

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**THE USE OF ELECTRICAL STIMULATION TO ENHANCE
WOUND HEALING**

ZEYNEP GERGİN

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF BIOENGINEERING**

ADVISOR: ASSOC. PROF. DR. BENGÜ ERGÜDEN

JUNE 2025

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**THE USE OF ELECTRICAL STIMULATION TO
ENHANCE WOUND HEALING**

ZEYNEP GERİN

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF BIOENGINEERING**

ADVISOR: ASSOC. PROF. DR. BENGÜ ERGÜDEN

JUNE 2025

**T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ**

**YARA İYİLEŞMESİNDE ELEKTRİK
STİMÜLASYONUNUN KULLANIMI**

ZEYNEP GERGİN

**YÜKSEK LİSANS TEZİ
BİYOMÜHENDİSLİK ANABİLİM DALI**

DANIŞMAN: DOÇ. DR. BENGÜ ERGÜDEN

HAZİRAN 2025

MASTER of SCIENCE JURY APPROVAL FORM

A thesis submitted by Zeynep GERGİN, defended on 19/06/2025 before the jury formed with the 16/06/2025 date and 2025/32 numbered decision of the GTU Graduate Administration Board, has been accepted as a MASTER of SCIENCE thesis in the Department of Bioengineering.

JURY

MEMBER

(THESIS ADVISOR) : Assoc. Prof. Bengü ERGÜDEN

MEMBER

: Assoc. Prof. Fatih TARLAK

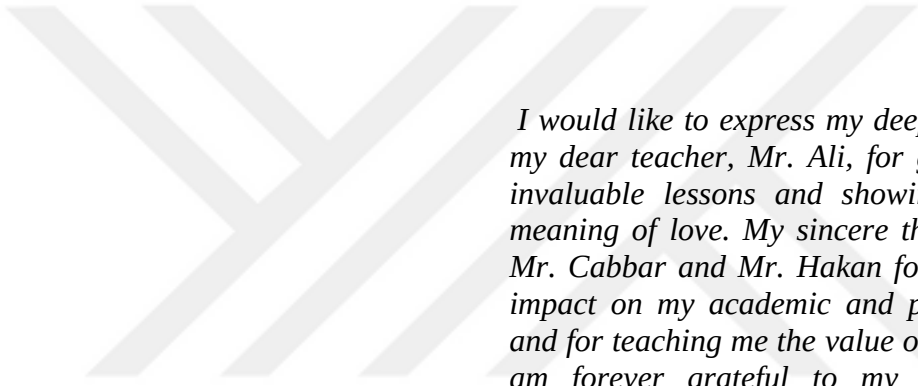
MEMBER

: Assist. Prof. Handan EMİŞOĞLU KÜLAHLI

APPROVAL

Gebze Technical University Graduate Administration Board
...../...../..... date and/..... numbered decision.

SIGNATURE/SEAL



I would like to express my deepest gratitude to my dear teacher, Mr. Ali, for guiding me with invaluable lessons and showing me the true meaning of love. My sincere thanks also go to Mr. Cabbar and Mr. Hakan for their profound impact on my academic and personal growth, and for teaching me the value of loving others. I am forever grateful to my beloved family, especially my parents, for their patience and sacrifices. Finally, I thank my second family — Aunt Ayşen, Melike, Tuğçe, Damla, Nadide, Merve, Aunt Nuriye, Aunt Sebahat, Aunt Mijde, Name, and Tuğcan — for their presence and support in my life.

ABSTRACT

The skin is the body's largest organ, with a three-layered structure that protects it from various thermal, chemical, and physical damage. A skin wound is damage to the normal structure and function of the skin. This condition also leads to changes in the local environment. Skin wounds can occur due to acute injuries and chronic conditions. The wound healing process is fundamentally divided into four phases: coagulation, inflammation, proliferation, and remodeling. The normal healing process of the skin should result in the restoration of continuity and proper function. However, some injuries may not heal in a timely and regular manner. The underlying causes of chronic wounds include necrosis, an increase in reactive oxygen species, abnormal re-epithelialization, infections, ongoing inflammation, bacterial colonization, and reduced angiogenesis. They are affected by factors classified into two main categories: local and systemic factors that influence wound healing. These include nutrition, hypoxic stress, chronic diseases, and infections. Among these, when considering infection, it is observed that microorganisms proliferate beneath the skin when a skin wound occurs, delaying the healing process. The application of electrical stimulation has recently emerged as a promising method to accelerate the wound healing process. Additionally, its effectiveness on bacteria was first tested by Rowley 30 years ago, but no records of optimization studies have been found. At this point, we applied 3V, 5V, 8V, and 10V DC electricity on the bacteria commonly found in the wound area, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *S. epidermidis*, and *Escherichia coli*, examining the diameters of the zones formed at different durations (30 seconds, 1 minute, 2 minutes, 5 minutes). It was observed that as the application time and voltage increased at the cathode, the zone diameter also increased. However, such a conclusive result could not be obtained at the anode. Furthermore, differences were observed in the fingerprint region and the CH₃, CH₂ regions in the FTIR results. This indicates that we need to focus on the effect of this electrical application on the bacterial cell wall and cell membrane.

Keywords: Electrical Stimulation, Antibacterial, Wound Healing, Direct Current

ÖZET

Cilt üç katmanlı yapısı ile vücudu çeşitli termal, kimyasal ve fiziksel hasardan koruyan bedenin en büyük organıdır. Cilt yarası cildin normal yapısına ve işlevine verilen zarardır. Bu durum yerel ortamın değişmesine de yol açar. Cilt yaraları akut yaralanmalar ve kronik durumlar nedeniyle meydana gelebilir. Yara iyileşme süreci temelde koagülasyon, inflamasyon, proliferasyon ve yeniden modellenme fazı olarak dörde ayrılmıştır. Cildin normal iyileşme süreci sürekliliğinin ve doğru işlevinin yeniden sağlanması ile sonuçlanmalıdır. Fakat bazı yaralanmalar zamanında ve düzenli bir şekilde iyileşmeyebilir. Kronik yaraların temelinde nekroz, reaktif oksijen türlerinin artışı, anormal re-epitelizasyon, enfeksiyonlar devam eden iltihaplanma, bakteriyel kolonizasyon ve azalmış anjiyogenez yer alır. Yara iyileşmesini etkileyen yerel ve sistemik faktörler olarak iki ana kategoriye sınıflandırılan faktörlerden etkilenir. Bunlar beslenme, hipoksik stres, kronik hastalıklar ve enfeksiyon olarak sıralanabilir. Bunların içerisinde enfeksiyon dikkate alındığında cilt yarası meydana geldiğinde mikroorganizmaların cilt altındaki dokulara yayılım gösterdiği ve yara iyileşmesini geciktirdiği görülmektedir. Yaraların iyileşme sürecini hızlandırmak için elektriksel uyarı uygulaması son zamanlarda umut verici bir yöntem olarak ortaya çıkmıştır. Ayrıca bakteriler üzerindeki etkinliği 30 yıl önce ilk kez Rowley tarafından denenmiş fakat optimizasyona yönelik çalışma kaydı bulunamamıştır. Bu çalışmada yara bölgesinde yoğunlukla bulunan *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *S.epidermidis* ve *Escherichia coli* bakterileri üzerinde 3V, 5V, 8V ve 10V DC elektrik uygulaması yapılarak farklı sürelerde (0.30 sn, 1 dk, 2 dk, 5 dk) oluşan zon çapları incelendi. Katot ucunda zon çaplarının uygulama süresi ve voltaj miktarı arttıkça zon çapının arttığı görüldü. Anot ucunda ise sağlıklı bir sonuç elde edilemedi. Bununla birlikte yapılan FTIR sonucunda parmak izi bölgesinde ve CH₃, CH₂ bölgelerinde farklılıklar görüldü. Bu elektrik uygulamasının bakterinin hücre duvarına yaptığı etki üzerinde durmamız gerektiğini göstermektedir.

Anahtar Kelimeler: Elektrik Stimülasyonu, Antibakteriyel, Yara İyileşmesi, Direkt Akım

ACKNOWLEDGEMENTS

I am deeply grateful to my thesis advisor, Assoc. Prof. Dr. Bengü Ergüden, for her guidance, continuous support, and assistance throughout the thesis writing process. My sincere thanks also go to Research Assistant Gözde Yeşiltaş, whose encouragement and support have been a great source of motivation for me. Lastly, I would like to thank the staff and authorities of the Chemistry Laboratory for their valuable help and contributions in the acquisition of the data used in this thesis.



TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	vi
ÖZET	vii
ACKNOWLEDGEMENTS	viii
TABLE OF CONTENTS	ix
LIST OF SYMBOLS AND ABBREVIATIONS	x
LIST OF FIGURES	xii
LIST OF TABLES	xv
1 INTRODUCTION	1
1.1. Skin Structure and Function	1
1.1.1 Epidermis	1
1.1.2 Dermis	2
1.1.3 Hipodermis	2
1.2. Background of Wound Healing	3
1.2.1. Definition of Wound	3
1.2.2. Phases of Wound Healing	3
1.2.2.1. The Coagulation Phase	4
1.2.2.2. The Inflammation Phase	5
1.2.2.3. The Proliferation Phase	6
1.2.2.4. The Remodelling Phase	8
1.2.3. Types of Wound	8
1.2.4. Factors Affecting Wound Healing	9
1.2.4.1. Nutrition	9
1.2.4.2. Hypoxic Stress	9
1.2.4.3. Chronic Disease	10
1.2.4.4. Infection	10
1.3. Challenges in Wound Healing	11
1.4. Role of Bioelectricity in Tissue Repair	12
1.5. Electrical Stimulation as a Therapeutic Strategy	14
2. PURPOSE OF THE STUDY	18
3. EXPERIMENT	19
3.1. Materials and Method	19
3.1.1. Preparation of Culture Media	19
3.1.2. DC Current	20
3.1.3. AC Current	21
3.1.4. FTIR Experiment	21
4. RESULTS	22
5. CONCLUSION	51
REFERENCES	59
BIOGRAPHY	72
PUBLICATIONS AND PRESENTATIONS FROM THE THESIS	73

LIST OF SYMBOLS AND ABBREVIATIONS

AC	:Alternating Current
CFU	:Colony Forming Unit
cm	:Centimeter
DC	:Direct Current
EC	:Electric Current
ECM	:Extracellular Matrix
ECMs	:Extracellular Matrices
EFs	:Electric Fields
ES	:Electrical Stimulation
e.g.	:Exemplia Gratia
E.coli	:Escherichia coli
FREMS	:Frequency Rhythmic Electrical Modulation Systems
FTIR	:Fourier Transform Infrared Spectroscopy
h	:Hour
HIF-1	:Hypoxia-Inducible Factor-1
HVPC	:High Voltage Pulsed Current
IL-1	:Interleukin-1
IL-23	:Interleukin-23
LIDC	:Low Intensity DC
LVPC	:Low-Voltage Pulsed Current
min	:Minute
mm	:Millimeter
mV	:Millivolt
μ A	:Microampere
μ m	:Micrometer
NETs	:Neutrophil Extracellular Traps
NT3	:Neurotrophin-3
P.aeruginosa	:Pseudomonas aeruginosa
PC	:Pulsed Current
PDGF	:Platelet-Derived Growth Factor
PEMF	:Pulsed Electromagnetic Field
pH	:Potential of Hydrogen
PMNs	:Polymorphonuclear Neutrophils
ROS	:Reactive Oxygen Species
S.aureus	:Staphylococcus aureus
S.epidermidis	:Staphylococcus epidermidis
SDF1/CXCL12	:Stromal Cell-Derived Factor 1
sec	:Second
TENS	:Transcutaneous Electrical Nerve Stimulation
TEP	:Trans-epithelial Voltage
TGF- β	:Transforming Growth Factor- β
TIMPs	:Tissue Inhibitor of Metalloproteinase
TLR	:Toll-like receptor
TNF- α	:Tumor Necrosis Alpha

UV	:Ultraviolet
V	:Voltage
VEGF	:Vascular Endothelial Growth Factor
V _m	:Membrane Potential
°C	:Celsius



LIST OF FIGURES

	<u>Page</u>
Figure 1.1: Skin layer.	3
Figure 1.2: Phases of wound healing.	4
Figure 1.3: Factors affecting wound healing.	11
Figure 3.1: The application of DC electric current to the anode and cathode terminals for specified durations.	20
Figure 3.2: Device used for DC electric current peqlab EV202.	21
Figure 4.1: The zone diameters formed by <i>E. coli</i> bacteria under different positive voltages and application durations.	30
Figure 4.2: The zone diameters formed under different positive voltages and application durations of <i>P. aeruginosa</i> bacteria.	30
Figure 4.3: The zone diameters formed under different positive voltages and application durations of <i>S. aureus</i> bacteria.	31
Figure 4.4: The zone diameters formed under different positive voltages and application durations of <i>S. epidermidis</i> bacteria.	31
Figure 4.5: The zone diameters formed by <i>E. coli</i> bacteria under different negative voltages and application durations.	32
Figure 4.6: The zone diameters formed under different negative voltages and application durations of <i>P. aeruginosa</i> bacteria.	32
Figure 4.7: The zone diameters formed under different negative voltages and application durations of <i>S. aureus</i> bacteria.	33
Figure 4.8: The zone diameters formed under different negative voltages and application durations of <i>S. epidermidis</i> bacteria.	33
Figure 4.9: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 3 volts at the anode.	34
Figure 4.10: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 5 volts at the anode.	34
Figure 4.11: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 8 volts at the anode.	35
Figure 4.12: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 10 volts at the anode.	35
Figure 4.13: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 3 volts at the cathode.	36
Figure 4.14: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 5 volts at the cathode.	36
Figure 4.15: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 8 volts at the cathode.	37

Figure 4.16: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to 30 seconds of electrical stimulation at 10 volts at the cathode.	37
Figure 4.17: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 3 Volts of electrical stimulation for 1 minute.	38
Figure 4.18: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 5 Volts of electrical stimulation for 1 minute.	38
Figure 4.19: Inhibition zones created by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 8 Volts of electrical stimulation for 1 minute.	39
Figure 4.20: Inhibition zones formed by exposing <i>E.coli</i> , <i>S.aureus</i> , <i>P.aeruginosa</i> and <i>S.epidermidis</i> bacteria to negative 10 volts of Electrical stimulation for 1 minute.	39
Figure 4.21: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 3 Volts of electrical stimulation for 1 minute.	40
Figure 4.22: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 5 Volts of electrical stimulation for 1 minute.	40
Figure 4.23: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 8 Volts of electrical stimulation for 1 minute.	41
Figure 4.24: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 10 Volts of electrical stimulation for 1 minute.	41
Figure 4.25: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 3 Volts of electrical stimulation for 2 minute.	42
Figure 4.26: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 5 Volts of electrical stimulation for 2 minute.	42
Figure 4.27: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 8 Volts of electrical stimulation for 2 minute.	43
Figure 4.28: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 10 Volts of electrical stimulation for 2 minute.	43
Figure 4.29: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 3 Volts of electrical stimulation for 2 minute.	44
Figure 4.30: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 5 Volts of electrical stimulation for 2 minute.	44
Figure 4.31: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 8 Volts of electrical stimulation for 2 minute.	45
Figure 4.32: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> ,	45

<i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 10 Volts of electrical stimulation for 2 minute.	
Figure 4.33: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 3 Volts of electrical stimulation for 5 minute.	46
Figure 4.34: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 5 Volts of electrical stimulation for 5 minute.	46
Figure 4.35: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 8 Volts of electrical stimulation for 5 minute.	47
Figure 4.36: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to negative 10 Volts of electrical stimulation for 5 minute.	47
Figure 4.37: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 3 Volts of electrical stimulation for 5 minute.	48
Figure 4.38: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 5 Volts of electrical stimulation for 5 minute.	48
Figure 4.39: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 8 Volts of electrical stimulation for 5 minute.	49
Figure 4.40: Inhibition zones formed by exposing <i>E. coli</i> , <i>S. aureus</i> , <i>P.aeruginosa</i> , and <i>S. epidermidis</i> bacteria to positive 10 Volts of electrical stimulation for 5 minute.	49
Figure 4.41: The FTIR spectra of the sample taken from the circumference of the zone formed in the culture of <i>the E.coli</i> bacteria exposed to electrical stimulation.	50
Figure 4.42: The FTIR spectra of the sample taken from the circumference of the zone formed in the culture of the <i>S.aureus</i> bacteria exposed to electrical stimulation.	50

LIST OF TABLES

	<u>Page</u>
Table 4.1: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over <i>E.coli</i> .	22
Table 4.2: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over <i>E. coli</i> .	23
Table 4.3: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over <i>P. aeruginosa</i> .	24
Table 4.4: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over <i>P. aeruginosa</i> .	25
Table 4.5: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over <i>S.aureus</i> .	26
Table 4.6: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over <i>S.aureus</i> .	27
Table 4.7: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over <i>S.epidermidis</i> .	28
Table 4.8: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over <i>S.epidermidis</i> .	29

1. INTRODUCTION

1.1. Skin Structure and Function

The skin, as the largest organ of the body [1], consists of a complex blend of lipids, proteins, water, and minerals [2]. The integumentary system is organized into three primary layers: the outer layer known as the epidermis, the middle layer called the dermis, and the innermost layer referred to as the hypodermis (Figure 1). Each of these layers has its own specific functions, but together they form a comprehensive shield—protecting the body from microbial invasion and various external hazards, reducing the risk of mechanical, thermal, and physical injuries, minimizing moisture loss, lessening the impact of ultraviolet (UV) radiation, and even contributing to vitamin D synthesis [3, 4].

1.1.1 Epidermis

Human epidermis is a multilayered epithelium derived from keratinocytes, composed of stacked squamous epithelial cells, keratinized (thus lacking blood vessels) and generally consist of approximately 40 to 50 layers. The epidermis typically occurs in four basic layers: stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The innermost layer stratum basale, consists of the youngest and least differentiated progenitor cells (keratinocyte) that constantly renews the epidermis. These basal keratinocytes, contribute to keratin production by differentiating when they divide vertically on a thin membrane called the basal lamina that separates the epidermis from the dermis, while their horizontal division provides support for post injury tissue repair [5].

The stratum spinosum and stratum granulosum, which are layers of the epidermis, consist of nucleated cells. They consist of approximately 15 to 20 layers in total. The stratum granulosum contains many granules responsible for high levels of keratin synthesis. Langerhans cells, which are located in every layer of the epidermis, are more densely located in the stratum spinosum layer. The intracellular organelles and the nucleus gradually decompose and the accumulation of keratin takes place, forming

the derm stratum corneum. With the progression of keratinization, more keratin accumulation is seen. Dead keratinocytes accumulate and form a flexible and strong skin barrier [5]. The structure consisting of keratinized keratinocytes with a thickness of 10-20 μm is called the stratum corneum [6].

1.1.2 Dermis

The dermis, which forms the middle layer of the skin, is a connective tissue consisting of fibers such as collagen and elastin, which are usually major elements of the skin [7, 138]. Unlike keratinocytes found in the epidermis, fibroblasts that produce fibers such as collagen and elastin, which make up connective tissue, are sparse [5]. It controls the entry of vascular and nerve networks, epidermal appendages, fibroblasts, mast cells, macrophages and other blood cells by allowing the induction of signals in the epidermis. The epidermal-dermal junction is a structure that allows the exchange of substances between the epidermis and the dermis. This structure is home to skin appendages such as sweat glands, sebaceous glands, and hair follicles [8].

1.1.3 Hipodermis

The hypodermis, which consists of connective tissue and adipose tissue under the dermis layer, is the last layer of skin before skeletal muscle [9]. The hypodermis is home to, large blood vessels, fat stromal cells, nerves and fat cells. It supports keratinocyte and fibroblast proliferation. Thermoregulation is important for the structures not to be affected by trauma, the energy reserve and the production of hormones such as leptin produced by adipose tissue [10, 139]. Age, hormones, genetic factors and where they are located in the body have a significant impact on the accumulation of fat cells in the hypodermis layer [11, 12, 13]. Depending on the diet, the size of the fat cells varies [14]. Macrophages, fibrous bands, collagen, and elastin are components that connect the hypodermis to the dermis. It is possible to see hair follicles embedded in the hypodermis [15].

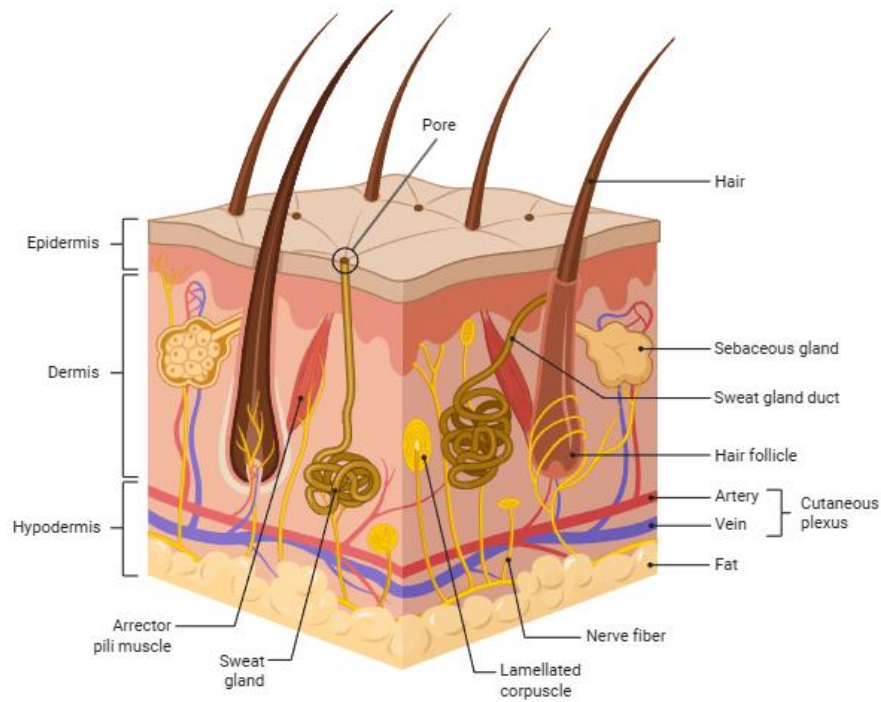


Figure 1.1: Skin layer (Created with Biorender).

1.2. Background of Wound Healing

1.2.1. Definition of Wound

The skin, as the body's largest external organ, serves as a primary shield against physical damage and harmful microorganisms, and any disruption to this protective barrier should ideally be swiftly restored [16]. A skin wound is the damage to the skin's normal structure and function, which also leads to the alteration of the local tissue environment [17]. A wound is any damage to living tissue that can affect not only the epidermal layer of the skin but also the underlying subcutaneous structures, depending on the severity of the injury [16]. Skin wounds can occur due to acute injuries (such as surgical wounds, skin abrasions) or other chronic conditions (such as diabetic ulcers, venous ulcers) [18]. Self-healing of wounds can occur in about two weeks. This is the case for most wounds [19].

1.2.2. Phases of Wound Healing

The wound healing process is typically divided into four phases: the coagulation phase, the inflammation phase, the proliferation phase, and the remodeling phase (Figure 1.2).

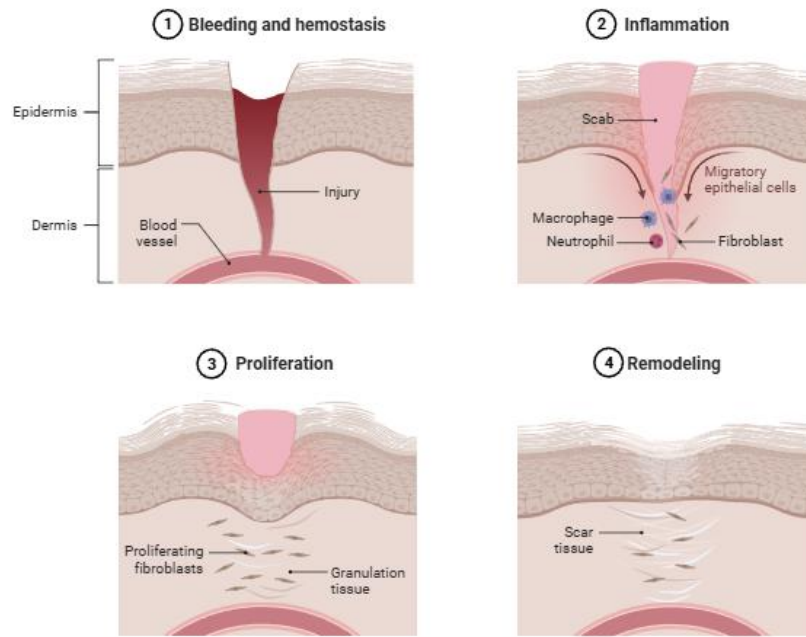


Figure 1.2: Phases of wound healing (Created with Biorender).

