HANEEN AYAD KHALEEL

ATILIM UNIVERSITY 2022

# THE PREPARATION AND ANTIBACTERIAL ANALYSIS OF SILVER NANOPARTICLES ADDED PEDOT

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
ATILIM UNIVERSITY

HANEEN AYAD KHALEEL

A MASTER OF SCIENCE THESIS
IN
THE DEPARTMENT OF CHEMICAL ENGINEERING

JANUARY 2022

# THE PREPARATION AND ANTIBACTERIAL ANALYSIS OF SILVER NANOPARTICLES ADDED PEDOT

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES OF
ATILIM UNIVERSITY

BY

HANEEN AYAD KHALEEL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE IN
CHEMICAL ENGINEERING

JANUARY 2022

Approval of the Graduate School of Natural and Applied Sciences, Atilim University.

Prof. Dr. Ender KESKİNKILIÇ
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of **Master of Science in Chemical Engineering and Applied Chemistry, Atilim University.**

Prof. Dr. Şeniz ÖZALP YAMAN
Head of Department

This is to certify that we have read the thesis THE PREPARATION AND ANTIBACTERIAL ANALYSIS OF SILVER NANOPARTICLE ADDED PEDOT submitted by Haneen Ayad Khaleel and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Prof. Dr. Murat KAYA
Co-Supervisor

Prof. Dr. Sultan Belgin İŞGÖR
Supervisor

**Examining Committee Members:**

Prof. Dr. Yasemin G. İşgör
Vocational School of Health Services, Medical Laboratory Techniques Program, Ankara University

Prof. Dr. S. Belgin İşgör
Chemical Engineering Department, Atilim University

Prof. Dr. Seha Tirkeş
Chemical Engineering Department, Atilim University

**Date**: **January 24, 2022**

I declare and guarantee that all data, knowledge and information in this document has been obtained, processed and presented in accordance with academic rules and ethical conduct. Based on these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name : Haneen Ayad Khaleel

Signature :

## ABSTRACT

### THE PREPARATION AND ANTIBACTERIAL ANALYSIS OF SILVER NANOPARTICLES ADDED PEDOT

Khaleel, Haneen Ayad

M.S., Chemical Engineering and Applied Chemistry

Supervisor: Prof. Dr. Sultan Belgin İŞGÖR

Co-Supervisor: Prof. Dr. Murat KAYA

January 2022, 44 pages

With a high hospital-acquired infection (HAIs) prevalence, the use of antibacterial coatings can help to reduce bacterial contamination at the hospital surfaces that are frequently interacted (e.g. keyboards, push plates, or bed rails) and commonly underlying the hospital-acquired infection. Nanoparticles and polymers combination shows strong antibacterial action against a number of gram-negative and Gram-positive bacteria. In this study, nanoparticles are incorporated with polymer as antibacterial surfaces to prevent HAIs. The aim of this study is to make a coating material to prevent infections by preparing PEDOT embedded with silver nanoparticles and testing the substance against common pathogens found in hospitals, such as *Escherichia Coli* and *Staphylococcus Aureus*. (PEDOT) was synthesized in the presence of EDOT and iron (III) chloride ($FeCl_3$), via a chemical polymerization process. By the liquid impregnation technique, silver nanoparticles were bonded to PEDOT then the substance was pressed to make pellets. While the antibacterial activity was tested by using disk diffusion methods, the materials prepared in this study show potential for HAIs reduction in hospitals. Due to the significant properties of the silver nanoparticles and PEDOT, the inhibition of bacterial growth can be done without any harmful effect on the human body. Further research should be conducted

to improve the substance's antibacterial efficacy for a wide range of medical use.

Keywords: Nanoparticle, Polymer, PEDOT, AgNPs, Antibacterial analysis, *Escherichia Coli*, *Staphylococcus Aureus*

# ÖZ

## GÜMÜŞ NANOPARÇACIK EKLENMİŞ PEDOT'UN HAZIRLANMASI VE ANTİBAKTERİYEL ANALİZİ

Khaleel, Haneen Ayad
Yüksek Lisans, Kimya Mühendisliği ve Uygulamalı Kimya
Danışman : Prof. Dr. Sultan Belgin İŞGÖR
Eş Danışman : Prof. Dr. Murat KAYA

Ocak 2022, 44 sayfa

Hastane aracılıklı enfeksiyon (HAE) prevalansı yüksek olduğunda, antibakteriyel kaplamaların kullanılması, sıklıkla etkileşime giren hastane yüzeylerinde (örneğin klavyeler, itme plakaları veya yatak rayları) ve genellikle hastane kaynaklı enfeksiyonun altında yatan bakteriyel kontaminasyonun azaltılmasına yardımcı olabilir. Nano. parçacıkler ve polimerler kombinasyonu, bir dizi gram negatif ve gram pozitif bakteriye karşı güçlü antibakteriyel etki gösterir. Bu çalışmada nanoparçacıklr, HAE' lari önlemek için antibakteriyel yüzeyler olarak polimer ile birleştirilmiştir. Bu çalışmanın amacı, gümüşnanoparçacıklar eklenmiş PEDOT hazırlayarak *Escherichia Coli* ve *Staphylococcus Aureus* gibi hastanelerde bulunan yaygın patojenlere karşı antimikrobiyal etkinliğinin test ederek enfeksiyonları önlemek için uygun bir kaplama malzemesi yapmaktır. (PEDOT) kimyasal polimerizasyon işlemi ile EDOT ve demir (III) klorür ($FeCl_3$) varlığında sentezlendi. Sıvı emprenye tekniği ile gümüş nanoparçacıklar PEDOT'a bağlandı ve daha sonra madde pelet yapmak için preslendi. Antibakteriyel aktivite disk difüzyon yöntemleri kullanılarak test edilirken, bu çalışmada hazırlanan malzemeler hastanelerde HAE larda azalma potansiyeli göstermektedir. Gümüş nanoparçacıkların ve PEDOT'un özelliklerinden dolayı, bakteri üremesinin önlenmesi insan vücudu üzerinde herhangi bir zararlı etki olmadan yapılabilir. Elde edilen malzemenin yaygın tıbbi kullanımı için antibakteriyel

etkinliğini arttırmak üzere daha fazla araştırma yapılmalıdır.

Anahtar Kelimeler: Nanopartikül, Polimer, PEDOT, AgNPs, antibakteriyel analiz, *Escherichia Coli*, *Staphylococcus Aureus*

*To my family, for their endless love, support and encouragement*

## ACKNOWLEDGMENTS

My sincere thanks to Prof. Dr. S. Belgin İşgör, for her constant support and guidance. Her levels of patience, knowledge, and ingenuity are something I will always aspire to. I would also like to express my deepest gratitude to my family and friends for their unfailing support and encouragement.

## TABLE OF CONTENTS

## LIST OF FIGURES

FIGURES

# CHAPTER 1

# INTRODUCTION

With the increase of bacterial infections in hospitals and the increase of antibiotic-resistant pathogenic bacteria, some of these antibiotics have become useless for treatment. Excessive use of antibiotics for acute and chronic diseases, incomplete treatment without recuperation, and the high dose of antibiotics used at the maximum allowed level may cause the bacteria to developed resistance against the antibiotics used. It is important to develop antibacterial agents with low side effects, high therapeutic efficiency, and also with benefits for patients economically. One of the alternatives to antibacterial agents is the nanoparticles, to which the pathogens cannot develop resistance as easily as they develop against chemical agents. Some of the nanoparticles (NPs) such as silver nanoparticles, may exert antibacterial activity by causing damage to the plasma membrane, by inhibiting DNA reproduction, and by inhibiting enzymes essential to bacteria. [1].

Studied with silver nanoparticles showed that the antibacterial activity of AgNPs can be enhanced with polymer addition such as poly-N-isopropylacrylamide (PNIPAM), poly-N-methylaniline (PNMA), or poly (3, 4-ethylenedioxythiophene) (PEDOT). In this study, a combination of PEDOT was used as a coatingmaterial of AgNPs to prevent bacterial infection. PEDOT was originally recognized for its outstanding conductivity and has been proven to be a promising material for many biomedical applications due to its essential biocompatibility, mechanical properties, and high electronic and ionic conductivity. Therefore, PEDOT may be used to kill bacteria [2].

## 1.1 Polymers

Polymer is a derivative of the Greek words *poly* (many) and *meros* (part). Polymers, also known as macromolecules, are massive molecules containing hundreds to millions of atoms [3]. Those are made by linking togethera huge number of smaller molecules called monomers. The structure of polystyrene, as an example, is shown in figure 1.1:

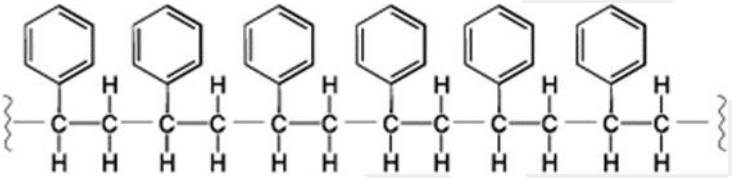

Figure 1.1: Structural formula of polystyrene.

It is also possible to write the repeating unit of the molecule as shown in figure 1.2, in which case the number of repeating units is indicated by a subscript "n".

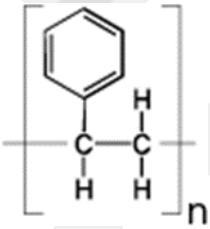

Figure 1.2: Repeat unit of polystyrene.

Depending on the polymer's molecular weight, n is generally in the range of a few hundred to several thousand units. In some cases, the polymer’s molecular weight might reach several million. To emphasize an extremely high molecular weight of a material, the term "high polymer" is sometimes used [3].

Polymers contain a wide range of physical and chemical characteristics, making them valuable in everyday use. Familiar polymers include artificial plastics (polystyrene), natural polymers (DNA), and proteins with biological characteristics and activities. Natural polymers generally are composed of monomers, whereas manufactured polymers are monomer-like. Polymers with just one monomer are called homopolymers, while polymers containing several monomer types are called

copolymers [4], copolymers represent a significant element of the latest technologies. Synthetic polymers like PEDOT, among others, are found almost everywhere in many consumer products like capacitors, antistatics, printed electronics, organic solar cells, organic light-emitting diodes, and touch panels [5].

