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Exploring the Effects of *Lucilia sericata* Larvae on Biofilm-forming Bacteria in Wounds

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Abstract

Objective: Biofilm-induced chronic wound infections are among the most significant causes of treatment failures. Healing chronic wounds with biofilm is a major issue worldwide due to high costs and adverse effects on patients. In this study, we aimed to investigate the impacts of *Lucilia sericata* larvae on biofilm-forming bacteria in chronic wounds.

Method: We recruited 30 patients applying to Istanbul University-Cerrahpasa, Traditional and Complementary Medicine Research and Application Center, Wound Healing Unit, between December 2019 and March 2021. We obtained swab cultures before and immediately after applying Larval Debridement Therapy (LDT). After identifying isolated bacteria, we examined all agents by susceptibility and biofilm formation.

Results: While isolating only a single bacterial genus/species in 19 patients, we could detect more than one bacterial genus/species in 11 of them. Moreover, we observed biofilm formation in bacteria isolated from 21 patients, but it was not the case in bacteria grown in 9 patients. Following LDT, we discovered that the biofilm layer on wounds was removed in a short time (two to three sessions).

Conclusion: Although many methods are adopted in healing chronic wounds with biofilm formation, they often remain ineffective. The therapy with *L. sericata* larvae, a simple and effective method, may promote rapid healing of biofilm-forming wounds.

Keywords: Biofilm, chronic wound, *Lucilia sericata*, larvae, bacteria

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Yaralarda Biyofilm İçeren Bakteriler Üzerine *Lucilia sericata* Larvalarının Etkisi

Öz

Amaç: Biyofilm kaynaklı kronik yara enfeksiyonları, günümüzde en önemli tedavi başarısızlığı nedenlerinin başında gelmektedir. Biyofilm içeren kronik yaraların tedavisi gerek yüksek maliyeti gerekse hastalarda oluşturdukları olumsuz etkileri nedeni ile tüm dünyada büyük bir sorundur. Bu çalışmada, kronikleşen yaralarda biyofilm içeren bakteriler üzerine *Lucilia sericata* larvalarının etkisinin araştırılması amaçlanmıştır.

Yöntemler: Aralık 2019 ve Mart 2021 tarihleri arasında İstanbul Üniversitesi-Cerrahpaşa, Geleneksel ve Tamamlayıcı Tıp Uygulama ve Araştırma Merkezi Yara Tedavi Ünitesine başvuran 30 hasta çalışmaya dahil edildi. Larva Debridman Tedavisi uygulamadan önce ve hemen sonra mikrobiyolojik kültür için sürüntü örnekleri alındı. İzole edilen bakterilerin identifikasyonu yapıldıktan sonra etkenlerin tümü antibiyotik duyarlılıkları ve biyofilm oluşumu açısından incelendi.

Bulgular: Hastaların 19'unda tek bir tür bakteri izole edilirken 11'inde birden fazla bakteri türü tespit edildi. Yirmi bir hastadan izole edilen bakterilerde biyofilm oluşumu gözlenirken, 9 hastada üreyen bakterilerde ise biyofilm oluşumu tespit edilemedi. Larvalar yaralara uygulandıktan sonra genel olarak iki-üç seans gibi kısa bir sürede biyofilm tabakasının bertaraf edildiği görüldü.

Sonuç: Biyofilm oluşumu gözlenen kronik yaraların tedavisinde birçok metot uygulanmakla birlikte çoğu zaman etkili olmamaktadır. *L. sericata* larvaları ile uygulanan tedavinin basit ve etkili bir yöntem olarak ele alınması biyofilm içeren yaraların hızlı bir şekilde iyileşmesini teşvik edebilir.

Anahtar kelimeler: Biyofilm, Kronik yara, *Lucilia sericata*, Larva, Bakteri.

INTRODUCTION

Chronic wounds are an important public health problem with devastating consequences for patients and significant costs on healthcare systems[1]. Although there are many factors having a role in prolonging the healing process of wounds, microbial colonization in wounds is the prominent one[2]. Previously, bacteria in chronic wounds were reported to form biofilms contributing to delayed healing[3].

