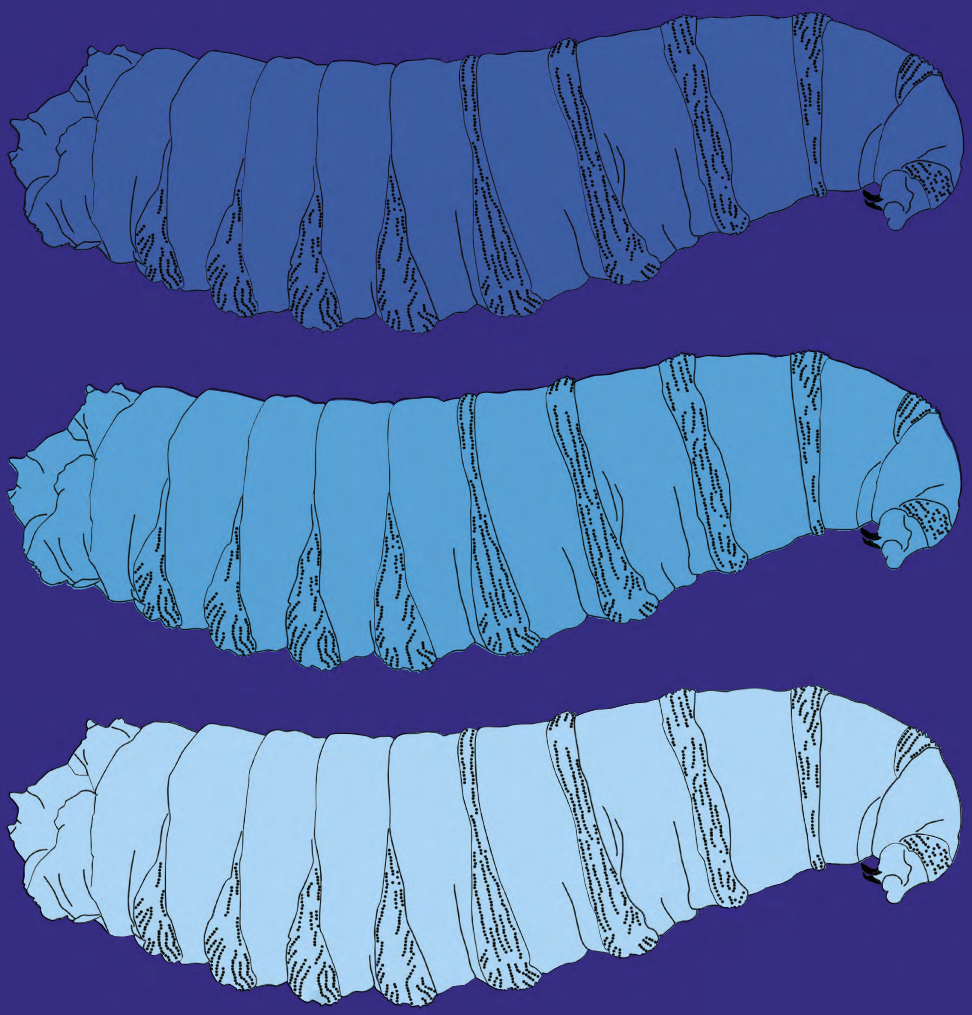
A COMPLETE GUIDE TO MAGGOT THERAPY

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



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Cover image: Line drawing of a green bottle blowfly (*Lucilia sericata*) maggot by Frank Stadler (2022), CC BY-NC. Cover design by Katy Saunders.

11. Bioprospecting and Testing of New Fly Species for Maggot Therapy

Patricia J. Thyssen, Franciéle S. Masiero and Frank Stadler

Lucilia sericata, the green bottle blowfly, has a long history of clinical use and an excellent safety record which makes it safe for therapeutic clinical use. In regions where it is naturally absent, maggot therapy cannot be offered to patients with chronic wounds unless an alternative local species is found. This chapter explains how new species are identified and tested for their therapeutic efficacy and clinical safety. The process involves the bioprospecting for candidate fly species, pre-clinical *in vitro* and animal studies to make sure they are therapeutically active and safe, and clinical trials of maggot therapy with human patients.

Introduction

The Diptera is one of the most taxonomically diverse insect orders. The flies also stand out with regard to the diversity of their feeding habits, including parasitism, saprophagy, predation, and omnivory. Flies can be found all over the planet, although many species may have only limited distribution due to physiological adaptations to survive in a certain biogeoclimatic region. For a detailed discussion of the natural history of medicinal flies, please refer to Chapter 7 of this book [1].

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Lucilia sericata (Meigen), the green bottle blowfly, has a long history of clinical use and an excellent safety record which makes it safe for therapeutic clinical use. In regions where it is absent, maggot therapy cannot be offered to patients with chronic wounds unless an alternative local species is found. This is because in most countries there are quarantine regulations in place for the introduction of living organisms, for whatever purposes, which is a necessary precaution to protect agricultural production and the environment from pests and diseases. How to establish laboratory colonies for medicinal maggot production and how to identify suitable species that have been previously used for maggot therapy is explained in Chapter 13 of this book [2]. Given that there are more than 1,500 blowfly species (Calliphoridae) known to date [3], it may seem that the identification of additional species for use in maggot therapy would be something simple, but this is not the case.

This chapter explains how new medicinal fly species are identified and tested for their therapeutic efficacy and clinical safety, so that maggot therapy is made possible in regions where *L. sericata* is not present. The process begins with bioprospecting which is the search for candidate fly species that possess the key characteristics required for clinical application. However, before shortlisted species can be tested on humans, they must first be subjected to pre-clinical trials to make sure they are therapeutically active and safe. Once there is evidence showing a good safety profile and at least effective debridement, which is a prerequisite for infection control and wound healing, clinical trials can be conducted to treat patients under controlled conditions with medicinal maggots from a new species. Such clinical trials are a prerequisite for the approval of maggot therapy in a country or block of countries with the same regulatory framework. After the efficacy and safety of maggot therapy have been demonstrated and the approval as a therapeutic good is obtained, medicinal maggots must be produced on a reliable basis, in line with good manufacturing practice, and at scale for country-wide delivery of maggot therapy services. Please refer to Chapters 12 to 18 of this book for guidance on the production and supply of medicinal maggots [2, 4–9].

