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Article

# Lyophilized Extract from Larvae of the Blowfly *Lucilia sericata* as a New Strategy for the Management of Chronic Wounds

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**Abstract: Background:** Chronic wounds represent a growing challenge in the aging population, significantly impairing quality of life, increasing the frequency of medical consultations, and imposing substantial healthcare costs. Chronic wounds are prone to complications, including local and systemic infections, and in severe cases, amputations. The therapeutic use of live larvae from the blowfly *Lucilia sericata* (biological debridement) has regained attention for its ability to debride necrotic tissue and stimulate granulation. Despite its benefits, this therapy is constrained by logistical challenges in producing and delivering live larvae and by patient adherence issues. **Objectives:** This study aimed to develop a lyophilized extract of *Lucilia sericata* larvae and evaluate its efficacy in treating chronic wounds. **Methods:** A lyophilized extract (Larveel<sup>®</sup>, Alpha-Biocare GmbH, Neuss, Germany) of the larvae of *Lucilia sericata* was produced under GMP conditions. In a total of ten patients with chronic refractory wounds the extract was used in individual therapeutic trials and its effect on bacterial colonization and wound healing was investigated. **Results:** Of ten patients, three discontinued treatment due to *P. aeruginosa* colonization. In seven patients, significant fibrin reduction, granulation, and wound healing occurred, with two achieving complete closure and four showing advanced epithelialization. **Conclusions:** In 7 of 10 patients, the application of the extract led to a marked reduction in wound slough, improved granulation, and progression of wound healing. These effects are likely attributed to the extract's ability to disrupt bacterial biofilm formation. The findings suggest that this novel therapeutic approach may provide a practical and effective alternative to live larval therapy for managing chronic wounds.

**Keywords:** Chronic wounds; Hard-to-heal wounds; Maggot therapy; Biological debridement; wound management; Biofilm; infections; blowfly; *Lucilia sericata*

## 1. Introduction

Chronic non-healing wounds represent an increasing challenge in the aging populations of industrialized nations, often resulting from multifactorial etiologies. Chronic wounds, typically defined as wounds that fail to demonstrate significant healing progress within three months, pose a substantial challenge to modern medicine. According to Körber et al., chronic wounds in the lower extremities frequently arise as complications of venous, arterial, or diabetic diseases [1]. In Germany, the prevalence of chronic wounds is estimated to be 2–3 million individuals, with leg ulcers affecting 0.7% of the general population and over 3.38% of individuals aged  $\geq 80$  years [2]. Chronic venous ulcers alone account for a substantial socio-economic burden, consuming approximately 2% of the national healthcare budget, with an average annual treatment cost of €6,600 per patient [3–5].

Effective management of chronic wounds is particularly challenging against the backdrop of increasing healthcare economization and cost containment. Optimal wound care necessitates a multifaceted approach that combines etiological treatments with appropriate local wound management. The selection of therapy is guided by factors such as wound size, location, exudate levels, and microbial colonization. Moist wound healing, the current gold standard, aims to convert chronic wounds into acute wounds to facilitate granulation and re-epithelialization [6].

However, certain wounds remain unresponsive or develop critical bacterial colonization despite conventional therapies, necessitating alternative treatment modalities. Biological treatment of chronic wounds, particularly biosurgery utilizing live *Lucilia sericata* larvae, has garnered renewed attention. Initially described during the European wars of the 19th and 20th centuries, biosurgery declined in popularity with the advent of antibiotics, but was rediscovered in the 1980s due to increasing antibiotic resistance and the rising prevalence of chronic wounds. Larval secretions and excretions have demonstrated the ability to debride necrotic tissue, eliminate bacteria, and stimulate granulation, rendering biosurgery a valuable therapeutic option [7].

Despite its efficacy, biosurgery presents several limitations. Patients frequently experience aversion or dysesthesia due to the visible movement of larvae on wounds [8]. The distinctive, unpleasant odor associated with larval excretions can impair compliance, while logistical challenges, such as the production, storage, and timely delivery of live larvae, further complicate its implementation. Biosurgery typically serves as a preparatory step in wound management, necessitating a transition to conventional therapies for complete healing. Contraindications such as coagulopathies, proximity to large vessels or body cavities, and *Pseudomonas aeruginosa* infections limit its applicability [9].

