K.G. A randomised controlled trial of larval therapy for the debridement of leg ulcers: Results of a multicenter, randomised, controlled, open, observer-blind, parallel-group study.Wound Repair Regen. 2014;22:43–5.
40. Wang S yu, Wang J ning, Lv D cheng, Diao Y peng, Zhang Z. Clinical research on the bio-debridement effect of maggot therapy for treating chronically infected lesions. Orthop Surg. 2010;2(3):201–6.
41. Sherman RA, Shapiro CE, Yang RM. Maggot therapy for problematic wounds: uncommon and off-label applications. Adv Skin Wound Care, 2007b;20:602–10.
42. Grassberger M, Frank C. Initial Study of Arthropod Succession on Pig Carrion in a Central European Urban Habitat, *Journal of Medical Entomology*. 2004;41(3):511–23.
43. Greenberg B, Kunıch J.C. Entomology and the Law: Flies as Forensic Indicators. First Ed., Cambridge University Press. Chapter 2. 2002.
44. Gupta A. A review of the Use of Maggots in Wound Therapy; MS: Ann Plas Surg; 2008;60:224-7.
45. Huberman L, Gollop N, Mumcuoglu KY, Block C, Galun R. Antibacterial properties of whole-body extracts and haemolymph of *Lucilia sericata* maggots. J Wound Care; 2007;16(3):123-7.
46. Sirekbasan S, Polat E, Cangel U. The Effect of Bacterial Colonization and Maggot Debridement Treatment on Wound Healing in Chronic Venous Leg Ulcers. Clin Lab. 2021 May 1;67(5) (doi: 10.7754/Clin.Lab.2020.201137).
47. Yan L, Chu J, Li M, et al. Pharmacological Properties of the Medical Maggot: A Novel Therapy Overview. *Evidence-Based Complementary and Alternative Medicine*, 2018, pp.1-11.
48. Gazi U, Taylan-Ozkan A, Mumcuoglu KY. The effect of *Lucilia sericata* larval excretion/secretion (ES) products on cellular responses in wound healing. Med Vet Entomol. 2021 Sep;35(3):257-66 (doi: 10.1111/mve.12497).
49. Cerovsky V, Slaninova J, Fucik V, et al. Lucifensin, a novel insect defensin of medicinal maggots: synthesis and structural study. Chembiochem 2011;12: 1352–61.

50. Calendar G, Pritchard D, Nigam Y, Jung W, Nibbering P. Multiple actions of *Lucilia sericata* larvae in hard-to-heal wounds. Bio Essays. 2013;35(12):1083-92.
51. Polat E, Cakan H, Aslan M. Detection of the anti-leishmanial effect of the *Lucilia sericata* larval secretions in vitro and in vivo on *Leishmania tropica:* First work. *Experimental Parasitology.* 2012;132(2):129-34.
52. Zhang W, Jiang W, Lan XJGmj. Exploration of the antitumor effects and mechanisms of Chrysomya megacephala extracts in H22 hepatomabearing mice. 2017;39(2):215-9.
53. Nigam Y. Morgan C. Does maggot therapy promote wound healing? The clinical and cellular evidence. Journal of the European Academy of Dermatology and Venereology, 2016;30:776–82.
54. Bazalinski D. Efficacy of Biological Therapy using Lucilia sericata Larvae in the Treatment of Chronic Wounds in Patients in Long-Term and Palliative Care. *Uniwersytet Rzeszowski: Rzeszów, Poland.* 2019.
55. Tourle R, Downie D.A. Villet, M.H. Flies in the ointment: A morphological and molecular comparison of *Lucilia cuprina* and *Lucilia sericata* (Diptera: Calliphoridae) in South Africa. Med. Vet. Entomol. 2009;23:6–14.
56. Tombulturk FK, Kanigur-Sultuybek G. A molecular approach to maggot debridement therapy with Lucilia sericata and its excretions/secretions in wound healing. Wound Repair Regen. 2021 Nov;29(6):1051-61. (doi: 10.1111/wrr.12961).
57. Sun X, Jiang K, Chen J, et al. A systematic review of maggot debridement therapy for chronically infected wounds and ulcers.International Journal of Infectious Diseases, 2014;25:32–7.
58. Cazander G, van Veen KE, Bouwman LH, Bernards AT, Jukema GN. The influence of maggot excretions on PAO1 biofilm formation on different biomaterials. Clin Orthop Relat Res 2009;467:536-45.
59. Barnes KM, Dixon RA, Gennard DEJJomm. The antibacterial potency of the medicinal maggot, Lucilia sericata (Meigen): variation in laboratory evaluation. 2010;82(3):234-7.
60. Nigam Y, Dudley E, Bexfield A, et al. The physiology of wound healing by the medicinal Maggot, *Lucilia sericata.* Adv Insect Physiol 2010;39:39–81.