Bioprospecting for Medicinal Fly Species

Bioprospecting is the process of searching for organisms with characteristics or properties that make them useful to humans as new

foods, new materials, new engineering applications, or new medicines. Fortunately, it is not all that difficult to prospect for potential new medicinal fly species. Eating habits such as necrophagy or parasitism can be discovered from observation in nature or at clinics that treat cases of myiasis in humans or domestic animals. Myiasis is the unintentional colonisation of a wound with fly maggots. Unless the species involved is parasitic and consumes living tissue, as is the case for both the New World screwworm *Cochliomyia hominivorax* (Coquerel) and the Old World screwworm *Chrysomya bezziana* (Villeneuve) [10], there is a good chance that the maggots found are therapeutically efficacious. Additionally, there are a large number of reports in the literature that can be useful to screen for and identify the appropriate feeding habit of a fly species, i.e., taking into account that the species must be obligatorily necrophagous, which means they must only feed on dead tissue. As is recommended for other scientific literature-based investigations, only data published in reputable, peer-reviewed and indexed scientific journals should be considered. In addition, public- or animal-health information held in regional public databases may also be useful in the search for new medicinal fly species.

A pre-selection of information in the literature can be very convenient to save time and to plan future research more effectively, but it should never be the only way to choose a fly species as a natural candidate for maggot therapy. There are a number of problems that are usually not evident at the time of screening. In most countries, reporting of a myiasis infestation in either animals or humans is not mandatory. For this reason, larvae of myiasis-causing flies are invariably removed and discarded, rather than properly diagnosed by specialists [11, 12]. An accurate diagnosis of the agent responsible for myiasis is necessary to gain more information about this disease presentation and to avoid misinformation. Flies that ingest the living tissue of their hosts (classified as causing obligatory myiasis, i.e., are parasites) should not be used for the treatment of wounds, since there is a risk that the damage caused will be greater than the benefit [13].

What about a species that is in transition from parasitism to necrophagy? *Lucilia eximia* (Wiedemann), known as carrion-breeding fly [14], is a good example of a parasitic transition phenomenon, since it has occasionally been incriminated as a facultative myiasis-causing fly (i.e., a fly with larvae that normally develop in carrion but can opportunistically parasitise and exploit living tissue or ingest

devitalised tissues from living hosts) in domestic and wild animals in the New World [15–17]. Another intriguing species is *Lucilia cuprina* (Wiedemann), which causes obligatory myiasis, predominantly in sheep, in Australia and New Zealand [18]. Stevens and Wall [19] studied the genetics of this species from specimens collected in Africa, Europe, Australasia, North America and the islands of Hawaii. The results supported the existence of an intraspecific genetic variation in *L. cuprina*, possibly related to geographic isolation [20] and strong climatic influence, which are factors that *a priori* determined which "species" would become predominant in different regions of the world [20]. Thus, in an independent evolution of the parasitic habit [21], one of the strains, *L. cuprina cuprina*, distributed in the Neotropical, Eastern and Southern regions of the Nearctic, would be responsible for facultative myiasis (therefore natural candidates for maggot therapy), while the other strain, *L. cuprina dorsalis*, present in the Australasian and Afrotropical regions, became an obligatory myiasis agent. Consequently, being able to correctly identify species is as important as knowing their biology. In Egypt, for example, *L. cuprina* larvae were accidentally used in two people to treat their wounds, but the initial intention was that *L. sericata* larvae were used [22]; the authors mentioned, without identifying the strain, that the treatment was safe and successful, but the outcome could have been unfavourable.

Competition between maggots from the same or different fly species in the same wound is another factor worth considering. *Chrysomya albiceps* (Wiedemann) is a calliphorid fly species originally from Africa and widely distributed in Asia, southern Europe, and several countries in South America. It is one of the species of great forensic relevance due to the frequency with which it colonises corpses [23] and it is known that its larvae are voracious predators of other fly larvae [24, 25]. While *Ch. albiceps* is not a parasitic species, its eligibility for maggot therapy is questionable considering that under adverse conditions (such as under temporary starvation during transport, for example) the larvae of this species could opt for cannibalism [24, 26], which would result in a reduction in the number of larvae placed in the wound and would consequently impair the evolution of the treatment.

Other characteristics that deserve to be highlighted when selecting a candidate species for maggot therapy are associated with (i) its

distribution, (ii) ease of breeding and maintenance, and (iii) the development cycle. The wound temperature is around 32°C [27–29], but in very warm climates this temperature may increase. Thus, it would be advisable to use local, warm-adapted species for treatment, since they may cope with these very high temperatures better than cold-climate species. Maggot therapy at scale will only be possible if the flies used can be easily mass-reared and maintained in the insectary. Mass rearing and efficient production, with a minimum of work and cost, is further supported by species that have a short development cycle of under four weeks from egg to egg.

Pre-clinical Trials to Assess Nutritional Strategy, Safety, and Therapeutic Efficacy of Medicinal Maggots

Assessing the nutritional strategy is the first step to making sure that a 'new' species of fly considered for maggot therapy can be used safely. After all, it would not be desirable to use fly species that are parasitic and consume living flesh. Such pre-clinical trials should be done using *in vitro* experimentation and *in vivo* with laboratory animals and real wounds. *In vitro* experimentation without laboratory animals has the advantage that (i) it does not involve vertebrate animals and therefore does not require animal ethics approval, (ii) with some exceptions, it is inexpensive regarding inputs and infrastructure, (iii) fewer people are involved in its execution, (iv) it allows for a greater number of replicates, and (v) the results can be obtained in a relatively short time.

The first pre-clinical trial that should be conducted is the *in vitro* testing to evaluate both the nutritional strategy of fly larvae and their debridement rate. The latter is determined by the amount of tissue that one larva is able to ingest per time spent on the wound.

Possible cytotoxic properties and mechanisms by which the larvae promote healing and control infection can be investigated through a series of *in vitro* tests using human or animal cell cultures from rats, mice, and monkeys (Vero cells, T lymphocytes, macrophages), or by the cultivation of microorganisms (multi-drug resistant bacteria, fungi that cause mycoses, or protozoa that cause cutaneous diseases such as leishmaniasis). In tests with cell cultures, larvae should not be used because their vigorous activity may disturb or damage the cell

culture. The larvae may also struggle to survive due to the lack of food and inappropriate environmental conditions (temperature, amount of oxygen and light). Instead, larval excretions and secretions rich in low- and medium-molecular weight proteins such as proteases and antimicrobial peptides are routinely extracted for this purpose. It is somewhat limiting, though, that for each hypothesis to be investigated, a separate test must be conducted to obtain the desired response (Table 11.1). Moreover, such testing can require special equipment and technical expertise with cell culture and experimental design. Inadequate setup of experimental groups, for example, can generate data with high bias and little scientific benefit.