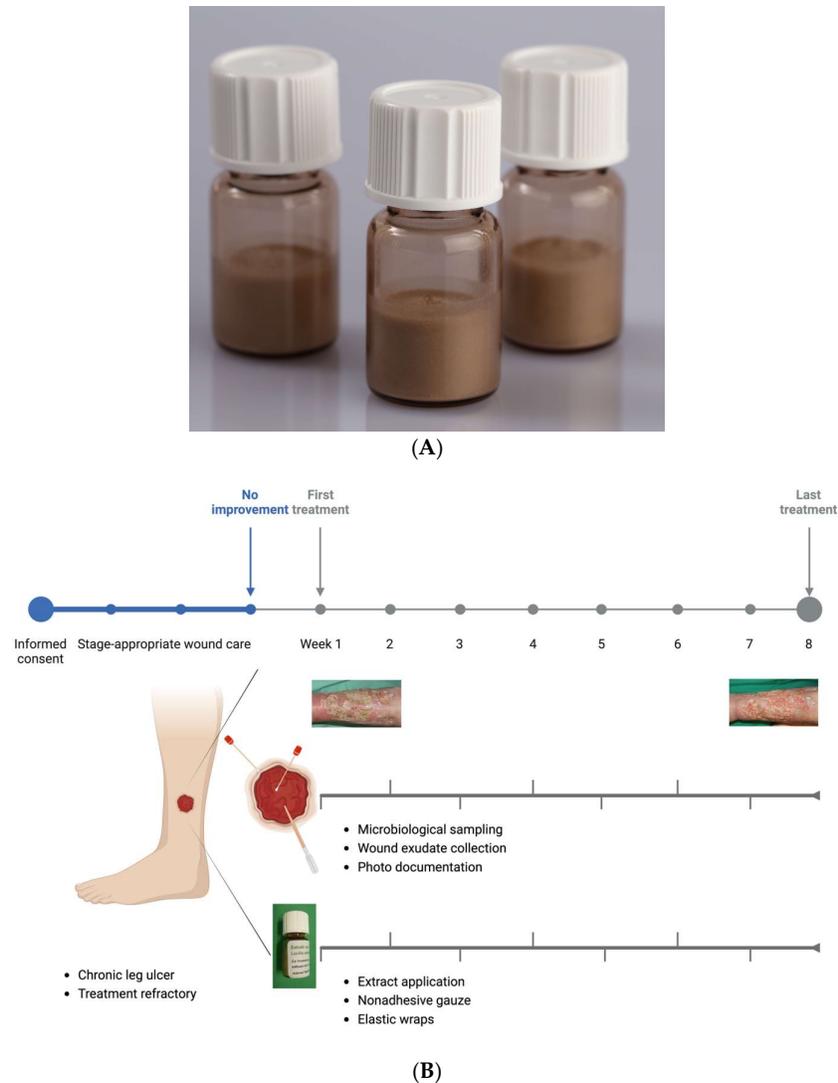
Given these challenges, research has focused on isolating and examining the individual components of *Lucilia sericata* larvae for their therapeutic potential. Studies have demonstrated that larval components exhibit beneficial effects on wound healing, suggesting their potential as an alternative to live larval therapy [10].

The objective of this study was to test a sterile, storable larval extract with comparable efficacy to live larvae for its effectiveness and safety, but without associated disadvantages. A larval extract can be applied as a conventional topical agent, minimizing odors, mitigating patient discomfort, and enabling treatment to continue until complete wound closure.

## 2. Materials and Methods

### 2.1. Development of a Lyophilized Maggot Extract

In accordance with the EU Good Manufacturing Practice (GMP) guidelines for human and veterinary medicinal products, a sterile lyophilized extract of *Lucilia sericata* larvae was developed (Larveel®, Alpha-Biocare GmbH, Düsseldorf) (Figure 1A). Third-instar larvae produced under GMP conditions for pharmaceutical excipients were utilized for extract preparation. The larvae were rapidly frozen in liquid nitrogen, homogenized with pharmaceutical-grade water to form a uniform emulsion, and subjected to thermal treatment at temperatures exceeding 60°C. Following sedimentation and filtration, a sterile-filtered ultrafine filtrate was obtained. The filtrate was subsequently transferred into glass vials and lyophilized, followed by  $\gamma$ -irradiation for sterilization. Prior to application, the extract was reconstituted in 2 ml of physiological saline. The safety of the extract was evaluated using the HET-CAM test, dermatological skin tests, pyrogen tests (rabbit test, LAL assay, and monocyte activation test), and in vitro toxicity assays, all of which indicated an absence of toxic or allergic reactions.



**Figure 1.** (A) Lyophilized extract of larvae of *Lucilia sericata* in 3.5 ml Glass-Vials. (B) Procedure for the Larveel Study. Patients with chronic wounds persisting for  $\geq 3$  months undergo a three-week period of stage-appropriate wound management without surgical intervention. If no improvement in wound condition is observed during this time, the patient is included in the study. Microbiological swab samples are collected from both the wound edge and center at predetermined intervals. Wound exudate is obtained using a pipette, and photo documentation is performed alongside the application of Larveel. Created in BioRender. Homey, B. (2025) <https://BioRender.com/i00k077>.

## 2.2. Application of Lyophilized Maggot Extract in Patients with Chronic Wounds

The extract was employed in individual therapeutic trials for chronic, therapy-resistant wounds following ethical approval by the Heinrich Heine University Düsseldorf Medical Faculty. The patients provided informed consent for treatment with Larveel<sup>®</sup>, wound exudate sampling, and scientific data generation. Ten patients with chronic wounds were included based on the following criteria:

1. Chronic leg ulcers
2. Refractory to treatment for at least three months
3. No underlying consumptive diseases (e.g., malignancies)
4. A three-week pretreatment phase with stage-appropriate wound care demonstrated no significant improvement

Following informed consent, the patients underwent the following protocol:

1. Comprehensive clinical and wound-specific history
2. Standardized photo documentation
3. Wound exudate collection
4. Microbiological sampling
5. Application of *L. sericata* extract
6. Dressing with sterile wound coverings

Exudate samples were collected prior to treatment initiation and at weekly intervals thereafter to analyze potential wound-healing mediators. For each 10 cm<sup>2</sup> wound surface, one vial of extract reconstituted in 0.9% saline was applied. Following a five-minute drying period, sterile dressings comprising nonadhesive gauze, gauze compresses, and elastic wraps were applied. Dressing changes were performed every other day, either independently or with caregiver assistance. Weekly clinical evaluations, including photographic documentation and adverse effect monitoring (e.g., pain), were conducted at the interdisciplinary ulcer clinic of the Dermatology Department, University Hospital Düsseldorf. The treatment regimen was maintained for a maximum duration of eight weeks (Figure 1B). Fifty percent of the patients received treatment thrice weekly at the clinic. Four patients managed dressing changes twice weekly at home with a weekly clinic visit. One patient independently performed all dressing changes.