### 1.1.1 Antimicrobial Polymers

Since 1965, when Dunraruma and Cornell reported that copolymers and polymers made from 2-methacryloxytroponones kill bacteria, antimicrobial polymers have been well recognized, for example, in the 1970s, polymers containingsalicylic acid, while in 1972 polymers were developed with quaternary ammonium groups [6-8]. So far, a vast number of these macromolecules have been identifiedand described, however not all of their functions are well-understood. Nevertheless, the number of FDA-approved disinfecting polymers has risen substantially in the pastdecade, indicating the need for alternatives to antibiotics and ecologically essential disinfectants. As a result, numerous evaluations have been published recently summarizing the current state [9, 10]. Antimicrobial polymers are characterized bytheir molecular weight, degree of alkylation, charge distribution, and the effect of these characteristics on the bactericidal activity were analyzed [11]. There are three types of antibacterial polymers, according to a recent study: 1) biocidal polymers, 2) polymeric biocides, and 3) biocide-releasing polymers. Biocidal groups linked to polymers function in the same way as similar low molecular weightchemicals, such that the repeating unit is a biocide, as illustrated in figure 1.3 [12].

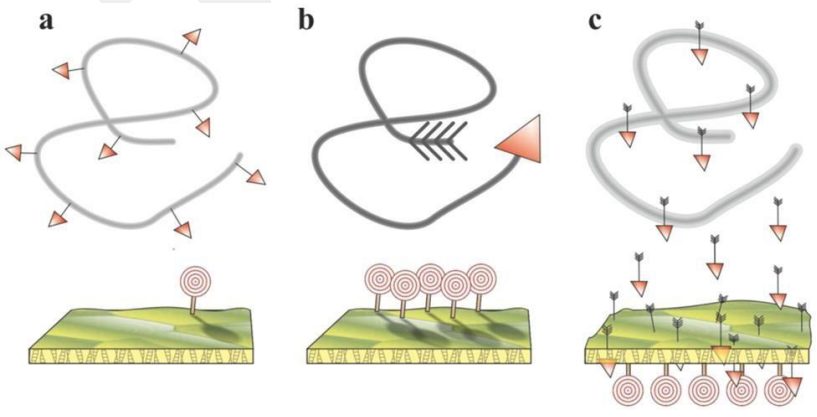


Figure 1.3: Antimicrobial polymers function as: (a) polymeric biocides (b) Biocidal polymers; (c) biocide-releasing polymers.

Polymericbiocides are expected to be less active than their low molecular weight counterparts considering the steric hindrance exerted by the backbone of the polymer. Biocidal polymers, on the other hand, have an active principle that is incorporated throughout the entire macromolecule, without the need for antimicrobial repeating units. The actual polymeric portion of biocide-releasing polymers does not operate; rather, it acts as a carrier for biocides that are somehow transported to the attacking microbial cells. These polymers are generally the most active systems because they may release biocides at high local concentrations near the cell [12].

In the past several years, a broad class of antimicrobial macromolecular systems, copolymers, and polymers which are functionalized with bioactive groups for example polymers with N-halamine active group like 3-bromo-1-chloro-5,5-dimethylhydantoin and1,3-dichloro-5,5-dimethyl hydantoin,have been studied, and efficiently employed as antimicrobial agents. In addition, novel moderately hydrophobic polymer structures containing protonatedprimary, secondary or tertiary amine groups with higher antibacterial activity than their quaternary amine groups have been created [11].

On the basis of their mechanism of action, antimicrobial polymers may be divided into active or passive polymers. Activated polymers such as 2-vinylthiophene reduce the adherence of microorganisms to surfaces, preventing their proliferation. Poly (sulfobetaine methacrylate), Poly (ethylene glycol), poly (dimethyl acrylamide), polyphenols are some of the examples. Cell walls of bacteria are disrupted by active polymers [13, 14]. Some examples of polymers with active properties are nisin immobilized organosilicon, polyurethane with quaternary ammonium, poly (dimethylamino) ethyl methacrylate with quaternary ammonium tethering, and poly (n, n-diethyl diamine diamine-cytosol-based acrylic) [15, 16]. For improved antimicrobial performance, the antimicrobial polymers should also have the following properties: hydrophobic functional group, amphipathic nature, low degree of polymerization, high molecular weight, and activity against a wide range of microorganisms [17, 18].

## 1.2 Poly (3, 4-ethylenedioxythiophene) (PEDOT)

PEDOT, a conjugated conducting polymer, has been widely employed in many applications because of its unique properties, including strong conductivity, high optical transparency, flexibility, chemical stability. On the other hand its inadequate biocompatibility and weak corrosion protection performance in the physiological environment are the most important obstacles to its application as a coating material [19]. In addition, due to its particular doping properties and doping chemistry, due to its biocompatibility PEDOT can be doped with many materials to increase its efficacy, PEDOT has gained much attention in biological applications [20]. Polymerization techniques for conducting conjugated PEDOT from EDOT monomers include electrochemical polymerization, chemical oxidative polymerization, and vapor phase polymerization [21- 23].

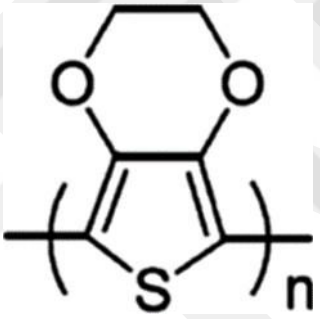


Figure 1.4: Chemical structure of PEDOT.

PEDOT, is an excellent candidate for the deposition of Ag nanoparticles. In addition to its outstanding environmental resilience, PEDOT polymer has good transparency and conductivity in thin oxidizedfilms, among other important properties [24]. It has been utilized in various types of advanced gadgets. The use of PEDOT and its nanocomposites seems to expand witheach breakthrough in synthesis [25, 26]. In 1988 chemically polymerized PEDOT was discovered. Its first commercial application was in photographic films as an antistatic layer, due to its ability to produce transparent and conductive films. As a result of PEDOT's success in other markets, including touch screens, solid electrolytecapacitors, packaging films, printed wiring boards (PCBs) and organic photovoltaics, and hole transport layers in organic light-emitting diodes (OLEDs), it has gained attention in other important fields, such

as and bioelectronics biomedical research [27]. As a result of its biocompatibility, mechanical characteristics, and strongelectrical and ionic conductivity, PEDOT can be utilized to destroy dangerous bacteria without causing harm to the human body, it is non-cytotoxic to cells and promotes cellular proliferation and differentiation [28].

### 1.2.1 The Evolution of PEDOT

It is the first conducting polymer to be reported with conductivities above 105 S $cm^{-1}$, close to the conductivity of copper, and transport characteristics equal to those of metal [29]. However, this material is sensitive to air and cannot be processed, making it unsuitable for industrial uses. There have been several attempts to overcome this problem by adding electron-donating heteroatoms such as nitrogen (N) or sulfur (S) either to the main chain or to the carbon substituents in heterocycles, but none of them have been successful. Researchers then focused on polyaniline, polypyrrole, and polythiophene [30- 32]. However, polythiophene derivatives have been produced that are more stable. The German Bayer Central Research Department wasparticularly active in its pursuit of stable conducting polymers, which are critical for industrial applications. Once their early attempts to stabilize polyacetylene failed andtheir work on polypyrrole was abandoned, they turned to monoalkoxy and 3, 4- dialkoxy substituted thiophenes to enhance stability, and to bicyclic rings to reduce steric hindrance [33]. The first attempts to synthesize 3,4-methylenedioxythiophene (MDOT) (Figure 1.5) failed due to the inability to separate a workable quantity ofthe monomer. They next turned their attention to 3,4-ethylenedioxythiophene (EDOT), which was an instant success. As a result of polymerization using an oxidative reagent, e.g. iron (III) chloride, a new conducting polymer, poly (3,4- ethylenedioxythiophene) (PEDOT), was discovered to be stable in its doped form andto have conductivities up to 200 S cm−1 [32, 34]. The synthesized PEDOT was immediately put to use in capacitors, as expected. They also studied the first electropolymerization of PEDOT in 1988, following the initial oxidative polymerization of PEDOT reported by Bayer AG researchers [35, 36].

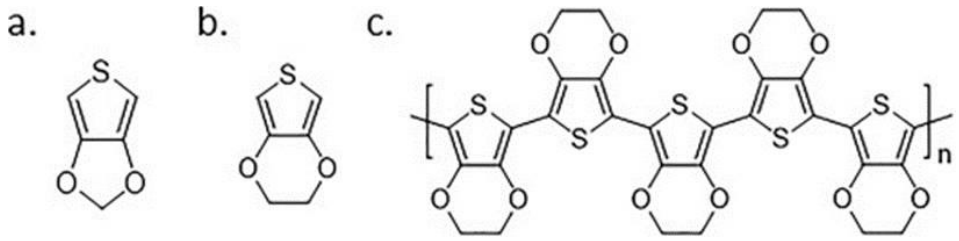


Figure 1.5: The structures of (a) MDOT, (b) EDOT, and (c) PEDOT.

Interestingly, PEDOT has high electrical conductivity, while still being stable in the air and water. Electrochemically produced PEDOT can only be created on conducting substrates. Whereas chemically manufactured PEDOT is black, infusible, and insoluble, an alternate option that could be processed was highly desired. Soon later, Bayer's scientists discovered it while working with Agfa's chemists who were searching for new antistatic coatings for photographic films. However, the sodium salt of polystyrene sulfonate (PSSNa), which they employed as an antistatic coating, was affected by humidity. They developed a stable PEDOT aqueous dispersion by oxidizing EDOT with persulfates and polymerizing in the presence of PSS in water. Subsequently, both industry and academia began to recognize the value of PEDOT: PSS and PEDOT as a whole [33, 37].

### 1.2.2 Chemical Properties of PEDOT

According to the researchers, there are just a few dozen monomer units in PEDOT chains. The scanning tunneling microscope (STM) of electro-polymerized PEDOT. The high angle annular dark-field (HAADF) scanning tunneling microscope of o-CVD PEDOT, and the transmission electron microscope (TEM) of solution-cast polymerized PEDOT have all shown short PEDOT chains with about 10 to 20 monomer units [38- 41]. When doped, PEDOT changes from an aromatic state to a quinoid state [Figure 1.6.].

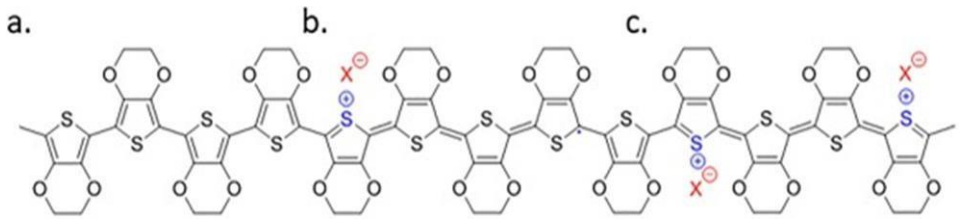


Figure 1.6: The structure of different states of doped PEDOT: (a) Neutral state; (b) Polaron state; and (c) Bipolaron state.