Wound debridement and application of antimicrobial agents are often considered traditional methods of eliminating wound infection. Yet, most chronic wounds contain more than one bacterial species and are characterized by polymicrobial biofilm infections[4]. It should be noted that a chronic wound should be regarded as a chronic infection containing a biofilm and that biofilm-associated conditions inherently require more than a single treatment strategy.

Larval therapy using sterile larvae of fly species, *Lucilia sericata*, (also known as maggot therapy or therapeutic myiasis) has been used successfully for many years in healing chronic and intractable wounds. These larvae, which do not harm healthy tissues, dissolve the necrotic tissues thanks to the enzymes they secrete and disinfect the wound by eating, killing, and stopping the reproduction of microorganisms. They also accelerate the healing of chronic wounds by stimulating granulation[5-8].

In this study, we evaluated the bacterial culture obtained from patients with chronic wounds and applying or referred to our unit for larval treatment and aimed to present a perspective on the effects of *L. sericata* larvae on biofilm-forming bacteria in chronic wounds.

METHOD

Research Design and Patients

We carried out the present research upon the approval of the Clinical Research Ethics Committee of Cerrahpaşa School of Medicine in Istanbul University (Date: 09.05.2019, Decision no: 133996). We prospectively investigated chronic wounds of 30 patients applying to Istanbul University-Cerrahpaşa, Traditional and

Complementary Medicine Research and Application Center, Wound Care Unit between December 2019 and March 2021 by microbiological culture and biofilm formation before and after larval therapy. We also recorded the patients' demographic information and clinical features of their wounds.

Bacteriological Analysis

We obtained swab cultures regularly before and immediately after each larval application session. We evaluated the samples after they were cultivated on Blood agar, Chocolate agar, and Mac Conkey agar media and kept in an oven at 37°C for 18-36 hours. We adopted routine, conventional methods to identify clinically significant bacteria and predicated on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for antibiotic susceptibility tests of isolated bacteria.

Detection of Biofilm Formation

We determined biofilm formation using the Congo red agar method; we prepared Congo red agar medium with sucrose, brain-heart infusion broth, Congo red, and agar. At the end of the incubation at 37°C for 24 hours, we evaluated the colonies by color change. At this point, we considered strains with black and dark red colonies to be positive and those with pink-red colonies to be negative for biofilm formation.

RESULTS

There were 23 male (76.7%) and 7 (23.3%) female patients in the study, and their mean age was 42.6±16.7 years (range: 28-73 years). Table I presents the demographic characteristics such as age, sex, underlying conditions, and features and localization of the wounds.

Table I: Patients' Demographic and Clinical Characteristics

Parameters	
Female/Male, n	7/23
Age (mean±SD)	42.6±16.7
Underlying condition	
Diabetes	20
Venous insufficiency	15
Osteomyelitis	1
Spina bifida	1
Wound localization	
Foot	28
Leg	2
Wound appearance	
Infected	15
Necrotic	10
Purulent	6
Macerated	1
Wound type	
Diabetic wound	20
Amputation wound	4
Pressure ulcer	2
Venous stasis ulcer	2
Ischemic wound	1
Burn wound	1
Treatment before LDT	
Antibiotic	30
Wet dressing	8
Hyperbaric oxygen	5
Ozone	5
Surgical debridement	4
VAC (Vacuum-Assisted Closure)	2
Other (Silver-Zinc Cream, Hirudin Therapy, Iodine, Rifadin)	7
Pain status	
Painful	19
Painless	11
Wound odor	
Yes	16
No	14

The most prevalent Gram-negative agents in the samples taken before LDT were *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Morganella morganii*, respectively. On the other hand, we determined that Gram-positive rods took first place among the isolated Gram-

positive bacteria, followed by *Staphylococcus aureus*.

A single bacterial genus/species was isolated in 19 (63.3%) patients, while more than one bacterial genus/species grew in 11 (36.7%). Besides, the number of bacterial colonies remained the same in the wounds of 22 (73.3%) patients after LDT. However, the number of bacterial colonies decreased by 50% in the wounds of 4 (13.3%) patients after LDT application twice. Also, we found the number of colonies was lowered by 50% in the wounds of 4 (13.3%) after applying LDT four times. In addition, after applying LDT three times in 13 (43.3%) patients, twice in 11 (36.7%), and four times in 4 (13.3%), we witnessed the growth of bacterial species in the wounds different than those the samples obtained before starting LDT. The bacterial genus/species remained the same in 2 (6.7%) patients after LDT.