### 2.3. Patient Demographics and Wound Characteristics

The study cohort comprised 10 patients (mean age, 66 years for females and 74 years for males). Ulcer duration ranged from three months to > three years, with a conservative mean duration of 15 months.

**Table 1.** Overview of the treated patients. The table shows an overview of age, sex, type of wounds and their duration and duration of treatment with maggot extract. Average age of the patients: ♀ = 66 years, ♂ = 74 years, ♀+♂ = 70 years.

Patient	Sex	Age (years)	Wound Type	Wound duration	Treatment duration (weeks)	Treatment
1	♀	46	Venous	12 months	8	Clinic
2	♀	72	Venous	12 months	7	Clinic
3	♀	74	Venous	6 months	2	Clinic
4	♂	84	Venous	12 months	2	Clinic
5	♂	78	arterial and venous	18 months	8	Patient/clinic
6	♂	65	Venous	24 months	8	Patient/clinic
7	♀	61	postoperative	3 months	8	Clinic
8	♀	79	Venous	36 months	8	Clinic
9	♂	73	Arterial	3 months	7	Patient/clinic
10	♂	72	Venous	24 months	8	Patient/clinic

### 2.4. Exudate Collection from Ulcers

Following dressing removal, the wounds were irrigated with sterile saline and dried. Saline was applied for two minutes, then aspirated into syringes. Samples were centrifuged at 500 × g for five minutes at 4°C to remove cells and debris, filtered through 0.45 µm and 0.22 µm syringe filters, and stored in liquid nitrogen.

### 2.5. Bacterial Colonization Analysis

Swab samples were obtained from the wound edges and center prior to treatment initiation and biweekly thereafter. Bacterial identification and resistance testing were performed at the Institute of

Medical Microbiology and Hospital Hygiene, Düsseldorf. The isolates were cultured for subsequent analysis.

#### 2.6. Native Larval Secretions and Extracts

Excretions/secretions (ES) from larvae were prepared according to the protocol described by Barnes et al. [11]. Briefly, third-instar larvae were incubated in demineralized water at 30°C, ES was centrifuged at  $7,826 \times g$  for five minutes, filtered (0.22 $\mu$ m) and stored in liquid nitrogen.

#### 2.7. Effect on Bacterial Biofilms

The extract's effect on bacterial biofilm formation was evaluated using methods described by van der Plas et al. and O'Toole and Kolter [12,13]. Biofilms were formed on microtiter plates containing *S. aureus* (MSSA and MRSA), *S. pyogenes*, and *P. mirabilis* reference strains. Biofilm density was quantified photometrically at 570 nm following crystal violet staining.

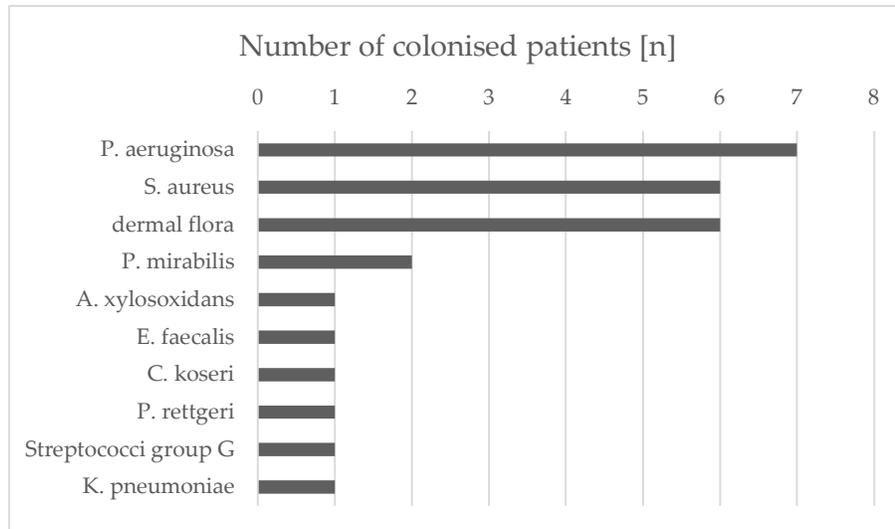
#### 2.8. Effect on Bacterial Growth

Bacterial isolates and reference strains were cultured with maggot extract and piperacillin/tazobactam, respectively. Growth inhibition was assessed by measuring the optical density (600 nm) after 24 h of incubation.