Table 11.1 List of some *in vitro* tests performed to (i) characterise the antimicrobial factors and investigate the activities of larvae and their products against bacteria, fungi and parasites; (ii) to evaluate the mechanisms of action that contribute to wound healing; and (iii) to investigate how to improve aspects related to production, survival and disinfection of immature (eggs and maggots).

***In vitro* tests performed to observe:**	**Target Species***
Characterisation of antimicrobial factors (peptides)	*Calliphora vicina* (Robineau-Desvoidy) (alloferon 1 and 2) [30], *Lucilia cuprina* (lucifensin II) [31], *L. eximia* (lucilin)[32], *L. sericata* (lucifensin, lucimycin)[33, 34], *Protophormia terraenovae* (Robineau-Desvoidy) (phormia A and B) [35], *Sarconesiopsis magellanica* (Le Guillou) (sarconesin, sarconesin II) [36, 37]
Antibacterial, antifungal and/or antileishmania activities of larval excretions/secretions, specific peptides, fat body or hemolymph extracts	*Ca. vicina* [30, 38, 39] *Chrysomya albiceps* [40], *Chrysomya megacephala* (Fabricius) [40, 41], *Chrysomya putoria* (Wiedemann) [40], *Chrysomya rufifacies* (Macquart) [42], *Co. macellaria* [42, 43], *L. cuprina* [31, 44–46], *L. eximia* [32], *L. sericata* [33, 34, 39, 47–49], *Musca domestica* Linnaeus [50], *P. terraenovae* [35], *S. magellanica* [35, 37, 39, 49, 51, 52]
Action against bacterial biofilm	*Ca. vicina* [53], *L. sericata* [54–57]
Synergism between larval excretions/secretions and antibiotics	*L. cuprina* [45], *L. sericata* [56, 58]

***In vitro* tests performed to observe:**	**Target Species***
Combined use of larvae and topical agents	*Co. macellaria* [59]
ES stimulus on fibroblast migration and components of the extracellular matrix	*L. eximia* [32], *L. sericata* [60]
ES and their ability to inhibit pro-inflammatory responses	*L. eximia* [32], *L. sericata* [61]
Angiogenesis stimulus	*L. sericata* [62, 63]
Transgenic larvae and their ability to express and secrete human platelet-derived growth factor	*L. sericata* [64]
Larval growth, survival, and debridement efficacy	*Co. macellaria* [59], *L. sericata* [65, 66]
Survival of embryos by cryopreservation	*L. sericata* [67]
Efficiency of egg disinfection	*Ch. megacephala* [68, 69], *Ch. putoria* [69], *Ch. rufifacies* [68], *Ca. vicina* [70], *Co. macellaria* [68, 69], *L. cuprina* [68, 71, 72], *L. Sericata* [68]

The most important question to be answered in pre-clinical trials is whether the larvae of the chosen species feed only on devitalised tissue or also feed on living flesh. This question must be answered using *in vivo* animal models, because *in vitro* tests cannot reproduce the complexity of biological, physiological, and immunological interactions between maggots and hosts. The evaluation of cytotoxicity in response to the products produced by the larvae is also of critical importance, but as seen previously, it can be appropriately observed through *in vitro* tests. If both results are promising, then it is demonstrated that maggot therapy is safe and development can enter the clinical trial phase.

In vivo tests can also be performed to help identify safety or even efficacy for treatments of wounds with specific aetiology, such as cutaneous parasitic infections, or those where the individual has some type of comorbidity such as diabetes (Table 11.2). In maggot therapy the animal models that have been most used are mice, rats, and rabbits. However, *in vivo* experiments should only be conducted when necessary because (i) they are expensive, with experimental animals raised in specialised laboratory animal facilities costing approximately USD10.00

and USD15.00 per pathogen-free (SPF) mouse and rat, respectively; and (ii) there are added training and animal welfare requirements for researchers to consider. The study design must be approved by a recognised ethics committee and experiments should be carried out with the fewest possible replicates (animals) to prevent the unnecessary sacrifice of laboratory animals.

Table 11.2 List of some *in vivo* tests performed (i) to assess the nutritional strategy and safety of larvae, (ii) to investigate the activities of larvae and their products against bacteria, viruses, and parasites, (iii) to investigate the capacity to enhance antitumoural activity, and (iv) to assess the mechanisms of action and effectiveness of different therapeutic approaches in wound healing.

***In vivo* tests performed for/to:**	**Target Species***
assess the nutritional strategy and the safe use of a "new species" for maggot therapy	*Co. macellaria* [73]
assess the capacity to enhance antitumoural activity	*Ca. vicina* [30]
assess the antibacterial, antiviral and/or antileishmania activities of larval excretions/secretions or peptides	*Ca. vicina* [30, 39], *M. domestica* [74], *L. sericata* [39, 75–77], *S. magellanica* [75].
assess the effectiveness of maggot therapy and/or larval products in wound healing in animals with or without diabetes	*Co. macellaria* [78, 79], *L. cuprina* [41, 80, 81], *L. sericata* [82–84], *S. magellanica* [82, 85]
compare the effectiveness between maggot therapy and conventional treatments for wound healing	*Co. macellaria* [79]
assess the effectiveness of maggot therapy between different fly species	*L. sericata* [82], *S. magellanica* [82]

From an animal ethics point of view and to avoid unnecessary sacrifice of experimental animals, it would be helpful to use animals, for example pets or farm animals, that have been accidentally injured. However, it is very difficult to ensure the homogeneity in species, age, sex, wound type, aetiology and other factors required for unbiased planning of the experiment and the analysis of results. Although, it is not impossible to plan a prospective-style trial with such animals, but the time it

would take to accumulate a meaningful case number of sufficiently homogeneous nature is probably impractical, unless the researchers have access to a regular supply of injured animals such as from large-scale farming activities or veterinary practice.

While small individual immunogenic differences may exist, when wounds are artificially induced within the same group of laboratory animals, the samples are still sufficiently homogeneous. There are several ways to induce wounds in anaesthetised laboratory animals, ranging from scarification of the skin [76, 81, 83, 84] to chemical burns [73]. Wounds may also be induced with wound-causing parasites [39]. Wounds may or may not be inoculated with pathogenic bacteria [52, 82]. For a comparative evaluation of the therapeutic action of fly larvae it is important to take into consideration how the wound was induced. Any initial variations in size and depth of the wound, in infection status, or microbe/parasite load must be accounted for in the analysis in order not to distort results and lead to wrong conclusions.

Before starting an *in vivo* test, it is mandatory to submit to an institutional ethics committee an application to conduct an experiment with animals, together with a very detailed design of the study. This application will be assessed by the committee against the guidelines of regional or international associations for guarantee animal welfare (e.g., World Organization for Animal Health (OIE), World Veterinary Association (WVA), United States Department of Agriculture (USDA)). These assessments can take time, particularly when the experimental design and methodologies lack detail and are poorly researched.

