Use of Fly Larvae (Insecta: Diptera) as an Alternative Treatment for Wounds

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Abstract

Larvotherapy, which, as the name suggests, is the use of larvae, in the case of flies, to heal wounds that resist healing. They act on the wound through four mechanisms: they remove dead necrotic tissue, disrupt the bacterial biofilm, an extremely organized community of microorganisms that greatly interfere with the ulceration repair process, promote the growth of healthy tissue, and eliminate bacteria that cause infection. The treatment is more efficient in some cases than traditional medications and scarring. The objective of this manuscript is to describe the use of fly larvae as an alternative therapy. The methodology used an integrative literature review and a synthesis process to develop the study to expand the understanding of knowledge and achieve the expected results. The integrative literature review is a method that aims to synthesize results obtained in research on a topic or issue, in a systematic, ordered, and comprehensive way. It is called integrative because it provides broader information on a subject/problem, thus constituting a body of knowledge. To carry out the study, a search for scientific articles was carried out through the Virtual Health Library, in the SCIELO, LILACS, and PUBMED databases, using the terminologies registered in the Health Sciences descriptors, maggots, flies, and wounds, healing. Regarding the inclusion criteria, national and international articles were used in full, in Portuguese, English, and Spanish. The exclusion criteria were articles that were presented in duplicate and did not meet the objectives of the investigation.

Keywords: Maggots, Microorganisms, Necrotic, Tissue, Ulceration.


Introduction

Larval Therapy is a biological debridement technique, that is, cleaning and removing dead tissue from wounds, reducing the number of microorganisms present in them and contributing to the healing process. Popularly applied in countries such as Germany, Holland, the United States, Israel, Canada, and Mexico, the technique consists of using live fly larvae, sterilized in the laboratory, and applied to wounds, which digest only the dead wound tissue, keeping the tissue intact. intact and stimulated to heal. The fly species used is called Chrysomya megacephala (Fabricius, 1794) (Diptera: Calliphoridae). It is a safe, low-cost, and effective procedure, very suitable for the Brazilian reality. Larvotherapy is indicated for the
treatment of various injuries that are difficult to heal, such as integumentary, soft tissue, bone regions (in cases of osteomyelitis), diabetic foot ulcers, infected post-surgical ulcers, decubitus ulcers, venous stasis and burns. Clinical indications vary, however, the larvae can be applied to chronic wounds (infected or not by multi-resistant microorganisms), specially indicated for those patients who do not respond to conventional and current treatments, including cases of co-morbidities, which make surgical interventions impossible (Evans et al., 2012; Almeida et al., 2013; Afonso, 2014; Masiero et al., 2015; Silva et al., 2017; UFSM, 2019; Von et al., 2020; Dalmedico et al., 2021; Hanzel and Sperotto, 2021; Monteiro et al., 2021; Maul et al., 2022).

Objective

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Methods

The methodology used an integrative literature review and a synthesis process to develop the study to expand the understanding of knowledge and achieve the expected results. The integrative literature review is a method that aims to synthesize results obtained in research on a topic or issue, in a systematic, ordered, and comprehensive way. It is called integrative because it provides broader information on a subject/problem, thus constituting a body of knowledge. To carry out the study, a search for scientific articles was carried out through the Virtual Health Library, in the SCIELO, LILACS, and PUBMED databases, using the terminologies registered in the Health Sciences descriptors, maggots, flies, and wounds, healing. Regarding the inclusion criteria, national and international articles were used in full, in Portuguese, English, and Spanish. The exclusion criteria were articles that were presented in duplicate and did not meet the objectives of the investigation.

Selected Articles

Larvae work to heal wounds because feeding on bacteria and necrotic tissue stimulates the production of scar tissue. They sterilize material passing through the digestive tract and secrete therapeutic agents such as allantoin and urea (Figure 1) (Silva et al., 2017; Silveira, 2019; Von et al., 2020).

![Figure 1. Direct Placement of Lucilia cuprina (Wiedemann, 1830) Larvae in Rat Wounds Induced and Infected by MRSA, to Compare with Routinely Used Techniques, such as Mechanical Debridement Using Saline and Gauze and Topical Application of Antibacterial Ointment](image)

Source: Janaína Brand Dillmann, 2019
Researchers at the Insects and Vectors Laboratory collect the flies in the field and then raise them in the laboratory, where they are fed a specific diet to stimulate the female's egg laying. From these eggs, the creation of larvae begins. The quantity of larvae used in each application depends on the size of the patient's lesion and the stock that the laboratory will make available. The larvae are applied to the lesion and then a bandage is made, placing non-adherent gauze moistened with saline solution over the larvae, followed by dry gauze and bandage. Patients sign a consent form that guarantees that they will undergo therapy and that they agree (Figures 2-4) (Almeida et al., 2013; Gov.br., 2014; Masiero et al., 2015; Silva et al., 2017; Von et al., 2020; Maul et al., 2022).