1.2.2.1. The Coagulation Phase

In the event of any skin injury, the primary and immediate task is to rapidly close the exposed tissue. This is achieved through local constriction of blood vessels (vasoconstriction) and the formation of a blood clot. The occurrence of the response is necessary both to prevent blood loss and to prevent pathogens from entering the tissue. With the narrowing of the blood arteries, the process of minimizing bleeding in the vascular system is entered. With the induction of vasoconstrictors such as endothelin to minimize bleeding, vascular smooth muscles begin to contract [20]. Catecholamines, including epinephrine and norepinephrine, along with prostaglandins, contribute significantly to the regulation of vasoconstriction. Platelets are a primary source of platelet-derived growth factor (PDGF). This signaling molecule triggers the stimulation of mesenchymal-derived cells, specifically vascular smooth muscle cells found within blood vessel structures [21].

A mesh-like fibrin structure that closes the injury site is generated through platelet activation and the initiation of blood clotting mechanisms. This blood clotting mechanism involves a sequential activation of several serine proteases. As a result, thrombin enzymatically converts fibrinogen, giving rise to fibrin fibers that form the

clot framework [22]. Although there are various pathways that initiate the clotting cascade, the internal pathway is one of the most important. This pathway becomes activated when factor XII interacts with certain negatively charged components, including collagen found at the injury site [23].

Any deficiency in the progression of coagulation can lead to the inability to stop bleeding in the injured area [24, 25]. Platelet degranulation enables the discharge of diverse soluble factors into the wound area, including transforming growth factor- β (TGF- β), PDGF, Stromal cell-derived factor 1 (SDF1/CXCL12), Vascular Endothelial Growth Factor (VEGF), and endostatin [26]. However, platelets are not absolutely necessary for repair, highlighting the redundancy and integration of many components that have evolved in this vital repair process [22]. For instance, the primary immune defense, inflammation, and blood clot formation are interconnected processes that mutually activate each other at the wound site [27, 28]. Molecular models are injury or pathogen-associated signals that stimulate the release of inflammation inducing mediators through Toll-like receptor (TLR) signaling pathways. This triggers the migration of diverse immune cells to the wound site, signaling the beginning of the inflammatory stage of healing process.

1.2.2.2. The Inflammation Phase

Leukocytes are made up of 50% to 70% neutrophils in adults. Neutrophils are important in the management of inflammation at the sites of infection. They are dormant and have a lifespan of 8-12 hours in the circulation and 1-2 days in the tissues. Stimuli such as macrophages, growth factors, chemokines and N-formyl peptides play a very important role in initiating the inflammatory phase in wound areas. Of the leukocytes, neutrophils are the first to arrive at the wound site [29]. It produces NETs, which are chromatin filaments that extend outside the cell and contain cytosolic proteins, histones and proteases by neutrophils, which are activated to capture and destroy bacteria, fungi and viruses that are external pathogens [30, 31]. They may setback the closure of microbiologically model wounds [32, 33]. During the course of physiological tissue repair, neutrophils have a key function in eliminating microorganisms. They also release mediators that reduce the further recruitment of neutrophils. However, through macrophages, a process is initiated that changes the state of the damaged epithelial tissue from a pro-inflammatory and non-proliferative

state to an anti-inflammatory and non-proliferative state [34]. In the defense against infection, microorganisms are eliminated through multiple pathways, including the activity of proteolytic enzymes, the action of antimicrobial peptides, and the generation of reactive oxygen species (ROS). Neutrophils have a central function in these defense strategies. These cells have a short lifespan and undergo programmed cell death (apoptosis) through pathways involving caspases and molecules such as cathepsin D [35, 36]. Neutrophils do not suppress the healing process; however, the deficiency of these cells can result in the formation of chronic and non-healing wounds. Neutrophils and immune cells such as inflammatory/M1 macrophages are essential components of the healing process and they are responsible for the elimination of pathogens, dead cells, and tissue debris. The shift from the inflammatory stage to the proliferative stage is largely supported by the phenotypic switch of M1 macrophages into the M2, anti-inflammatory subtype. These M2 macrophages secrete multiple bioactive substances—such as matrix metalloproteinases (MMPs), signaling proteins, and tissue growth mediators—that assist in attracting various cell populations to the wound area.

1.2.2.3. The Proliferation Phase

The lack or inability to function of macrophages, which play an important role in the wound healing process, significantly prevents the wound from closing. Studies show that macrophages depleted during wound healing can lead to inability to remove necrotic tissue, reduce the number of fibroblasts, and inhibit progress in the healing process. Studies demonstrate that modulating inflammatory response may assist in decreasing scar formation in wound healing. With this disruption of macrophages activation at the wound area may prevent dissolution inflammation particularly diabetic wound. This regular inflammation situation, put at risk for wound closure and especially diabetic wounds scar formation may get worse. Constantly inflammation prevents phenotype resolution [37-39]. Lipid mediators such as resolvins, protectins, lipoxins and maresins play a role in the regulation of inflammation. These mediators control wound healing. They play a role in controlling and resisting inflammation. B and T lymphocytes, which initiate an immune response against foreign substances and pathogens, are important for wound healing [40]. Mast cells play an important role in initiating the inflammatory process while also terminating inflammation through

apoptosis. In addition its effects are seen in the wound healing process and in the fibrosis phase. Available data suggest that the findings regarding the associations between abnormal healing processes observed in mice and keloid and hypertrophic scar development warrant further investigation of these structures in terms of their functional roles and treatment approaches [39]. Therapeutic intervention is key especially when it comes to chronic or damaged wounds acceleration of wound closure and reduction of scar formation, understanding immune cells' function in wound healing. Fibroblasts located in the dermal layers represent a diverse group of cells with highly versatile structures [41, 42]. Fibroblasts respond to soluble extracellular signals, such as growth factors and cytokines, undergoing an activation that triggers cellular proliferation and regulation of metalloproteinases. During the wound healing process, fibroblasts migrating to the granulation tissue begin collagen synthesis and replace the temporarily formed fibrin matrix [43, 44]. Myofibroblasts contribute to increased collagen accumulation and initiate wound contraction. Fibroblasts can sense mechanical loads in terms of both magnitude and direction, and transduce these signals into appropriate adaptive responses [43, 45]. Keratinocytes undergo activation triggered by exposure with hydrogen peroxide, growth factors, cytokines and pathogens along with changes in electrical gradients and mechanical tension. This activation occurs usually within 12 hours after injury. This stimulus causes keratinocytes at the wound edge to go through a migratory and sharp phenotypic limited epithelial-mesenchymal transition [46, 47]. A transition occurs, where polarity shifts from a top-down direction to a front-reinforced direction. This shift indicates the movement of leading keratinocytes in a process referred to as re-epithelialization. To regenerate the epidermal layer, keratinocytes migrate laterally through the wounded area. Keratinocytes modulate their intercellular adhesion through PCKa-mediated alterations in desmosomes and electric field-induced changes in adherens junctions, facilitating their reorganization by the migrating epithelial layer [48]. In the neo-epidermis, keratinocytes release matrix metalloproteinases (MMPs), which facilitate their migration, promote the synthesis of new extracellular matrix components, and aid in reconstructing the basal membrane [49, 50]. Keratinocytes multiply along the wound margins and move across the newly generated matrix, thereby contributing to wound closure and re-establishing the skin's barrier function. Wound closure indicates the beginning of the remodeling or scar maturation phase.

1.2.2.4. The Remodelling Phase

The remodeling and maturation phase is a long-term procedure that can last for months or even years. This process has a significant impact on scar formation and the likelihood of wound reappearance. The remodeling phase allows the neovascular system to regress and the tissue to remodel from newly formed tissue to fibrous tissue [51]. The presence of collagen III is first seen in the granulation tissue and is replaced by collagen I over time. Throughout this process, myofibroblasts in the granulation tissue actively synthesize matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitor of metalloproteinase (TIMPs), which are essential for the targeted breakdown of specific extracellular matrix components [51, 52]. An irregular synthesis of TIMPs and MMPs can result in an imbalance in Extra cellular matrix (ECM) remodeling, potentially playing a role in the formation of chronic wounds [51, 53]. Macrophages play a crucial role in wound remodeling, as they are tasked with breaking down excess ECM and clearing debris through phagocytosis [54].

1.2.3. Types of Wound

Wounds can be classified into two types based on their causes and outcomes: acute and chronic wounds [55]. Normal or acute wounds heal through a systematic and structured process aimed at restoring the body's physical and functional stability, ultimately resulting in full wound recovery [56, 57]. Standard acute wounds generally recover within 4 to 6 weeks with few complications [58]. The normal healing process of the skin should result in the reinstatement of its continuity and proper function.

However, some wounds may not heal in a timely and orderly manner [59, 60]. Chronic wounds are defined as wounds that fail to advance through the healing stages within a timeframe of 42 days or less [61, 62]. The primary characteristics of chronic wounds include necrosis, elevated production of reactive oxygen species, abnormal re-epithelialization, infections, exudation, ongoing inflammation, bacterial colonization, and reduced angiogenesis [63-66]. Chronic wounds are typically classified into three primary types: pressure ulcers, diabetic foot ulcers and vascular ulcers [67]. There are multiple factors that affect wound healing rates in preventing acute wounds from healing quickly and properly and from progressing to chronic wounds. Wound healing

can be influenced by factors that are classified into two main categories: local factors and systemic factors.

1.2.4. Factors Affecting Wound Healing

1.2.4.1. Nutrition

Proper nutrition is critical at all stages of recovery. Whether at the micro or macro level, it is necessary to take these nutrients into the body at an adequate level. Proteins are necessary for the production of collagens that aid macrophage function and for the accumulation of extra cellular matrices (ECMs). Certain amino acids like arginine, are essential for inflammation. Carbohydrate and lipid consumption is necessary for cellular proliferation. Lipids play an important role in increasing immune responses and reducing oxidative stress. It also helps coagulation by facilitating the absorption of vitamins A, D, E, K [68]. Impaired cytokine release and subsequent immune response have been associated with malnutrition. Malnutrition plays a role in the infection and irregular wound healing process of patients [69]. Nutritional supplementation of malnourished patients results in less postoperative infection and short-term hospitalization [70]. Malnutrition is common in diabetic, elderly and obese individuals who are prone to chronic wound development. Considering this situation, it becomes a vital necessity to provide adequate and balanced nutrition in order for wound repair to proceed effectively [71].

1.2.4.2. Hypoxic Stress

Acute tissue hypoxia occurs when the vessels around the wound site are injured and adequate oxygenation is not provided. This often leads to increased expression of hypoxia-inducible factor-1 (HIF-1), which plays a role in processes such as the regulation of immune response, vessel formation, and activation of growth factors, in order to contribute to the wound repair process [72]. In chronic tissue hypoxia, overexpression of HIF-1 is accompanied by inflammation, ROS production, and poor ECM accumulation. The effects of hypoxia include deterioration in angiogenesis, inhibition of cell migration to the wound site, and granulation tissue formation. As a

result, wound healing slowed down [73]. For this reason, long-term hypoxia should be prevented and tissue perfusion should be ensured in order to ensure timely recovery.

1.2.4.3. Chronic Disease

Chronic diseases can have a chain of processes that take time to resolve in wound healing. An example of these diseases is diabetes. There is a proven impairment in the healing of acute wounds seen in individuals with diabetes. Chronic nonhealing diabetic foot ulcers occur in an estimated 15% of all people with diabetes. Diabetic foot ulcers, which are serious complications of diabetes, lead 84% of all lower leg amputations associated with diabetes [74]. High levels of metalloprotease, which is one of the hallmarks of diabetic foot ulcers, are 60 times higher in chronic wounds than in acute wounds. The increase in protease activation is effective in inhibiting normal repair and promoting tissue destruction [75, 76].

1.2.4.4. Infection

When a skin injury occurs, microorganisms normally found on the skin surface reach the underlying tissues. Classification of microorganisms according to the proliferation and infection development in the wound allows the wound status to be evaluated as contamination, colonization, local infection/critical colonization and spreading invasive infection.

Contamination refers to the presence of non-proliferating organisms on the wound, while colonization refers to the proliferation of microorganisms in the wound without tissue damage. Local infection or critical colonization represents a stage in which local tissue responses begin with the increase in microorganisms. Invasive infection is defined as the occurrence of damage to host tissues with the proliferation of microorganisms in the interior of the wound [77].

The second stage of the healing process, the inflammation stage, plays a critical role in removing microorganisms that may cause infection. If effective decontamination is not achieved, the inflammatory process may be prolonged. Both bacteria and endotoxins can lead to prolonged increases in proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), and prolong the inflammation phase. If this situation continues, the likelihood of the wound becoming

chronic and the healing process being compromised increases. Persistent inflammation also causes increased levels of matrix metalloprotease (MMP) family proteases that can degrade the Extracellular Matrix. This increased protease activity, together with a decrease in the amount of natural protease inhibitors, leads to rapid degradation of growth factors in chronic wounds [77, 78]. *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and β -hemolytic streptococci are common bacteria in infected and clinically non-infected wounds [77, 79]. *P. aeruginosa* and *S. aureus* appear to play an important role in bacterial infection of wounds. Many chronic ulcers fail to heal, probably because of the presence of biofilms containing *P. aeruginosa*, which protect the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds [80].

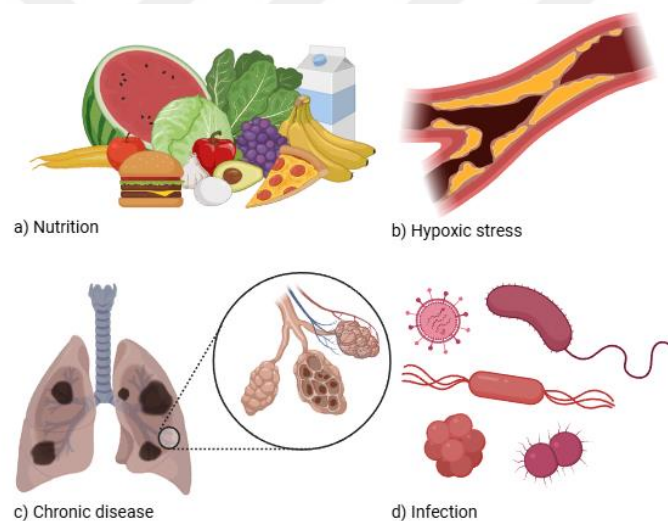


Figure 1.3: Factors affecting wound healing (Created with Biorender).

1.3. Challenges in Wound Healing

Wound healing technologies and scar treatment products have become a growing industry area worth billions of dollars. The market for scar reduction products is estimated to be worth over \$12 billion, while healing technology products are worth over \$15 billion. It is estimated that between 2 and 4.5 million people in the United States suffer from chronic wounds. This situation creates an economic burden of approximately \$25 billion each year, significantly straining the healthcare system. The

increase in healthcare spending, the aging of society, and the prevalence of chronic diseases such as diabetes play important roles in this increase in cost [81]. Tissue damage caused by traumas or operational procedures disrupts the integrity of the skin and creates a suitable environment for the development of infection. The healing of such injuries occurs through a chain of biological processes that are very precisely regulated. However, this process can be interrupted by various obstacles such as inadequate tissue perfusion, ongoing inflammation, pressure, infection and nutritional deficiencies. Vascularization, especially in the wound area, is vital for the successful completion of the treatment by ensuring adequate oxygen and nutrient transport [82]. As a promising method, the application of electrical stimulation to accelerate wound healing has recently emerged as a treatment method. In addition, important studies have been recorded in the creation and development of flexible electronic devices aimed at shortening recovery times [83].

1.4. Role of Bioelectricity in Tissue Repair

Our body's several parts (heart, brain, muscles and bones) are known to produce natural electrochemical impulses as an endogenous bioelectric system. We recognize the endogenous electrical potential, which we sometimes call the skin battery, with its effects on the healing process of wounds [84-85]. In the untraumatized fence, there is a natural endogenous electrical potential between the epidermal and sub-epidermal layers. This potential is between 10 and 60 mV [85]. The electric potential between these layers is also called the transepithelial electric potential. The transepithelial electric potential is the result of the electrogenic activity generated by ion transport of Na^+/K^+ ATPase pumps in epidermal cells [86]. It creates an electric potential that is positive at the wound edges and negative at the wound center. The potential difference of 10-60 mV can be measured within 2-3 mm of the wound [86-88]. Electric current can be sustained in humid conditions. However, with the drying of the wound surface, conductivity ends. In 1983, the relationship between ion motion and injury-induced electric current and tissue repair rate was determined. In one study, increasing the uptake of Cl^- and Na^+ ions into the damaged corneal epithelium of rats by applying $AgNO_3$ led to a significant increase in the injury current, which contributed to the acceleration of the healing process.

A decrease in injury flow occurred in rat corneal wounds containing furosemide, a component that inhibits the outflow of Cl^- . The decrease in this flow has also created broken corneal wounds. Clinical tests have shown that there is a voltage difference of 100-150 mV/mm between the wound locations and intact skin [89-91]. These intra-body electrical fields are of vital importance in the tissue repair process [89, 90]. Endogenous currents, which are a guide for cellular migration, also promote the healing of wounds [90]. It has been shown in many studies that electrical stimulation directs cell migration, angiogenesis, known as the formation of blood vessels, and nerve growth during the wound healing process, and plays a role in this process by accelerating wound healing [92-96]. Electrical stimulation has been shown to increase protein synthesis and blood circulation, but it has a bactericidal effect in a study conducted in 1987 [97].

The stage called 0 hours after the occurrence of the injury is hemostasis. Studies investigating the effect of electrical stimulation on the process at this stage are rare. However, it has been shown to activate the platelet surface procoagulant markers and promote the production of platelet-derived microparticles [98]. Activation of complement cascades occurs after platelet degranulation. With the activation of inflammatory cells, the elimination of bacteria is achieved [99].

The second stage, which is 1-3 days after the injury, is inflammation. At this stage, cells such as macrophages and neutrophils, which are involved in the early phase of the immune response, come into play.

At this stage, EF can be an important participant in wound healing as well as biochemical factors. Endogenous EFs have been shown to potentiate cell phagocytosis and autolysis by increasing the electrical chemotaxis of neutrophils and macrophages using the change of strength of electrical stimulation [100]. Electric fields (EFs) have been shown to enhance macrophages' ability to recognize target cells and at the same time significantly increase the expression of proinflammatory cytokines such as $\text{TNF-}\alpha$, Neurotrophin-3 (NT3), IL-1 and Interleukin-23 (IL-23) [101, 102]. The results show that electrical stimulation has an effect on the inflammatory phase.

The third stage, which is 4-21 days after the injury, is the proliferation stage. Regeneration of angiogenesis and granulation with the migration of keratinocytes at the wound edge plays a fundamental role in re-epithelialization [103]. Many cell types

respond to EFs via a phenylalanine nomenon called directional cell migration, galvanotaxy, or electrotaxy. EFs promote fibroplasia with the onset of the proliferative phase by directing fibroblasts to the injured area. One study showed that with the application of EF of 50-100 mV/mm, primary and cell line cultures of human dermal fibroblasts moved slowly and directionally towards the anode [95]. In another study, the electric current applied to the fibroblasts in the anterior cruciate ligament of the calf by the pulsed direct current found that these cells were positioned perpendicularly against the increase in migration rate and electrical stimulation [104].

In the last stage, remodeling, accelerating wound shrinkage and epithelialization, EF directs the migration of myofibroblasts, epidermal cells, and keratinocytes close to the injured area. RhoA-regulated F-actin redistribution has been found to mediate EF-induced fibroblast alignment. [105]. In an EF performed in vitro, endogenous wound EFs and keratinocytes migrate in the same direction towards the cathode [86,106, 107,108,109,110].

1.5. Electrical Stimulation as a Therapeutic Strategy

Electrical stimulation (ES) treatment has emerged as an alternative method to accelerate wound healing. The first discovery of the existence of bioelectricity was seen in the experiments carried out by the Italian biologist L.A. Galvani. Then, Meteucci discovered the damage potential by measuring the damage current at the separating end of the nerve and muscle of a frog's leg. E. J. Lund found that the direction of the endogenous bioelectric field is triggered and controlled by the bioelectric polarity of the ion current [111]. H. S. Burr studied the relationship between potential gradients in various species and organs and the developmental processes of these structures [112, 113]. In the mid to late 20th century, Jaffe and his colleagues made significant contributions to the field by demonstrating that electric field stimulation within physiological ranges could guide not only the migration of various cell types—such as fibroblasts, nerve cells, osteoblasts, osteoclasts, epidermal cells, macrophages, leukocytes, and amoeba—but also influence the growth direction of structures including nerves, mouse eggs, myoblasts, coelenterates, sea squirts, decidua, and fungi [114-120]. The membrane potential (V_m) between the two sides of the cell membrane in the smallest building block of the organism is called the potential difference. This potential difference is due to the charge gradient inside and outside

the membrane. Cells have different cell membrane potentials depending on their type, tissue and degree of differentiation. Cells that have undergone terminal differentiation are typically strongly negatively polarized, with membrane potentials ranging between -40 mV and -90 mV. In contrast, embryos, stem cells, and cancer cells tend to be more depolarized, showing less negative potentials—for instance, -8.5 mV in fertilized eggs, -23 mV in 4-cell stage embryos, and around -25 mV in 16-cell stage embryos [121, 122]. The natural electrical potential between the epidermal and sub-epidermal layers of undamaged skin is between 10-60 mV [123]. This trans-epithelial voltage (TEP) increases greatly around a wound. Disruption of the epithelium by an injury short-circuits the TEP and directs positive electrical flow toward the wound [90, 91]. Injuries produce electric current [124] and clinical studies have shown that the voltage difference between the wound area and the undamaged skin varies between 100 and 150 mV/mm [89-91]. The application of external electrical stimulation has been carried out by scientists and its effects on fractures, wounds and pain have been investigated. These various electrical applications include Direct current (DC), Alternating current (AC), HVPC (high voltage pulsed current) and low intensity DC (LIDC). Direct current is the continuous flow of electrical charge in one direction in a single phase waveform. Currents of 20–200 μ A can be provided at low voltage [125]. AC has a biphasic waveform consisting of two alternating symmetrical electrical pulses. Voltages are 50-150 V depending on tissue hydration [125, 126]. Pulsed electromagnetic field (PEMF) is the most commonly used treatment for nonunions, and transcutaneous electrical nerve stimulation (TENS) is the most commonly used treatment for pain control. FREMS (frequency rhythmic electrical modulation systems) is a type of transcutaneous electrotherapy that uses ES to continuously vary the amount of stimulation (pulse, frequency, duration, and voltage) [127-129]. Pulsed current (PC) refers to the short-term passage of ions or electrons in a specific direction, either unidirectional or bidirectional, and is usually followed by short-term pulses followed by longer periods of no current. Each pulse is considered an independent electrical event separated by specific time intervals. According to a study, pulsed current stimulation usually consists of pulses lasting less than 1 millisecond or in the form of a square waveform that remains active for only 5% of the total time (low duty cycle). This structure prevents polarization of the signal while also helping to reduce the discomfort that continuous current can cause and the accumulation of acidic or basic substances around the electrode [130]. Studies have been conducted to

investigate the effects of electrical stimulation on bacterial colonization in the wound area with different electrical currents. In one study, it was reported that 100 μ A DC did not have an inhibitory effect on any bacterial strain by exposing *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* to alternating current (AC) or direct current (DC). However, 20 mA AC showed a 38% decrease in the number of viable cells (CFU) per unit that could form colonies in *P. aeruginosa* ($p < 0.05$) [131]. There has been one study in which it was reported that the growth of three bacteria isolated from open wounds exposed to HVPC at a voltage of 250 V or higher for 2 hours was inhibited at the anode and cathode [132]. In another study focusing on chronic wounds, the team exposed three strains obtained from chronic venous ulcers to constant diphasic (FD-B) current and high voltage monophasic current (HVMC) for 30 and 60 minutes, respectively, and FD-B completely inhibited the colony forming units (CFU) of all three strains within 30 minutes ($p < 0.05$), while HVMC caused an average of 50% CFU reduction on all bacteria except *E. coli* [133]. Daeschlein et al. [134], experimenting with electric current on three Gram-negative species (*E. coli*, *P. aeruginosa*, *Klebsiella pneumonia*) and three Gram-positive species (*S. aureus*, *Staphylococcus epidermidis*, *Escherichia faecium*) that frequently colonize chronic wounds, reported that 42 mA monophasic low-voltage pulsed current (LVPC) for 30 minutes caused a significant decrease in CFU in all pathogens tested ($p < 0.01$), while no antibacterial effect was recorded in the control group. However, it was stated that applying the current with positive polarity caused a greater bacteriostatic effect than with negative polarity. Barranco et al. [135] exposed *S. aureus* to DC for 48 h using different electrodes (silver, platinum, gold and steel) and varying currents of 0.4, 4, 40 and 400 μ A and reported that the positive silver anode led to the highest reduction in bacterial growth at the lower current range (0.4 and 4 μ A).