Preclinical Research Protocols

The following three protocols explain (i) how to conduct an *in vitro* test to assess the nutritional strategy and debridement efficacy of medicinal maggots, (ii) how to assess the antimicrobial action of larval excretions and secretions (ES) against bacteria, and (iii) how to conduct *in vivo* experiments to assess the therapeutic safety and efficacy of fly species after preliminary *in vitro* testing. Each protocol is accompanied by a schematic summary of the process (Figures 11.1 to 11.3)

How to Conduct an In Vitro Test to Assess the Nutritional Strategy and Debridement Efficacy of Medicinal Maggots

1. Establish fly colonies as described in Chapter 13 [2] of this book. For guidance on colony maintenance in the insectary, please refer to Chapter 14 [7].
2. Harvest eggs from established colonies with some raw minced liver.
3. Remove eggs from the liver bait and disinfect by washing them for 3 min with 1% sodium hypochlorite (NaOCl) [69].
4. Place disinfected eggs on filter paper into a Petri dish and incubate them at 25°C until the larvae hatch.
5. Prepare the *in vitro* wound model: place a 20 g portion of raw minced pork muscle (this meat should be used for the wound model because it resembles closely human tissue and results in larval growth very similar to that of human tissue [87, 88]) and a few drops of artificial blood (250 g portion of fresh bovine liver triturated in 50 mL of distilled water) into a small plastic container (approx. 145 mL—use inexpensive containers with a tightly fitting lid).
6. Introduce 30 newly hatched larvae to each wound model replicate (consider that maggots debride approximately 150 mg of necrotic tissue per day [27]). The maggots used should always be of the same age and developmental stage.
7. If performance at the point of care is to be simulated then maggots to be tested should be packaged as per regular shipment and left for 24 h (or the typical time it takes for delivery) before testing.
8. If the debridement efficiency of bagged maggots is to be tested then the bag is placed onto the meat and a tie should be applied to ensure close contact between the bag and the meat surface.
9. A piece of fine-weave chiffon fabric or other synthetic mesh fabric may be sandwiched between container and lid to confine maggots. The lid needs to be fitted with a hole about

4 cm^2, large enough to permit ventilation but small enough to prevent excessive moisture loss from the chamber.

10. Incubate at 33±1°C. This temperature range corresponds to the wound bed temperature range of 31–35°C [27, 28].
11. The incubation time to assess the nutritional strategy and/or debridement activity will depend on whether free-range or bagged application is simulated. Free-range application is much faster, necessitating removal of maggots after 24, 48 or 72 h of application (the duration of each experiment corresponds to common maggot therapy treatment times [89–91]. Bagged application is less efficient and bags may remain on the wound for up to 96 h [92, 93].
12. After the incubation period, debridement efficacy can be established with the measurement of the: (i) weight of the remaining meat (measured to the nearest mg); (ii) number of surviving maggots; (iii) developmental stage (instar) of surviving maggots; (iv) weight or length of maggots (since weight and size are correlated it will not be necessary to measure both). In both cases, measurements should be taken after maggots have been quickly euthanised by rapid immersion in near boiling water (approximately 70ºC)—it is important to consistently stick to one method for comparability of results over time.
13. With adequate replication (there should be at least three replicates for each experimental group), the data generated will allow descriptive statistical analysis and also analysis of variance between assay runs. This can reveal whether debridement efficacy is maintained, increases or declines over time.

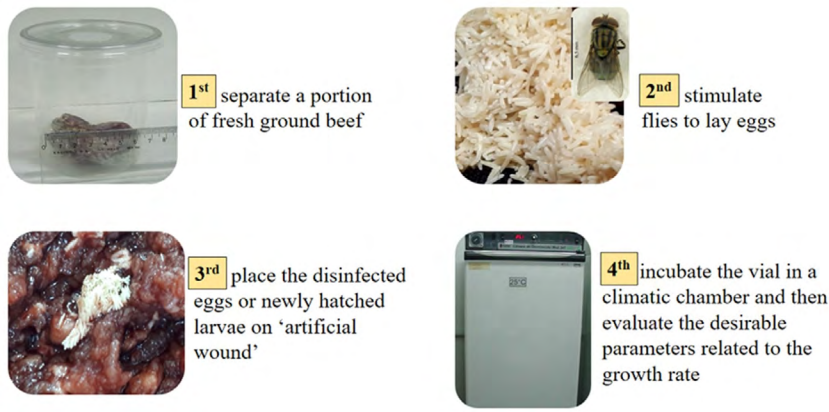

Figure 11.1 Summary model and protocol of an *in vitro* test to assess the nutritional strategy and debridement activity of medicinal larvae (adapted from Masiero and colleagues [59]).

How to Assess the Antimicrobial Action of Larval Excretions/Secretions (ES) against Bacteria

1. Establish fly colonies as described in Chapter 13 [2] of this book. For guidance on colony maintenance in the insectary, please refer to Chapter 14 [7].
2. Harvest eggs from established colonies with some raw minced liver.
3. Remove eggs from the liver bait and disinfect by washing them for 3 min with 1% sodium hypochlorite (NaOCl) [69].
4. Place disinfected eggs on filter paper and incubate them at 25°C until the larvae hatch.
5. Transfer newly hatched larvae to a container with minced beef at a dose of 1 larva per gram of beef.
6. Allow larvae to feed for 72 h.
7. Remove larvae from the meat and wash them 3 times in sterile distilled water.
8. Add 25 live larvae to 800 μL of sterile distilled water, held in a 1.5 mL microtube. Incubate for 1 h in the dark at 37±2°C.

9. Remove larvae from the microtube, discard them and centrifuge the resultant liquid at 4000 x g at 4°C for 15 min.
10. Sterilise the liquid, i.e., the excretions/secretions (ES) by passing them through a 0.45 μm filter.
11. Refrigerate ES at 10±2°C for up to 24 h.

Agar plate test:

1. Different concentrations of ES and bacteria can be tested on agar plates with results measured in colony-forming unit (CFU). Experimental groups may be divided into: (i) ES; (ii) ES + bacterial inoculum; (iii) bacterial inoculum; and (iv) control, i.e., bacterial inoculum plus an antimicrobial agent for which efficacy is known.
2. Seeding and reading of the plates: aliquots of 25 μL of each experimental group should be plated by spreading onto Petri dishes containing BHI agar (Brain Heart Infusion) with three replicates for each treatment. Seedings are done at 0 h, 1 h, 2 h, 4 h and 12 h after the extraction of ES.
3. Petri dishes are incubated at 37°C, according to the reading period set.
4. Count CFU using a colony counter. Plates presenting more than 300 CFUs are considered uncountable.