Polymerization achieves doping levels of around 33% (1 dopant for every 3 monomer units). NMR spectroscopy cannot be utilized to characterize PEDOT since it is insoluble in any common solvent. As a result, characteristics such as molecular weight are typically left undetermined. On thick or thin films, vibrational and electronic spectroscopy can be used to determine information such as elemental composition, energy gap, chemical state, or doping level [43, 33].

## 1.3 Nanoparticles

In the past few of years, a new term has emerged into the light and has become the focus point of the world's attention. This term is "nanotechnology", and this technology has resulted in a huge leap in all areas of science and engineering. In addition to numerous applications in the fields of economic, medical, electronic, informatics, computational, and petrochemical fields. In addition to military, biological, environmental, agricultural, and others. [43].

Nanotechnology is a modern technology that has been recognized as an advanced field in various installations, devices, and systems consisting of infinitesimal units, involving production, processing, and application. The idea of nanotechnology arose from the Greek word meaning dwarf, as nanoparticles defined the area of individual particles, whose dimensions do not exceed 100 nanometers. The unique characteristics of nanoparticles are due to their small size as well as their composition and surface structure. This unique characteristic and the physical changes of different chemicals at the nanoscale have resulted in a real increase and influential applications in industry and medicine [44].

Although nanotechnology is quite recent, the presence of devices with nano dimensional objects is not new, the existence of nanostructures goes back to the Earth's age and the start of life on it living cells are a significant example of natural nanotechnology. There are known to be a few extremely small devices produced in the human body which exceed the limits of the nanoscale. A live cell is a key reservoir for a vast number of biological nano-sized devices called ribosomes that make cell proteins as coupled lines that are formed by another nanoscale apparatus called the Golgi apparatus. In addition, the enzymes themselves are nanoscale machines which activate chemical processes according to cell requirements, thus the manufactured nanomachines to interact inside the cell and perform different functions, such as the delivery of a drug, analysis of cell content, or its destruction when harmful [45].

With the increase in the development of nanoscience and its use in most fields, the interest in using nanoparticles in medicine as an alternative method to control the surface properties to prevent bacterial attachment, especially to hospital equipment, has increased. Among those nanoparticles is the silver nanoparticle, which is one of the most common nanoparticle types known for multiple applications at lower cost, in addition to possessing the anti-efficacy of a wide range of antibiotic resistant pathogenic bacteria, as some of these antibiotics have become useless in treating diseases, especially acute and chronic diseases, such as excessive use of antibiotics, or an overdose of the permissible, or Cutting off the treatment without completing it allowed the bacteria to become resistant to these antibiotics, which led to the expansion and development of bacterial resistance to the point that resulted in strains resistance to all types of antibiotics available in the treatment [1].

### 1.3.1 Silver Nanoparticles (AgNPs)

Silver is a white, glossy transition metal that is both hard and soft with a high thermal and electrical conductivity rating. Silver, either alone or in combination with other techniques, has been recognized for its medical and therapeutic advantages, which have been recognized for longer than recorded history, even before the discovery of microorganisms as infectious agents. In addition to coins, vessels, and solutions, it is employed in sutures, foils, and collides as lotions and ointments, among other applications. For infectious illnesses and surgical infections, it is a leading therapeutic

agent in the field of medicine. Compared to risk factors, silver has greater advantages [46, 47]. To enhance the physical, chemical, and biological characteristics of Ag, silver nanoparticles (AgNPs) can be produced via nanotechnology, Exploring AgNPs' antibacterial properties arose as a natural consequence of the development of nanotechnology [48, 49].

Silver nanoparticles (AgNp) have attracted a lot of attention in the field of nanomedicine due to their unique physicochemical characteristics, which include chemical stability, high thermal and electrical conductivity, and enhanced surface Raman scattering [50]. They also have a wide spectrum of bactericidal and fungicidal characteristics [51], making them useful in a wide range of products, from textiles and plastics to targeted drug delivery systems. Scientists and industry have been drawn to AgNPs because of their remarkable antibacterial properties. Many infectious and harmful species, including multidrug-resistant bacteria, are susceptible to the antibacterial action of AgNPs [52, 53]. However, even though AgNPs have been intensively studied for their very strong antibacterial activity, their mechanism of action is still unclear. Indeed, the powerful antibacterial and broad-spectrum action against morphologically and metabolically diverse microorganisms appear to be associated with a complex mechanism by which nanoparticles interact with microorganisms. Moreover, their specific shape and the diverse forms of contact with bacterial surfaces may give a novel antibacterial mechanism that has not been fully explored yet. From a structural standpoint, AgNPs have at least one dimension in the range of 1 to 100 nm, and more significantly, as particle size lowers, the surfacearea-to-volume ratio considerably rises. As a result, the bulk material's physical (i.e. increased absorption or diffusion rates), chemical (i.e. increased reaction rate), and biological characteristics (i.e. accumulation within the cell tissue) are dramatically different [54].

### 1.3.2 The Development of Silver Nanoparticles in Medicine

When ancient Romans and Phoenicians (before the 5th century) kept drinking water in silver vessels, its antibacterial qualities were well-known. Until the discovery of antibiotics, Von Naegele is thought to be the first individual to utilize silver as an

antibacterial or anti-infective agent [55, 56]. During the time of Hippocrates (about 300 BC), silver compounds were used to cure ulcers and speed up wound healing. Antibacterial uses of silver before the 20th century included eye drops for newborns against gonorrhea and other bacterial/fungal infections, silver baths to prevent biofilm formation in burn patients and the treatment of syphilis and brain infections with silver salvarsan, among other uses for ionic silver during this time period. Even today, silver nitrate eye drops, which include silver, are used to treat infections. Because silver was cytotoxic and affected living animal tissues to a certain extent, the use of antibiotics replaced silver as the preferred therapy. Most medications, however, have led to the creation of antibiotic-resistant bacterial strains in the past ten years, decreasing their efficiency. [57, 58]. Since antibiotics were no longer effective in treating infections, silver nanoparticles and their antibacterial capabilities were found. Despite being non-cytotoxic, it has been demonstrated to be antibacterial (affecting both gram-positive and gram-negative bacteria), antiviral, and antifungal [56, 58]. Despite the fact that the specific mechanism of action for silver's antibacterial activity is unknown, the application has been extensively investigated and documented. Today's studies are primarily focused on verifying AgNP's efficacy and safety so that it may be used in a therapeutic environment to combat germs. Understanding the mechanism of antibacterial action, on the other hand, might assist in minimizing the limits of silver and AgNPs. However, the interactions between AgNP and microorganisms (such as bacteria) are highly dependent on both their surface composition (e.g. gram-negative bacteria or gram-positive bacteria) and their outer membrane characteristics (e.g. gram-negative bacteria or gram-positive bacteria) [56]. By providing strength, shape, and rigidity, the bacterial cell wall is meant to protect the cell against osmotic rupture and mechanical damage. It is possible to classify bacterial cell walls based on whether they are gram-positive or gram- negative. Chemically and physically, gram-negative bacteria have more complicated cell wall structures compared to gram-positive bacteria. Cell membranes of gram-negative bacteria have lipopolysaccharides that boost their negative charge and help to preserve the bacterium's structure (Figure 1.7) [59]. In addition, gram-negative bacteria are more resistant to hydrophobic substances (e.g. detergents). Like photo-sensitizers, the structure of the bacterial cell wall influences the capacity of nanoparticles to enter the cell wall and cause damage [58-61].

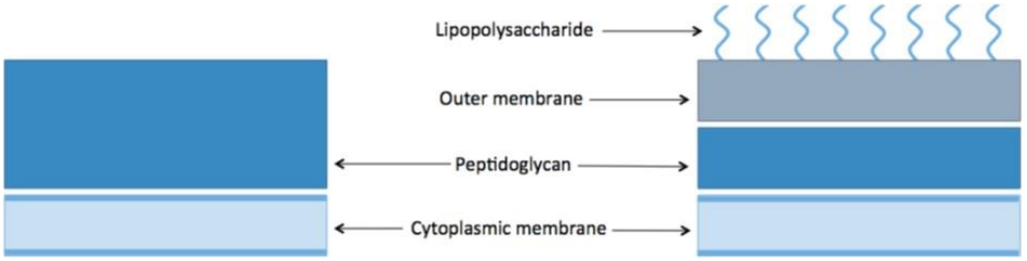


Figure 1.7: The schematic representation of the cell wall structure of gram-positive and gram-negative bacteria.

Bacterial sensitivity to nanoparticles can be influenced by other variables, such as the kind of nanoparticle and the rate of bacterial growth. Smaller, faster-growing bacteria are more vulnerable to nanoparticles, probably because the expression of stress-response genes influences a slow-growing bacterium's tolerance [62-64]. For example, studies have revealed the AgNPs were more effective in killing gram-negative bacteria than they were against gram-positive. Their investigations indicated that the minimum inhibitory concentration (MIC) needed for gram-negative bacteria (E. *coli*) was ten times lower than that necessary for gram-positive bacteria (S. *aureus*) [65].

### 1.3.3 Antibacterial Mechanism of Silver Nanoparticles

There is still a lot to learn about nanoparticles' antimicrobial mechanism. Numerous methods of killing bacteria have been described utilizing nanoparticles, including disruption/penetration of the cell, electrostatic contact, or generation of reactive oxygen species (ROS) that cause permanent damage and ultimately cell death (Figure 1.8) [66- 68].