Falcone and Spadaro [136] also reported that the electrically active silver anode exhibited significant bacteriostatic effects even at low direct current (4 μ A). Asadi and Torkaman summarize the antibacterial effects of ES as a combination of direct effects (disruption of bacterial membranes and inhibition of bacterial cell proliferation) and indirect effects (pH changes, i.e., increased pH at the cathode and decreased pH at the anode; temperature changes; electrolysis products, e.g., toxic species, chlorine, H_2O_2 , radicals; and galvanotaxis, increased migration of white blood cells, such as macrophages and leukocytes, to the infected wound site) [137]. There are some points

to consider when supporting wound healing with electrical stimulation. First of all, the electrode to be used must be biocompatible, must not create a toxic effect in the environment it is in, and must support wound healing. However, the material used in wound healing must have the capacity to sustain the electric current for the duration of use until the wound closes, which can vary from a few hours to days.



2. PURPOSE OF THE STUDY

Wound infections are significant clinical problems that prolong the healing process and can lead to serious complications. Nowadays, antibiotics are commonly used to combat these infections; however, antibiotic resistance complicates treatment processes. Alternatively, studies have been conducted on the inhibition or killing of bacteria through electrical stimulation, and this approach has shown promising results. However, in the literature, there are limited studies in which the effectiveness of treatment parameters (voltage, current, duration) in applications using DC (direct current) electrical stimulation has been systematically optimized. This situation prevents the method from reaching adequate standards for clinical use. In this study, the antibacterial effect of DC electrical stimulation was optimized, and then the chemical and structural changes induced by this application in bacterial cell membranes were analyzed using FTIR spectroscopy. Thus, it was attempted to reveal not only the killing of bacteria but also how this effect occurs at the molecular level. The obtained data is important in terms of grounding the electrical treatment parameters scientifically and making this method a safe and effective alternative in wound healing.

3. EXPERIMENT

3.1. Materials and Method

Sodium chloride (Sigma Aldrich Lot#SZBD1280V)

Tryptone D, Type IV (HiMedia, RM193-500G)

Yeast Extract Powder, Type I (HiMedia, RM668-500G)

Peptone, Bacteriological HiMedia RM001, 500g

Petri dish

Electrode

Peqlab Electrophoresis power supply (EV 202)

Stainless steel 316L

Incubator

Ruler

Escherichia coli (ATCC® 25922™)

Pseudomonas aeruginosa (ATCC® 27853™)

Staphylococcus aureus ATCC® 25923™)

Staphylococcus epidermidis (ATCC® 12228™)

3.1.1. Preparation of Culture Media

A solid medium mixture was created by adding 2 grams of Tryptone D, Type IV (HIMEDIA, RM193-500G), 1 gram of Yeast Extract Powder, Type I (HIMEDIA,

RM668-500G), 1 gram of Sodium chloride (Sigma Aldrich Lot#SZBD1280V) and 3 grams of Peptone (Bacteriological HiMedia RM001) to 200 ml of distilled water. From this mixture, enough was prepared for 64 petri dishes. The prepared mixtures were taken to a solid autoclave. After 1 hour and 20 minutes, the ready media were poured onto the solid media without solidifying. The media were left to cool. Of the cooled media, 16 were separated for *E.coli*, 16 for *P.aeruginosa*, 16 for *S.aureus*, and 16 for *S.epidermidis*. Each petri dish allocated for each bacterium was labeled with how much voltage and for how long it would be exposed to DC current. After the *E.coli* inoculation, it was expected to settle on the medium for 10 minutes, and then electrical currents were applied. After the application of the electrical current, it was left to grow in the incubator at 37 °C for 18 hours. The same procedures were applied on other days for the other bacteria.

3.1.2. DC Current

Stainless steel 316L wires with a length of 20 mm were preferred as the electrode material. In the application of electrodes, the spacing of the anode and cathode was realized as 45 mm (Figure 3.1). 3V electrical application was performed for 30 sec, 1 min, 2 min and 5 min, and each of these applications was applied as 3 repetitions in the same petri dish. Electrical application was performed as 3V, 5V, 8V and 10V for 30 sec, 1 min, 2 min and 5 min for each voltage (Figure 3.2). After exposure to ES, each plate was incubated at 37 °C for 18 h. Using a millimeter ruler, it was measured how much bacterial growth was prevented in the area where the electrode was applied.



Figure 3.1: The application of DC electric current to the anode and cathode terminals for specified durations.



Figure 3.2: Device used for DC electric current peqlab EV202.

3.1.3. AC Current

LB agar medium was created and bacteria were planted respectively. 16 plate media were created for each bacterium. A total of 64 media were created. Stainless steel 316L wires with a length of 20 mm were preferred as the electrode material. In the application of electrodes, the spacing of the anode and cathode was maintained at 45 mm. 3V electrical application was performed for 30 sec, 1 min, 2 min and 5 min, and each of these treatment was applied as 3 repetitions within the same Petri dish. Electrical application was performed as 2V, 4V, 6V, 8V and 10V for 30 sec, 1 min, 2 min and 5 min for each voltage. After exposure to ES, each plate was incubated at 37 °C for 18 h. Using a millimeter ruler, it was measured how much bacterial growth was prevented in the area where the electrode was applied.

3.1.4. FTIR Experiment

Swabs were taken from control bacteria to which no electricity was applied and placed in eppendorfs. Swabs were taken from the bacteria remaining at the edges of the zone formed during the 5V 5 minute application of *E. coli* and *S. aureus* bacteria to which DC electricity was applied. These bacterial swabs were taken from two different zones as anode application and cathode application and placed in eppendorfs. These controls and samples were kept in an oven overnight and dried. The dried controls and samples were ready for Fourier Transform Infrared Spectroscopy (FTIR) measurements. FTIR measurements were performed in transmission mode using the Perkin Elmer Spectrum 4000 in the range of 800 to 4000 cm^{-1} .

4. RESULTS

AC electric current did not affect bacteria growth. The diameters of the inhibition zones created by the application of DC electric current on *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* bacterial strains at the anode and cathode are given in **Tables 4.1-4.8**.

Table 4.1: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over *E.coli*.

DC Electricity Current Application to <i>E. coli</i> Bacteria (CATHODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0.4 cm	0.4 cm	0.4 cm
1 min	0.4 cm	0.4 cm	0.4 cm
2 min	0.5 cm	0.4 cm	0.5 cm
5 min	0.7 cm	0.7 cm	0.7 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0.3 cm	0.3 cm	0
1 min	0.7 cm	0.7 cm	0.6 cm
2 min	0.8 cm	0.8 cm	0.8 cm
5 min	1.5 cm	1.5 cm	1.3 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0.3 cm	0.4 cm	0.4 cm
1 min	0.9 cm	0.8 cm	0.8 cm
2 min	1.2 cm	1.8 cm	1.8 cm
5 min	2.1 cm	2.1 cm	2.1 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.6 cm	0.6 cm	0.8 cm
1 min	0.8 cm	1 cm	0.7 cm
2 min	1.6 cm	1.6 cm	1.5 cm
5 min	2.2 cm	2.3 cm	2.3 cm

Table 4.2: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over *E. coli*.

DC Electricity Current Application to <i>E. coli</i> Bacteria (ANODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0	0	0
5 min	0	0	0
5 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0	0	0
5 min	0	0	0
8 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0.6 cm	0.6 cm	0.6 cm
2 min	0	0	0
5 min	1.1 cm	1.2 cm	1.1 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0	0	0
5 min	1.4 cm	1.5 cm	1.5 cm

Table 4.3: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over *P. aeruginosa*.

DC Electricity Current Application to <i>P. aeruginosa</i> Bacteria (CATHODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0.3 cm	0.4 cm	0.4 cm
1 min	0.5 cm	0.5 cm	0.5 cm
2 min	0.6 cm	0.5 cm	0.6 cm
5 min	1 cm	1.1 cm	1.1 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0.5 cm	0.4 cm	0.4 cm
1 min	0.7 cm	0.6 cm	0.7 cm
2 min	0.9 cm	0.8 cm	0.8 cm
5 min	1.7 cm	1.6 cm	1.6 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0.8 cm	0.7 cm	0.7 cm
1 min	0.9 cm	0.9 cm	1 cm
2 min	1.3 cm	1.4 cm	1.4 cm
5 min	1.9 cm	2.1 cm	2 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.6 cm	0.7 cm	0.8 cm
1 min	0.9 cm	1 cm	0.9 cm
2 min	2.1 cm	1.9 cm	1.5 cm
5 min	2.7 cm	2.8 cm	2.8 cm

Table 4.4: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over *P. aeruginosa*.

DC Electricity Current Application to <i>P. aeruginosa</i> Bacteria (ANODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0	0	0
5 min	0.6 cm	0.7 cm	0.6 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0	0.3 cm	0.3 cm
1 min	0.4 cm	0.5 cm	0.5 cm
2 min	0.6 cm	0.6 cm	0.7 cm
5 min	1.2 cm	1.1 cm	1.1 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0	0.1 cm	0.5 cm
1 min	0.6 cm	0.6 cm	0.7 cm
2 min	0.8 cm	1 cm	0.9 cm
5 min	1.4 cm	1.5 cm	1.4 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0.5 cm
1 min	0	0	0
2 min	0	0.7 cm	0.9 cm
5 min	1.4 cm	1 cm	0.6 cm

Table 4.5: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over *S.aureus*.

DC Electricity Current Application to <i>S. aureus</i> Bacteria (CATHODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0.4 cm	0.5 cm	0.5 cm
1 min	0.6 cm	0.6 cm	0.6 cm
2 min	0.8 cm	0.8 cm	0.8 cm
5 min	0.9 cm	1 cm	1 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0.6 cm	0.7 cm	0.7 cm
1 min	0.8 cm	0.8 cm	0.7 cm
2 min	0.9 cm	0.9 cm	0.9 cm
5 min	1.5 cm	1.8 cm	1.7 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0.7 cm	0.8 cm	0.6 cm
1 min	0.9 cm	0.8 cm	0.8 cm
2 min	1.55 cm	1.55 cm	1.3 cm
5 min	2 cm	2.2 cm	2.1 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.7 cm	0.6 cm	0.5 cm
1 min	1.1 cm	1.2 cm	1.1 cm
2 min	1.8 cm	1.55 cm	1.6 cm
5 min	2.4 cm	2.5 cm	2.6 cm

Table 4.6: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over *S.aureus*.

DC Electricity Current Application to <i>S. aureus</i> Bacteria (ANODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0.55 cm	0.6 cm	0.55 cm
5 min	0.7 cm	0.8 cm	0.8 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0.7 cm	0.75 cm	0.7 cm
5 min	1 cm	1.1 cm	1.1 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0.5 cm	0.5 cm	0.6 cm
1 min	0.7 cm	0.7 cm	0.7 cm
2 min	1 cm	1 cm	0.95 cm
5 min	1.4 cm	1.5 cm	1.4 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.35 cm	0.55 cm	0.5 cm
1 min	0.55 cm	0.75 cm	0.8 cm
2 min	1 cm	0.95 cm	1 cm
5 min	1.6 cm	1.7 cm	1.7 cm

Table 4.7: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the cathode side over *S.epidermidis*.

DC Electricity Current Application to <i>S. epidermidis</i> Bacteria (CATHODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0.4 cm	0.4 cm	0.4 cm
1 min	0.4 cm	0	0.4 cm
2 min	0.6 cm	0.6 cm	0.6 cm
5 min	0.9 cm	1 cm	1 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0.3 cm	0.3 cm	0.3 cm
1 min	0.7 cm	0.7 cm	0.6 cm
2 min	1 cm	0.9 cm	0.9 cm
5 min	1.2 cm	1.5 cm	1.5 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0.4 cm	0.3 cm	0.4 cm
1 min	0.9 cm	0.9 cm	0.9 cm
2 min	1.2 cm	1.2 cm	1.1 cm
5 min	1.75 cm	2.1 cm	1.95 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.6 cm	0.6 cm	0.55 cm
1 min	1 cm	0.9 cm	0.9 cm
2 min	1.3 cm	1.5 cm	1.4 cm
5 min	2 cm	2.1 cm	2.1 cm

Table 4.8: Measurements of the inhibition zones created by DC electric current at different durations and voltages on the anode side over *S.epidermidis*.

DC Electricity Current Application to <i>S. epidermidis</i> Bacteria (ANODE)			
3 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0.4 cm	0.4 cm	0.4 cm
5 min	0.6 cm	0.65 cm	0.7 cm
5 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0.55 cm
2 min	0.5 cm	0.4 cm	0.6 cm
5 min	0.9 cm	0.9 cm	0.9 cm
8 Volt			
Time	1. application	2. application	3. application
30 sec	0	0	0
1 min	0	0	0
2 min	0.5 cm	0.4 cm	0.6 cm
5 min	1.3 cm	1.3 cm	1.2 cm
10 Volt			
Time	1. application	2. application	3. application
30 sec	0.3 cm	0.3 cm	0.4 cm
1 min	0	0	0
2 min	0.8 cm	0.9 cm	0.8 cm
5 min	1.25 cm	1.45 cm	1.32 cm

The bar graphs below shows the data obtained by taking the average of the diameters of each zone created by the bacteria as 3 replicas at the cathode of the DC current (Figures 4.1-4.4).

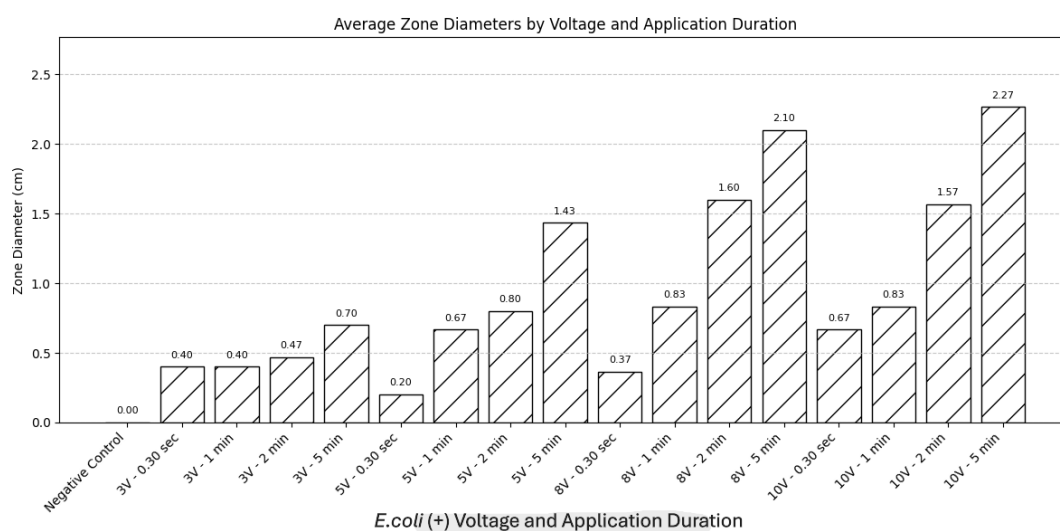


Figure 4.1: The zone diameters formed by *E.coli* bacteria under different positive voltages and application durations.

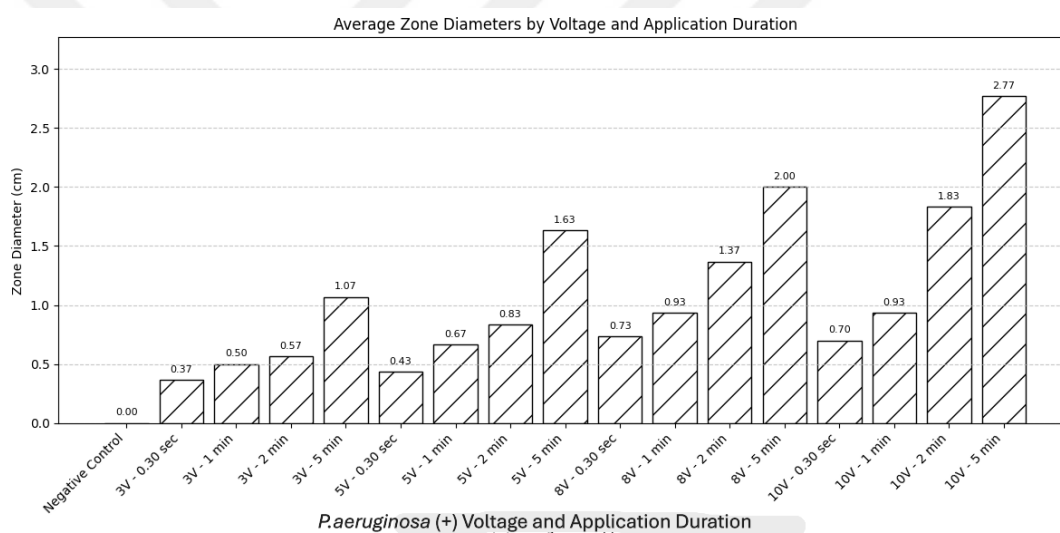


Figure 4.2: The zone diameters formed under different positive voltages and application durations of *P. aeruginosa* bacteria.

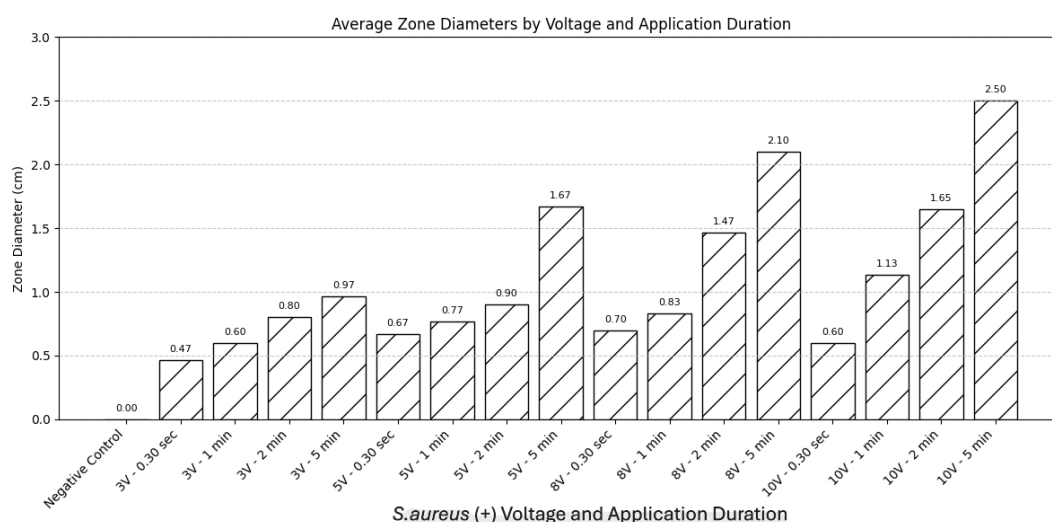


Figure 4.3: The zone diameters formed under different positive voltages and application durations of *S. aureus* bacteria.

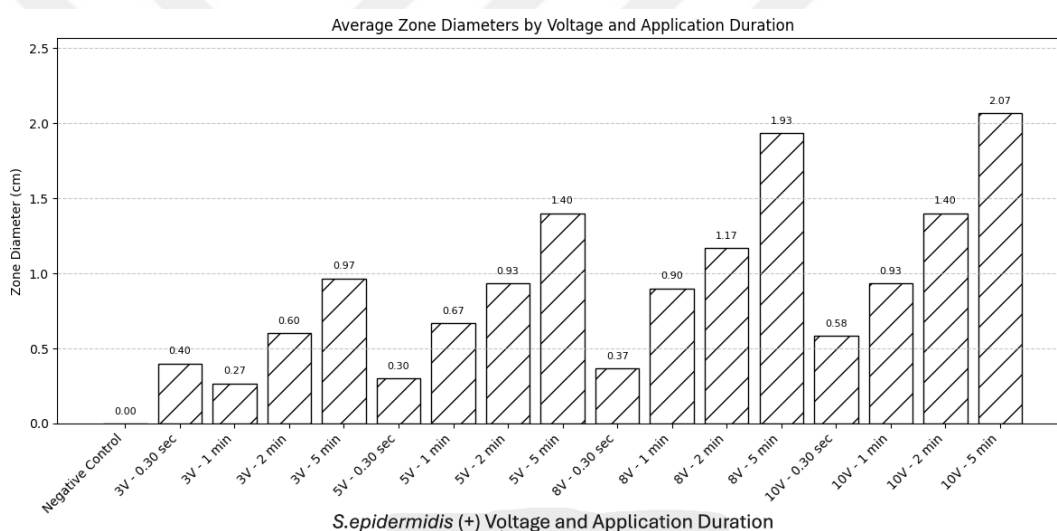


Figure 4.4: The zone diameters formed under different positive voltages and application durations of *S. epidermidis* bacteria.

The bar graphs below shows the data obtained by taking the average of the diameters of each zone created by the bacteria as 3 replicas at the anode of the DC current (Figures 4.05-4.08).

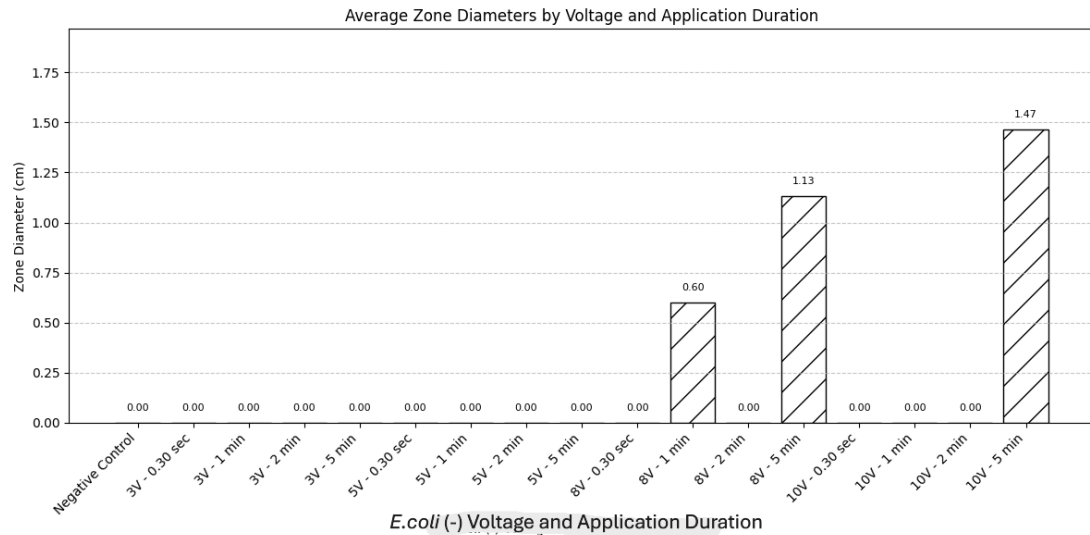


Figure 4.5: The zone diameters formed by *E. coli* bacteria under different negative voltages and application durations.