Figure 2. Stages of the Cycle of Flies Eggs, Larvae, Pupae, and Adults
Source: Janaína Brand Dillmann, 2019.

Figure 3. The Colony is where Flies Live and Mature. Distributed on Shelves, at a Temperature of 25°C and an Air Humidity of 70%, Each Colony is Kept in PVC Pipes, Covered with Polypropylene Plastic Bags. There, the Insects are Fed Commercial Pasty Dog Food, Honey and Water ad Libitum. Some of the Larvae are Kept in the Colony for Renewal. After the Three Larval Stages, it Turns into a Pupa in the Sand and, from This Stage, Adult Males or Females are Born, Starting the Cycle All Over Again;
Source: Janaina Brand Dillmann, 2019.
In the laboratory process: Sterilized eggs of blowflies from the Calliphoridae family are used, such as the species 
*Sarconesia chlorgaster* (Wiedemann, 1830) and *Lucilia cuprina* (Wiedemann, 1830). With a portion of fresh beef liver, the fly is encouraged to posture, which is the act of laying eggs. Subsequently, these eggs are removed and taken to the laboratory for sterilization against microorganisms, which is confirmed when they are placed in Mueller Hinton microbiological culture medium. In this process, the surface of the egg is analyzed. If no growth of microorganisms has occurred in this medium, the egg is considered sterile and the hatched larvae can be prepared for shipping when the healthcare professional receives and applies it (Masiero et al., 2015; Martins Junior, 2017; Silva et al., 2017; Silveira, 2019; Von et al., 2020; Hanzel and Sperotto, 2021; Dillmann, 2022).

Larvotherapy, which, as the name suggests, is the use of larvae, in the case of flies, to heal wounds that resist healing. They act on the wound through four mechanisms: they remove dead necrotic tissue, disrupt the bacterial biofilm, an extremely organized community of microorganisms that greatly interfere with the ulceration repair process, promote the growth of healthy tissue, and eliminate bacteria that cause infection. The treatment is more efficient in some cases than traditional medications and scarring (Almeida et al., 2013; Masiero et al., 2015; Martins Junior, 2017; Von et al., 2020; Monteiro et al., 2021).

Larvae incubated for 24 hours with a bacterial suspension of *Staphylococcus aureus* Rosenbach 1884 (Bacillales: Staphylococcaceae), *Pseudomonas aeruginosa* (Schroeter 1872) (Pseudomonadales: Pseudomonadaceae), or methicillin-resistant MRSA, and antimicrobial analysis using Colony Forming Unit (CFU) assay in two incubation times 0 and 6 hours with groups of pre-treated and untreated larval extracts (PBS), compared to a bacterial growth control group. Hematological evaluation. It was therefore concluded that *L. cuprina* larvae are safe and effective in healing the lesion and eliminating MRSA (Figure 5) (Meldau, 2009; Masiero et al., 2015; Reyes et al., 2020; Von et al., 2020; Monteiro et al., 2021; Dillmann, 2022).

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Figure 5. The Insect that is Sent to Treat the Lesions is the Sterilized Larval Stage of the Fly (L1), almost Invisible to the Naked Eye. As it Feeds, it Becomes L2 and then L3 (Larva 3). When it Reaches this Stage, which is Already Very Visible, it will Leave the Food (Injury), as It No Longer Needs It. At this Point, it Moves Towards the Surface of the Wound, the Opening of which Must Not Be Suffocated and Extremely Closed, as the Larva Needs to Breathe as It is an Aerobic Animal. At this Point, It is Removed

Sources: Mútua Terrassa and https://www.mutuaterrassa.com/es/terapia-larval-had-farmacia-cirugia-vascular

The larvae produce small peptide molecules, one of which is sarconesin, discovered by a Colombian researcher. The name comes from the species of fly she studies S.a magellanica. The objective now is to use sarconesin as an active ingredient in medicine. As it is a relatively small molecule, it can be artificially synthesized in the laboratory or produced by genetic engineering, through the introduction of the DNA bases that encode it into a host bacterium (Figures 6-7) (Almeida et al., 2013; Gov.br., 2014; Silva et al., 2017; Diaz-Roa et al., 2019; Von et al., 2020; Monteiro et al., 2021).