Microtiter plate and optical density test:

1. Reading by OD: dose a 96-well microtiter plate so that each vertical well row represents one treatment (ES, ES + bacterial inoculum, bacterial inoculum, bacterial inoculum + control antibiotic, and sterile Luria-Bertani broth.
2. The experimental groups are tested at least in triplicate. A total volume of 200 μL (1:1 in ES + bacterial inoculum and bacterial inoculum + control antibiotic groups) is introduced into each well.
3. Incubate the microplate at 37°C.
4. Measure changes in the OD at 540 nm (wavelength) with a microplate reader spectrometer. Measure every 1 h (for up to 6 h) and then after 24 h at 540 nm.

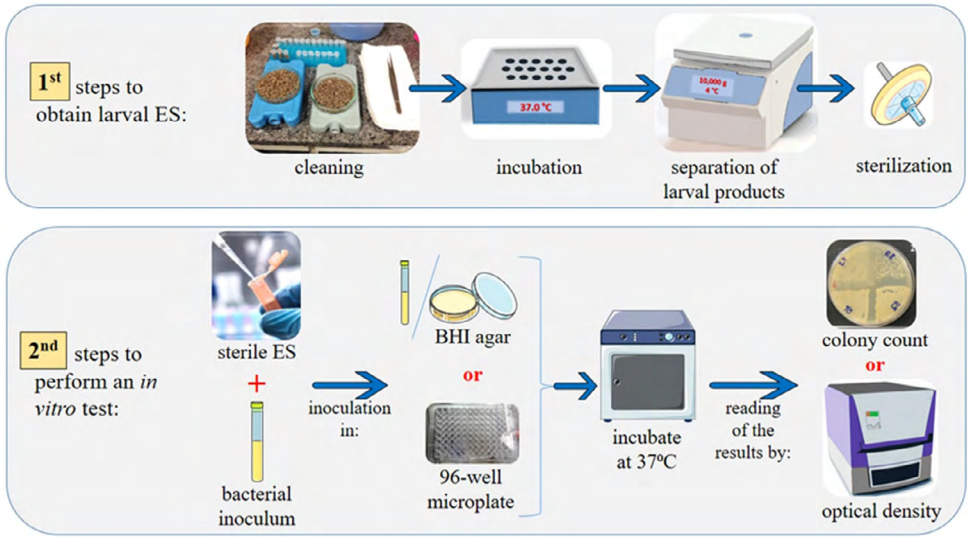

Figure 11.2 Summary model of *in vitro* test to assess the antimicrobial action of larval excretions/secretions of medicinal blowfly larvae against bacteria (adapted from Masiero and colleagues [43]).

How to Conduct In Vivo Experiments to Assess Therapeutic Safety and Efficacy of Fly Species after Preliminary In Vitro Testing

1. Establish fly colonies as described in Chapter 13 [2] of this book. For guidance on colony maintenance in the insectary, please refer to Chapter 14 [7].
2. Harvest eggs from established colonies with some raw minced liver.
3. Remove eggs from the liver bait and disinfect by washing them for 3 min with 1% sodium hypochlorite (NaOCl) [69].
4. Place disinfected eggs on filter paper and incubate them at 25°C until the larvae hatch.
5. Transfer new hatched larvae to a container containing 1 g sterile diet [94] or blood agar per transferred larva.
6. Allow larvae to feed on diet for 12 h before depositing them on the wound.
7. Obtain around 13 male (or female) 12-week-old Wistar rats (*Rattus norvegicus alvinus*, Rodentia, Mammalia), weighing approximately 350 g.
8. Keep the rats in individual cages at a temperature of 22 ± 2°C and a 12-h photoperiod.
9. Before lesions are induced, administer anaesthesia and analgesia with ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg) both intraperitoneal.
10. Shave the dorsal region and administer 0.2 mL of a 1:4 solution of hydrochloric acid and distilled water subcutaneously.
11. Lesions will open on their own within three days. Analgesic medicine should be given systematically to control discomfort.
12. Randomly divide animals into at least two groups (N= 6 in each group)
13. Treat one group with maggot therapy (5 larvae/cm^2) and the other, being the control group, with a treatment for which efficacy is known and supported by strong evidence. During

treatment, the lesions must be covered with a confinement dressing made of polyurethane dressing, sterile gauze, and tape to prevent escape and suffocation of larvae [79].

14. Administer treatments for 48–72 h.
15. Evaluate lesions daily, from the beginning of the application of the treatments until the end of the experiment or complete healing. Possible assessment metrics include:
    a. Healing progress, wound shape, wound edge characteristics, wound depth, quantity of necrotic tissue, type and quantity of exudate, and amount of granulation tissue.
    b. The wounds can also be evaluated according to Pressure Ulcer Scale for Healing (PUSH) (National Pressure Ulcer Advisory Panel, 1998). Particular attention (and a more detailed examination) should be paid on the initial day of treatment (day 0) and on days 3, 7, and 12 after treatment, to capture significant changes in the lesions [73].
    c. Additionally, photographic records before, during, and after each treatment are recommended in order to document the results and to assess the ratio of wound healing (RWH). RWH represents the reduction percentage of the wound in relation to its size before the beginning of the treatment [95]. It is calculated by the following formula, where: A(i) = wound area at day zero, i.e., prior to treatment; A(f) = wound area on the day of evaluation. Areas can be calculated using any software suitable for this purpose.

$$RWH = \frac{A(i) - A(f)}{A(i)} \times 100$$

    d. Collect histopathological samples at 0 h (before application of any therapeutic treatment) and at 3, 7, and 12 days after administration of treatments.

e. On day 0, euthanise the one animal not assigned to a treatment group and obtain tissue samples. At days 3, 7, and 12, euthanise two randomly selected animals from each group for tissue sampling. Use a scalpel to excise a 5mm tick section of wound extending 5mm beyond the wound margin to include normal skin tissue.

f. Fix the samples for 24 h in 10% paraformaldehyde and dehydrate in 70% ethanol.

g. Then embed samples in paraffin and cut 5 μm sections.

h. Stain sections with hematoxylin-eosin (HE), and examine under an optical microscope for signs of inflammation, epithelial regeneration, and blood vessel formation.