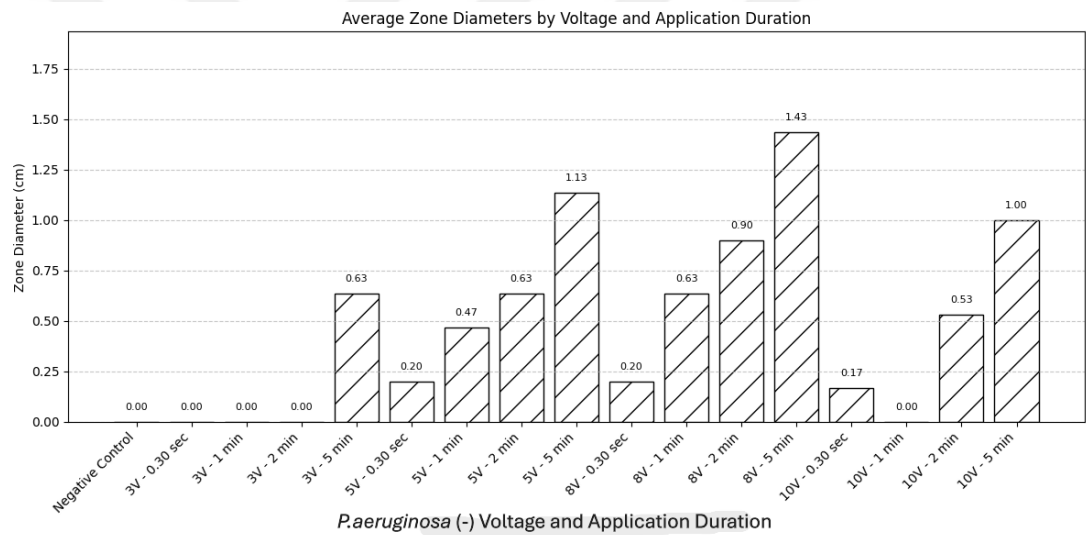


Figure 4.6: The zone diameters formed under different negative voltages and application durations of *P. aeruginosa* bacteria.

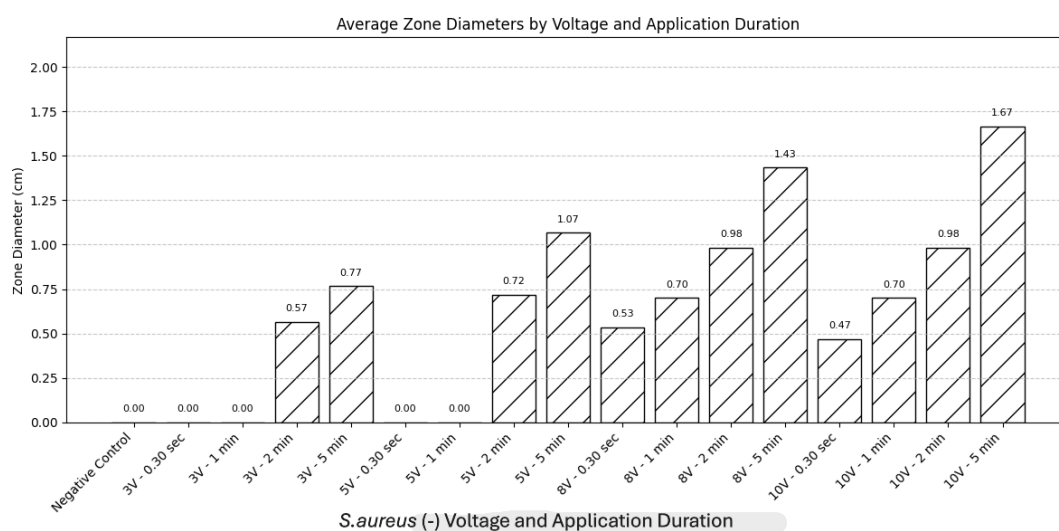


Figure 4.7: The zone diameters formed under different negative voltages and application durations of *S. aureus* bacteria.

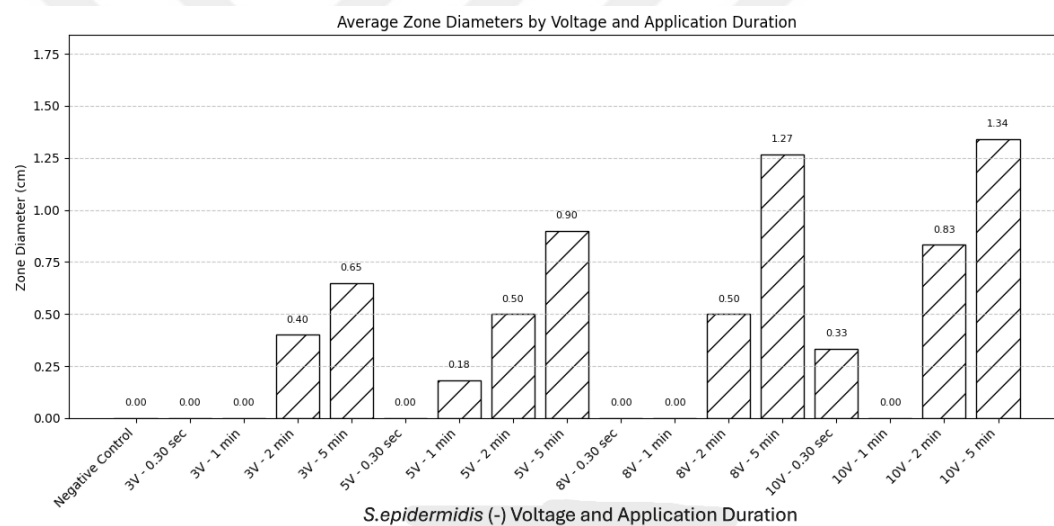


Figure 4.8: The zone diameters formed under different negative voltages and application durations of *S. epidermidis* bacteria.

The images of the inhibition zones at the anode and cathode during the application of 3V, 5V, 8V, and 10V electrical stimulation of bacterial strains for a duration of 30 seconds are provided below (**Figures 4.9-4.24**).

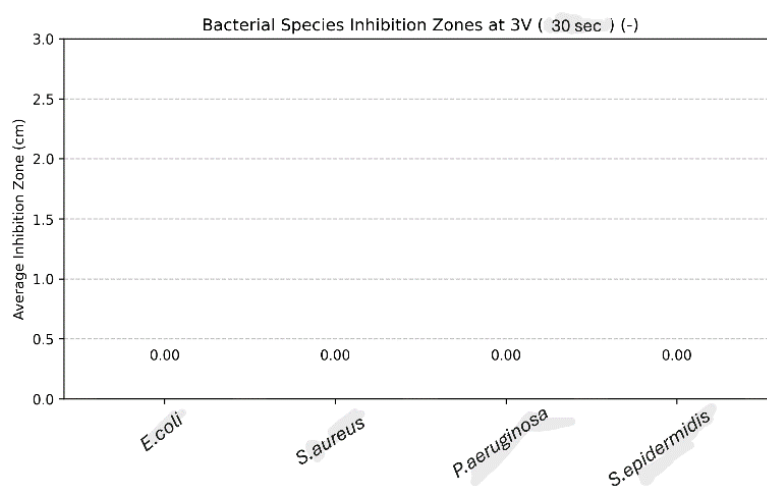


Figure 4.9: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 3 volts at the anode.

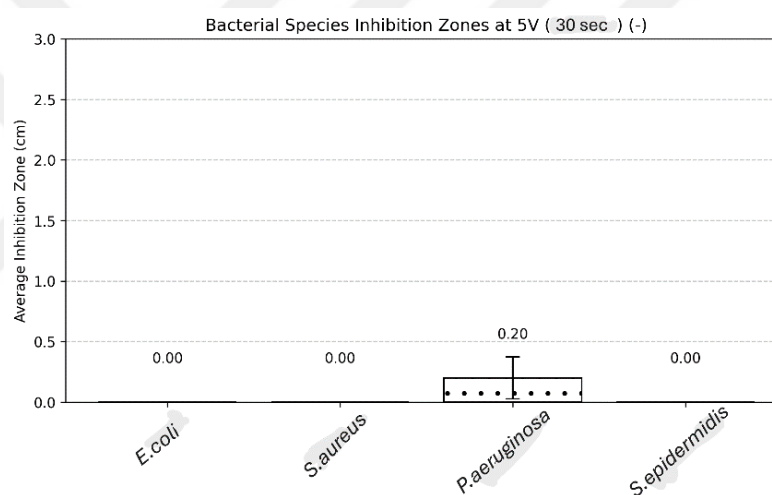


Figure 4.10: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 5 volts at the anode.

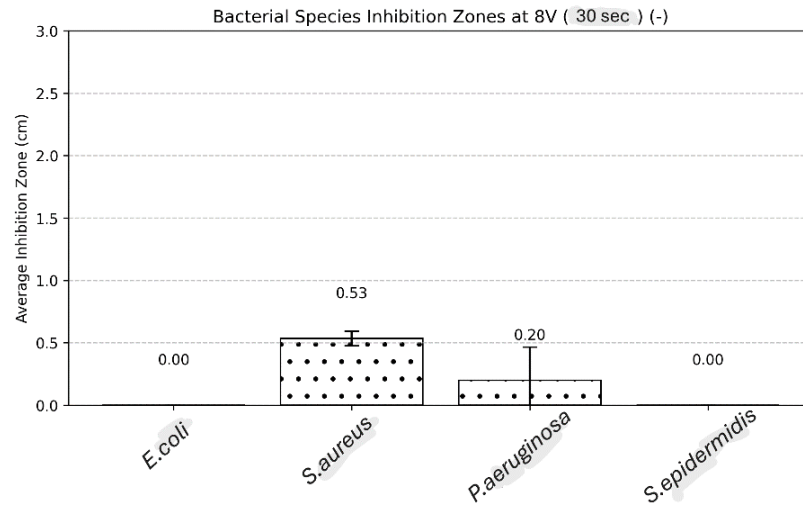


Figure 4.11: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 8 volts at the anode.

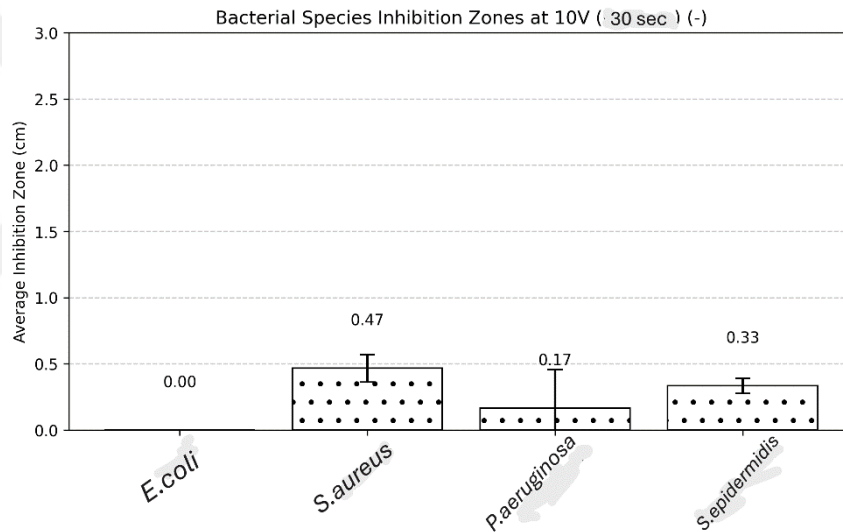


Figure 4.12: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 10 volts at the anode.

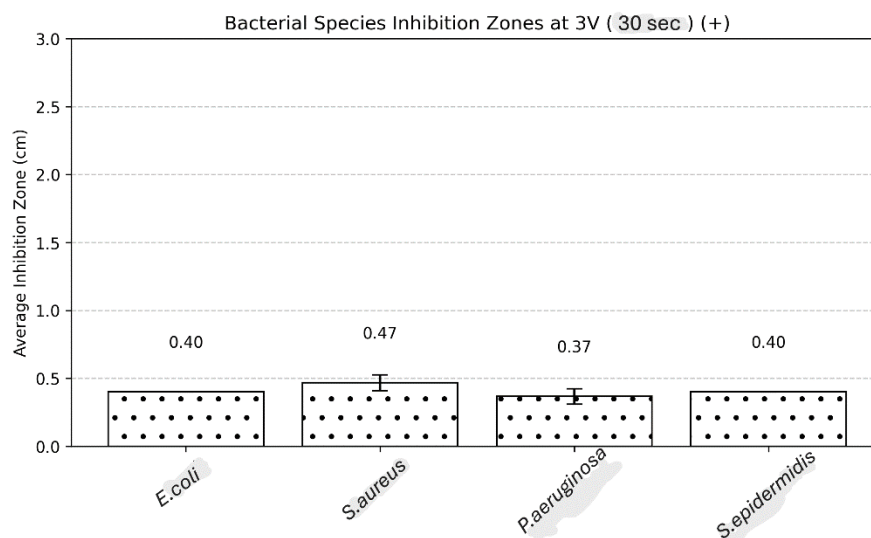


Figure 4.13: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 3 volts at the cathode.

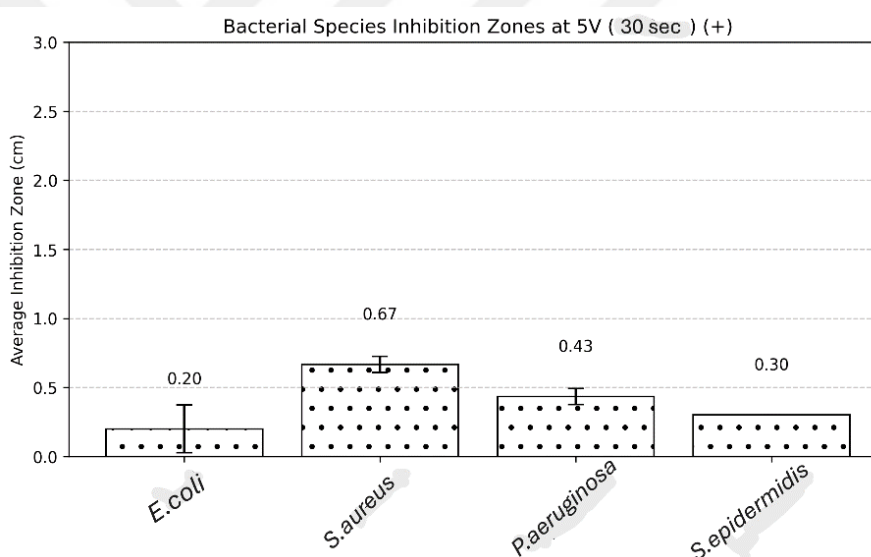


Figure 4.14: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 5 volts at the cathode.

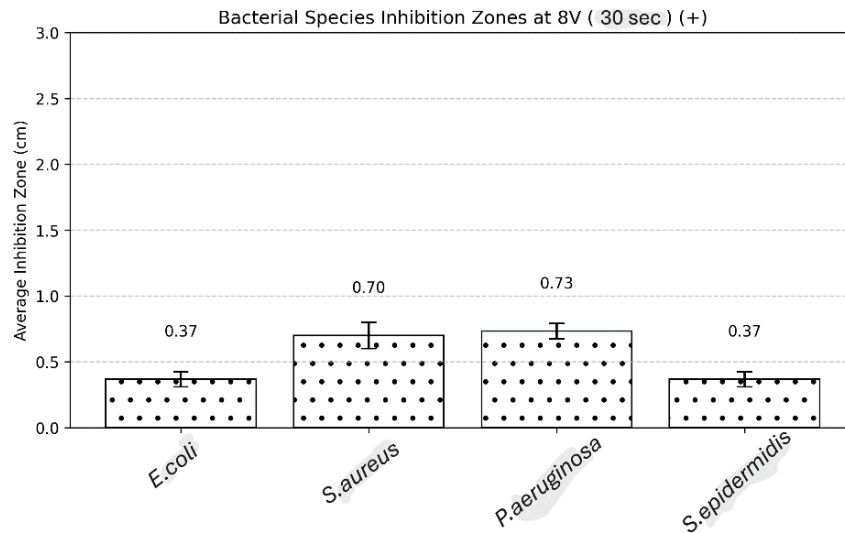


Figure 4.15: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 8 volts at the cathode.

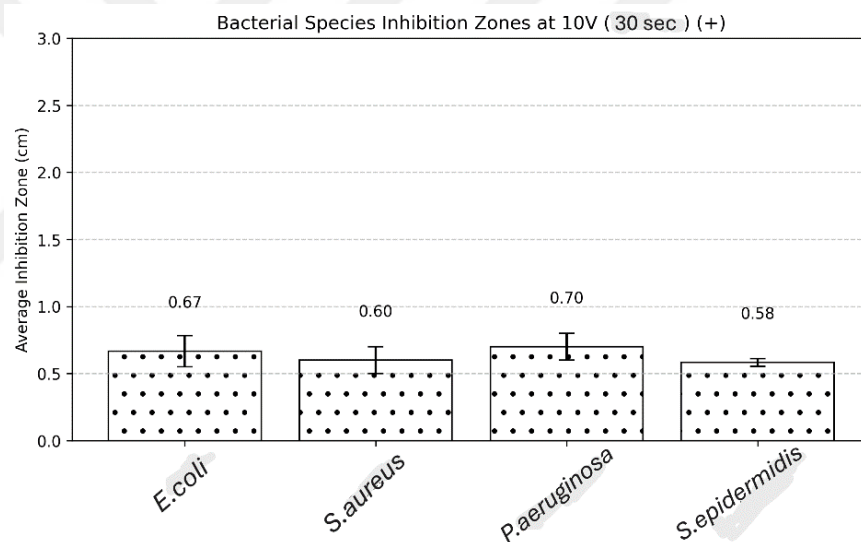


Figure 4.16: Inhibition zones created by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to 30 seconds of electrical stimulation at 10 volts at the cathode.

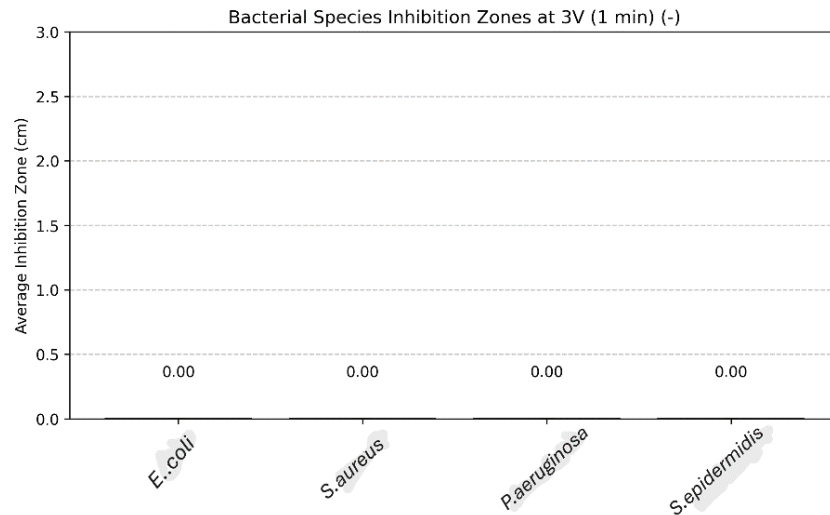


Figure 4.17: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 3 Volts of electrical stimulation for 1 minute.

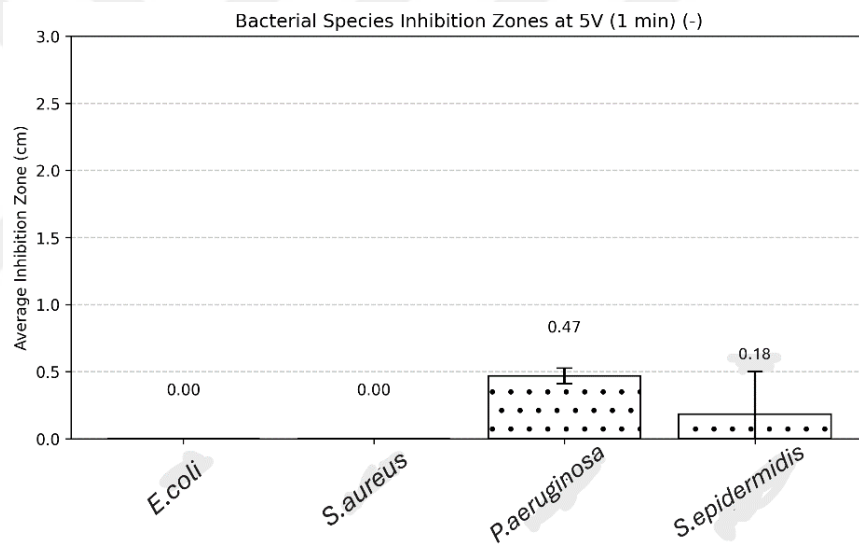


Figure 4.18: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 5 Volts of electrical stimulation for 1 minute.

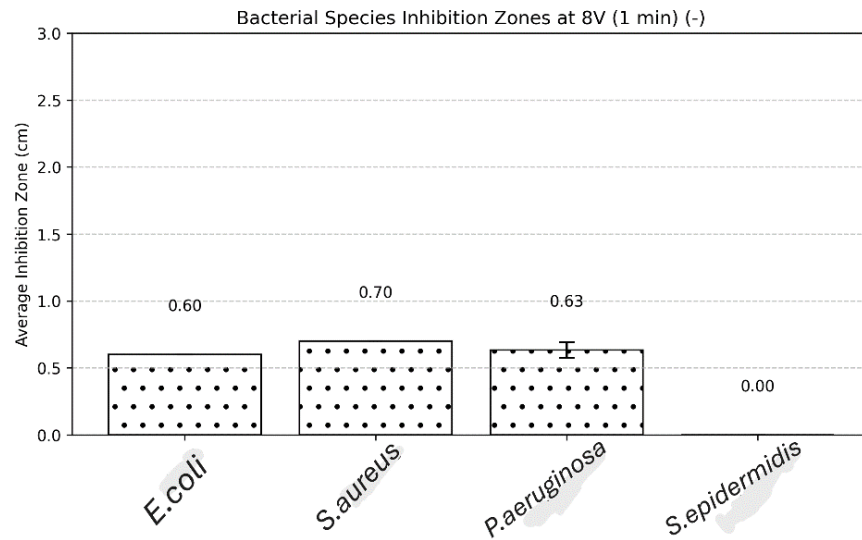


Figure 4.19: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 8 Volts of electrical stimulation for 1 minute.

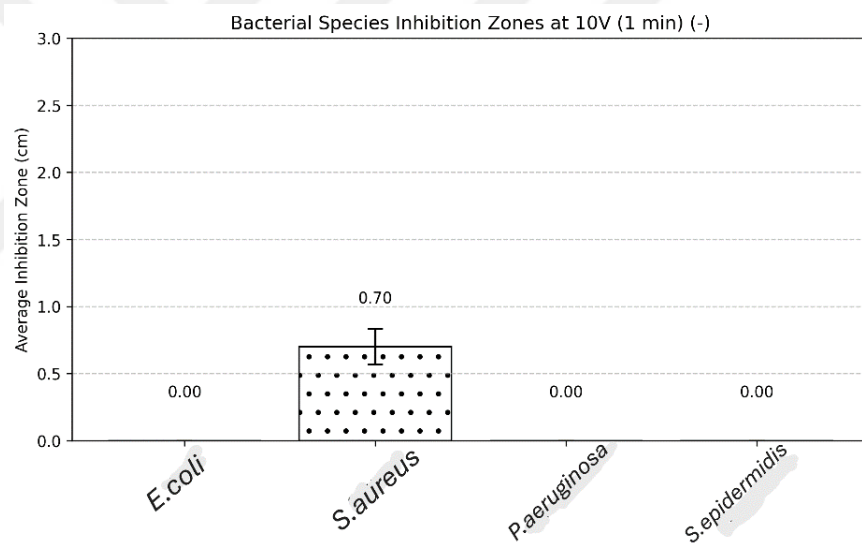


Figure 4.20: Inhibition zones formed by exposing *E.coli*, *S.aureus*, *P.aeruginosa* and *S.epidermidis* bacteria to negative 10 Volts of electrical stimulation for 1 minute.