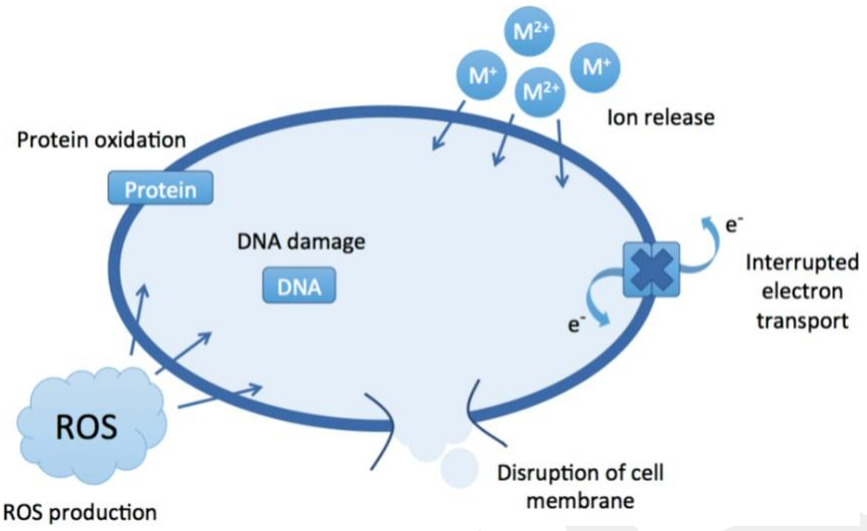


Figure 1.8: The possible mechanisms of nanoparticles bacterial toxicity: Several methods of nanoparticle toxicity against bacteria have been proposed, including ROS generation, disruption of the cell membrane, ion release, and protein and DNA damage.

Antibacterial activity of silver nanoparticles (Ag NPs) depends on the particles size and is mediated via ROS generation, which leads to structural alterations in the cell membrane and, eventually, cell death. Although the release of silver ions is thought to be the basic proposed mechanism of action of Ag NPs, there are other theories. Ag nanoparticles have been found to be highly efficient in inhibiting the formation of biofilms in E. *coli* and S. *aureus* while exhibiting no substantial cytotoxicity in mammalian cells [69, 70].

## 1.4 Bacterial Resistance

Bacteria are single-cell organisms found both inside the human body and outside it. Bacteria may be widely categorized into the categories of gram-positive and gram-negative bacteria based on their cellular composition. The major difference between them is that gram-positives have a thick exterior peptidoglycan protective layer with no lipid membrane, whereas gram-negatives have a thin peptidoglycan layer and have an outer lipid membrane [71, 72]. *Staphylococcus aureus* is one example of a gram-positive bacterium, while *Escherichia coli* is an example of a gram-negative bacterium. Actually, most microorganisms are really useful. The *Lactobacillus*

*acidophilus*, for example, the L.*acidophilus* in the intestines, can assist to digest food [73]. However, some pathogenic bacteria can produce diseases like strep throat [74].

In the 1930s, antimicrobial agents and antibiotics were discovered as drugs to treat bacterial infections. Specifically, antibiotics are used against bacteria to kill the bacteria or to create a hostile environment in which the bacteria cannot multiply anymore [75]. A crucial issue in the treatment of infections has been reached over 90 years later since new medicines are not being created quickly enough to maintain the natural ability of bacteria to adapt as they protect themselves against antibiotics [76]. As a result, the most potent medicines are becoming ineffective and antibiotic resistance is becoming one of the world's biggest health problems. Every year, nearly 2 million Americans are infected with multidrug resistance infections, which result in approximately 99,000 deaths [77]. Gram-negative bacteria a high number of Hospital-Acquired Infections (HAIs) are caused by multi-drug resistant bacteria, including urinary tract infections (UTIs), pneumonia, and bloodstream infections [78]. Patients with drug-resistant infections are more likely to have severe clinical consequences and die than those with non- resistant forms of the same bacterium, and they require more hospital resources [77].The bacteria's ability to resist the effects of a medication consequence of whicheither bacterial death or the stopping of bacterial development, is called antibiotic resistance. In the presence of antibacterial products, these resistant pathogens can live so normal therapy becomes ineffective, enabling infection to spread and danger to others [79]. Our capacity to cure common diseases decreases with emerging resistancemechanisms, leading to disability, long-term disease, higher cost, and a higher chance of death [80]. The process of antibiotics resistance shown in figure 1.9 [81].

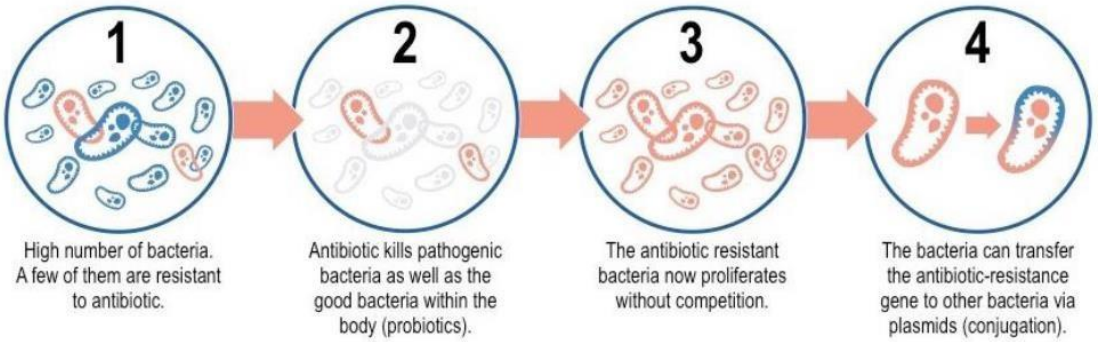


Figure 1.9: The schematic representation of antibiotics resistance: An infographic from the CDC briefly shows the process through which the resistance of antibiotics takes place.

Three basic mechanisms scientifically established by which bacteria gain resistance: (1) Enzymatic breakdown of antibacterial medications; (2) changes in the permeability of membranes to antibiotics; and (3) bacteria's altering of antimicrobial target characteristics [82].

Antibiotic resistance is caused by inappropriate application, as medicines are not given at the right time and dosage and needlessly provided (i.e. for viral or already resistant bacterial infections). People may use residual antibiotics from prior prescriptions and prescription drugs in order to quickly relieve the symptoms of their disease, regardless of the cause [83]. Furthermore, the misuse and use of antibiotics that are unnecessary for therapy is commonly prescribed for patients [80]. Antibiotic resistance cycle is shown in figure 1.10 [70].

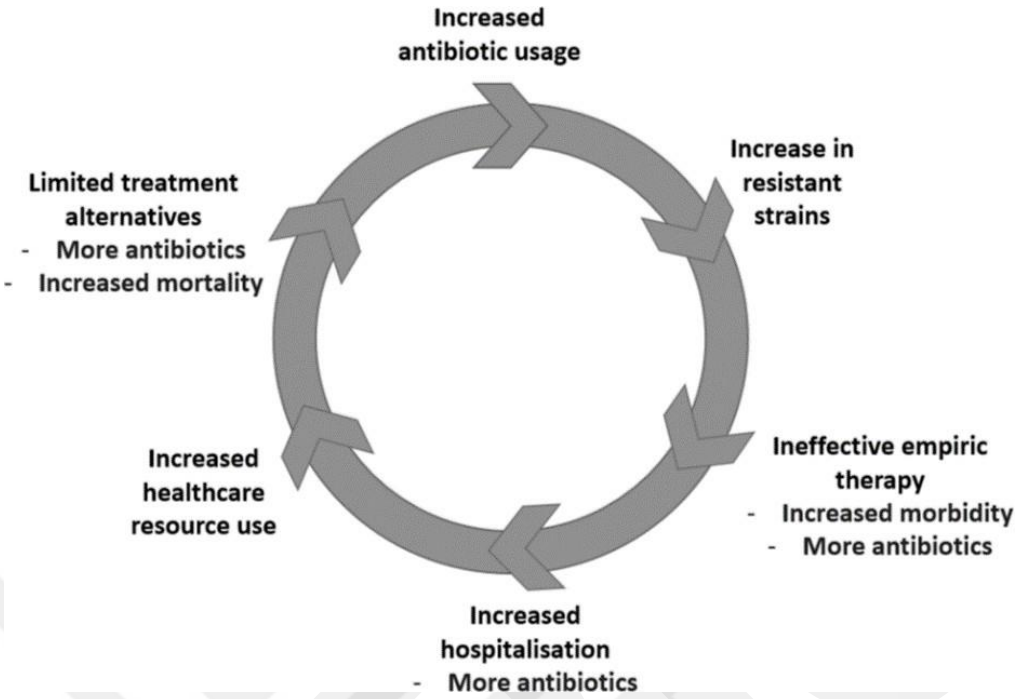


Figure 1.10: Antibiotic resistance cycle.

### 1.4.1 Common Multi-Drug Resistant Bacteria

Globally, antibiotic resistance in public health care systems has become a serious problem. Some bacteria are naturally resistant to specific types of antibiotics, but the majority of bacteria acquire resistance through genetic mutations (changes either in their chromosomal DNA/RNA) or by obtaining resistance genes from other bacteria or drug overuse. These characteristics are passed on when bacteria mate. Over the course of a lifetime, bacteria can develop a variety of resistance characteristics, making them resistant to different types of medicines [84]. Among those bacteria, *Staphylococcus aureus* and *Escherichia coli* represent the greatest threat to human health due to their increasing resistance to antibiotics.

In humans and other animals, E. *coli*, which is a gram-negative bacteria, is found in the intestines, where it is required for bowel function. In contrast, septicemia and meningitis can be caused by it when it enters our bloodstream or body tissues. You may find it in a wide range of medical products like humidifiers, endotracheal tubes,

and other medical devices [72, 85]. 1 in 2000 instances of newborn meningitis is caused by E. *coli*, which enters the circulation from the nasopharynx and travels to the meninges. Bloody diarrhea is the most common complication since some E.*coli* strains can cause intestinal illnesses. The bacteria E. *coli* is also responsible for 90% of urinary tract infection (UTIs). As a result of E-*coli* infection, some people, particularly children under the age of five, are more likely to develop hemolytic uremic syndrome, a condition that destroys red blood cells and leads to kidney failure [70, 86].

When it comes to bacterial infections, *Staphylococcus aureus* is a gram-positive bacterium, often known as S. *aureus*, is one of the most common disease-causing bacteria. Infections such as wound infections, osteomyelitis, endocarditis, and septicemia are frequent both in the community and in hospitals, and treatment is difficult owing to the advent of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*) [87]. S. *aureus* may be found in the environment as well as in the typical human flora on the skin and mucous membranes (most frequently the nasal region) of most healthy people. However, if it enters the circulation or internal tissues, it can cause various diseases that might be life-threatening. In most cases, transmission occurs when two people come into close touch. Some illnesses, on the other hand, can be transmitted in different ways [88, 89].