Clinical Trials

Any investigative procedure performed with human subjects with the aim of providing information on the safety and efficacy of drugs and other medical therapies is called a clinical trial. As illustrated in Figure 11.4, these trials are generally divided into three phases starting with a small number of participants (phase I) where aspects related to safety are evaluated, followed by studies with a larger number of participants (phase II), which often compare the 'new' product or protocol with a currently prescribed treatment. Once favourable safety and efficacy data are gathered, the phase III trials are conducted, covering a much larger number of participants. Phase IV clinical trials follow the approval of a medical product or therapy by a regulatory agency (or health authority) and once placed on the market. In this phase, the long-term safety and efficacy of the therapy is being investigated using thousands of participants. It is important to remember that all clinical trials irrespective of the phase can only be carried out with the approval of the relevant ethics committee and respective local health authorities, because the trial involves either human or animal subjects (for veterinary therapies).

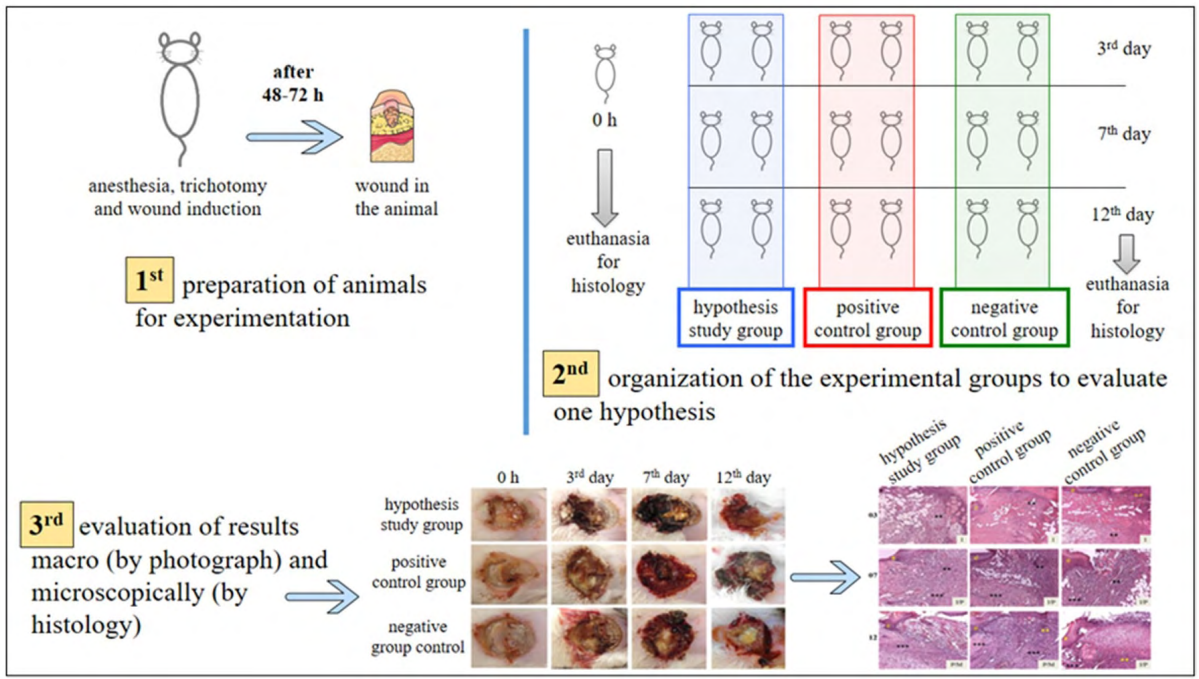

Figure 11.3 Summary model for *in vivo* experimentation to assess the safety and efficacy of blowfly species after preliminary *in vivo* experimentation and pre-selection for maggot therapy (adapted from Nassu and Thyssen [73] and Masiero and colleagues [95]).

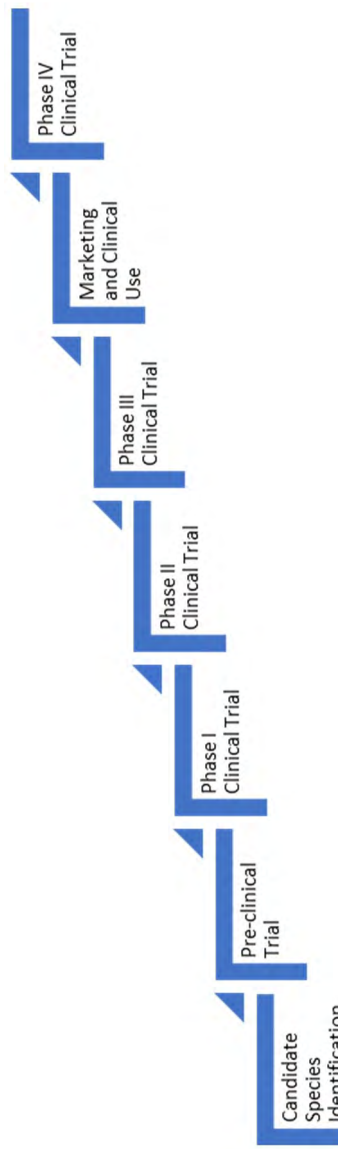

Figure 11.4 Step-wise process for the evaluation of a fly species for maggot therapy from bioprospecting to clinical use and beyond.

There are other relevant issues to consider when planning a clinical trial. They may be carried out in a single centre (usually phase I and II trials) or in several (multicentre trials). The latter are commonly phase III trials because of the number of participants involved. As for the methodology for acquiring sample data, the trials can be classified as blind, double-blind, randomised controlled, retrospective, or prospective. In a blind study, neither the study subject nor the examiner knows what treatment (response variable) is administered at any stage. It is commonly seen as one of the most effective and efficient mechanisms to guarantee the quality, reliability, integrity and consistency of the results obtained. For somewhat obvious reasons, trials with maggot therapy cannot be properly blinded because there is no way to "eliminate" the sensation of larvae moving in the wound, which is likely to be perceived by the patient, even if maggots are applied in biobags. As for the visual appearance of the dressing, practitioners will invariably notice even young maggots during application of biobags or free-range maggots on gauze pads. At the end of treatment during dressing removal, there is no hiding which wound received medicinal maggots. Consequently, blind maggot therapy trials are only possible if participating patients commit to secrecy and do not reveal what treatment they have received. Moreover, the researchers evaluating the therapeutic outcome must remain ignorant with regard to the treatment and do not witness dressing application and removal. The randomised controlled clinical trial (better known as RCT) is often used to test the effectiveness of a product or therapeutic approach in a given population compared to a control treatment, i.e., a product/approach with known effects. Randomisation with regard to which patient gets what treatment is a strategy used to increase the validity of the results obtained. The term control indicates that the participants will not receive a single intervention factor, otherwise it would be a descriptive study of effects with limited scientific value. It is the author's opinion that RCTs would be the preferred way to validate the use of maggot therapy. In Brazil, for example, the national health authority does not authorise the wide-scale use of a medical product or therapy like maggot therapy without consistent results from a RCT.