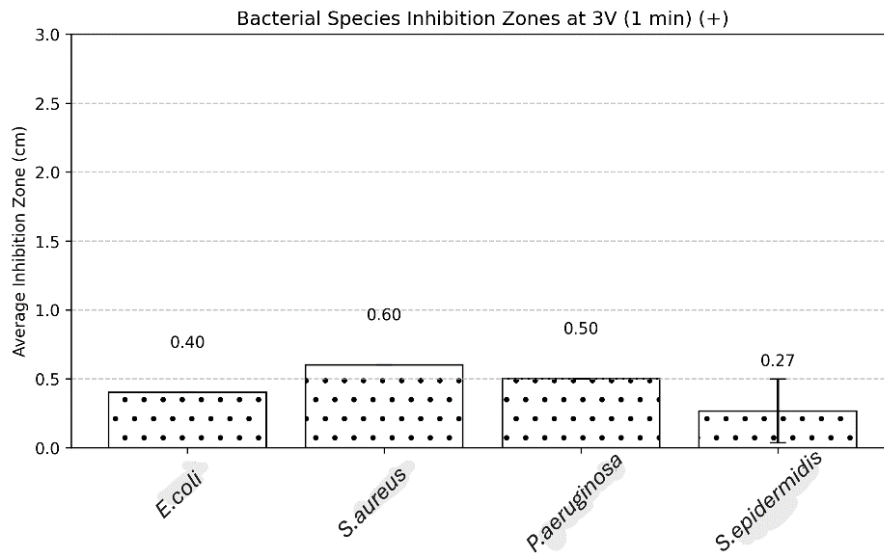


Figure 4.21: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 3 Volts of electrical stimulation for 1 minute.

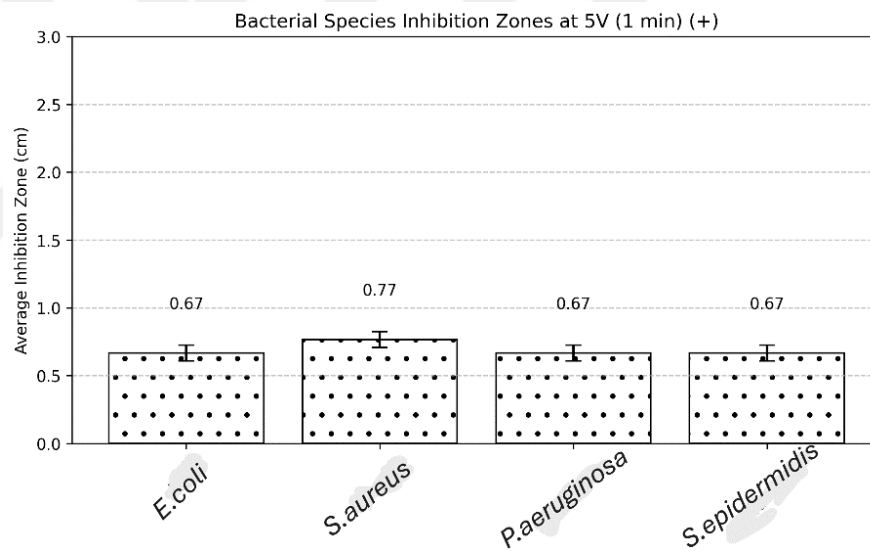


Figure 4.22: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 5 Volts of electrical stimulation for 1 minute.

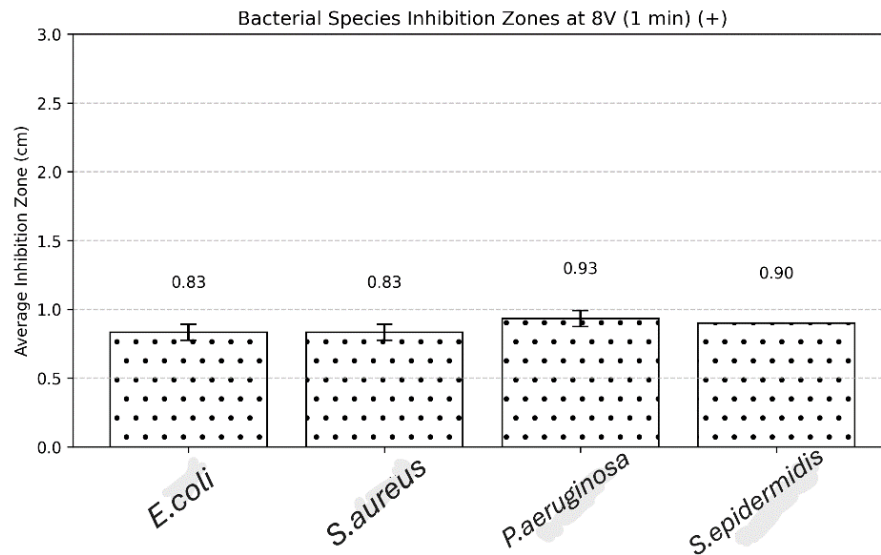


Figure 4.23: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 8 Volts of electrical stimulation for 1 minute.

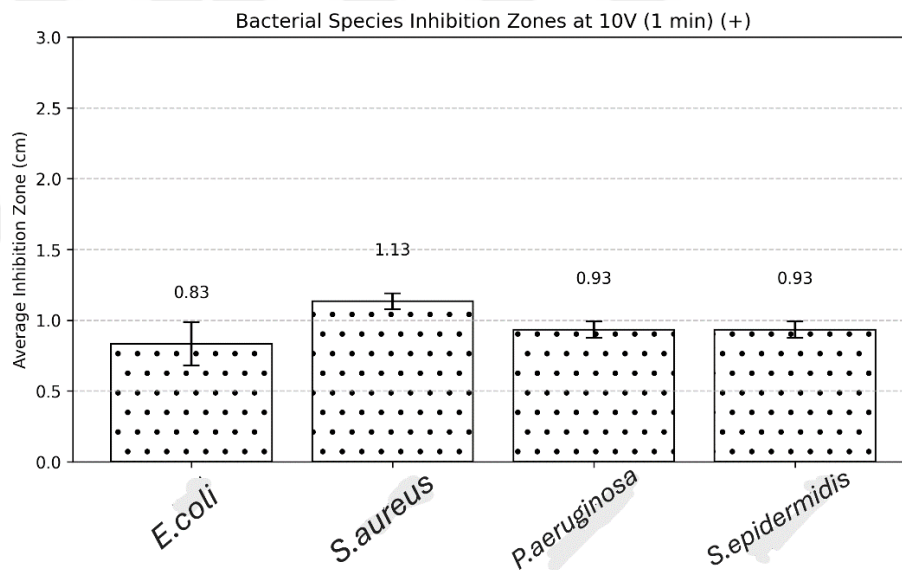


Figure 4.24: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 10 Volts of electrical stimulation for 1 minute.

The representation of the inhibition zones of bacterial strains exposed to the same voltage for the same durations is shown in the graphs below (**Figures 4.25-4.32**).

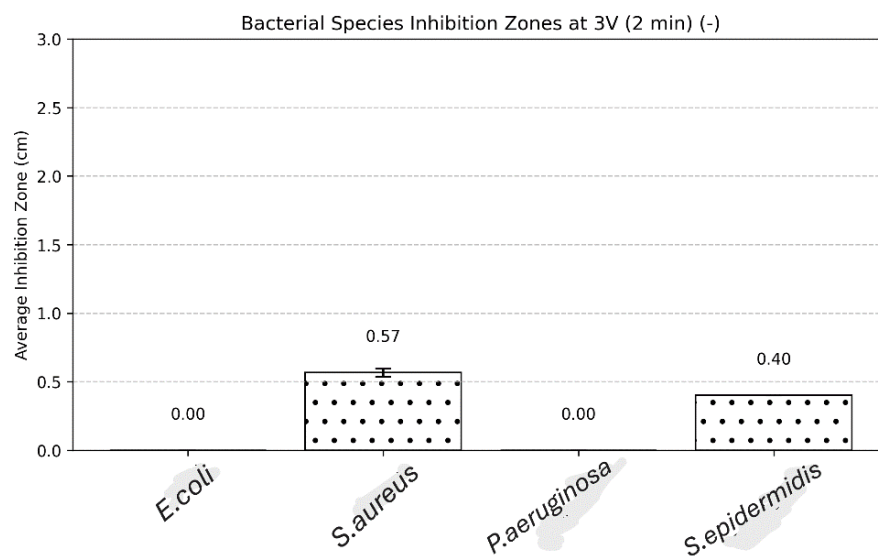


Figure 4.25: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 3 Volts of electrical stimulation for 2 minute.

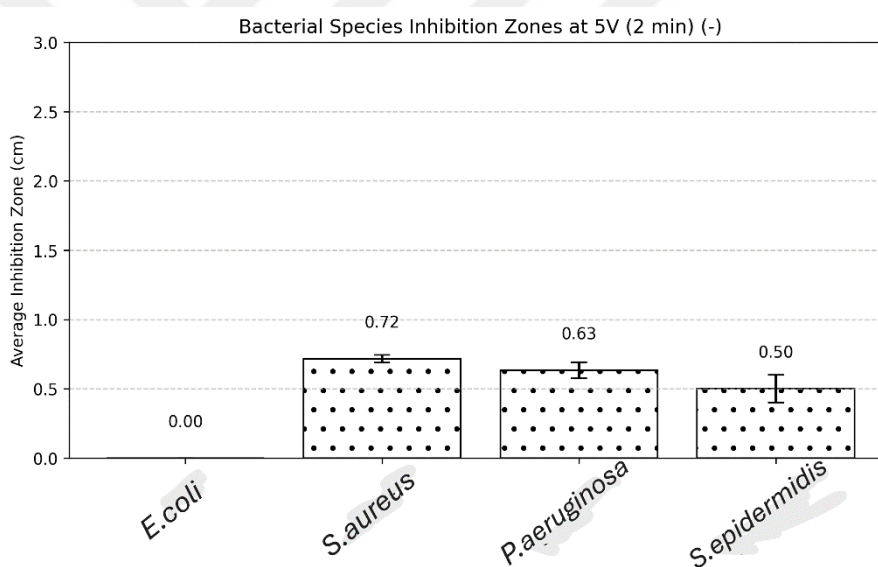


Figure 4.26: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 5 Volts of electrical stimulation for 2 minute.

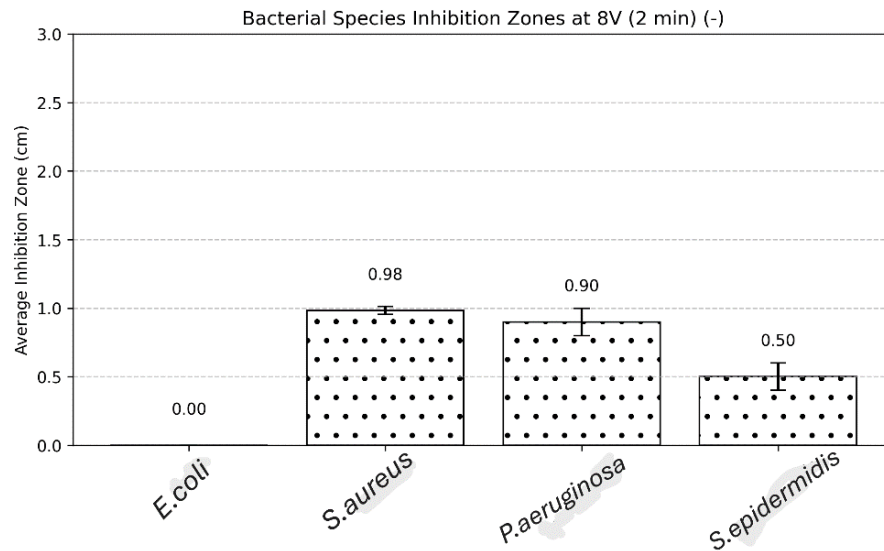


Figure 4.27: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 8 Volts of electrical stimulation for 2 minute.

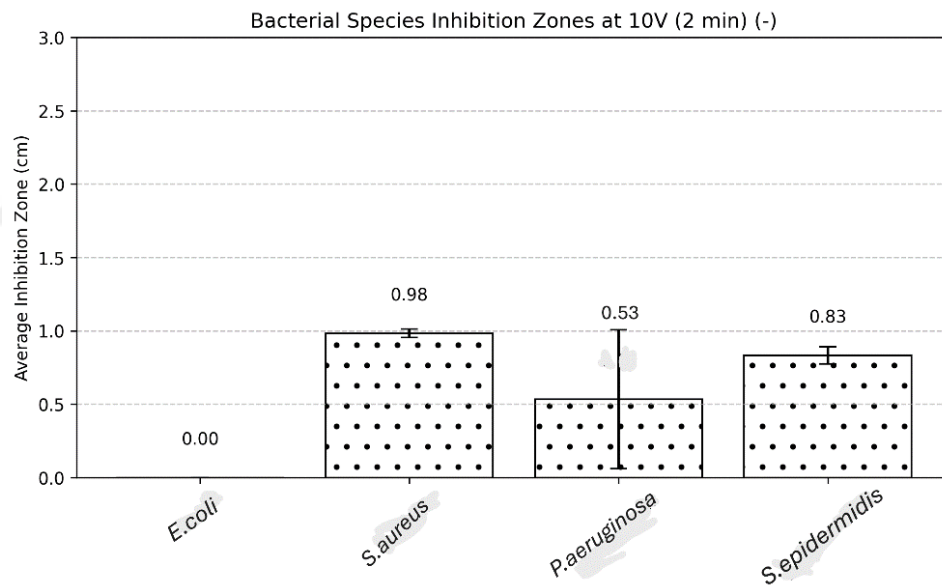


Figure 4.28: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 10 Volts of electrical stimulation for 2 minute.

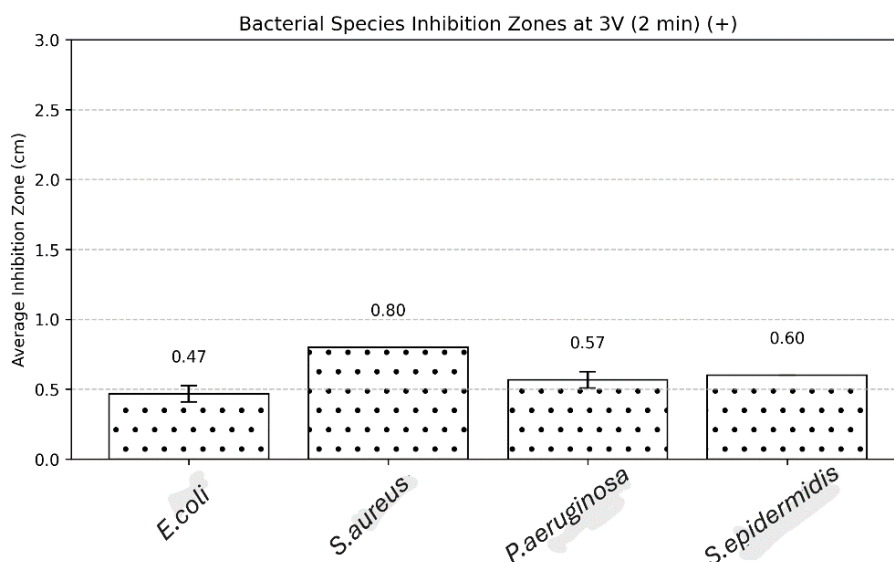


Figure 4.29: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 3 Volts of electrical stimulation for 2 minute.

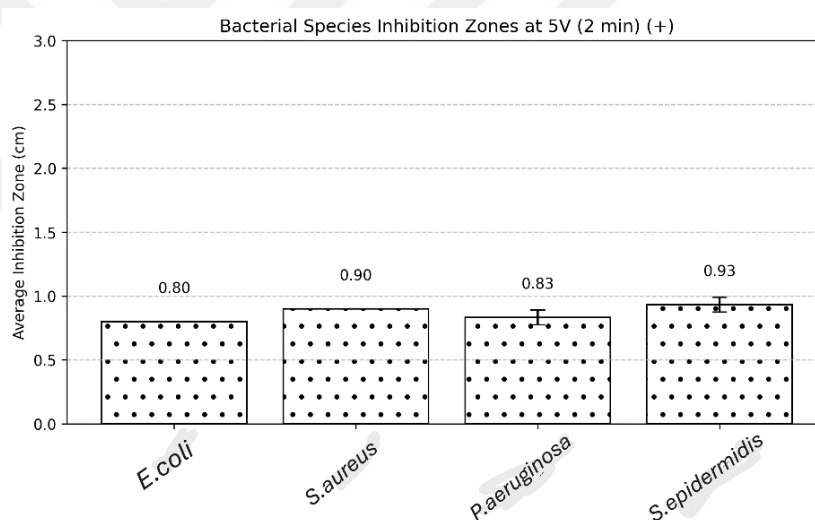


Figure 4.30: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 5 Volts of electrical stimulation for 2 minute.

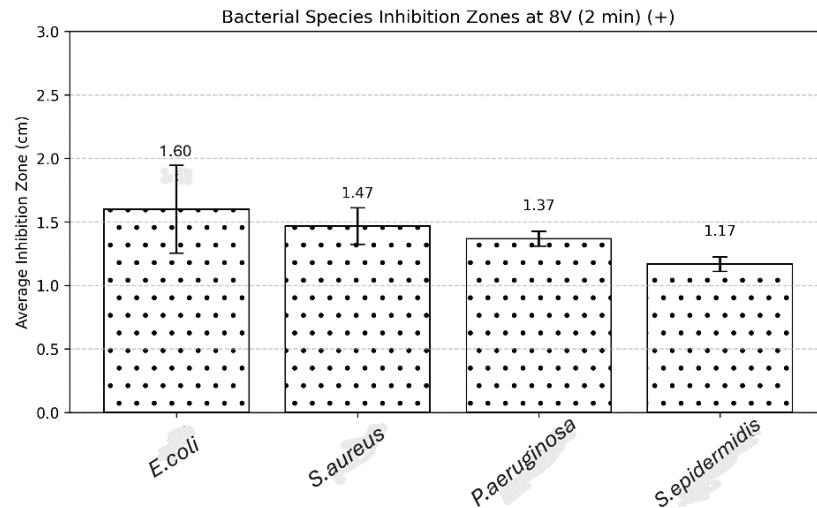


Figure 4.31: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 8 Volts of electrical stimulation for 2 minute.

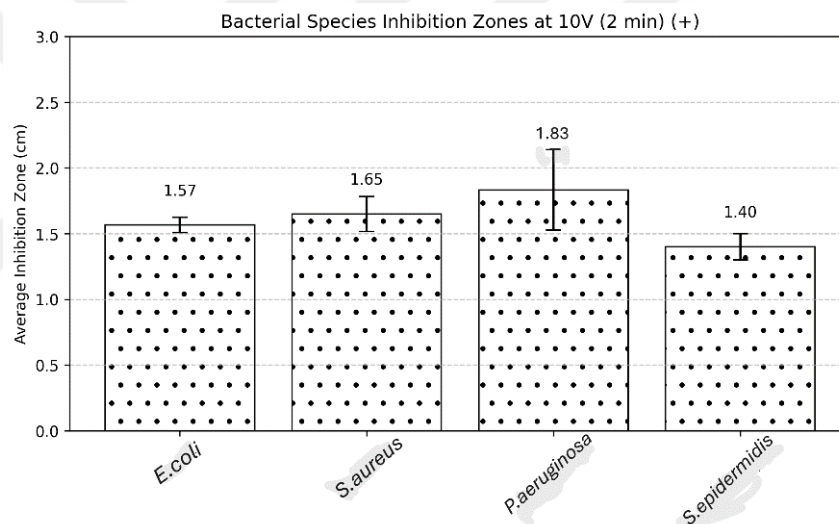


Figure 4.32: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 10 Volts of electrical stimulation for 2 minute.

The representation of the inhibition zones of bacterial strains exposed to the same voltage for the same durations is shown in the graphs below (**Figures 4.33-4.40**).

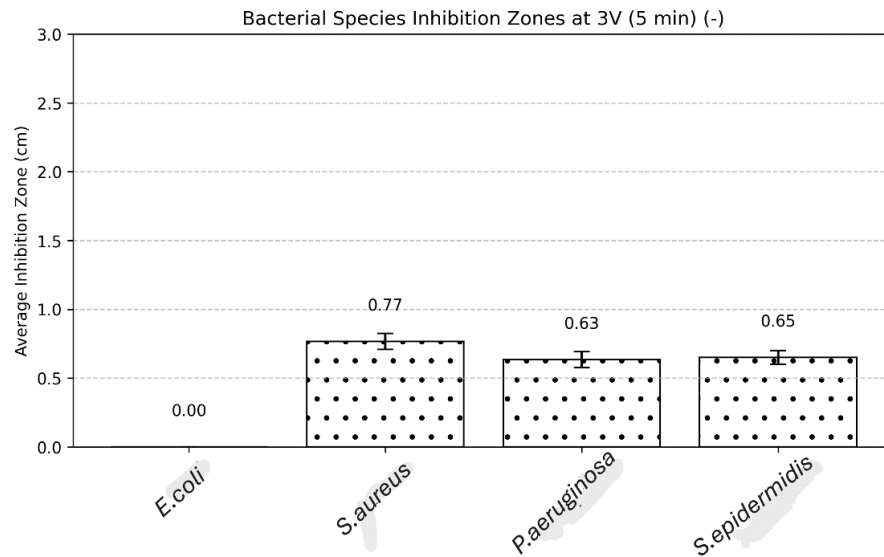


Figure 4.33: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 3 Volts of electrical stimulation for 5 minute.

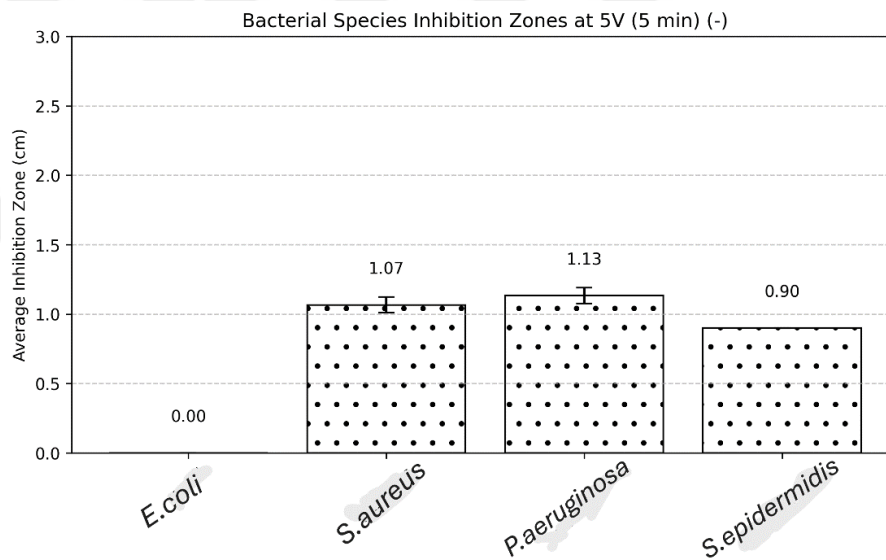


Figure 4.34: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 5 Volts of electrical stimulation for 5 minute.

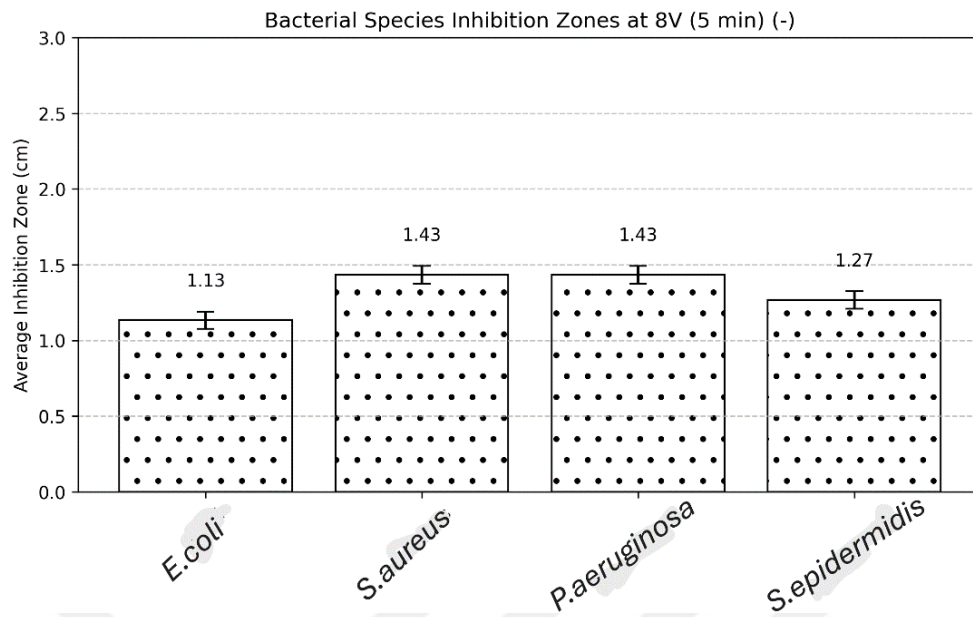


Figure 4.35: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 8 Volts of electrical stimulation for 5 minute.