### 3. Results

#### 3.1. Isolated Bacterial Species and Effects of Maggot Extracts on Bacterial Colonization In Vivo

The wounds of all patients were permanently or intermittently populated by various bacterial species (Figure 2). Differentiation of isolates from the same species was based on antibiograms. The most commonly isolated bacterial species were *Pseudomonas aeruginosa* (7/10 wounds), *Staphylococcus aureus* (6/10 wounds), and *Proteus mirabilis* (2/10 wounds) (Table 2). All *S. aureus* isolates were methicillin sensitive (MSSA). The species *Achromobacter xylosoxidans*, *Enterococcus faecalis*, *Citrobacter koseri*, *Providencia rettgeri*, Group G streptococci and *Klebsiella pneumoniae* were each isolated only from wounds of one patient. In addition to these single and multiple findings, the smears included (in part temporal) other bacterial species that were germs of the normal dermal flora and therefore not used for further analysis and assessment. Essentially, a continuous decrease in the bacteria colonizing the wound could be detected after treatment with maggot extract. After only two weeks of treatment, for example, in patient 1, *A. xylosoxidans* and *E. faecalis*, could be found neither at the edge of the wound nor in the middle of the wound. This condition persisted until the last sampling after six weeks. Interestingly, no significant differences were found between the bacterial colonization of the wound margin and the wound center (Table 2). The initial *P. aeruginosa* isolate from patient 3 was found to be resistant to three out of four lead antibiotics (acylureidopenicillins, third- and fourth-generation cephalosporins, and quinolones), but was sensitive to all carbapenems tested and was therefore classified as 3MRGN (multidrug-resistant gram-negative rods). The treatment of the patient with maggot extract was discontinued in week 3 in favor of antibiotic therapy so that no further samples could be taken. At the beginning of treatment in the fourth patient, the treated wound was colonized with *P. aeruginosa*. Owing to an increase in colonization, systemic antibiotics was initiated. In all other patients, a decrease in wound colonization by *S. aureus* was detected. The decrease in colonization could also be correlated with improved clinical findings. A detailed overview of wound colonization during therapy is presented in Table 2.



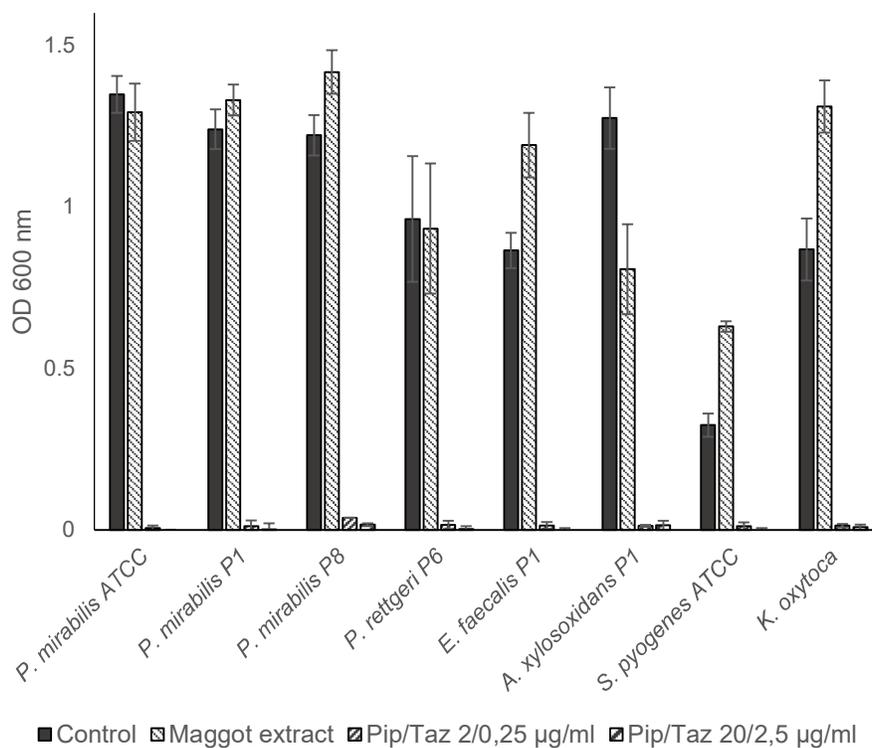
**Figure 2.** Frequencies of different bacterial species from patient wounds during the treatment with maggot extract. Swabs were taken from the wound edges and wound centers of the patient's wounds prior to treatment and every other week of treatment. The characterization as an individual isolate was carried out via antibiograms.

**Table 2.** Frequency and localization of isolation of bacterial species during treatment with maggot extract.

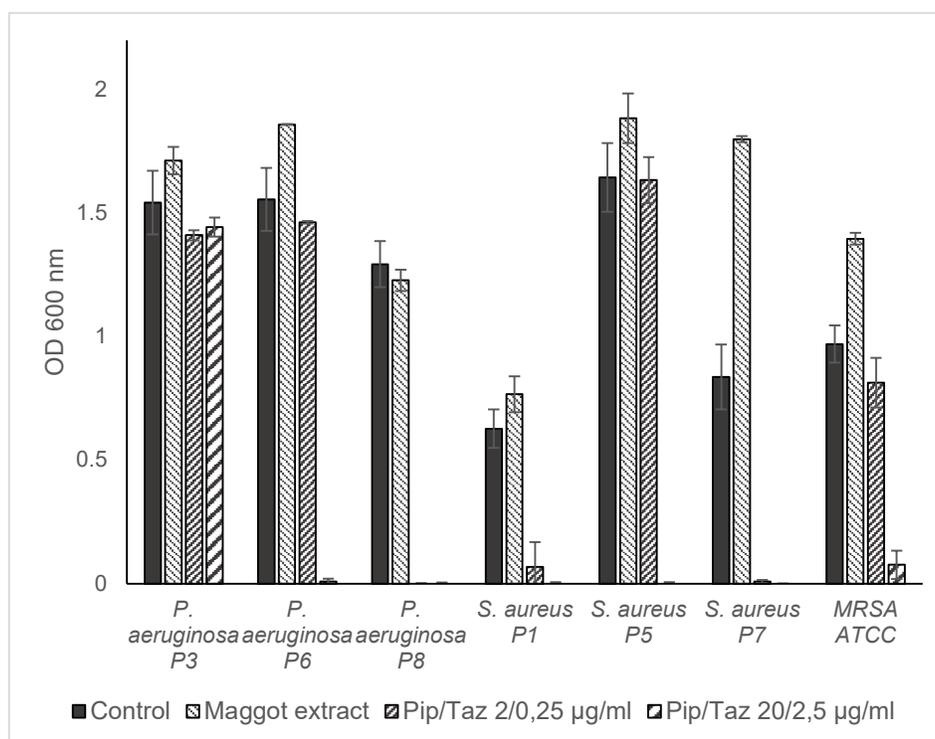
	week 0 n=10		week 2 n=9		week 4 n=8		week 6 n=8		week 8 n=5	
	margin	center	margin	center	margin	center	margin	center	margin	center
<i>P. aeruginosa</i>	7	6	6	5	6	4	3	4	3	3
<i>S. aureus</i>	6	5	2	2	2	2	4	4	1	1
<i>P. mirabilis</i>	2	1	1	1	0	0	2	1	1	1
<i>A. xylosoxidans</i>	1	1	0	0	0	0	0	0	0	0
<i>E. faecalis</i>	1	1	0	0	0	0	0	0	0	0
<i>P. rettgeri</i>	1	0	0	0	0	0	0	0	0	0
<i>C. koseri</i>	1	1	1	1	1	1	0	0	0	0
<i>K. pneumoniae</i>	0	0	0	0	0	0	1	1	0	0
Gr. G Streptococci	1	0	1	0	1	0	1	0	1	0
Dermal flora	4	3	3	2	0	0	2	2	2	2