As mentioned above, studies can also be retrospective (in which the researcher studies participants based on an outcome) or prospective (when the outcome has not yet occurred). The type of study or

experimental design must be chosen to minimise the chance of researcher bias. If retro- or prospective studies are undertaken then researchers must be careful to not only search for evidence that is consistent with their hypothesis but also to disregard those that may actually be more relevant [96]. In addition, Hanson [97] provides step-by-step guidance on how to design, conduct and report clinical research, including helpful tables and checklists.

The global burden of chronic and difficult-to-heal wounds is steadily growing. Most patients receiving maggot therapy are diabetics due to the frequency with which they are affected by difficult-to-heal wounds and the high number of unsuccessful treatments that often lead to infections, amputation of extremities, or death. It is estimated that the number of people with diabetes worldwide affected by complications of the lower limb is between 40 and 60 million [98]! Maggot therapy is very promising both in terms of its efficacy and affordability, particularly in austere environments [99, 100]. However, there appear to be only few large and well-designed clinical trials developed or under way that seek to assess the efficacy of maggot therapy in terms of its ability to debride wounds, control infections, and promote wound healing, although these therapeutic benefits have long been observed in practice and in the laboratory. Some of the most significant studies and clinical trials conducted to date (where the number of participants is equal to or $>$ 10), including nonrandomised retrospective studies, nonrandomised prospective studies, RCTs (multicenter or blind) and meta-analysis, are shown in Table 11.3.

Table 11.3 Examples of some of the most significant studies and clinical trials conducted to assess the efficacy of maggot therapy taking into account the main objectives to be achieved and the target species of blowflies used for maggot therapy. Those with sample size less than ten have been excluded.

Type of study/ clinical trial design	**Main goal**	**Country and Reference**
Nonrandomised retrospective study	To compare the healing rate between wounds treated with maggot therapy (*L. sericata*) (n = 14) and conventional treatment (n = 6).	USA [101]
	To compare the treatment of infected wounds with maggot therapy (the fly species used was not mentioned, but was most likely *L. sericata*) and antibiotics.	USA [102]
	To compare time and healing rate in the treatment of infected pressure ulcers or diabetic foot ulcers (n = 23) with maggot therapy (*L. sericata*) and conventional treatment (n = 20).	China [103]
	To assess the healing evolution in decubitus (pressure) ulcers in the sacral region (n = 36). No control group was included.	Turkey [47]
Multicentre retrospective study	To assess the healing rate in the treatment of wounds (n = 723) with maggot therapy (*L. sericata*) by loose or bagged larvae.	Israel [104]
Nonrandomised prospective study	To compare the treatment of infected chronic diabetic foot ulcers in individuals aged 18–80 years with maggot therapy (*L. sericata*) and antibiotics. No control group was included.	UK [105]
	To compare the healing rate between wounds treated with maggot therapy (*L. cuprina*) (n = 29) and conventional debridement (n = 30).	Malaysia [106]
	To assess the treatment of chronic ulcers of distinct aetiologies in individuals aged 32–87 years with maggot therapy (*L. eximia*). No control group was included.	Colombia [91]
	To compare the treatment of infected lower limb wounds with maggot therapy (*L. sericata*) (n = 80)—in this case, considering two aspects: the treated area (legs or feet) and number of applied larvae (5 or 10 larvae/cm2)—and ozone therapy (n = 49).	Poland [107]

Type of study/ clinical trial design	**Main goal**	**Country and Reference**
Meta-analysis	To assess the outcomes and median costs of diabetic ulcer treatments with either maggot therapy or conventional treatment by pooling four previous cohort studies.	Thailand [108]
	To assess the time and healing rate in the treatment of wounds with mixed aetiologies. Twelve studies were included in the meta-analysis.	China [109]
RCT	To compare the median percentage area reduction of venous leg ulcers treated with four-layer compression bandaging (group I, n = 20) or four-layer compression bandaging + larvae (*L. sericata*) (group II, n = 20).	UK [110]
	To compare the treatment of infected (by *Staphylococcus aureus* or *Pseudomonas aeruginosa*) diabetic foot ulcers (n = 50) with maggot therapy (*L. sericata*) and conventional treatment (antibiotic therapy, debridement, and offloading).	Iran [111]
Three-arm RCT	To compare cost-effectiveness, time, and healing rate in the treatment of venous ulcers in which participants (n = 267) were assigned to one of three treatment groups: 1) loose larvae (*L. sericata*), 2) bagged larvae (*L. sericata*), and 3) hydrogel.	UK [112, 113]
Blinded RCT	To compare debridement rate and reduce the bacterial load and wound area in venous ulcers treated with maggot therapy (*L. sericata*) or surgical debridement followed by one topical application of silver sulfadiazine per week (for four weeks). It was mentioned that the statistician was not aware of the origin of the data that was received for analysis.	Mexico [114]

Type of study/ clinical trial design	Main goal	Country and Reference
Multicentre blinded RCT	To compare the debridement rate of venous ulcers, with 40 cm2 (or smaller) and > 2 cm deep (n= 119), treated with maggot therapy (bagged *L. sericata* larvae) and conventional treatment (surgical debridement + dressing of hydrogel or alginate) in individuals during a two-week hospital stay.	France [115]
	To compare the debridement speed of venous or arterial/venous ulcers (n = 64) treated with maggot therapy (*L. sericata*) or hydrogel.	UK [116]

Regulatory Approval

Unfortunately, in most countries (particularly those in the southern hemisphere) the use of maggot therapy is still limited to clinical research studies which prevent the treatment of a large number of wound patients who would benefit from maggot therapy (Table 11.3). The reasons for this include:

- MT is subject to regulatory and bureaucratic limitations before it reaches the patient. Under such circumstances, a very detailed research proposal must be presented to a local ethics committee, which will authorise its execution, provided the wellbeing and safety of patients are assured.
- In addition, it is also necessary to obtain the consent of every patient participating in the study. The human ethics of maggot therapy is discussed in detail in Chapter 19 of this book [117].
- A single or even multiple treatment centres may not have a large number of patients with wounds that are amenable to maggot therapy.
- Likewise, the treatment centres may lack appropriate infrastructure to cater for a large number of patients within the timeframe of a clinical trial.

To market and use medicinal maggots or wound care products derived from medicinal maggots on a routine basis, the medical product must be approved for use by the relevant ministries of health or their regulatory agencies. Health regulatory agencies are usually independent statutory bodies under the law with the main objective to regulate and inspect products or activities to protect healthcare consumers. Some of the best-known regulatory bodies and agencies are, for example, the Food and Drug Administration in the United States, the Medicines and Healthcare products Regulatory Agency in the United Kingdom, and the European Medicines Agency.