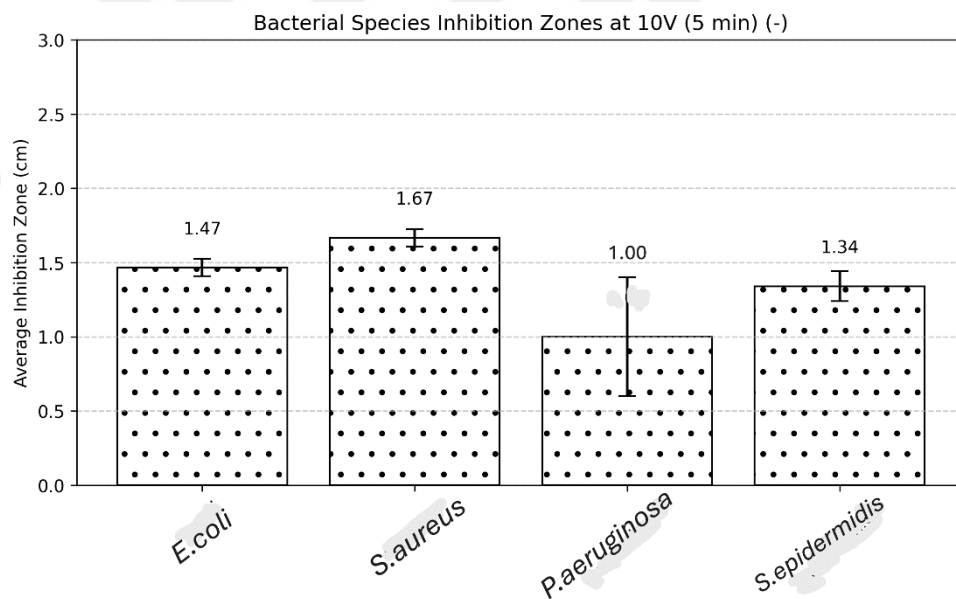


Figure 4.36: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to negative 10 Volts of electrical stimulation for 5 minute.

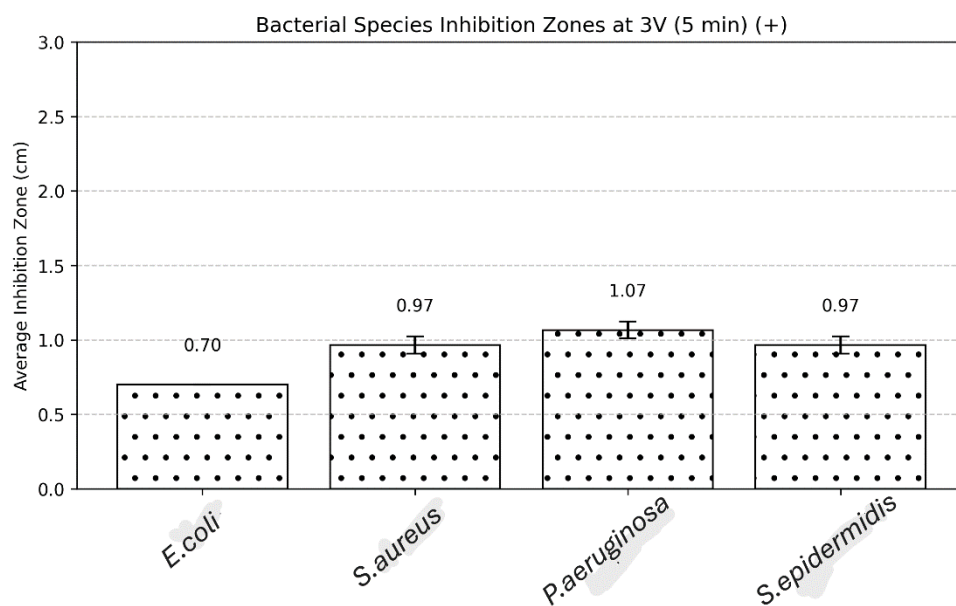


Figure 4.37: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 3 Volts of electrical stimulation for 5 minute.

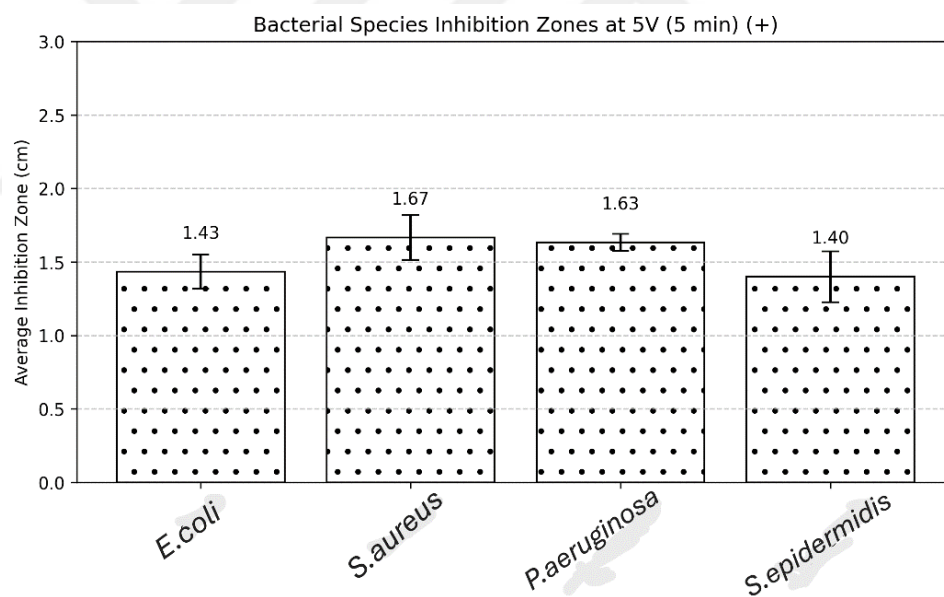


Figure 4.38: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 5 Volts of electrical stimulation for 5 minute.

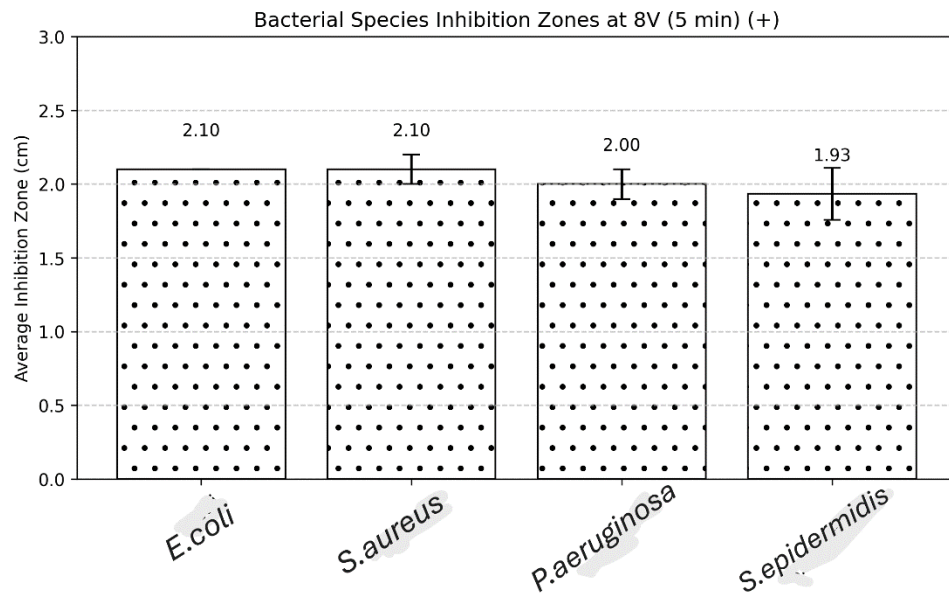


Figure 4.39: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 8 Volts of electrical stimulation for 5 minute.

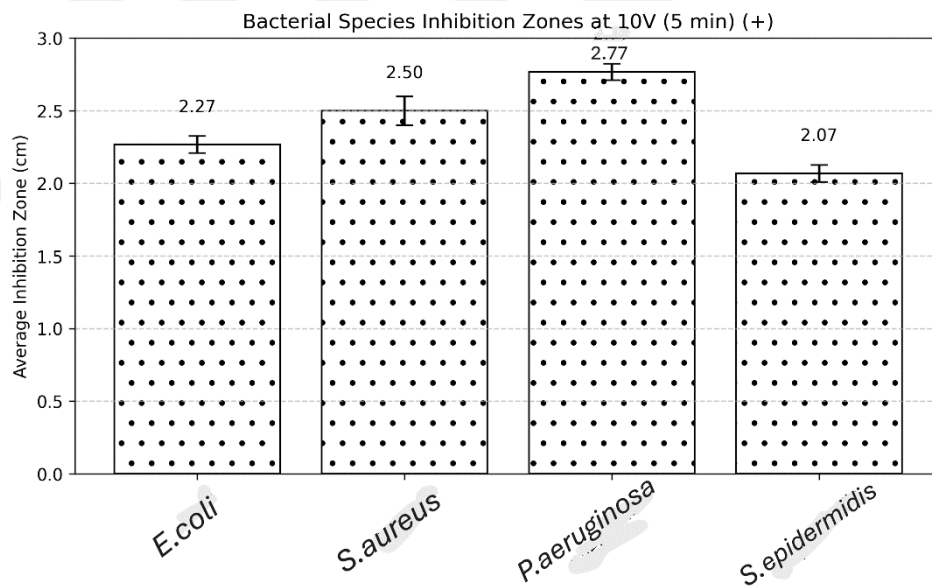


Figure 4.40: Inhibition zones formed by exposing *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. epidermidis* bacteria to positive 10 Volts of electrical stimulation for 5 minute.

The FTIR spectra of *E. coli* and *S. aureus* cells treated with the ES and the untreated control cells are given in **Figure 4.41** and **Figure 4.42**.

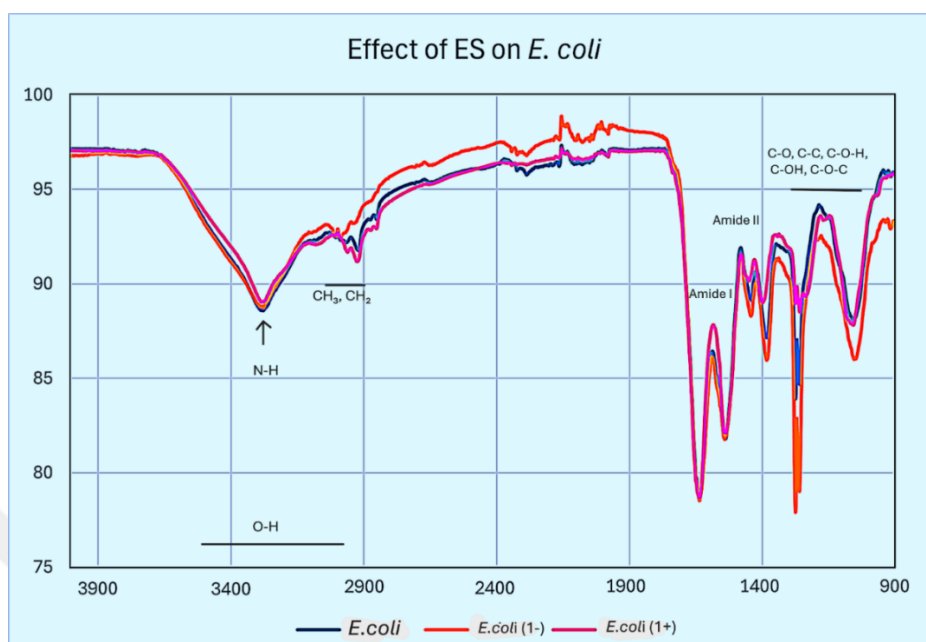


Figure 4.41: The FTIR spectra of the sample taken from the circumference of the zone formed in the culture of the *E. coli* bacteria exposed to electrical stimulation.

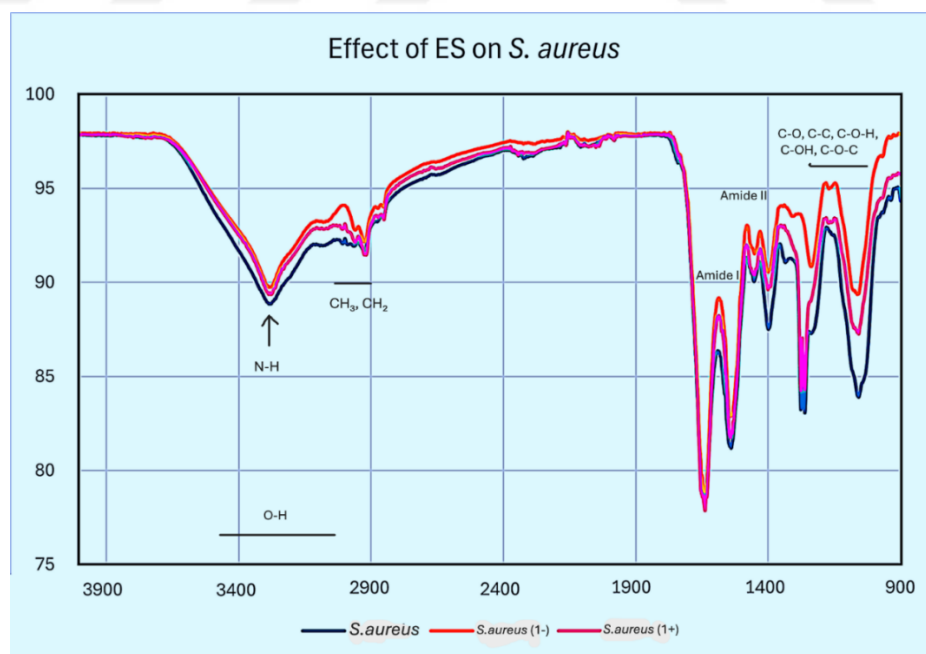


Figure 4.42: The FTIR spectra of the sample taken from the circumference of the zone formed in the culture of the *S. aureus* bacteria exposed to electrical stimulation.

5. CONCLUSION

Figure 4.1, created from the data presented in **Table 4.1**, demonstrates the effect of DC current on *E. coli* cells at the cathode, where a gradual increase in the zone diameter for each applied time except for the durations of 3V 30 seconds and 3V 1 minute during the application of electrical stimulation at voltages of 3, 5, 8, and 10 volts is observed. There is no significant difference observed between the application duration of 30 seconds at 3V and the 1-minute application for *E. coli* bacteria.

Figure 4.5, created from the data obtained from **Table 4.2**, demonstrates the effect of DC current on *E. coli* cells at the anode. It is observed that the DC current application conducted on *E. coli* bacteria yielded significant results only in 3 applications at the anodic end. In general, the effect of DC current on *E. coli* cells at the anode is less effective compared to the cathode. Moreover, some inconsistency exists in the effect observed for the anode.

Figure 4.2, created from the data obtained from **Table 4.3**, it can be seen that with the application of DC current to *P. aeruginosa* bacteria at different voltages and durations, the zone diameter increases proportionally with the increase in duration at each voltage at the cathodic end.

In **Figure 4.6**, created from data obtained from **Table 4.4**, there is no effect observed until the 5 minute of application of a 3V DC electric current to the *P. aeruginosa* bacteria at the anode. Significant increases are observed with 5V and 8V applications. However, the 30 second application of the 8V treatment shows high variability. A stable and consistent result has not been achieved. 10V application data also shows discrepancies as, there was no effect observed within the 1-minute application. However, during one of the three replicates of a 30-second duration application, there was a killing effect observed, while no effect was observed in the others. In the 2-minute applications of 10V, no effect was observed in one instance, while the values obtained in the 5-minute application are very distant from each other, showing high variability. Again, the effect of DC current on *P. aeruginosa* cells at the anode is less effective and inconsistent compared to the cathode.

In **Figure 4.3**, created from data obtained from **Table 4.5**, it is seen that the zone diameter increases in proportion to the duration increase at each voltage for the *S. aureus* bacteria subjected to DC electric current at the cathode.

In **Figure 4.7**, created from data obtained from **Table 4.6**, at 3V and 5V, there is no effect observed in 30 seconds and 1 minute for the DC electric current applied to the *S. aureus* bacteria at the anode. However, a gradual increase in zone diameter is observed with increasing duration for the remaining voltages. The inhibition zones increases gradually at both cathode and anode, but the effect of DC current on *P. aeruginosa* cells at the anode is less effective compared to the cathode.

In **Figure 4.4** created with the data obtained from **Table 4.7**, during the application of DC electric current to *S. epidermidis* bacterium at the cathode, an average zone diameter of 0.40 cm is observed with an application time of 30 seconds and a zone diameter of 0.27 cm is observed with a 1-minute application. Although there is a significant increase in duration and voltage at the cathode, a decrease is observed here. This may have occurred due to experimental error. Because while a zone diameter was obtained in two applications of 3 replicates, no effect was seen in one application.

In **Figure 4.8** created with the data obtained from **Table 4.8**, there is no effect observed at the anode during the application of DC electric current to *S. epidermidis* bacterium at 3V for 30 seconds and 1 minute. At 5V, no effect is observed at 30 seconds, while in a 1-minute application, no effect is seen in 2 of the 3 replicates, whereas a zone diameter of 0.55 cm was formed in the 3rd replicate. No effect was observed at 8V during 30 seconds and 1 minute. No effect was observed during a 1-minute application at 10V. However, distinct effects have been shown in shorter and longer duration applications. Once more, the effect of DC current on *P. aeruginosa* cells at the anode is less effective compared to the cathode and some inconsistency exists at the anode.

When the effects of the same duration and voltage on all bacteria are examined, the responses of the bacteria to electrical stimulation with respect to each other have been investigated. In **Figure 4.9**, during the 30-second application at 3V, no zone diameter was formed at the anode for any of the bacteria. In **Figure 4.10**, during the 30-second application at 5V, an average zone diameter of 0.20 cm was formed at the anode for *P. aeruginosa* bacteria (**Table 4.4**).

In **Figure 4.11**, during the 30-second application at 8V, a zone diameter of 0.53 cm was formed in *S. aureus* bacteria, while an average zone diameter of 0.20 cm was observed in *P. aeruginosa* bacteria. In **Figure 4.12**, during the 30-second application at 10V, a zone diameter of 0.47 cm was seen in *S. aureus* bacteria. In *S. epidermidis* bacteria, a zone diameter of 0.33 cm was formed. Since the obtained values are close to each other and consistent, this value can be considered significant. From **Figure 4.09** to **Figure 4.12**, despite the increase in voltage in *E. coli* bacteria, it has been observed that there was no effect at the anode during the 30-second application.

In **Figure 4.13**, during the application of DC current for 30 seconds at 3V, inhibition zones of 0.40 cm in diameter were observed in *E. coli* bacteria, 0.47 cm in *S. aureus* bacteria, 0.37 cm in *P. aeruginosa* bacteria, and 0.40 cm in *S. epidermidis* bacteria at the cathode. When the obtained values are compared with those in **Tables 1, 3, 5, and 7**, they are considered significant because they are close and consistent. Among these four bacteria, *S. aureus* showed the most inhibition, while the lowest inhibition amount was seen in *P. aeruginosa*.

In **Figure 4.14**, during the application of DC current for 30 seconds at 5V, a zone diameter of 0.3 cm was formed in 2 out of 3 replicates for *E. coli*, while no zone diameter was observed in 1 replicate. In *S. aureus* bacteria, an inhibition zone of 0.67 cm, 0.43 cm in *P. aeruginosa*, and 0.30 cm in *S. epidermidis* was observed. More inhibition was seen in *S. aureus*, while the lowest average inhibition was observed in *E. coli*.

In **Figure 4.15**, when the effects of the 30 seconds application at 8V on the bacteria were examined, inhibition diameters of 0.37 cm in *E. coli*, 0.70 cm in *S. aureus*, 0.73 cm in *P. aeruginosa*, and 0.37 cm in *S. epidermidis* were observed. In **Figure 4.16**, the effects of the 30 seconds application at 10V on the bacteria at the cathode were examined. Inhibition zone diameters of 0.67 cm in *E. coli*, 0.60 cm in *S. aureus*, 0.70 cm in *P. aeruginosa*, and 0.58 cm in *S. epidermidis* were formed.

In **Figure 4.17**, under the application of 3V for 1 minute, no zone diameter formation was observed at the anode with DC current for any bacteria.

In **Figure 4.18**, under the application of 5V for 1 minute, while no zone diameter was observed at the anode for *E. coli* and *S. aureus* bacteria, a zone of 0.47 cm in diameter was formed in *P. aeruginosa* and an average zone of 0.18 cm in *S. epidermidis* bacteria.

When examining the 3 replicates of *S. epidermidis* bacteria, it was noted that no zone formation was observed in 2 replicates, while 1 replicate showed a zone diameter of 0.55 cm. This resulted in a very high standard deviation.

In **Figure 4.19**, under the application of 8V for 1 minute, zone formations of 0.60 cm in *E. coli*, 0.70 cm in *S. aureus*, and 0.63 cm in *P. aeruginosa* were observed at the anode. The highest inhibition diameter was observed in *S. aureus*, while no zone formation was observed in *S. epidermidis*.

In **Figure 4.20**, under the application of 10V for 1 minute, zone diameter formation was only observed for *S. aureus* at the anode. While a zone diameter of 0.63 cm was observed in *P. aeruginosa* in **Figure 4.19**, the absence of zone diameter formation with increasing voltage indicates that an application above a certain amount does not have an effect.

In **Figure 4.21**, during the 3V 1-minute application at the cathode, an average zone formation with a diameter of 0.40 cm for *E. coli* bacteria, 0.60 cm for *S. aureus* bacteria, 0.50 cm for *P. aeruginosa* bacteria, and 0.27 cm for *S. epidermidis* bacteria is observed. Among these, in the case of *S. epidermidis* bacteria, a zone diameter of 0.4 cm is obtained in 2 out of 3 replicates, while no zone diameter is observed in the other replicate. Therefore, the standard deviation value is high.

In **Figure 4.22**, during the 5V 1-minute application at the cathode, zone diameters of 0.67 cm for *E. coli* bacteria, 0.77 cm for *S. aureus* bacteria, 0.67 cm for *P. aeruginosa* bacteria, and 0.67 cm for *S. epidermidis* bacteria are observed. Since the replica values obtained are close and consistent with each other, it is a significant result.

In **Figure 4.23**, during the 8V 1-minute application at the cathode, average zone diameters of 0.83 cm for *E. coli* bacteria, 0.83 cm for *S. aureus*, 0.93 cm for *P. aeruginosa*, and 0.90 cm for *S. epidermidis* are shown. When these values are compared, the highest inhibition is observed in *P. aeruginosa* bacteria. *E. coli* and *S. aureus* have the lowest and the same values among these measurements.

In **Figure 4.24**, during the 10V 1-minute application at the cathode, average zone diameters of 0.83 cm for *E. coli* bacteria, 1.13 cm for *S. aureus* bacteria, 0.93 cm for *P. aeruginosa*, and 0.93 cm for *S. epidermidis* bacteria are noted. The highest inhibition appears in *S. aureus* bacteria, while the lowest inhibition value is observed in *E. coli* bacteria.

In **Figure 4.25**, during the 3V 2-minute application, no zone formation is observed at the anode for *E. coli* and *P. aeruginosa* bacteria, while a zone of 0.57 cm in diameter is observed for *S. aureus* bacteria and a zone of 0.40 cm in diameter for *S. epidermidis* bacteria.

In **Figure 4.26**, during the 5V 2-minute application, there is no zone formation at the anode for *E. coli* bacteria. Zone formations of 0.72 cm for *S. aureus* bacteria, 0.63 cm for *P. aeruginosa* bacteria, and 0.50 cm for *S. epidermidis* bacteria are observed.