### 3.2. Effects of Maggot Extracts on Bacterial Growth In Vitro

Studies on growth inhibition in the form of a zone of inhibition assay showed no inhibition with different extract batches and the native ES, neither in the isolated strains nor in the reference strains. In microbroth dilution assays with patient isolates and ATCC strains for the first group of bacteria (*P. mirabilis* isolates and an ATCC strain, *P. rettgeri*, *E. faecalis*, *A. xylosoxidans*, *S. pyogenes* ATCC and a clinical isolate of *K. oxytoca*) no significant growth inhibition was found even in the highest possible/solvable extract concentration. The same applies to the other isolates and strains tested (*P. aeruginosa* from wounds of patients 3, 6, and 8; *S. aureus* from wounds of patients 1, 5, and 7; and one MRSA ATCC strain). In some isolates, even a slight but not significant increase in growth compared to the control experiments could be observed (Figures 3 and 4). Total growth inhibition only occurred according to specific antibiograms at high concentrations of piperacillin/tazobactam. All three patient isolates of *S. aureus* were sensitive to 20/2.5 µg/ml and in part also to 2/0.25 µg/ml piperacillin/tazobactam, whereas the MRSA strain expectedly did not respond to these concentrations with complete growth inhibition.



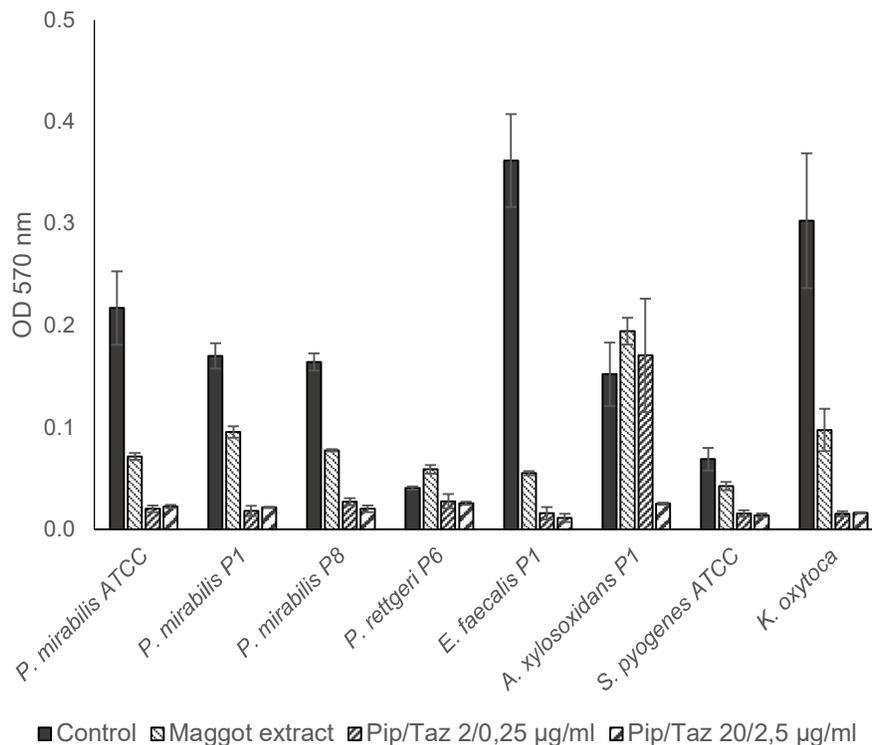
**Figure 3.** Microbroth dilution assay for *P. mirabilis* and other isolates. Isolates were cultivated in TSB (control), maggot extract dissolved in TSB or pip/taz diluted in TSB for 24 h at 37°C. Shown are mean values minus non-inoculated vehicle controls and the standard deviation from each n = 3 measurements. OD = optical density; P = Patient + number; Pip/Taz = Piperacillin/Tazobactam; TSB = tryptic soy broth.