Figure 6. The Search for New Antimicrobial Drugs to Counteract This Problem is a Priority for Health Institutions and Organizations, Both Globally and in Individual Countries. Sarconesia magellanica (Le Guillou, 1842) (Diptera, Calliphoridae) Blowfly Larval Excretions and Secretions (ES) are an Important Source for Isolating Antimicrobial Peptides (AMPs);

Sources: Díaz-Roa, A., et al., 2019
Figure 7. Sarconesin II MIC Effect on *Escherichia coli* (Theodor Von Escherich (1885). (Bacteria: Enterobacteriaceae) Bacterial Membrane by Scanning Electron Microscopy (SEM). Untreated *E. coli* (A) had a Normal Smooth Surface, while Treatment with Sarconesin II (B) Gave an Elongated Pattern, Membrane Disruption, and Blebbing on the Outer Face (arrows). Some Bacteria had Variable Length, Rough Cell Surfaces, or Globular Protrusions on Their Surfaces. These Images Revealed that Sarconesin II Could Induce Alterations in Cell Morphology; Sources: Díaz-Roa, A., et al., 2019

The use of *Musca domestica* L., 1758 (Diptera: Muscidae) is promising in the larval therapy of chronic lesions, as compared to other fly species used in TL-Leishmania, non-scavenging flies are rare, as they can cause myiasis in humans. and the few times it has been reported were accidental cases, it has a shorter life cycle, cosmopolitan geographic distribution, greater population abundance, and is a synanthropic species that is easy to collect and has a diet based on a synthetic diet, which facilitates its maintenance in laboratory conditions with low production costs (Figure 8) (Fu et al., 2009; Martiradonna et al., 2009; Meldau, 2009; Jun-Cheng et al., 2012; Reyes et al., 2020; Hanzel and Sperotto, 2021).

Figure 8. The Illustration Shows a Wound Caused by Leishmaniasis Above, the Parasites that Cause the Disease and, Below, the Infected Human Cells; Source: Photo: Getty Images/BBC News Brasil
Despite its effectiveness, easy applicability, and low cost, larval therapy has been little used in Brazil. In the country, studies are restricted to the use of experimental animal models, and only recently was this therapy used for the first time in humans. Considering that larval application for the treatment of injuries in animals, there is currently a study that intends to evaluate the treatment using it on larvae of Cochliomyia macellaria (Fabricius, 1775) (Diptera, Calliphoridae) (Meldau, 2009; Jun-Cheng et al., 2012; Silva et al., 2017; Maul et al., 2022).

There are studies that demonstrate the massive use of M. domestica for the treatment of chronic skin lesions in humans, without evidence of side effects in its direct use. Furthermore, their larval extractions and secretions were characterized, determining a powerful antitumor, antiviral, and antibacterial activity of the larval protein fractions, as well as their antiplasmodial effects on Plasmodium berghei Vincke et Zips, 1948 (Apicomplexa: Aconoidasida: Haemosporida), which represents the first evidence of action in a mesaxonic parasite (Figure 9) (Li et al., 2008; Fu et al., 2009; Jun-Cheng et al., 2012; Reyes et al., 2020; Dillmann, 2022).

Figure 9. Packaging in Which the Larvae are Sold;
Source: UFSM, 2023

The Larval Therapy (LT) is an alternative method for treatment hard healing wounds. It uses immature flies to clean these wounds, decreasing the need for amputations. A more efficient use of LT considers the wound area. For this calculation invasive techniques are usually used, like manual planimetry with plastic film (Marques et al. 2007; Meldau, 2009; Silva et al., 2017).

According to Meldau (2009), the application of fly larvae to the lesion must follow the following steps:

1. Perform the aseptic technique before, during, and after the procedure.
2. Mark the edge of the lesion with a sterile plastic sheet.
3. Make a hole in the flora, exposing only the lesion.
4. Add 0.9% saline solution to the bottle containing the larvae.
5. Prepare a layer of gauze and then a layer of nylon gauze, which allows air to enter so that the larvae can move in the exudate.
6. Place the contents of the bottle in the center of the gauze.
7. Apply the gauze to the wound, fixing the nylon gauze to the sheet with adhesive tape.
8. Wrap the bandage so that air enters the larva.
9. Assess the lesion daily, especially the exudate. Although it is recommended that the maggot dressing remains on the wound bed for 24 to 72 hours, it produces a large amount of exudate, which may require changing the dressing.