### 1.4.2 Hospital-Acquired Infections

Hospital-Acquired Infections (HAIs) are infections that arise in patients at the hospital or in healthcare facilities that were not present during admission and are also called nosocomial infections. Infections can occur both in the hospital and after discharge [90]. Since 1980, the incidence of HAI has increased rapidly due to the development of multidrug-resistant bacteria [91], developed as well as developing countries worldwide. In developed countries, 7 out of every 100 hospitalized patients will have at least one HAI, while 10 in developing countries [92]. Over 1.4 million people suffer from lower respiratory tract infections surgical wounds, and urinary tract infections (UTIs) acquired in hospitals [93]. Different pathogenic agents attach themselves to medical devices and implants, such as venous and urinary catheters, which lead to

increases in hospital costs and patient discomfort when changed [94]. Healthcare workers can potentially take the virus via direct contact with an infected patient or a colonized surface. Once the surfaces are contaminated, bacterial contamination is also a risk to patients and surrounding surfaces in close proximity, as they may serve as a reservoir of pathogenic bacteria in the environment [95]. Several factors increase HAIs are shown in figure 1.11 [95].

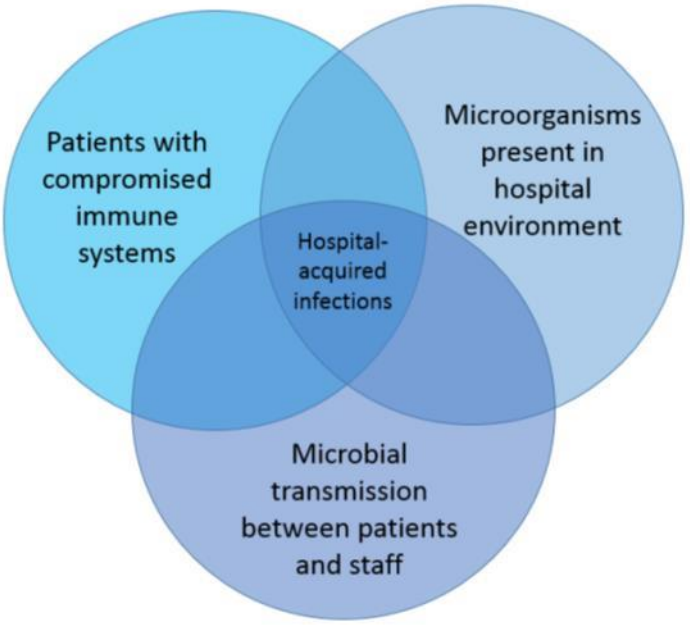


Figure 1.11: Factors affecting the spread of bacteria in hospitals.

Hundreds of millions of people each year are affected by the endemic burden of HAIs, causing disabilities, emotional stress, and lower quality of life [96]. It is also one of the leading causes of death, resulting in significant financial and economic costs for healthcare [51]. Prolonged hospital stays of infected patients are a major contributor to these expenses, which also increase the demand for medicine, isolation, and usage of other laboratory equipment. The emergence of new microbes and increased antibiotic resistance will make nosocomial infections much more of a problem for public health [97]. This makes the search for a new treatment that is more effective than the common treatments an absolute necessity, figure 1.12 explains the role of antibacterial surface in decreasing HAIs [51].

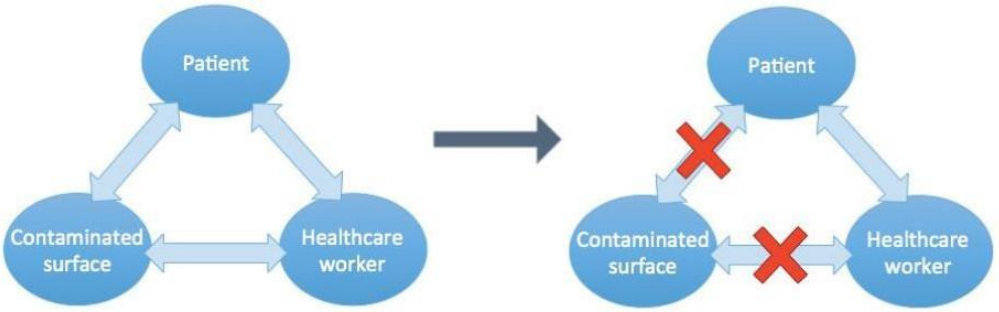


Figure 1.12: Antibacterial surfaces role in preventing HAIs from the direct transfer of microorganisms.

## 1.5 Antibacterial Study of PEDOT- AgNPs

The effect of antibacterial PEDOT: PSS-AgNP is tested against *Streptococcus mutans* and*Escherichia coli* to represent two common oral pathogens, gram-positive and gram-negative, respectively, through a study conducted in 2019 the results show that these permanent antifungals and antibacterial coatings can help to prevent bacterial infections when applied to orthodontic appliances of stainless steel [2].

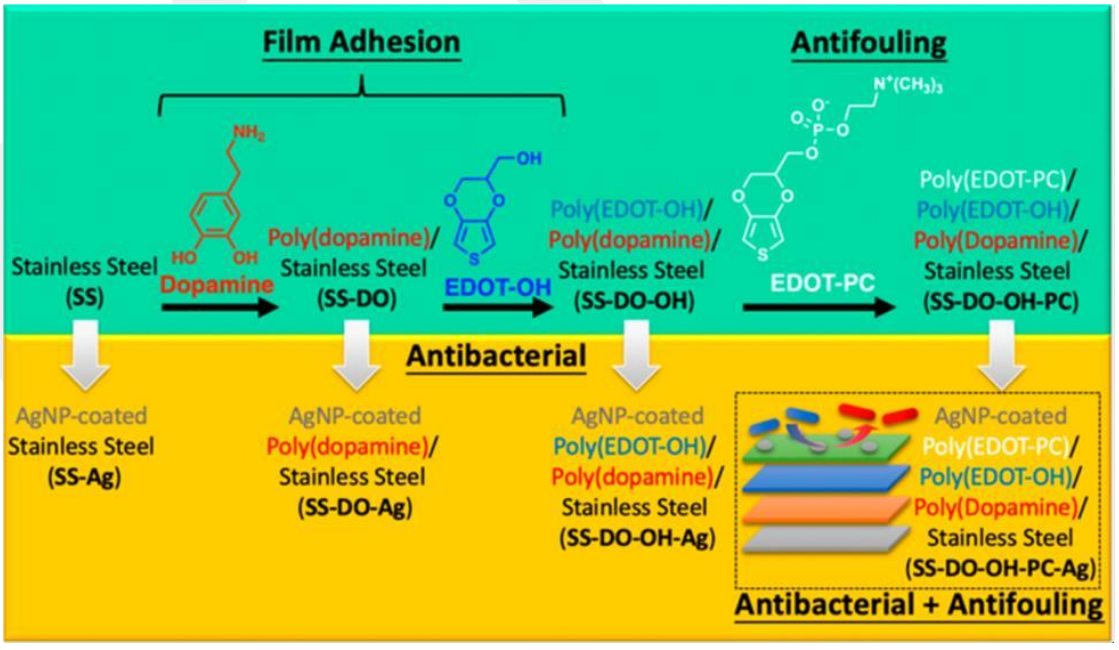


Figure 1.13: Polydopamine and Poly (EDOT-OH) Adhesion Enhancement, Zwitterionic Poly (EDOT-PC) Surface Antifouling, and Silver Nanoparticles (AgNPs) Antibacterial Coating.

As shown in figure 1.13, PDA and poly-(EDOT-OH) layers were used to increase adhesion, while zwitterionic poly (EDOT-PC) layers were used to reduce bacterial binding, making this nanocomposite coating a successful method to reduce bacterial growth on stainless steel with no harmful effect on the human body [2].

# CHAPTER 2

# EXPERIMENTAL

## 2.1 Materials

In the preparation of polymer, 3, 4 ethelenedioxythiophene (EDOT), iron (III) chloride, unhydrous ($FeCl_3$), chloroform ($CHCl_3$) was used. The addition of silver nanoparticles was performed with silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$). Lysogeny Broth (LB) media, Chromogenic TBX agar and Baired Parker Agar were used (Laborlar Biyoteknoloji, Istanbul, Türkiye) for bacterial studies.

## 2.2 Characterization and Instrumentation

Quanta 400 FE-SEM from FEI, Field Emission Scanning Electron Microscopy (FE-SEM) was first performed to identify the morphology of the produced particles.
Transmission Emission Microscopy (TEM) with a JEOL JEM-2010F was utilized for the thorough characterization to determine the particle size and shape. With a maximum accelerating voltage of 200 kV, the device is an advanced field emission electron microscope. SEM and TEM were used in conjunction with an energy dispersive X-ray analyzer (EDX) to determine the elemental composition of the particles. SEM, TEM, and EDX studies were performed by dropping sample suspensions onto grids covered with carbon tape. The samples were then dried overnight before being examined. With the use of ICP-OES, Elan, and Perkin Elmer, it was possible to determine the silver loading percentage. Press (Optosense) was used to prepare NPs pellets. $CO_2$ incubator (New Brunswick), centrifuge (Hettich) and temperature controlled shaker were used for bacterial growth. Antibiotic discs (Sigma) were used for growth inhibition analysis.

## 2.3 Preparation of PEDOT

PEDOT was synthesized via chemical polymerization in the presence of EDOT and iron (III) chloride ($FeCl_3$).0.550 g Iron (III) chloride ($FeCl_3$) was first dissolved in 5 ml of $CHCl_3$. EDOT was then added to 5 ml of $CHCl_3$ in a separate beaker. On a magnetic stirrer, the first solution was added to the monomer solution drop by drop. PEDOT was obtained by mixing the final mixture for 3 hours at room temperature. In order to precipitate the polymer, 30 mL of methanol was added to the mixture at the end of this period, and the mixture was then refrigerated for 24 hours.

Soxhlet extraction apparatus was used to wash filter paper with methanol for two days, removing the precipitated PEDOT from the solution after it had been recovered by filtering. At room temperature, the product was dried. Figure 2.1 illustrates the preparation process.

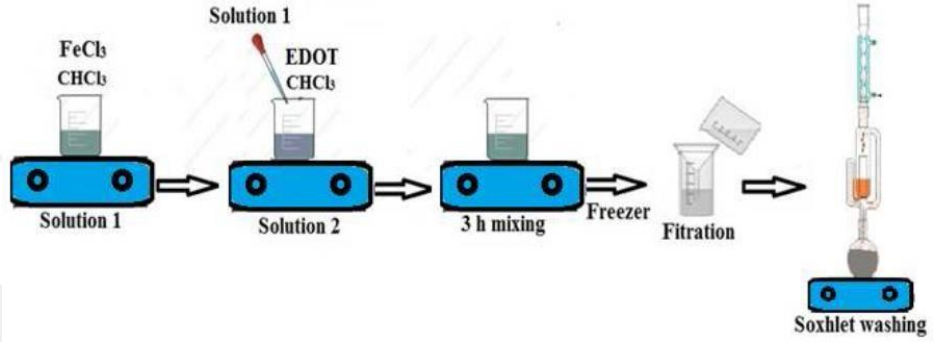


Figure 2.1: PEDOT is prepared through chemical polymerization.