**Figure 4.** Microbroth dilution assay for *P. aeruginosa* and *S. aureus* isolates. Isolates and ATCC strains were cultivated in TSB (control), maggot extract dissolved in TSB or pip/taz diluted in TSB for 24 h at 37°C. Shown are mean values minus non-inoculated vehicle controls and the standard deviation from each n = 3 measurements. OD = optical density; P = Patient + number; Pip/Taz = Piperacillin/Tazobactam; TSB = tryptic soy broth.

### 3.3. Effects of Maggot Extracts on Bacterial Biofilm Formation In Vitro

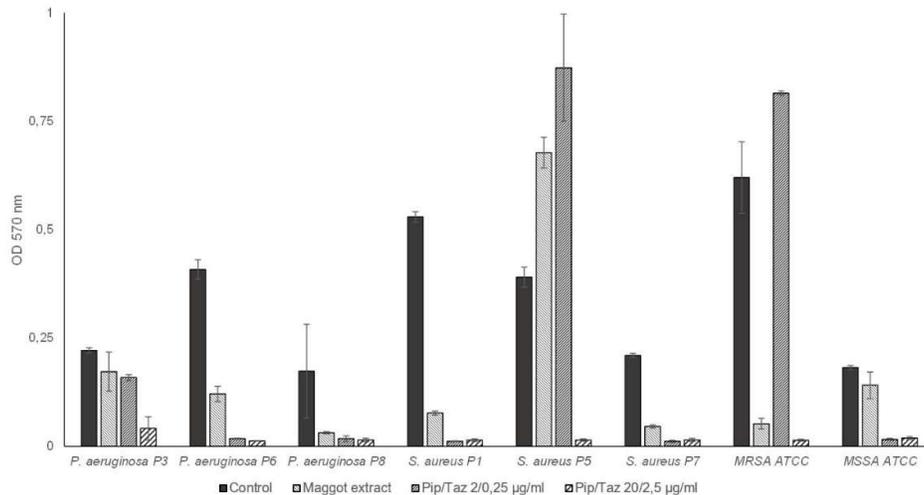
The investigation into the potential influence of a maggot extract on the formation of bacterial biofilms demonstrated, in the first set of bacteria (*P. mirabilis* isolates and ATCC strain, *P. rettgeri*, *E. faecalis*, *A. xylosoxidans*, *S. pyogenes* ATCC and a clinical *K. oxytoca* isolate), that for all *P. mirabilis* isolates/strain, *E. faecalis*, *S. pyogenes* and *K. oxytoca*, the formation of biofilms in the presence of the maggot extract was inhibited. In contrast, bacterial growth and biofilm formation were completely inhibited by piperacillin/tazobactam concentrations of 2/0.25 µg/ml. For *A. xylosoxidans*, biofilm formation could only be prevented by a piperacillin/tazobactam concentration of 20/2.5 µg/ml. The lower piperacillin/tazobactam concentration (2/0.25 µg/ml) or the maggot extract resulted in approximately equally strong biofilm formation compared to the control (Figure 5).



**Figure 5.** Biofilm formation by *P. mirabilis* and other isolates. Isolates and ATCC strains were cultivated in TSB (control), maggot extract dissolved in biofilm-medium or pip/taz diluted in biofilm-medium for 24 h at 37°C. Biofilms were stained with crystal violet. Shown are mean values minus non-inoculated vehicle controls and the standard deviation from each n = 3 measurements. OD = optical density; P = Patient + number; Pip/Taz = Piperacillin/Tazobactam.

In the second set of bacteria tested (*P. aeruginosa* from wounds of patients 3, 6, and 8, *S. aureus* from wounds of patients 1, 5, and 7, as well as MRSA and MSSA ATCC strains), maggot extract resulted in a slight increase in biofilm formation in one *S. aureus* isolate (Figure 6). Minimal or no inhibitory effect on biofilm formation was observed for the *P. aeruginosa* isolate of patient 3 and the MSSA strain, with a comparable effect of piperacillin/tazobactam 2/0.25 µg/ml for *P. aeruginosa* from