10. When changing the dressing, clean it with 0.9% saline solution and remove the remaining larvae with tweezers.

11. Assess the injury. If there is no longer a need to apply biotherapy, continue treatment with another coverage. (Figure 10) (Li et al., 2008; Jun-Cheng et al., 2012; Hanzel and Sperotto, 2021; Seo et al., 2021; Maul et al., 2022).

![Figure 10. Schematic of Maggot Therapy and Sterilization to Prevent Related Infections](Source: Ahmadnejad, & Kaboudari, 2020)

Although larvae from different species of flies have already been used in this form of treatment, the most used today are the larvae of *L. sericata*, very common in countries in the northern hemisphere. In Brazil, larvae from the Calliphoridae and Muscidae families are the most used in biotherapy, as they present biological behavior similar to that of *L. sericata* (Figure 11) (Meldau, 2009; Martins Junior, 2017).

![Figure 11. Allantoin Molecule Glyoxylureido, is Used in Cosmetics. Structural Chemical Formula and Molecule Model. Larvae Decrease Healing Time and Stimulate the Growth of Viable Tissues for Healing Through the Production of Allantoin and Urea, in Addition, They Favor the Proliferation and Migration of Fibroblasts and Keratinocytes, Promote Angiogenesis, Reduce the Inflammatory Response and the Complement System (C3 and C4); Source: myloview.com.br, n.d](Source: myloview.com.br, n.d)
Scientists from the Butantan Institute have identified two proteins with great potential to combat degenerative diseases in the venom of the caterpillar *Lonomia obliqua* Walker, 1855 (Lepidoptera, Saturniidae), popularly known as firecracker. The insect presents risks to humans when the venom comes into contact with the skin, resulting in burns, hemorrhages, and even kidney failure (Figure 12) (Filho, 2023).

According to the researchers who participated in the study, published in the journal Frontiers in Molecular Biosciences, these proteins, called rLosac and rLopap, can be explored to treat degenerative diseases and develop products aimed at healing and regeneration. Proteins prevent cell death. Losac, in addition to activating blood coagulation factor, has a neuroprotective function, while Lopap, which activates prothrombin, also induces the production of extracellular matrix molecules, such as collagen and fibronectin, related to regeneration (Ana Marisa Chudzinski-Travassos- Butantan Institute) (Filho, 2023).

Considering that wounds have a great impact on patients who carry them, they last for months and can lead to functional impairment and decreased quality of life due to the occurrence of reactions such as pain, reduced movement, depression, loss of self-esteem, and social isolation, with a significant increase in morbidity (Silva et al., 2017; Zubir et al., 2020).

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**Figure 12. Photomicrographs of the *Lonomia obliqua* Walker, 1855 (Lepidoptera: Saturniidae) at the Sixth Larval Instar Stage. a- General View. b- Details of Scoli from the Median Dorsal Region. c- Scolus Isolated from the Median Dorsal Region. Note that the Scolus has a Central Axis from which Originate Lateral Branches (spines) with Different Colors and Sizes. d- Apical Region of the Spine where It was Broken Off (Scissors) and Subapical Region where It was Pressured (Arrow) to Collect Secretions. e- Details of a Spine Broken at Its Tip Showing a Secretion Derived from the Interior of the Spine. f- Observe a Small Drop with a Green Color on the Left and Orange on the Right that Exuded from the Interior of Different Spines.
Sources: Spadacci-Morena, et al., 2016**
Conclusion

Larvotherapy, as the name suggests, is the use of larvae, in the case of flies, to heal wounds that resist healing. They act on the wound through four mechanisms: they remove dead necrotic tissue, disrupt the bacterial biofilm, an extremely organized community of microorganisms that greatly interfere with the ulceration repair process, promote the growth of healthy tissue, and eliminate bacteria that cause infection.

References


UFSM. (2019). UFSM laboratory is the only one in the country to carry out biotherapy, a treatment that prevents amputations and heals injuries. Retrieved from https://www.ufsm.br/2023/05/11/laboratorio-da-ufsm-e-o-unico-a-fazer-bioterapia-no-pais-tratamento-que-evita-amputations-and-scar-injuries