## 2.4 Addition of Silver Nanoparticles onto PEDOT

In this section, silver nanoparticles were attached to PEDOT (PEDOT-AgNPs) materials. Silver ions were fixed onto PEDOT using a liquid impregnation technique. Initially, 100 mg of PEDOT was added to 10 mL of water and dispersed by mixing using a magnetic stirrer. Solid silver nitrate ($AgNO_3$) was added to the original dispersion at a rate of 3% (w/w) and blended for an additional 5 hours. Using an external magnet, the silver ions bound to PEDOT were separated from the mixture.

After that, water was used to wash away the nonattached silver ions and other contaminants from the separated particles. In order to determine the ultimate loading quantity of silver ions onto the PEDOT composite using ICP-OES analyses, supernatants were collected during the separation and washing stages, The silver ions ($Ag^{+}$) were reduced using sodium borohydride ($NaBH_4$) after loading. This was achieved by dispersing silver ion-loaded PEDOT composite material in 10 mL of water. NaBH4 was then added to the dispersion and stirred for 1 hour with magnetic stirring. Afterward, the final particles were collected and rinsed multiple times with deionized water. The particles were then dried and utilized in photocatalytic studies. The loading method is shown in Figure 2.2.

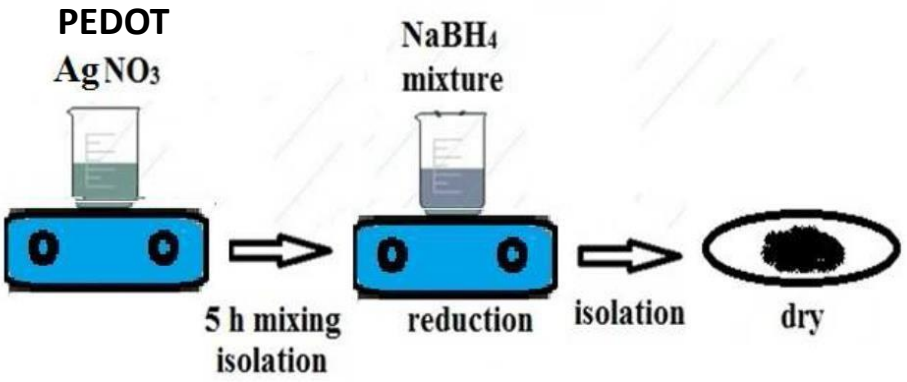


Figure 2.2: Addition of silver nanoparticles to a composite compound consisting of $SiO_2$-$CoFe_2O_4$/PEDOT

## 2.5 Pellets Production

The nanoparticles were used to make the pellets since the NPs are insoluble in most solvents. To make pellets, the press shown in the image below was employed (Optosense, Orlando, Florida, USA). The prepared pellets were then applied onto the plate previously inoculated bacteria together with the antibiotic disc which contains 20 uL penicilline / streptomycine (Pen/strep) antibiotics.

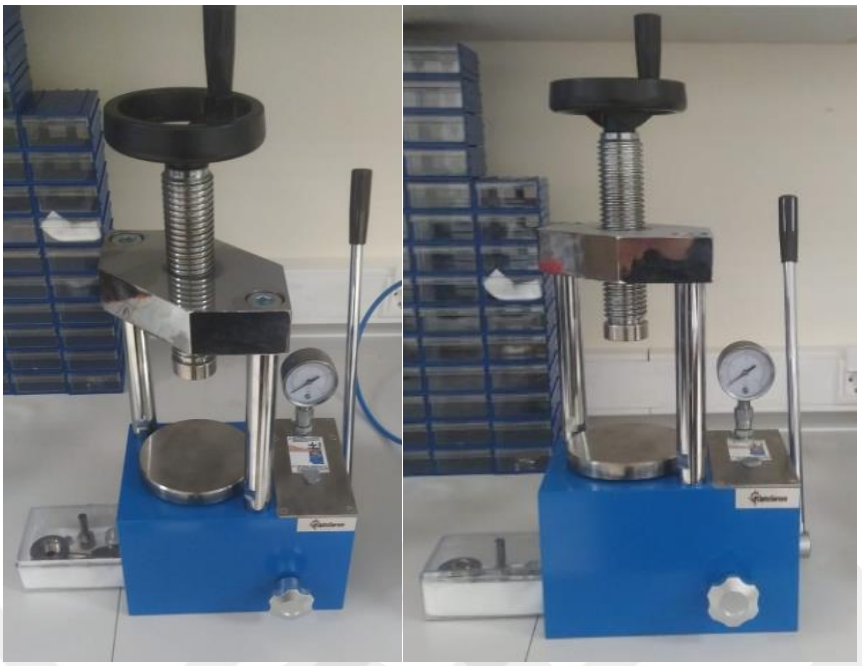

Figure 2.3: A press is used to prepare nanoparticle pellets.

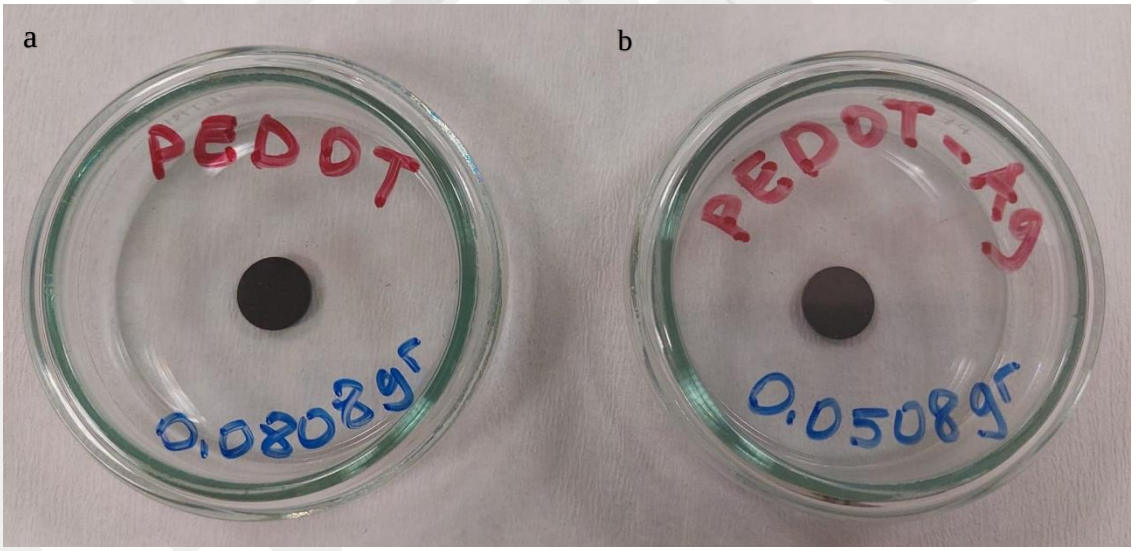


Figure 2.4: pellets of (a) PEDOT with 0.0808g weight, (b) PEDOT-Ag with 0.0508g weight.

## 2.6 Bacterial Growth and Antibacterial Study Protocol:

A single colony was taken from a newly streaked plate and used to inoculate a starter culture in sterile flasks with 3 to 5 mL of Lysogeny Broth (LB) medium. The flasks are incubated at 37 °C for 8 hours while shaking at 250-300 rpm. To inoculate an

overnight culture, the starting culture was used. In a large flask with the necessary volume of medium, the starting culture was diluted 1:500 to 1:1000 and incubated for 12 to 16 hours at 37 °C while shaking at 250-300 rpm. From this media the concentration were calculated by OD measurement. And then the concentration of bacteria were diluted to $2.5X10^{4}$ colonies. All these studies were performed by using sterile medium and under aseptic conditions.

S. *aureus* (ATCC 29213) and E.*coli* (ATCC 25922) were grown in LB medium. In the laboratory, E.*coli* and S.*aureus* bacteria were cultured in Lysogeny Broth (LB) agar under an aseptic environment, the bacterial colonies were diluted to $2.5X10^{4}$ colonies using LB agar and inoculated.

Commercially available Chromogenic TBX agar and Baired Parker Agar were used (Laborlar Biyoteknoloji, Istanbul, Türkiye), the Chromogenic TBX (Tryptone Bile X-glucuronide) agar was inoculated with E.*coli,* while Baired Parker Agar was used to inoculate S.*aureus*.

Antibiotic discs were used to apply antibiotics as reference for antibacterial study.

The discs were placed on to the plate previously inoculated by bacteria and the 20 uL antibiotic (pen/strep) were applied on to the discs. The growth inhibition zone of nanoparticles and antibiotics were compared.


Figure 2.5: a) Agar plates are used to inoculate bacteria. b) E.*coli* and S.*aureus* are grown in LB Medium.

# CHAPTER 3

# RESULTS AND DISCUSSION

PEDOT, $SiO_2$ $CoFe_2O_4$/PEDOT, and $SiO_2$-$CoFe_2O_4$/PEDOT-AgNPs nanocomposite materials were analyzed structurally and morphologically using SEM, transmission electron microscopy, and energy-dispersive X-ray spectroscopy (EDX) in combination with TEM and SEM.