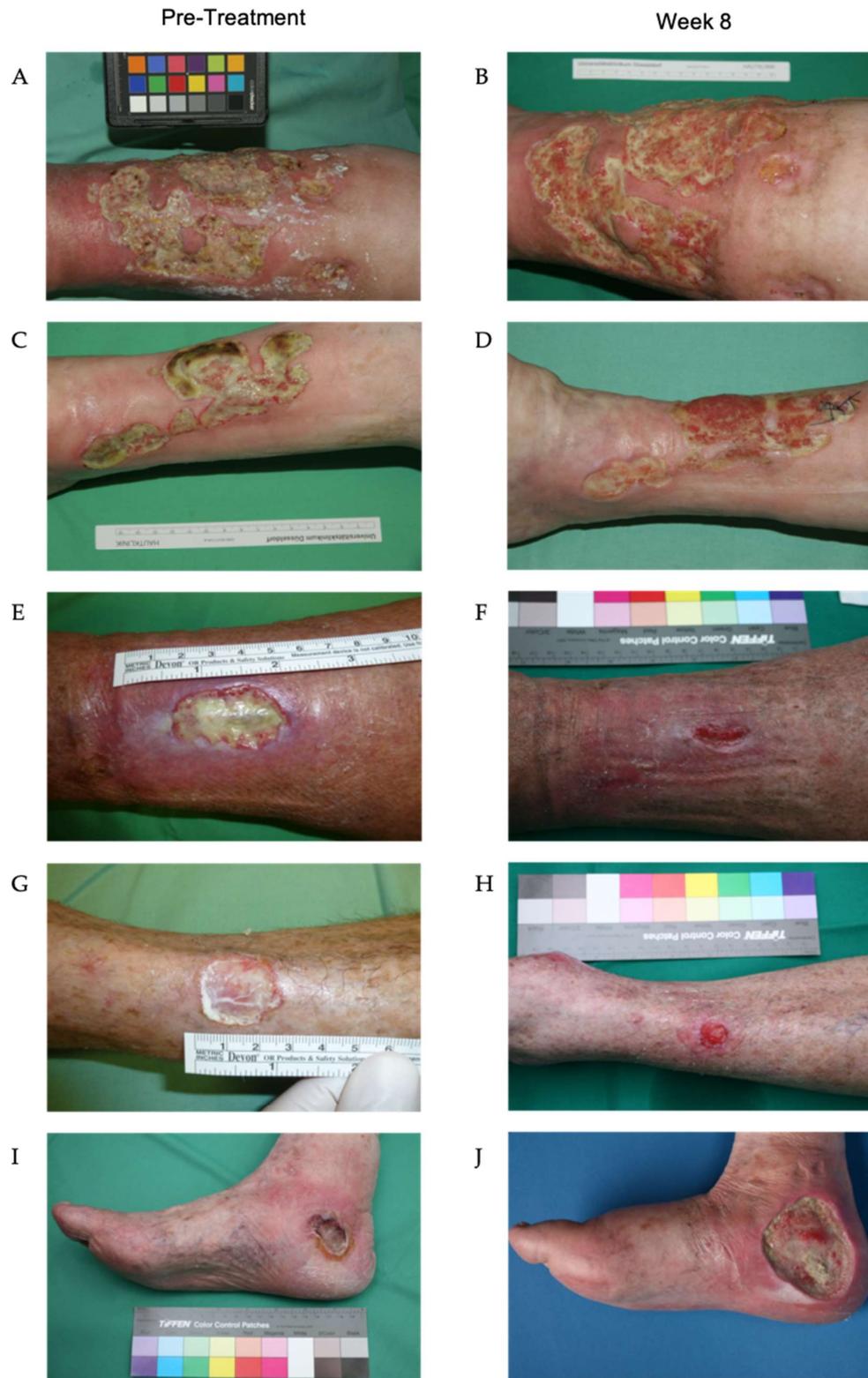
patient 3. However, higher concentrations of 20/2.5  $\mu\text{g/ml}$  piperacillin/tazobactam resulted in complete prevention of biofilm formation in both strains. The extract demonstrated a significant inhibitory effect on biofilm formation by the *P. aeruginosa* isolates from patient 6 as well as the *S. aureus* isolate of patient 1, and an almost complete inhibitory effect on biofilm formation by the *P. aeruginosa* isolate of patient 8 and the *S. aureus* isolate of patient 7, comparable to that of piperacillin/tazobactam at a concentration of 2/0.25  $\mu\text{g/ml}$ . For the strong biofilm-forming MRSA strain, biofilm formation was almost completely prevented with maggot extract, comparable to high-dose piperacillin/tazobactam. Lower doses of piperacillin/tazobactam resulted in enhanced biofilm formation.



**Figure 6.** Biofilm formation by *P. aeruginosa* and *S. aureus*. Isolates and ATCC strains were cultivated in TSB (control), maggot extract dissolved in biofilm-medium or pip/taz diluted in biofilm-medium for 24 h at 37°C. Biofilms were stained with crystal violet. Shown are mean values minus non-inoculated vehicle controls and the standard deviation from each  $n = 3$  measurements. OD = optical density; P = Patient + number; Pip/Taz = Piperacillin/Tazobactam.

#### 3.4. Clinical Course of Lower Leg Ulcerations During Treatment with Maggot Extract

In two patients, the treatment regimens were discontinued after two weeks due to inadequate therapeutic response or due to the increasing colonization with *P. aeruginosa*, necessitating antibiotic therapy. Another patient (patient 9) was transitioned to an alternative therapeutic approach after approximately seven weeks due to critical colonization with *P. aeruginosa*. In the remaining seven patients, a significant reduction in fibrin deposits and an increase in granulation tissue were observed after only two weeks. Nearly complete wound closure was achieved in patients 7 and 8 by the conclusion of the treatment period after 8 weeks. The ulcerations of patients 1, 2, 5, 6 and 10 exhibited signs of secondary wound healing, including epithelial islets, re-epithelialization of wound edges, fibrin depletion, and consecutive increases in granulation tissue, which were not attained via treatment prior to this investigation (Figure 7).



**Figure 7.** Wounds of patients during treatment with maggot extract. The wounds of the patients were treated three times a week. Photographs before treatment (A, C, E, G, I) and after eight weeks (B, D, F, H) of treatment. Treatment with Larveel significantly reduced fibrin deposits and enhanced granulation tissue formation (B, D, F, H). In patients 7 and 8 (E, F, G, H), a marked reduction in wound size was observed after 8 weeks, with near-complete healing and epithelialization progressing from the wound margins (Figures F and H). In contrast,

patient 9 (Figures I and J) experienced a critical colonization by *Pseudomonas aeruginosa*, which was associated with an increase in wound size.