In **Figure 4.27**, during the 8V 2-minute application, there is no zone formation for *E. coli* bacteria at the anode, while zone formations of 0.98 cm for *S. aureus* bacteria, 0.90 cm for *P. aeruginosa* bacteria, and 0.50 cm for *S. epidermidis* bacteria are observed.

In **Figure 4.28**, during the 10V 2-minute application, no zone formation is noted for *E. coli* bacteria at the anode, while a zone formation of 0.98 cm is observed for *S. aureus* bacteria. When we look at *P. aeruginosa* bacteria, a high standard deviation is observed. This is because, in one of the three replicates, zone formation was seen, while in the 2nd and 3rd applications, the values were 0.7 cm and 0.9 cm respectively. The average zone diameter for *S. epidermidis* bacteria is 0.83 cm. The bacteria showing the highest inhibition diameter among them is *S. aureus*.

In **Figure 4.29**, during the 3V application for 2 minutes, a zone formation with a diameter of 0.47 cm was observed in *E. coli* bacteria, 0.80 cm in *S. aureus* bacteria, 0.57 cm in *P. aeruginosa* bacteria, and 0.60 cm in *S. epidermidis* bacteria at the cathode tip. The highest inhibition was seen in *S. aureus* bacteria, while the lowest inhibition was observed in *E. coli* bacteria.

In **Figure 4.30**, during the 5V application for 2 minutes, zone formation with a diameter of 0.80 cm was observed in *E. coli* bacteria, 0.90 cm in *S. aureus* bacteria, 0.83 cm in *P. aeruginosa* bacteria, and 0.93 cm in *S. epidermidis* bacteria at the cathode tip. The highest inhibition was observed in *S. epidermidis* bacteria, while the lowest inhibition was noted in *E. coli* bacteria.

In **Figure 4.31**, during the 8V application for 2 minutes, zone formation with a diameter of 1.60 cm was observed in *E. coli* bacteria, 1.47 cm in *S. aureus* bacteria, 1.37 cm in *P. aeruginosa* bacteria, and 1.17 cm in *S. epidermidis* bacteria at the cathode tip.

In **Figure 4.32**, during the 10V application for 2 minutes, zone formation with a diameter of 1.57 cm was observed in *E. coli* bacteria, 1.65 cm in *S. aureus* bacteria, 1.83 cm in *P. aeruginosa* bacteria, and 1.40 cm in *S. epidermidis* bacteria at the cathode tip. When the values in *E. coli* were examined in **Table 4.1**, it was seen that the zone diameter in the 8V 2-minute application was lower compared to the others. This resulted in a high standard deviation.

In **Figure 4.33**, no zone formation was observed at the anode for *E. coli* bacteria in the 3V 5-minute application. However, a zone diameter of 0.77 cm was seen for *S. aureus* bacteria, 0.63 cm for *P. aeruginosa* bacteria, and 0.65 cm for *S. epidermidis* bacteria. The highest inhibition was observed in *S. aureus* bacteria.

In **Figure 4.34**, no zone diameter formation was observed at the anode for *E. coli* bacteria in the 5V 5-minute application. The zone diameters observed were 1.07 cm for *S. aureus*, 1.13 cm for *P. aeruginosa*, and 0.90 cm for *S. epidermidis*. The highest inhibition among them was observed in *P. aeruginosa* bacteria. Despite the voltage increase in the previous **Figure 4.33**, the zone diameter for *S. aureus* bacteria decreased.

In **Figure 4.35**, an application of 8V for 5 minutes resulted in a zone diameter of 1.13 cm for *E. coli* bacteria at the cathode. The zone diameters observed were 1.43 cm for *S. aureus*, 1.43 cm for *P. aeruginosa*, and 1.27 cm for *S. epidermidis*.

In **Figure 4.36**, for the 10V 5-minute application, a zone diameter of 1.47 cm was observed for *E. coli* bacteria at the anode. The zone diameters observed were 1.67 cm for *S. aureus*, 1 cm for *P. aeruginosa*, and 1.34 cm for *S. epidermidis*. Considering the high standard deviation observed in *P. aeruginosa* bacteria, it is seen that the obtained values are not consistent.

In **Figure 4.37**, during a 5-minute application at 3V, a zone formation of 0.70 cm was observed for *E. coli* bacteria, 0.97 cm for *S. aureus* bacteria, 1.07 cm for *P. aeruginosa* bacteria, and 0.97 cm for *S. epidermidis* bacteria at the cathode.

In **Figure 4.38**, at 5V for a 5-minute application, the zone formations at the cathode were 1.43 cm for *E. coli* bacteria, 1.67 cm for *S. aureus* bacteria, 1.63 cm for *P. aeruginosa* bacteria, and 1.40 cm for *S. epidermidis* bacteria. The highest inhibition was observed for *S. aureus* bacteria while the lowest inhibition was seen for *S. epidermidis* bacteria.

In **Figure 4.39**, during an 8V 5-minute application, the zone diameters at the cathode were 2.10 cm for *E. coli* bacteria, 2.10 cm for *S. aureus* bacteria, 2 cm for *P. aeruginosa* bacteria, and 1.93 cm for *S. epidermidis* bacteria. The highest inhibition was found in *E. coli* and *S. aureus* bacteria, while the lowest inhibition was in *S. epidermidis* bacteria.

In **Figure 4.40**, during a 10V 5-minute application, zone formation at the cathode showed 2.27 cm for *E. coli* bacteria, 2.50 cm for *S. aureus* bacteria, 2.77 cm for *P. aeruginosa* bacteria, and 2.07 cm for *S. epidermidis* bacteria. The largest zone diameter was seen in *P. aeruginosa* bacteria, whereas the smallest zone diameter was seen in *S. epidermidis* bacteria.

When the graphs are analyzed, it is observed that bacteria exposed to the same duration and voltage do not show significant differences among themselves. We cannot say that *S. aureus* bacteria consistently developed a larger zone diameter or that *E. coli* did not create a zone diameter. Although these values were obtained at certain durations and voltages, this situation is not constant.

Gram-positive bacteria lack regular outer membranes and instead have thickened peptidoglycan cell walls. The structure of the cell wall contains two types of extra cell wall glycopolymers (CWG). Extra cell wall glycopolymers differ in terms of sugar building blocks and non-carbohydrate residues. According to net charge, CWGs can be classified as zwitterionic, anionic, and neutral polymers. Classical wall teichoic acids or lipoteichoic acids are zwitterionic due to negatively charged phosphates and positively charged amino groups [140]. Due to the negative charge it harbors, it can be seen as the reason for the inhibition of bacterial growth at the cathode rather than the anode during applied electrical stimulation. Similarly, the study conducted by Raetz and colleagues proved that the phosphoryl groups found in the lipopolysaccharide region of Gram-negative bacteria render them negatively charged. This explains the reason for the more meaningful and distinctive bactericidal effect of the cathode application on Gram-negative bacteria [141].

FTIR spectroscopy is sensitive not only to the molecular structures of functional groups and 'scaffolds' in biomolecules, but also to intermolecular interactions (even weak ones) that are known to play a vital role in biological processes. This thesis reports the FTIR spectroscopic analyses of *E. coli* and *S. aureus* bacteria subjected to

5V DC current for 5 minutes, comparing them with *E. coli* and *S. aureus* bacteria that were not exposed to electrical application. In **Figure 4.41** and **Figure 4.42**, the Amide I band ($\sim 1645\text{--}1655\text{ cm}^{-1}$, peptide bonds in proteins) was used as a normalization standard for the FTIR spectra. Changes in the fingerprint region in the range of $500\text{--}1000\text{ cm}^{-1}$ have been observed when applying cathode treatment to *E. coli* bacteria. In *S. aureus* bacteria, both negative and positive applications are more pronounced compared to the cases without electrical application. In **Figure 4.41**, when looking at the range of $1485\text{--}1000\text{ cm}^{-1}$ (C–O, C–C, C–O–H, C–O–C in polysaccharides and polyesters), it has been observed that there is a difference in the peak formed as a result of positive electricity exposure in *E. coli* bacteria. When examining the range of $3700\text{--}2700\text{ cm}^{-1}$ (the region of stretching vibrations of O–H and N–H groups), differences are also observed. In the CH_3 , CH_2 region, at a wavelength of 2960 cm^{-1} , both *E. coli* and *E. coli* (1+) bacteria subjected to positive electricity show a distinct peak, while the non-dominant peak of *E. coli* (1-) showing a negative peak suggests a possible formation of a new bond. The image may have arisen due to reactions involving CH_3 groups, with new groups being added to the structure, replacing CH_3 with other groups. The changes in the CH_2 , CH_3 groups of the fatty acid groups of the bacterial cell membrane may result in changes in the fluidity of the membrane and deterioration leading to leakage of vital ions and small molecules. In **Figure 4.42**, the same differences are also observed in *S. aureus* bacteria. In *S. aureus* bacteria, the difference in the range of $1485\text{--}1000\text{ cm}^{-1}$ is actually seen as a second peak formation in the ones subjected to electricity compared to the untreated bacteria. This may indicate the formation of new bonds.

Overall, it can be concluded from the FTIR data that the electrical stimulation affect the biochemical composition of both *E. coli* and *S. aureus* bacteria cells, at both the cathode and the anode. Moreover, changes in the fingerprint region and the CH_3 , CH_2 regions suggest further examination of the effect of electrical stimulation on the bacterial cell wall and cell membrane.

REFERENCES

- [1]. Liu, Y., Pharr, M., & Salvatore, G. A. (2017). Lab-on-Skin: A Review of Flexible and Stretchable Electronics for Wearable Health Monitoring. *ACS nano*, 11(10), 9614–9635. <https://doi.org/10.1021/acsnano.7b04898>
- [2]. Al-Khafaji, Z., Brito, S., & Bin, B. H. (2022). Zinc and Zinc Transporters in Dermatology. *International journal of molecular sciences*, 23(24), 16165. <https://doi.org/10.3390/ijms232416165>
- [3]. Jablonski, N. G. (2008). *Skin: A natural history*. University of California Press.
- [4]. Holick M. F. (2008). Sunlight, UV-radiation, vitamin D and skin cancer: how much sunlight do we need?. *Advances in experimental medicine and biology*, 624, 1–15. https://doi.org/10.1007/978-0-387-77574-6_1
- [5]. Brito, S., Baek, M., & Bin, B.-H. (2024). Skin Structure, Physiology, and Pathology in Topical and Transdermal Drug Delivery. *Pharmaceutics*, 16(11), 1403. <https://doi.org/10.3390/pharmaceutics16111403>
- [6]. Souto, E. B., Fangueiro, J. F., Fernandes, A. R., Cano, A., Sanchez-Lopez, E., Garcia, M. L., Severino, P., Paganelli, M. O., Chaud, M. V., & Silva, A. M. (2022). Physicochemical and biopharmaceutical aspects influencing skin permeation and role of SLN and NLC for skin drug delivery. *Heliyon*, 8(2), e08938. <https://doi.org/10.1016/j.heliyon.2022.e08938>
- [7]. Lai-Cheong, J. E., & McGrath, J. A. (2021). Structure and function of skin, hair and nails. *Medicine*, 49(5), 337–342. <https://doi.org/10.1016/j.mpmed.2021.02.010>
- [8]. Briggaman R. A. (1982). Biochemical composition of the epidermal-dermal junction and other basement membrane. *The Journal of investigative dermatology*, 78(1), 1–6. <https://doi.org/10.1111/1523-1747.ep12497841>
- [9]. Lopez-Ojeda W., Pandey A., Alhadj M., Oakley A.M., *Anatomy, Skin (Integument)*, StatPearls Publishing; Treasure Island, FL, USA: 2021.
- [10]. Wong, R., Geyer, S., Weninger, W., Guimberteau, J. C., & Wong, J. K. (2016). The dynamic anatomy and patterning of skin. *Experimental dermatology*, 25(2), 92–98. <https://doi.org/10.1111/exd.12832>
- [11]. Rivera-Gonzalez, G., Shook, B., & Horsley, V. (2014). Adipocytes in skin health and disease. *Cold Spring Harbor perspectives in medicine*, 4(3), a015271. <https://doi.org/10.1101/cshperspect.a015271>
- [12]. Frank, A. P., de Souza Santos, R., Palmer, B. F., & Clegg, D. J. (2019). Determinants of body fat distribution in humans may provide insight about obesity-

related health risks. *Journal of lipid research*, 60(10), 1710–1719. <https://doi.org/10.1194/jlr.R086975>

[13]. Lee, M. J., & Fried, S. K. (2017). Sex-dependent Depot Differences in Adipose Tissue Development and Function; Role of Sex Steroids. *Journal of obesity & metabolic syndrome*, 26(3), 172–180. <https://doi.org/10.7570/jomes.2017.26.3.172>

[14]. Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A. E., Cushman, S. W., & Periwai, V. (2009). Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. *PLoS computational biology*, 5(3), e1000324. <https://doi.org/10.1371/journal.pcbi.1000324>

[15]. Williams, R., Pawlus, A. D., & Thornton, M. J. (2020). Getting under the skin of hair aging: the impact of the hair follicle environment. *Experimental dermatology*, 29(7), 588–597. <https://doi.org/10.1111/exd.14109>

[16]. Tortora, G. J., & Derrickson, B. H. (2016). *Principles of anatomy and physiology* (15th ed.). Wiley.

[17]. Robson, M. C., Steed, D. L., & Franz, M. G. (2001). Wound healing: biologic features and approaches to maximize healing trajectories. *Current problems in surgery*, 38(2), 72–140. <https://doi.org/10.1067/msg.2001.111167>

[18]. Velnar, T., Bailey, T., & Smrkolj, V. (2009). The wound healing process: an overview of the cellular and molecular mechanisms. *The Journal of international medical research*, 37(5), 1528–1542. <https://doi.org/10.1177/147323000903700531>

[19]. Martin P. (1997). Wound healing--aiming for perfect skin regeneration. *Science (New York, N.Y.)*, 276(5309), 75–81. <https://doi.org/10.1126/science.276.5309.75>

[20]. Goffin, J. M., Pittet, P., Csucs, G., Lussi, J. W., Meister, J. J., & Hinz, B. (2006). Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. *The Journal of cell biology*, 172(2), 259–268. <https://doi.org/10.1083/jcb.200506179>

[21]. Park, J. E., & Barbul, A. (2004). Understanding the role of immune regulation in wound healing. *American journal of surgery*, 187(5A), 11S–16S. [https://doi.org/10.1016/S0002-9610\(03\)00296-4](https://doi.org/10.1016/S0002-9610(03)00296-4)

[22]. Peña, O. A., & Martin, P. (2024). Cellular and molecular mechanisms of skin wound healing. *Nature reviews. Molecular cell biology*, 25(8), 599–616. <https://doi.org/10.1038/s41580-024-00715-1>

[23]. de Bont, C. M., Boelens, W. C., & Pruijn, G. J. M. (2019). NETosis, complement, and coagulation: a triangular relationship. *Cellular & molecular immunology*, 16(1), 19–27. <https://doi.org/10.1038/s41423-018-0024-0>

[24]. Berntorp, E., Fischer, K., Hart, D. P., Mancuso, M. E., Stephensen, D., Shapiro, A. D., & Blanchette, V. (2021). Haemophilia. *Nature reviews. Disease primers*, 7(1), 45. <https://doi.org/10.1038/s41572-021-00278-x>

- [25]. Peyvandi, F., Bolton-Maggs, P. H., Batorova, A., & De Moerloose, P. (2012). Rare bleeding disorders. *Haemophilia : the official journal of the World Federation of Hemophilia*, 18 Suppl 4, 148–153. <https://doi.org/10.1111/j.1365-2516.2012.02841.x>
- [26]. Golebiewska, E. M., & Poole, A. W. (2015). Platelet secretion: From haemostasis to wound healing and beyond. *Blood reviews*, 29(3), 153–162. <https://doi.org/10.1016/j.blre.2014.10.003>
- [27]. Gaertner, F., & Massberg, S. (2016). Blood coagulation in immunothrombosis-At the frontline of intravascular immunity. *Seminars in immunology*, 28(6), 561–569. <https://doi.org/10.1016/j.smim.2016.10.010>
- [28]. Burzynski, L. C., Humphry, M., Pyrillou, K., Wiggins, K. A., Chan, J. N. E., Figg, N., Kitt, L. L., Summers, C., Tatham, K. C., Martin, P. B., Bennett, M. R., & Clarke, M. C. H. (2019). The Coagulation and Immune Systems Are Directly Linked through the Activation of Interleukin-1 α by Thrombin. *Immunity*, 50(4), 1033–1042.e6. <https://doi.org/10.1016/j.immuni.2019.03.003>
- [29]. Mamun, A. A., Shao, C., Geng, P., Wang, S., & Xiao, J. (2024). Recent advances in molecular mechanisms of skin wound healing and its treatments. *Frontiers in immunology*, 15, 1395479. <https://doi.org/10.3389/fimmu.2024.1395479>
- [30]. Jorch, S. K., & Kubes, P. (2017). An emerging role for neutrophil extracellular traps in noninfectious disease. *Nature medicine*, 23(3), 279–287. <https://doi.org/10.1038/nm.4294>
- [31]. Yipp, B. G., & Kubes, P. (2013). NETosis: how vital is it?. *Blood*, 122(16), 2784–2794. <https://doi.org/10.1182/blood-2013-04-457671>
- [32]. Dovi, J. V., Szpaderska, A. M., & DiPietro, L. A. (2004). Neutrophil function in the healing wound: adding insult to injury?. *Thrombosis and haemostasis*, 92(2), 275–280. <https://doi.org/10.1160/TH03-11-0720>
- [33]. Martin, P., D'Souza, D., Martin, J., Grose, R., Cooper, L., Maki, R., & McKercher, S. R. (2003). Wound healing in the PU.1 null mouse--tissue repair is not dependent on inflammatory cells. *Current biology : CB*, 13(13), 1122–1128. [https://doi.org/10.1016/s0960-9822\(03\)00396-8](https://doi.org/10.1016/s0960-9822(03)00396-8)
- [34]. Singer, A. J., & Clark, R. A. (1999). Cutaneous wound healing. *The New England journal of medicine*, 341(10), 738–746. <https://doi.org/10.1056/NEJM199909023411006>
- [35]. Petreaca, M. L., Yao, M., Liu, Y., Defea, K., & Martins-Green, M. (2007). Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability. *Molecular biology of the cell*, 18(12), 5014–5023. <https://doi.org/10.1091/mbc.e07-01-0004>
- [36]. Schraufstatter, I. U., Chung, J., & Burger, M. (2001). IL-8 activates endothelial cell CXCR1 and CXCR2 through Rho and Rac signaling pathways. *American journal of physiology. Lung cellular and molecular physiology*, 280(6), L1094–L1103. <https://doi.org/10.1152/ajplung.2001.280.6.L1094>

- [37]. Gillitzer, R., & Goebeler, M. (2001). Chemokines in cutaneous wound healing. *Journal of Leukocyte Biology*, 69(4), 513–521. <https://doi.org/10.1189/jlb.69.4.513>
- [38]. Jakubzick, C., Gautier, E. L., Gibbings, S. L., Sojka, D. K., Schlitzer, A., Johnson, T. E., Ivanov, S., Duan, Q., Bala, S., Condon, T., van Rooijen, N., Grainger, J. R., Belkaid, Y., Ma'ayan, A., Riches, D. W., Yokoyama, W. M., Ginhoux, F., Henson, P. M., & Randolph, G. J. (2013). Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity*, 39(3), 599–610. <https://doi.org/10.1016/j.immuni.2013.08.007>
- [39]. Roberts, H. R., & Tabares, A. H. (1995). Overview of the coagulation reactions. *Molecular Basis of Thrombosis and Hemostasis*, 58, 35–50. <https://doi.org/10.4103/0019-5049.144643>
- [40]. Conus, S., Perozzo, R., Reinheckel, T., Peters, C., Scapozza, L., Yousefi, S., & Simon, H. U. (2008). Caspase-8 is activated by cathepsin D initiating neutrophil apoptosis during the resolution of inflammation. *The Journal of experimental medicine*, 205(3), 685–698. <https://doi.org/10.1084/jem.20072152>
- [41]. Tracy, L. E., Minasian, R. A., & Caterson, E. J. (2016). Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. *Advances in wound care*, 5(3), 119–136. <https://doi.org/10.1089/wound.2014.0561>
- [42]. Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound Healing: A Cellular Perspective. *Physiological reviews*, 99(1), 665–706. <https://doi.org/10.1152/physrev.00067.2017>
- [43]. Wynn, T. A., & Barron, L. (2010). Macrophages: master regulators of inflammation and fibrosis. *Seminars in liver disease*, 30(3), 245–257. <https://doi.org/10.1055/s-0030-1255354>
- [44]. Greaves, N. S., Ashcroft, K. J., Baguneid, M., & Bayat, A. (2013). Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing. *Journal of dermatological science*, 72(3), 206–217. <https://doi.org/10.1016/j.jdermsci.2013.07.008>
- [45]. Eckes, B., Zweers, M. C., Zhang, Z. G., Hallinger, R., Mauch, C., Aumailley, M., & Krieg, T. (2006). Mechanical tension and integrin alpha 2 beta 1 regulate fibroblast functions. *The journal of investigative dermatology. Symposium proceedings*, 11(1), 66–72. <https://doi.org/10.1038/sj.jidsymp.5650003>
- [46]. Shaw, T. J., & Martin, P. (2016). Wound repair: a showcase for cell plasticity and migration. *Current opinion in cell biology*, 42, 29–37. <https://doi.org/10.1016/j.ceb.2016.04.001>
- [47]. Li, J., Chen, J., & Kirsner, R. (2007). Pathophysiology of acute wound healing. *Clinics in dermatology*, 25(1), 9–18. <https://doi.org/10.1016/j.clindermatol.2006.09.007>

- [48]. Preetam, S., Ghosh, A., Mishra, R., Pandey, A., Roy, D. S., Rustagi, S., & Malik, S. (2024). Electrical stimulation: A novel therapeutic strategy to heal biological wounds. *RSC Advances*, 14(48), 32142–32173. <https://doi.org/10.1039/D4RA04258A>
- [49]. Nunan, R., Campbell, J., Mori, R., Pitulescu, M. E., Jiang, W. G., Harding, K. G., Adams, R. H., Nobes, C. D., & Martin, P. (2015). Ephrin-Bs Drive Junctional Downregulation and Actin Stress Fiber Disassembly to Enable Wound Re-epithelialization. *Cell reports*, 13(7), 1380–1395. <https://doi.org/10.1016/j.celrep.2015.09.085>
- [50]. Rousselle, P., Braye, F., & Dayan, G. (2019). Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Advanced drug delivery reviews*, 146, 344–365. <https://doi.org/10.1016/j.addr.2018.06.019>
- [51]. Caley, M. P., Martins, V. L., & O'Toole, E. A. (2015). Metalloproteinases and Wound Healing. *Advances in wound care*, 4(4), 225–234. <https://doi.org/10.1089/wound.2014.0581>
- [52]. Bos, J. D., Teunissen, M. B., Cairo, I., Krieg, S. R., Kapsenberg, M. L., Das, P. K., & Borst, J. (1990). T-cell receptor gamma delta bearing cells in normal human skin. *The Journal of investigative dermatology*, 94(1), 37–42. <https://doi.org/10.1111/1523-1747.ep12873333>
- [53]. Desmoulière, A., Redard, M., Darby, I., & Gabbiani, G. (1995). Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *The American journal of pathology*, 146(1), 56–66.
- [54]. Gill, S. E., Pape, M. C., Khokha, R., Watson, A. J., & Leco, K. J. (2003). A null mutation for tissue inhibitor of metalloproteinases-3 (Timp-3) impairs murine bronchiole branching morphogenesis. *Developmental biology*, 261(2), 313–323. [https://doi.org/10.1016/s0012-1606\(03\)00318-x](https://doi.org/10.1016/s0012-1606(03)00318-x)
- [55]. Karimi, K., Odhav, A., Kollipara, R., Fike, J., Stanford, C., & Hall, J. C. (2017). Acute Cutaneous Necrosis: A Guide to Early Diagnosis and Treatment. *Journal of cutaneous medicine and surgery*, 21(5), 425–437. <https://doi.org/10.1177/1203475417708164>
- [56]. Summer, M., Ali, S., Fiaz, U., Hussain, T., Khan, R. R. M., & Fiaz, H. (2024). Revealing the molecular mechanisms in wound healing and the effects of different physiological factors including diabetes, age, and stress. *Journal of Molecular Histology*, 55(4), 637–654. <https://doi.org/10.1007/s10735-024-10223-3>
- [57]. Monaco, J. L., & Lawrence, W. T. (2003). Acute wound healing an overview. *Clinics in plastic surgery*, 30(1), 1–12. [https://doi.org/10.1016/s0094-1298\(02\)00070-6](https://doi.org/10.1016/s0094-1298(02)00070-6)
- [58]. Wallace HA, Basehore BM, Zito PM. Wound Healing Phases. [Updated 2023 Jun 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470443/>