## 3.1 The SEM and EDX Measurement of PEDOT

The morphological characteristics of PEDOT was done by using SEM. (Figure 3.1)

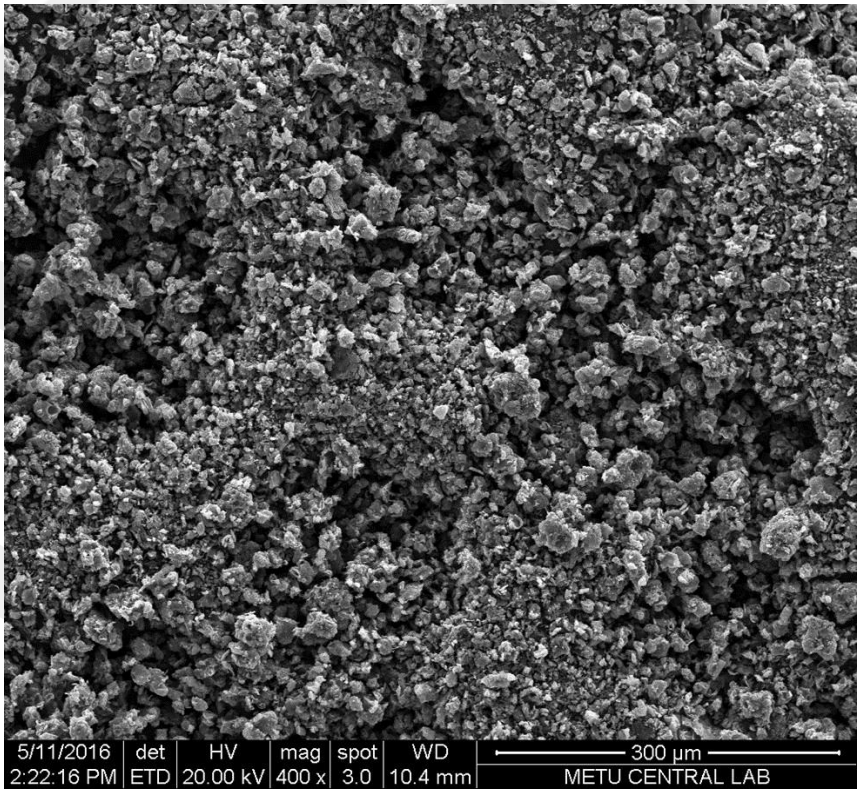


Figure 3.1: PEDOT as seen in a SEM.

From the result it has concluded that the particles width is 10.4mm on spot size 3.0 under 400x magnification with focus depth of 300 µm and accelerating voltage for the electrons (HV) equals 20.00kV

EDX was used to determine the elemental composition of the produced polymer.

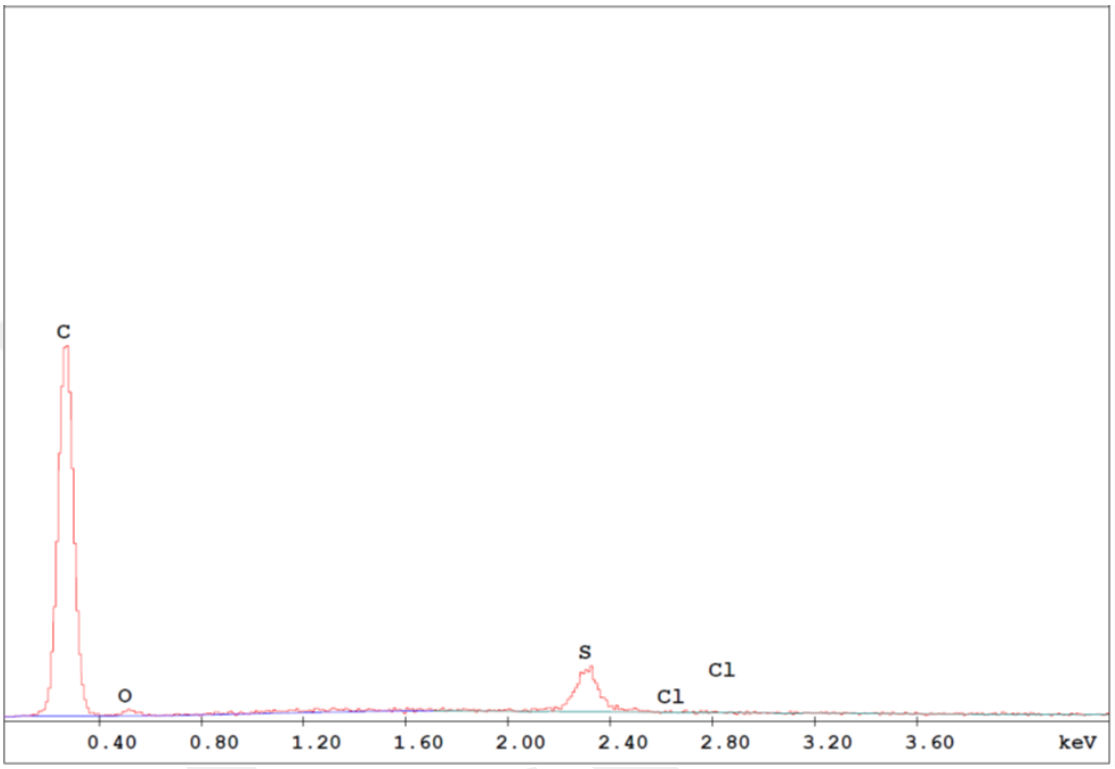


Figure 3.2: PEDOT EDX pattern.

In the EDX pattern, we can detect the distinctive peak of PEDOT linked to a sulfur-based structure. The chlorine peak is due to the FeCl3 employed in PEDOT's preparation.

### 3.2 The TEM and EDX Measurement of PEDOT-AgNPs

Last but not least, the addition of silver nanoparticles to PEDOT was studied using both TEM and EDX. Figures 3.3 and 3.4 show the findings obtained from the experiment.

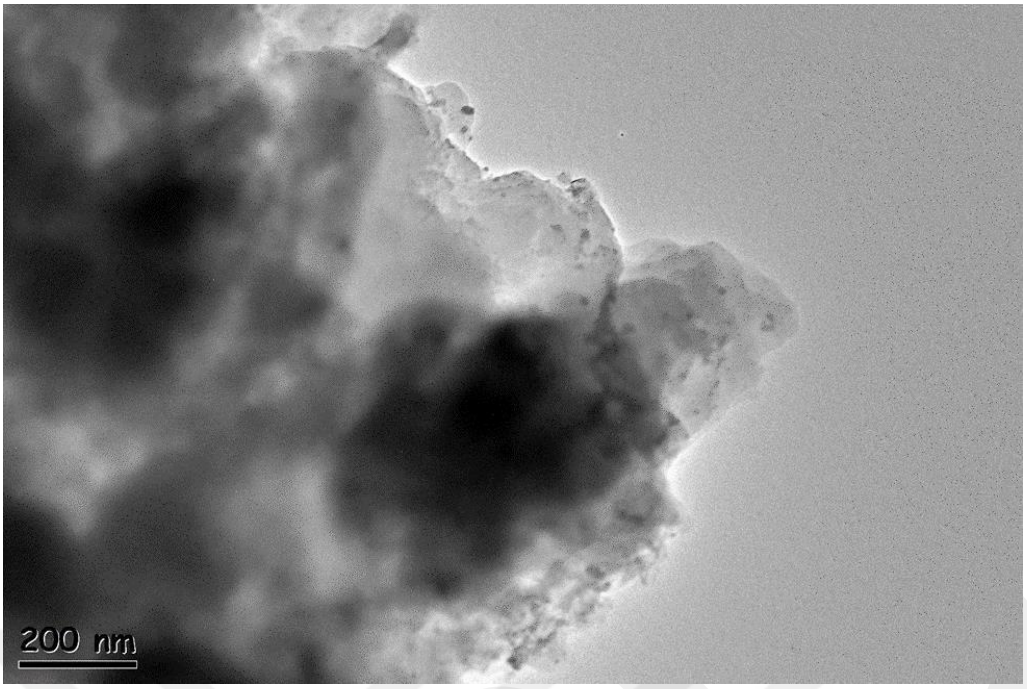


Figure 3.3: PEDOT-AgNPs composite material seen under a TEM.

TEM tests confirmed the addition of silver nanoparticles to the PEDOT-AgNPs range in size from 5 to 10 nm. The PEDOT polymer holds the whole structure together while providing a surface for the insertion of silver nanoparticles. To verify the presence of silver in the final construction, an EDX measurement was done afterward. Figure 3.4 shows the obtained EDX pattern.

The EDX pattern of PEDOT AgNPs reveals the presence of S and Ag. ICP-OES measurements were also used to determine the final amount of silver ions loaded onto PEDOT after the addition of AgNPs. In accordance with the acquired ICP-OES results, the PEDOT-AgNPs composite material contains 1.78% (w/w) silver metal.

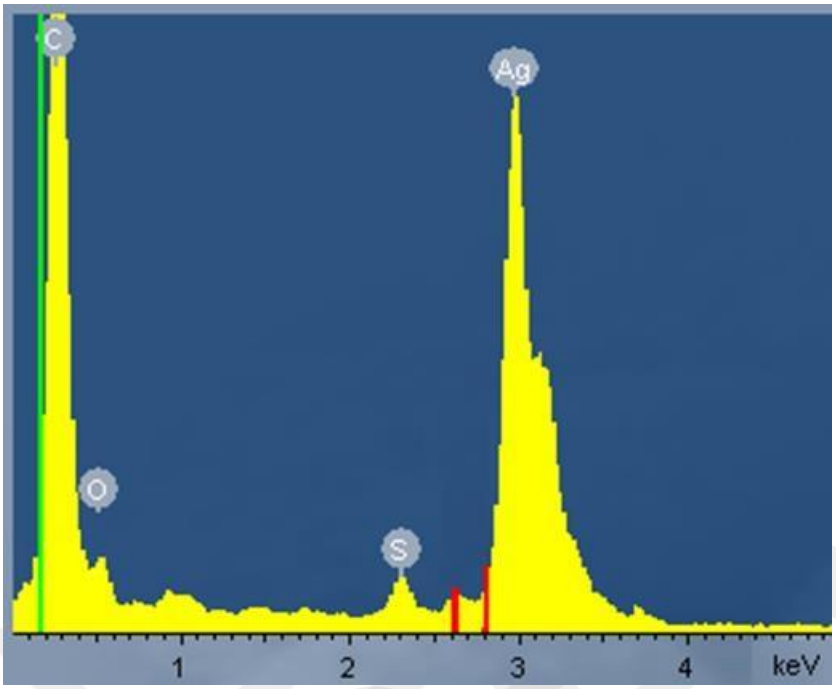


Figure 3.4: PEDOT-AgNPs EDX.

This data obtained was supported by the different previous studies which showed the structure and characterization of PEDOT, AgNPs, and PEDOT-AgNPs [98, 99].

### 3.3 Antibacterial Effect of $AgNO_3$

As a reference for antibacterial effect the antibiotic penicilline / streptomycine were chosen. To show the antibacterial activity of Pen/Strep and as the reference material of nanoparticles , $AgNO_3$ was tested by using antibiotic discs.

The bactaria plate containing the antibiotic discs loaded with 20 uL of $AgNO_3$ (17ug/ml), and 20 uL antibiotics Penicilline/Streptomycin (Biological Industries, USA), were incubated overnight in incubator at 37 °C. The growth inhbition profile of reference $AgNO_3$ and pen/strep is given below (Figure 3.4).