#### 4. Discussion

In a systematic comparison of conventional treatment methods for chronic ulcerations, it has been found that maggot therapy significantly accelerates the healing of wounds, particularly diabetic foot syndrome, but also of other ulcers, and increases the likelihood of healing [14]. Given this context, the objective of this study was to investigate the possibility of making the beneficial effects of this biosurgical debridement accessible to a patient population by circumventing the adverse effects of the extracts. The extracts from the larvae of *Lucilia sericata* significantly differed from the method of biosurgical debridement with living larvae of this species. Locally administered maggots continuously release their excretions/secretions into the wound and thus to the bacteria present there. It has been established that the larvae of *Lucilia sericata* possess various proteases and antibacterial peptides (AMP), such as seraticin, and evidence from in vitro experiments indicates that living maggots are capable of affecting their bacterial environment by expressing certain antibacterial peptides, such as putative *Lucilia* defensin, dipterin, and various proline-rich antibacterial peptides [15–19].

The absence of bactericidal effect in this maggot extract compared to living fly maggots is likely attributable to the production-related denaturation/absence of these peptides and proteases. Examination of the smears of the wounds treated with maggot extract revealed a uniform distribution of bacterial colonization between the wound edge and center. The number of patients treated in this study (n = 10) was relatively small but demonstrated similarities regarding isolated species with a retrospective ten-year study conducted in Germany. The study by Jockenhofer et al., which examined wounds from 100 patients, identified *S. aureus* (53%) and *P. aeruginosa* (25%) as the most common wound-colonizing species [20]. In the present study, *S. aureus* was found in six of ten patients and *P. aeruginosa* in eight of ten patients. Notably, *P. aeruginosa* isolates exhibited remarkable intrinsic antibiotic resistance in this study, with one isolate being multi-drug resistant to three out of four antibiotic classes (3MRGN), which can render colonization particularly critical.

During the course of the study, it was observed that patients with a short ulcer persistence (approximately 3 months) exhibited no colonization with *P. aeruginosa*. The ulcer with the shortest duration colonized by *P. aeruginosa* had existed for six months (patient 3). Given the relatively short six-month duration of the ulcer, along with the increasing green plaque formation and the detection of *P. aeruginosa*, this colonization can be postulated as the cause of the ulcer's persistent nature. A longitudinal study comparable to this investigation has demonstrated that the duration of the ulcer increases the likelihood of colonization by *P. aeruginosa*, and this colonization can result in ulcer enlargement and further delayed wound healing [21]. This phenomenon was also clinically evident in the three patients who had to prematurely discontinue the study due to increased local colonization by *P. aeruginosa*. Nevertheless, with consistent application of the maggot extract in a total of seven patients, a clear clinical improvement was observed; therefore, it can be inferred that the positive effects on wound healing are not primarily mediated by a direct antimicrobial effect of the extract. Our analyses also revealed that the extract exhibited a remarkable inhibitory effect on the formation of bacterial biofilms. These biofilms play an essential role in the impaired healing of chronic wounds as they lead to prolonged inflammation and concomitant delayed wound healing by inhibiting local immune reactions [22]. This result is supported by the results of Becerikli et al. who, in experiments with the same larval extract, found that the stability of biofilms of *P. aeruginosa* and *S. aureus* is significantly impaired, especially in the presence of antibiotics [23].

It is important to note that improvement in wound healing is not necessarily measured solely by a reduction in the ulcer area. Additional factors, such as the formation of granulation tissue at the wound base and consequent reduction in wound depth, as well as the status of the proteinases and growth factors present in the wound exudates, reflect alterations in wound status. For instance, a decrease in biofilm could lead to a positive change in the wound environment with regard to

improved wound healing [24,25]. Specifically, the colonization of biofilm-forming species, some of which exhibit pronounced resistance to antibiotics, are also likely to negatively influence wound healing through various virulence factors. These biofilms, consisting primarily of extracellular polymeric substances, provide bacteria with protection from external influences, such as antibiotics [26]. The dissolution of such biofilms or the prevention of their formation is demonstrated for almost all isolated species in this study (Figures 5 and 6); therefore, it appears to be of essential importance for the healing of chronic wounds. Consequently, it is highly probable that the improved clinical situation of the chronic wound of the patients in this study was attributable to the reduction of biofilm formation, leading to enhanced accessibility of the host immune system and reduced proliferation of colonizing bacteria.

## 5. Conclusions

In summary, this study demonstrates for the first time that *Lucilia sericata* extract has a sustained positive effect on wound cleansing and conditioning. This extract can be administered on an outpatient and inpatient basis, at any dressing change, without any time limitation, and can also be readily utilized after brief instruction by non-medical or nursing staff. Based on the observations of our applications, it is anticipated that this extract will expand treatment options for chronic wounds, serving as an alternative to traditional biosurgery. However, given the limited number of cases in this initial study, a larger randomized controlled trial is warranted.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, Norman-Philipp Hoff and Falk Gestmann; methodology, Norman-Philipp Hoff and Falk Gestmann; software, Norman-Philipp Hoff, Falk Gestmann, Bernhard Homey, Theresa Jansen; validation, Bernhard Homey, Heinz Mehlhorn and Peter Arne Gerber; formal analysis, Norman-Philipp Hoff and Falk Gestmann; investigation, Norman-Philipp Hoff, Falk Gestmann and Theresa Jansen; writing—original draft preparation, Norman-Philipp Hoff, Falk Gestmann, Theresa Jansen, Sarah Janßen; writing—review and editing, Norman-Philipp Hoff, Falk Gestmann, Heinz Mehlhorn, Bernhard Homey and Peter Arne Gerber; visualization, Norman-Philipp Hoff and Falk Gestmann; supervision, Bernhard Homey, Heinz Mehlhorn and Peter Arne Gerber. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Heinrich Heine University Düsseldorf, Medical Faculty for studies involving humans.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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## Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

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