- [59]. Singh, A. V., Gemmati, D., Kanase, A., Pandey, I., Misra, V., Kishore, V., Jahnke, T., & Bill, J. (2018). Nanobiomaterials for vascular biology and wound management: A review. *Veins and Lymphatics*, 7, Article 7196. <https://doi.org/10.4081/vl.2018.7196>
- [60]. Dwivedi, C., Pandey, H., Pandey, A. C., Patil, S., Ramteke, P. W., Laux, P., Luch, A., & Singh, A. V. (2019). In Vivo Biocompatibility of Electrospun Biodegradable Dual Carrier (Antibiotic + Growth Factor) in a Mouse Model—Implications for Rapid Wound Healing. *Pharmaceutics*, 11(4), 180. <https://doi.org/10.3390/pharmaceutics11040180>
- [61]. Fonder, M. A., Lazarus, G. S., Cowan, D. A., Aronson-Cook, B., Kohli, A. R., & Mamelak, A. J. (2008). Treating the chronic wound: A practical approach to the care of nonhealing wounds and wound care dressings. *Journal of the American Academy of Dermatology*, 58(2), 185–206. <https://doi.org/10.1016/j.jaad.2007.08.048>
- [62]. Singh, A., Halder, S., Menon, G. R., Chumber, S., Misra, M. C., Sharma, L. K., & Srivastava, A. (2004). Meta-analysis of randomized controlled trials on hydrocolloid occlusive dressing versus conventional gauze dressing in the healing of chronic wounds. *Asian journal of surgery*, 27(4), 326–332. [https://doi.org/10.1016/S1015-9584\(09\)60061-0](https://doi.org/10.1016/S1015-9584(09)60061-0)
- [63]. Landén, N. X., Li, D., & Ståhle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and molecular life sciences : CMLS*, 73(20), 3861–3885. <https://doi.org/10.1007/s00018-016-2268-0>
- [64]. Larouche, J., Sheoran, S., Maruyama, K., & Martino, M. M. (2018). Immune Regulation of Skin Wound Healing: Mechanisms and Novel Therapeutic Targets. *Advances in wound care*, 7(7), 209–231. <https://doi.org/10.1089/wound.2017.0761>
- [65]. Huang, Y. Z., Gou, M., Da, L. C., Zhang, W. Q., & Xie, H. Q. (2020). Mesenchymal Stem Cells for Chronic Wound Healing: Current Status of Preclinical and Clinical Studies. *Tissue engineering. Part B, Reviews*, 26(6), 555–570. <https://doi.org/10.1089/ten.TEB.2019.0351>
- [66]. Zhao, R., Liang, H., Clarke, E., Jackson, C., & Xue, M. (2016). Inflammation in Chronic Wounds. *International journal of molecular sciences*, 17(12), 2085. <https://doi.org/10.3390/ijms17122085>
- [67]. Piipponen, M., Li, D., & Landén, N. X. (2020). The Immune Functions of Keratinocytes in Skin Wound Healing. *International journal of molecular sciences*, 21(22), 8790. <https://doi.org/10.3390/ijms21228790>
- [68]. Seth, I., Lim, B., Cevik, J., Gracias, D., Chua, M., Kenney, P. S., Rozen, W. M., & Cuomo, R. (2024). Impact of nutrition on skin wound healing and aesthetic outcomes: A comprehensive narrative review. *JPRAS open*, 39, 291–302. <https://doi.org/10.1016/j.jpra.2024.01.006>

- [69]. González-Torres, C., González-Martínez, H., Miliar, A., Nájera, O., Graniel, J., Firo, V., Alvarez, C., Bonilla, E., & Rodríguez, L. (2013). Effect of malnutrition on the expression of cytokines involved in Th1 cell differentiation. *Nutrients*, 5(2), 579–593. <https://doi.org/10.3390/nu5020579>
- [70]. Bikker, A., Wielders, J., van Loo, R., & Loubert, M. (2016). Ascorbic acid deficiency impairs wound healing in surgical patients: Four case reports. *International Journal of Surgery Open*, 2, 15–18. <https://doi.org/10.1016/j.ijso.2016.03.002>
- [71]. Herberger, K., Müller, K., Protz, K., Zyriax, B. C., Augustin, M., & Hagenström, K. (2020). Nutritional status and quality of nutrition in chronic wound patients. *International wound journal*, 17(5), 1246–1254. <https://doi.org/10.1111/iwj.13378>
- [72]. Hong, W. X., Hu, M. S., Esquivel, M., Liang, G. Y., Rennert, R. C., McArdle, A., Paik, K. J., Duscher, D., Gurtner, G. C., Lorenz, H. P., & Longaker, M. T. (2014). The Role of Hypoxia-Inducible Factor in Wound Healing. *Advances in wound care*, 3(5), 390–399. <https://doi.org/10.1089/wound.2013.0520>
- [73]. Bai, Q., Gao, Q., Hu, F., Zheng, C., Sun, N., Chen, W., Liu, J., Zhang, Y., Wu, X., & Lu, T. (2022). Reoxygenation Modulates the Adverse Effects of Hypoxia on Wound Repair. *International journal of molecular sciences*, 23(24), 15832. <https://doi.org/10.3390/ijms232415832>
- [74]. Brem, H., & Tomic-Canic, M. (2007). Cellular and molecular basis of wound healing in diabetes. *The Journal of clinical investigation*, 117(5), 1219–1222. <https://doi.org/10.1172/JCI32169>
- [75]. Woo, K., Ayello, E. A., & Sibbald, R. G. (2007). The edge effect: current therapeutic options to advance the wound edge. *Advances in skin & wound care*, 20(2), 99–119. <https://doi.org/10.1097/00129334-200702000-00009>
- [76]. Keylock, K. T., Vieira, V. J., Wallig, M. A., DiPietro, L. A., Schrementi, M., & Woods, J. A. (2008). Exercise accelerates cutaneous wound healing and decreases wound inflammation in aged mice. *American journal of physiology. Regulatory, integrative and comparative physiology*, 294(1), R179–R184. <https://doi.org/10.1152/ajpregu.00177.2007>
- [77]. Edwards, R., & Harding, K. G. (2004). Bacteria and wound healing. *Current opinion in infectious diseases*, 17(2), 91–96. <https://doi.org/10.1097/00001432-200404000-00004>
- [78]. Menke, N. B., Ward, K. R., Witten, T. M., Bonchev, D. G., & Diegelmann, R. F. (2007). Impaired wound healing. *Clinics in dermatology*, 25(1), 19–25. <https://doi.org/10.1016/j.clindermatol.2006.12.005>
- [79]. Davis, S. C., Ricotti, C., Cazzaniga, A., Welsh, E., Eaglstein, W. H., & Mertz, P. M. (2008). Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. *Wound repair and regeneration : official publication of the*

Wound Healing Society [and] the European Tissue Repair Society, 16(1), 23–29.
<https://doi.org/10.1111/j.1524-475X.2007.00303.x>

[80]. Bjarnsholt, T., Kirketerp-Møller, K., Jensen, P. Ø., Madsen, K. G., Phipps, R., Krogfelt, K., Høiby, N., & Givskov, M. (2008). Why chronic wounds will not heal: a novel hypothesis. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 16(1), 2–10.
<https://doi.org/10.1111/j.1524-475X.2007.00283.x>

[81]. Web 1, (2025), <https://www.fortunebusinessinsights.com/industry-reports/wound-care-market-101920>, (Access date: 06/05/2025).

[82]. Guo, S., & Dipietro, L. A. (2010). Factors affecting wound healing. *Journal of dental research*, 89(3), 219–229. <https://doi.org/10.1177/0022034509359125>

[83]. Jeong, S.-H., Kim, S., Lee, J., Lee, K., Kim, H., Lee, Y., & Kim, S. (2021). Accelerated wound healing with an ionic patch assisted by a triboelectric nanogenerator. *Nano Energy*, 79, 105463.
<https://doi.org/10.1016/j.nanoen.2020.105463>

[84]. Nobre, J., et al. (2024). *Electrical stimulation: a novel therapeutic strategy to heal biological wounds*. *RSC Advances*, 14(48), 32142–32173.
<https://doi.org/10.1039/D4RA04258A>

[85]. Rajendran, S. B., Challen, K., Wright, K. L., & Hardy, J. G. (2021). Electrical Stimulation to Enhance Wound Healing. *Journal of functional biomaterials*, 12(2), 40.
<https://doi.org/10.3390/jfb12020040>

[86]. Zhao, M., Song, B., Pu, J., Wada, T., Reid, B., Tai, G., Wang, F., Guo, A., Walczysko, P., Gu, Y., Sasaki, T., Suzuki, A., Forrester, J. V., Bourne, H. R., Devreotes, P. N., McCaig, C. D., & Penninger, J. M. (2006). Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. *Nature*, 442(7101), 457–460. <https://doi.org/10.1038/nature04925>

[87]. Foulds, I. S., & Barker, A. T. (1983). Human skin battery potentials and their possible role in wound healing. *The British journal of dermatology*, 109(5), 515–522.
<https://doi.org/10.1111/j.1365-2133.1983.tb07673.x>

[88]. Reid, B., Song, B., McCaig, C. D., & Zhao, M. (2005). Wound healing in rat cornea: the role of electric currents. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 19(3), 379–386.
<https://doi.org/10.1096/fj.04-2325com>

[89]. Sun Y. S. (2017). Electrical Stimulation for Wound-Healing: Simulation on the Effect of Electrode Configurations. *BioMed research international*, 2017, 5289041.
<https://doi.org/10.1155/2017/5289041>

[90]. Zhao M. (2009). Electrical fields in wound healing-An overriding signal that directs cell migration. *Seminars in cell & developmental biology*, 20(6), 674–682.
<https://doi.org/10.1016/j.semcd.2008.12.009>

- [91]. Nuccitelli R. (2003). Endogenous electric fields in embryos during development, regeneration and wound healing. *Radiation protection dosimetry*, 106(4), 375–383. <https://doi.org/10.1093/oxfordjournals.rpd.a006375>
- [92]. Zhao, Z., Qin, L., Reid, B., Pu, J., Hara, T., & Zhao, M. (2012). Directing migration of endothelial progenitor cells with applied DC electric fields. *Stem cell research*, 8(1), 38–48. <https://doi.org/10.1016/j.scr.2011.08.001>
- [93]. Wang, X. F., Li, M. L., Fang, Q. Q., Zhao, W. Y., Lou, D., Hu, Y. Y., Chen, J., Wang, X. Z., & Tan, W. Q. (2020). Flexible electrical stimulation device with Chitosan-Vaseline® dressing accelerates wound healing in diabetes. *Bioactive materials*, 6(1), 230–243. <https://doi.org/10.1016/j.bioactmat.2020.08.003>
- [94]. Zimolag, E., Borowczyk-Michałowska, J., Kędracka-Krok, S., et al. (2017). Electric field as a potential directional cue in homing of bone marrow-derived mesenchymal stem cells to cutaneous wounds. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1864(2), 267–279. <https://doi.org/10.1016/j.bbamcr.2016.10.005>
- [95]. Guo, A., Song, B., Reid, B., Gu, Y., Forrester, J. V., Jahoda, C. A., & Zhao, M. (2010). Effects of physiological electric fields on migration of human dermal fibroblasts. *The Journal of investigative dermatology*, 130(9), 2320–2327. <https://doi.org/10.1038/jid.2010.96>
- [96]. Kloth L. C. (2005). Electrical stimulation for wound healing: a review of evidence from in vitro studies, animal experiments, and clinical trials. *The international journal of lower extremity wounds*, 4(1), 23–44. <https://doi.org/10.1177/1534734605275733>
- [97]. Bourguignon, G. J., & Bourguignon, L. Y. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 1(5), 398–402. <https://doi.org/10.1096/fasebj.1.5.3678699>
- [98]. Frelinger, A. L., 3rd, Gerrits, A. J., Garner, A. L., Torres, A. S., Caiafa, A., Morton, C. A., Berny-Lang, M. A., Carmichael, S. L., Neculaes, V. B., & Michelson, A. D. (2016). Modification of Pulsed Electric Field Conditions Results in Distinct Activation Profiles of Platelet-Rich Plasma. *PloS one*, 11(8), e0160933. <https://doi.org/10.1371/journal.pone.0160933>
- [99]. Sinno, H., & Prakash, S. (2013). Complements and the wound healing cascade: an updated review. *Plastic surgery international*, 2013, 146764. <https://doi.org/10.1155/2013/146764>
- [100]. Cho, M. R., Thatte, H. S., Lee, R. C., & Golan, D. E. (2000). Integrin-dependent human macrophage migration induced by oscillatory electrical stimulation. *Annals of biomedical engineering*, 28(3), 234–243. <https://doi.org/10.1114/1.263>
- [101]. Hoare, J. I., Rajnicek, A. M., McCaig, C. D., Barker, R. N., & Wilson, H. M. (2016). Electric fields are novel determinants of human macrophage

functions. *Journal of leukocyte biology*, 99(6), 1141–1151.
<https://doi.org/10.1189/jlb.3A0815-390R>

[102]. Kindzelskii, A. L., & Petty, H. R. (2000). Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phase-matched with metabolic oscillators. *Biochimica et biophysica acta*, 1495(1), 90–111. [https://doi.org/10.1016/s0167-4889\(99\)00148-2](https://doi.org/10.1016/s0167-4889(99)00148-2)

[103]. Akita S. (2019). Wound Repair and Regeneration: Mechanisms, Signaling. *International journal of molecular sciences*, 20(24), 6328.
<https://doi.org/10.3390/ijms20246328>

[104]. Chao, P. H., Lu, H. H., Hung, C. T., Nicoll, S. B., & Bulinski, J. C. (2007). Effects of applied DC electric field on ligament fibroblast migration and wound healing. *Connective tissue research*, 48(4), 188–197.
<https://doi.org/10.1080/03008200701424451>

[105]. Liu, J., Guo, X., Ren, X., Tian, H., Liang, Y., Luo, Z., Wang, W., Wang, Y., Zhang, D., Huang, Y., & Zhang, J. (2018). A novel FPCL model producing directional contraction through induction of fibroblast alignment by biphasic pulse direct current electric field. *Experimental cell research*, 371(2), 426–434.
<https://doi.org/10.1016/j.yexcr.2018.09.003>

[106]. Allen, G. M., Mogilner, A., & Theriot, J. A. (2013). Electrophoresis of cellular membrane components creates the directional cue guiding keratocyte galvanotaxis. *Current biology : CB*, 23(7), 560–568.
<https://doi.org/10.1016/j.cub.2013.02.047>

[107]. Huang, L., Cormie, P., Messerli, M. A., & Robinson, K. R. (2009). The involvement of Ca²⁺ and integrins in directional responses of zebrafish keratocytes to electric fields. *Journal of cellular physiology*, 219(1), 162–172.
<https://doi.org/10.1002/jcp.21660>

[108]. Trollinger, D. R., Isseroff, R. R., & Nuccitelli, R. (2002). Calcium channel blockers inhibit galvanotaxis in human keratinocytes. *Journal of cellular physiology*, 193(1), 1–9. <https://doi.org/10.1002/jcp.10144>

[109]. Pullar, C. E., Isseroff, R. R., & Nuccitelli, R. (2001). Cyclic AMP-dependent protein kinase A plays a role in the directed migration of human keratinocytes in a DC electric field. *Cell motility and the cytoskeleton*, 50(4), 207–217.
<https://doi.org/10.1002/cm.10009>

[110]. Nishimura, K. Y., Isseroff, R. R., & Nuccitelli, R. (1996). Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. *Journal of cell science*, 109 (Pt 1), 199–207.
<https://doi.org/10.1242/jcs.109.1.199>

[111]. Lund, E. (1947), “Bioelectric Fields and Growth”, *Soil Science*, 64, 253.

- [112]. Burr, H. S., & Northrop, F. S. C. (1935). The electro-dynamic theory of life. *The Quarterly Review of Biology*, 10(3), 322–333.
- [113]. Burr H. S. (1944). The Meaning of Bio-Electric Potentials. *The Yale journal of biology and medicine*, 16(4), 353–360.
- [114]. Jaffe, L. F., & Nuccitelli, R. (1974). An ultrasensitive vibrating probe for measuring steady extracellular currents. *The Journal of cell biology*, 63(2 Pt 1), 614–628. <https://doi.org/10.1083/jcb.63.2.614>
- [115]. Jaffe, L. F., & Nuccitelli, R. (1977). Electrical controls of development. *Annual review of biophysics and bioengineering*, 6, 445–476. <https://doi.org/10.1146/annurev.bb.06.060177.002305>
- [116]. Jaffe L. F. (1981). The role of ionic currents in establishing developmental pattern. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 295(1078), 553–566. <https://doi.org/10.1098/rstb.1981.0160>
- [117]. Borgens R. B. (1982). What is the role of naturally produced electric current in vertebrate regeneration and healing. *International review of cytology*, 76, 245–298. [https://doi.org/10.1016/s0074-7696\(08\)61793-3](https://doi.org/10.1016/s0074-7696(08)61793-3)
- [118]. Hotary, K. B., & Robinson, K. R. (1994). Endogenous electrical currents and voltage gradients in *Xenopus* embryos and the consequences of their disruption. *Developmental biology*, 166(2), 789–800. <https://doi.org/10.1006/dbio.1994.1357>
- [119]. Nuccitelli R. (1992). Endogenous ionic currents and DC electric fields in multicellular animal tissues. *Bioelectromagnetics, Suppl* 1, 147–157. <https://doi.org/10.1002/bem.2250130714>
- [120]. Borgens R. B. (1983). The role of ionic current in the regeneration and development of the amphibian limb. *Progress in clinical and biological research*, 110 Pt A, 597–608.
- [121]. Levin M. (2007). Large-scale biophysics: ion flows and regeneration. *Trends in cell biology*, 17(6), 261–270. <https://doi.org/10.1016/j.tcb.2007.04.007>
- [122]. Funk R. H. (2015). Endogenous electric fields as guiding cue for cell migration. *Frontiers in physiology*, 6, 143. <https://doi.org/10.3389/fphys.2015.00143>
- [123]. Jaffe, L. F., & Vanable, J. W., Jr (1984). Electric fields and wound healing. *Clinics in dermatology*, 2(3), 34–44. [https://doi.org/10.1016/0738-081x\(84\)90025-7](https://doi.org/10.1016/0738-081x(84)90025-7)
- [124]. Nuccitelli, R., Nuccitelli, P., Li, C., Narsing, S., Pariser, D. M., & Lui, K. (2011). The electric field near human skin wounds declines with age and provides a noninvasive indicator of wound healing. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 19(5), 645–655. <https://doi.org/10.1111/j.1524-475X.2011.00723.x>

- [125]. Adegoke, B. O., & Badmos, K. A. (2001). Acceleration of pressure ulcer healing in spinal cord injured patients using interrupted direct current. *African journal of medicine and medical sciences*, 30(3), 195–197.
- [126]. Taylor, K., Mendel, F. C., Fish, D. R., Hard, R., & Burton, H. W. (1997). Effect of high-voltage pulsed current and alternating current on macromolecular leakage in hamster cheek pouch microcirculation. *Physical therapy*, 77(12), 1729–1740. <https://doi.org/10.1093/ptj/77.12.1729>
- [127]. Kloth, L. C., & Feedar, J. A. (1988). Acceleration of wound healing with high voltage, monophasic, pulsed current. *Physical therapy*, 68(4), 503–508. <https://doi.org/10.1093/ptj/68.4.503>
- [128]. Santamato, A., Panza, F., Fortunato, F., Portincasa, A., Frisardi, V., Cassatella, G., Valente, M., Seripa, D., Ranieri, M., & Fiore, P. (2012). Effectiveness of the frequency rhythmic electrical modulation system for the treatment of chronic and painful venous leg ulcers in older adults. *Rejuvenation research*, 15(3), 281–287. <https://doi.org/10.1089/rej.2011.1236>
- [129]. Carley, P. J., & Wainapel, S. F. (1985). Electrotherapy for acceleration of wound healing: low intensity direct current. *Archives of physical medicine and rehabilitation*, 66(7), 443–446.
- [130]. Thawer, H. A., & Houghton, P. E. (2001). Effects of electrical stimulation on the histological properties of wounds in diabetic mice. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 9(2), 107–115. <https://doi.org/10.1046/j.1524-475x.2001.00107.x>
- [131]. Petrofsky, J., Laymon, M., Chung, W., Collins, K., & Yang, T.-N. (2008). Effect of electrical stimulation on bacterial growth. *Journal of Orthopaedic and Neurological Surgery*, 31, 43.
- [132]. Kincaid, C. B., & Lavoie, K. H. (1989). Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic, pulsed current. *Physical therapy*, 69(8), 651–655. <https://doi.org/10.1093/ptj/69.8.651>
- [133]. Gomes, R. C., Brandino, H. E., de Sousa, N. T., Santos, M. F., Martinez, R., & Guirro, R. R. (2015). Polarized currents inhibit in vitro growth of bacteria colonizing cutaneous ulcers. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 23(3), 403–411. <https://doi.org/10.1111/wrr.12296>
- [134]. Daeschlein, G., Assadian, O., Kloth, L. C., Meinl, C., Ney, F., & Kramer, A. (2007). Antibacterial activity of positive and negative polarity low-voltage pulsed current (LVPC) on six typical Gram-positive and Gram-negative bacterial pathogens of chronic wounds. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, 15(3), 399–403. <https://doi.org/10.1111/j.1524-475X.2007.00242.x>

- [135]. Barranco, S. D., Spadaro, J. A., Berger, T. J., & Becker, R. O. (1974). In vitro effect of weak direct current on *Staphylococcus aureus*. *Clinical orthopaedics and related research*, (100), 250–255.
- [136]. Falcone, A. E., & Spadaro, J. A. (1986). Inhibitory effects of electrically activated silver material on cutaneous wound bacteria. *Plastic and reconstructive surgery*, 77(3), 455–459. <https://doi.org/10.1097/00006534-198603000-00022>
- [137]. Asadi, M. R., & Torkaman, G. (2014). Bacterial Inhibition by Electrical Stimulation. *Advances in wound care*, 3(2), 91–97. <https://doi.org/10.1089/wound.2012.0410>
- [138]. Matinong, A. M. E., Chisti, Y., Pickering, K. L., & Haverkamp, R. G. (2022). Collagen Extraction from Animal Skin. *Biology*, 11(6), 905. <https://doi.org/10.3390/biology11060905>
- [139]. Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, 395(6704), 763–770. <https://doi.org/10.1038/27376>
- [140]. Weidenmaier, C., & Peschel, A. (2008). Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nature reviews. Microbiology*, 6(4), 276–287. <https://doi.org/10.1038/nrmicro1861>
- [141]. Raetz, C. R., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual review of biochemistry*, 71, 635–700. <https://doi.org/10.1146/annurev.biochem.71.110601.135414>

BIOGRAPHY

Zeynep GERGİN is currently pursuing her studies in the Bioengineering Department at the Faculty of Sciences, Gebze Technical University (February 2023- present) in Gebze, Kocaeli. She completed her Bachelor's degree in Molecular Biology and Genetics at Muğla Sıtkı Koçman University between September 2018 and June 2022. Her secondary education was completed at Alp Oğuz Anatolian High School in İzmir,Turkey (September 2012- June 2016).



PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

[1] Gergin Z., Tarlak F., Ergüden B “Electrical Current Is an Effective Sanitizer for Both Gram-Positive and Gram-Negative Bacteria Cells”, 9. Lisansüstü Araştırmalar Sempozyumu, Kocaeli, Türkiye, 26-27 May.