Obtaining authorisation for the use of a medical product or therapy can be quite challenging, because stringent safety and efficacy criteria must be met [118]. Such requirements are necessary as an additional measure that protects not only patients but also their treating physicians from harm and liability. In recent decades, medicine has advocated evidence-based practice [119], i.e., clinical decision making based on data obtained from systematic reviews of primary research as conducted by Cochrane [120]. Another mechanism to assess the overall quality of evidence for clinical decision making is GRADE which stands for Grading of Recommendations, Assessment, Development and Evaluations [96]. It is the most widely adopted tool for grading the quality of evidence and for making recommendations, endorsed by over 100 organisations worldwide. GRADE classifies the evidence as very low, low, moderate or high considering the following criteria: limitation of study design, imprecision, inconsistency, indirectness and bias of publication. As can be seen in Table 11.3, most studies conducted to evaluate the therapeutic efficacy of maggot therapy do not meet such high standards. The main reasons why most maggot therapy studies are not eligible for inclusion in systematic reviews or meta-analyses include: non-random allocation, non-blind evaluation of results, absence or poor description of the control group, simultaneous intervention, lack of clarity or non-follow-up of the outcome, studies with inadequate sample sizes, heterogeneity of the treated groups, and the lack of standardisation in the use of maggot therapy. For example, in the latest systematic review of the Cochrane Database on the debridement of diabetic foot ulcers to assess the rate of healing [121], only one RCT with maggot therapy was eligible for inclusion in the analysis.

Having said this, the existing substantive body of evidence [108, 109, 111, 116] and clinical experience leaves maggot therapy practitioners and patients with little doubt that maggot-assisted wound care is highly efficacious. It is therefore important that future clinical trials are designed according to best practice. To that end, researchers planning such studies are encouraged to consider the recommendations on clinical data collection, prepared by the European Wound Management Association's Patient Outcome Group [122], that describe criteria for producing rigorous outcomes in both RCTs and clinical studies, and describe how to ensure studies are consistent and reproducible. Another helpful read is a protocol proposed by Fan and colleagues [123] on how to conduct a systematic review and meta-analysis, which draws attention to particularly topical issues, such as the choice of participants for a given clinical trial (the inclusion of very heterogeneous wounds has been common) and poorly standardised practice in relation to the use of maggot therapy. For example, there may be significant differences in the effect of debridement with variations only in these two parameters.

We do not provide detailed guidance on how to apply for authorisation to use maggot therapy from regulatory agencies, as the list of documents and their order of presentation tend to vary significantly from country to country. In Brazil, as in other jurisdictions, specialised consultants can be hired to initiate this process at the Brazilian National Health Surveillance Agency (ANVISA), and we have observed that this action avoids delays in the evaluation of requests.

Clinical Use and Commercial Production

In the 1930s and '40s after William S. Baer introduced maggot therapy to modern medicine, medicinal maggots were produced in US, Canadian, and European hospitals for in-house use and Lederle Laboratories (Pearl River, NY) supplied medicinal maggots commercially. However, with the advent of antibiotics, maggot therapy fell out of favour [124]. However, since the late 1980s and early '90s, interest in maggot therapy has been steadily growing again. Today, two examples of successful commercialisation of *L. sericata* larvae for therapeutic purposes stand out. In Europe, BioMonde is the main supplier of medicinal maggots with origins dating back to 1994. In Europe, medicinal maggots are

produced in accordance with current European Good Manufacturing Practice requirements. Until December 2020, BioMonde offered two products, loose (free-range) larvae and BioBag™ dressings. Due to regulatory and supply restrictions, as well as commercial unfeasibility, BioMonde discontinued the supply of free-range maggots in January 2021. In the US, medicinal maggots have been commercially produced and supplied by MonarchLabs since 2005. As far as regulatory approval is concerned, the Food and Drug Administration considers medicinal maggots a medical device [125].

It is important to note that in both cases the enterprises that commercialised medicinal maggots were spin-offs from research programmes at prestigious universities [126–128]. This is not a surprise because it is a long and costly research process until medicinal maggots can be commercially produced, marketed and applied in the clinical setting, especially for new medicinal fly species (but also for *L. sericata* in jurisdictions where maggot therapy is not yet approved). Unfortunately, partnerships between universities and the commercial sector in low- and middle-income countries are impractical for a number of reasons, including low institutional productivity and inadequate professional qualification. The absence of reliable, fast, and affordable delivery services for highly perishable maggots is also a barrier to commercialisation in low- and middle-income countries. Recent efforts by MedMagLabs [129] to locate medicinal maggot production at the point of care to avoid supply chain interruptions are promising. Regardless, these barriers to maggot therapy may explain why there has been little progress as far as maggot therapy in developing countries is concerned [130], despite the fact that highly efficacious maggot therapy is generally cheaper than other treatments for hard-to-heal wounds, especially in the low- and middle-income country context [113, 131].

In the US and Europe educational resources and training activities are offered by maggot therapy producers [132], researchers [133], professional bodies [134], and foundations [135]. The positive impact on patients' quality of life through qualitative studies of patient perceptions and experiences is still little explored but the few studies report favourable outcomes. For example, positive experiences among patients who used maggot therapy were reported by Kithching [136] and Silva and colleagues [137]. The latter study was carried out in South

America using *Ch. megacephala*, a newly selected medicinal fly species [90]. The patients who received the treatment perceived maggot therapy as the most successful treatment yet on their journey to wound healing.

Summary

It is highly desirable that a number of fly species representing all major biogeographic regions are available for maggot therapy [2]. This will ensure that maggot therapy can be implemented globally. This is because national quarantine authorities will be reluctant to permit the importation of non-native fly species, especially if they are considered agricultural pests. Moreover, it is conceivable that this bioprospecting will result in new fly species with superior or different therapeutic properties compared to those found in *L. sericata*.

The selection of a new fly species for maggot therapy must take into account the life-cycle of the species, which should be short so it facilitates mass rearing. The environmental requirements and the species' adaptability to maintenance in the insectary must also be considered. Any new target species with favourable characteristics must be assessed, either *in vitro* or in animal models, for their safety and efficacy. For example, they must not consume living tissue and pre-clinical testing must indicate likely therapeutic benefit to the patient. When development proceeds to clinical trials, it is important that these are planned and executed according to best practice to ensure that the data generated is acceptable to the medical regulators and demonstrates clearly the benefits and safety of maggot therapy with the species under investigation.

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