Overall, while we detected biofilm formation in bacteria isolated from 21 (70%) patients, it was not the case in bacteria grown in 9 (30%) patients.

DISCUSSION

The increasing number of chronic wounds worldwide, especially patients with infected diabetic foot ulcers, has become a significant health problem. Recent studies have shown that the biofilm layer, leading wounds to be chronic, prolongs healing[9]. The relevant field is continuously engaged in research to prevent biofilm formation or eradicate the biofilm layer in wounds. Besides, the most successful treatment strategy in recent years is considered a frequent application of debridement. Among the debridement methods used in wound healing, besides enzymatic and physical debridement, the biological debridement approach with *L. sericata* larvae has also attracted attention in recent years[4].

Bacteria with multi-antibiotic resistance are frequently detected in diabetic foot infections, which causes significant problems in treatment. The presence and complexity of bacterial biofilms in chronic wounds have long been recognized as a remarkable aspect of nonhealing wounds. Many studies show that biofilms can be prevented, inhibited, and degraded by larvae[10-14].

Cowan et al.[15] documented the promising efficacy of LDT in destroying bacterial biofilm in chronic wounds. Similarly, our results demonstrated that the larvae used in the treatment have the ability to destroy the biofilm in a chronic wound environment in as soon as 48 hours.

In another study, it was shown that larval secretions used successfully for healing chronic wounds are also effective against the biofilms of *S. aureus* and *P. aeruginosa*[10]. The researchers reported that larval secretion blocked biofilm formation caused by *S. aureus* and *P. aeruginosa* within 2 hours and after 10 hours, respectively. Some in vitro studies also concluded that biofilms caused by *P. aeruginosa* and *S. aureus* were eliminated within 48 hours after larvae application[15,16]. In parallel with these findings, we found that the larvae applied on the wounds overall removed the biofilm layer in a short time (two to three sessions).

In their study, Smith et al.[17] revealed that hydrogel dressing with *L. sericata* larval secretions significantly accelerated wound healing. Accordingly, controlled larval secretion after LDT may be an effective therapeutic option for stimulating tissue regeneration in wound management and accelerating wound healing. Pinherio et al.[18] applied LDT to a patient with an infected diabetic foot wound for 43 days and found that necrotic tissues were significantly reduced after the treatment. Sun et al.[19] also demonstrated that LDT reduced the healing time of chronic wounds and ulcers; therefore, LDT can be used as a supportive treatment in wound management. In our study, we confirmed that LDT shortens the healing time in

patients with chronic wounds without causing any damage to healthy tissues, which may also contribute to patients' quality of life.

In a literature review evaluating the 20-year microbiological profile of diabetic foot infections in Turkey, Hatipoglu et al.[20] determined that half of the isolated aerobic bacteria were Gram-positive, and the other half were Gram-negative bacteria. Nevertheless, it is noteworthy that the rate of Gram-negative bacteria among the isolated agents was considerably higher than Gram-positive bacteria in our study, which may be because we collected the samples in accordance with the guidelines to prevent contamination with the skin flora.

CONCLUSION

To sum, we concluded that chronic wound infections are associated with biofilms-forming bacteria. Bacteria remain protected from antibiotics and the immune system of the host thanks to their ability to form biofilms, allowing them to continue their pathogenicity. While treatment methods are limited to microorganisms in an extracellular polymeric matrix that attach and multiply irreversibly, we suggested LDT can be used as an economical, fast, and effective method in the treatment of wounds that do not heal clinically, have a chronic course, and do not respond to surgical treatment.

We knew that all of the patients were using antibiotics. Therefore, the increase of antibiotic-resistant bacteria and biofilm-forming abilities of these bacteria led us to adopt the LDT method in healing wounds. It is well-known that the biofilm layer contributes to resistance to antibiotics, but we discovered that the larvae eliminated the biofilm layer in wounds in a short time, two to three sessions. As the larvae showed promising efficacy in destroying bacterial biofilm from chronic wounds, we thought that LDT could be an effective means to treat nonhealing wounds and achieve maximum therapeutic results.

Ethics Committee Approval: This study was approved by Istanbul University-Cerrahpaşa, Faculty of Medicine local ethics committee (09.05.2019/133996).

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