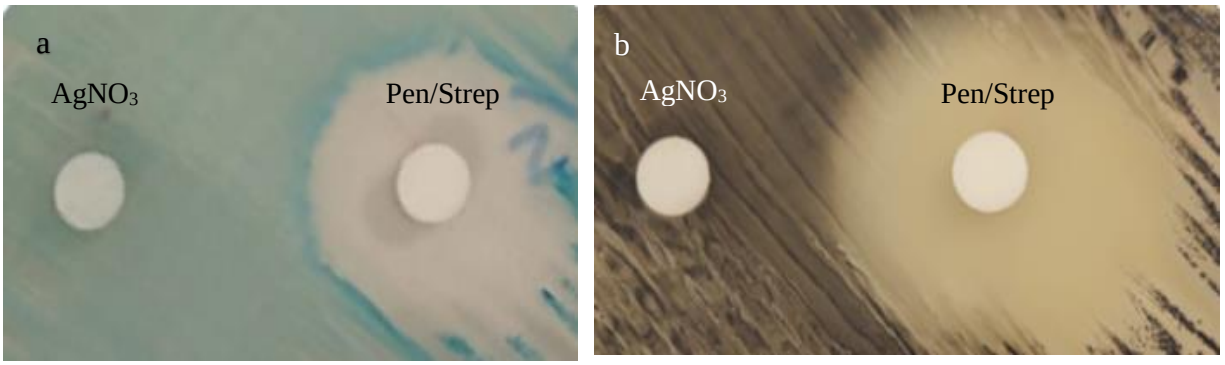


Figure 3.5: Antibacterial effect of $AgNO_3$ against: (a) *E.coli* (b) *S.aureus*

As shown in figure 3.5 there is no significant effect of $AgNO_3$ on growth inhibition of *E.coli* and *S.aureus* compared to the antibiotic that shows a clear inhibition zone on both bacteria.

Although it is known that $AgNO_3$ shows an antibacterial response against E.*coli* and S.*aureus*, the reason for no growth inhibition of the $AgNO_3$ zone was concluded that the concentration was not enough for $AgNO_3$ as a chemical compound with an ionic bond between the silver cation ($Ag^+$) and the nitrate anion ($NO_3^-$) not in nanoparticle form, as a nanoparticle the surface area to volume ratio is increased so the antibacterial effect will be magnified.

While the antibiotic pen/strep has a greater effect on S.*aureus* because of penicillin content which inhibits the formation of peptidoglycan layer of cell membrane of bacteria, making the cells leaky and weak. Furthermore, penicillin, like other beta-lactam antibiotics that attack the cell wall peptidoglycan, is ineffective against gram-negative bacteria such as E.*coli* because their chemical compositions prevent them from efficiently penetrating the outer membrane [100].

### 3.4 Antibacterial Effect of PEDOT and PEDOT-Ag

Effect of PEDOT-Ag pellet on S.*aureus* and E.*coli* bacterial colonies were tested by placing the pellets directly on to the plate (by the same principle of disc diffusion method) and the results are represented by the inhibition zone around the pellet that are shown in figure 3.5 for S.*aureus* and in figure 3.6 for E.*coli*.

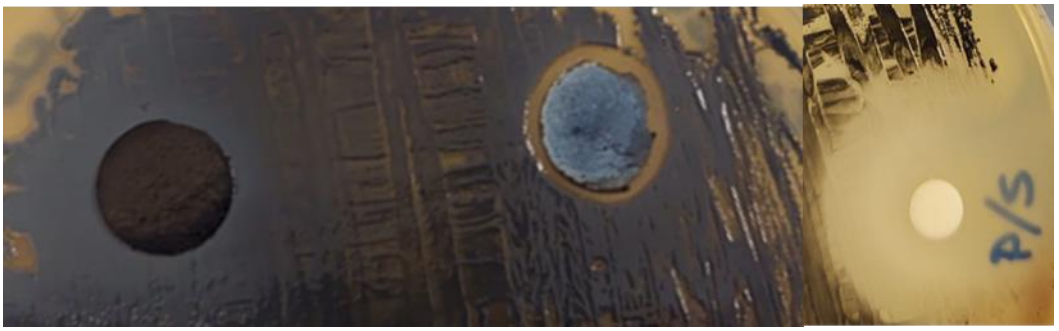


Figure 3.6: (a) PEDOT (b) PEDOT-Ag (c) Pen/strep (S.*aureus*)

Figure 3.7: (a) PEDOT (b) PEDOT-Ag (c) Pen/strep (E.*coli*)

As shown in figures, although the PEDOT pellets did not give a significant result in both cultures there is a tiny zone observed around the pellet on S.*aureus* plate. But there is a clear visible inhibition zone around the PEDOT-Ag pellets with a different zone size between S.*aureus* and E.*coli* cultures, clearly, the zone of inhibition is larger in E.*coli* culture than in S.*aureus,* that might be due to the bacterial structure and differences in cell wall composition between gram-positive and gram-negative bacteria. As it was explained previously in this study as well as other studies [101, 102] the results confirmed that the gram-negative bacteria (E.*coli*) have a higher antibacterial sensitivity compared to that of gram-positive bacteria (S.*aureus*).

Although there are several studies that show the antibacterial effect of PEDOT and AgNPs [103- 105], during this study the effect of PEDOT-AgNPs together was represented for the first time depending on different studies that showed the medical potential of both substances over time. Also, in this research, pellets were used instead of solutions or powder because pellets were easier to use and reusable. Pellets were exposed to UV light for 60 minutes before the antibacterial test to enhance antibacterial activity instead of visible light. Furthermore, this material has shown

antbacterial action against a significantly larger bacterial load than that observed in a hospital, with $2.5x10^4$ bacteria utilized in this study unlike an average of around 1 x $10^2$ CFU/$cm^{-2}$ in medical settings [106]

.

# CHAPTER 4

# CONCLUSION

For the first time PEDOT-AgNPs antibacterial activity were evaluated in this in vitro study since there is no study conducted on the antibacterial effect of PEDOT-AgNPs together before. PEDOT were successfully prepared using chemical polymerization procedure in the presence of EDOT and iron (III) chloride ($FeCl_3$) while the addition of Silver Nanoparticles on to PEDOT were done using liquid impregnation technique (Examples include impregnation fluids, dissolved polymers, hexadecane, vacuum pump oils, fluorocarbons, shear thinning fluids, shear thickeners, and others). Once all studies have been performed and data gathered from the antibacterial analysis by disk diffusion method (DDM) with PEDOT-AgNPs pellets, it can be stated that PEDOT-AgNPs showed significant antibacterial activity against selected gram-negative bacteria (E.*coli*) with less effect on gram-positive bacteria (S.*aureus*) due to the difference in cell wall composition. Thus, PEDOT-AgNPs might be a viable antibacterial coating material to reduce multidrug-resistant bacterial infection. PEDOT-AgNPs might be useful in antibacterial systems and medical devices, among other disciplines. Although additional studies are required to explore ways to make it an extremely strong technique against pathogens.

## REFERENCES


[1] Arvizo, Rochelle R, "Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future." *Chemical Society Reviews,* vol. 41. pp. 2943-2970, 2012.

[2] Lee, Bor-Shiunn, "Engineering Antifouling and Antibacterial Stain- less Steel for Orthodontic Appliances through Layer-by-Layer Deposition of Nanocomposite Coatings." *ACS Applied Bio Materials,* vol. 3. pp. 486-494, 2019.

[3] Sarih, Norazilawati Muhamad, "*Synthesis of Well-Defined Multi-End Functionalized Polymers via Living Anionic Polymerization*." Weinheim: Wiley-VCH Verlag GmbH & Company KGaA, 2010, pp. 1.

[4] So, Soonyong, and Timothy P. Lodge, "Size control and fractionation of ionic liquid filled polymersomes with glassy and rubbery bilayer membranes." *Langmuir,* vol. 32. pp. 4959-4968, 2016.

[5] Gomez-Carretero, "Salvador. Prevention of Bacterial Colonization in Hospital-acquired Infections Using Electrically Conducting Polymers. " PhD. thesis, Karolinska Institutet, Sweden, 2017.

[6] Cornell, Robert J., and L. Guy Donaruma. "2-Methacryloxytropones. Intermediates for the synthesis of biologically active polymers." *Journal of medicinal chemistry,* vol. 8. pp. 388-390, 1965.

[7] Vogl, Otto, and David Tirrell. "Functional polymers with biologically active groups." *Journal of Macromolecular Science—Chemistry,* vol. 13. pp. 415-439, 1979.

[8] Panarin, E. F., M. V. Solovskii, and O. N. Ekzemplyarov, "Synthesis and antimicrobial properties of polymers containing quaternary ammonium groups." *Pharmaceutical Chemistry Journal,* vol. 5. pp. 406-408, 1971.

[9] Tiller, Joerg C, "Antimicrobial surfaces," *Bioactive surfaces*, vol. 240. pp. 193-217, 2010.

[10] Tew, Gregory N., "De novo design of antimicrobial polymers, foldamers, and small molecules: from discovery to practical applications." *Accounts of chemical research*, vol.43. pp. 30-39, 2009.

[11] Timofeeva, Larisa, and Natalia Kleshcheva, "Antimicrobial polymers: mechanism of action, factors of activity, and applications." *Applied microbiology and biotechnology*, vol. 89. pp. 475-492, 2010.

[12] Siedenbiedel, Felix, and Joerg C. Tiller, "Antimicrobial polymers in solution and on surfaces: overview and functional principles." *Polymers*, vol. 4. pp. 46-71, 2012.

[13] Yu, Kai, "Engineering biomaterials surfaces to modulate the host response." *Colloids and Surfaces B: Biointerfaces*, vol, 124. pp. 69-79, 2014.

[14] Shahzad, Muhammad, "Selected dietary (poly) phenols inhibit periodontal pathogen growth and biofilm formation." *Food and function*, vol. 6. pp. 719-729, 2014.

[15] Duday, David, "Atmospheric pressure plasma modified surfaces for immobilization of antimicrobial nisin peptides." *Surface and Coatings Technology*, vol. 218. pp. 152-161, 2013.

[16] Yuen, John WM, Terence WK Chung, and Alice Y. Loke, "Methicillin-resistant Staphylococcus aureus (MRSA) contamination in bedside surfaces of a hospital ward and the potential effectiveness of enhanced disinfection with an antimicrobial polymer surfactant." *International journal of environmental research and public health*, vol.12. pp. 3026-3041, 2015.

[17] Arora, Ankita, and Abhijit Mishra, "Antibacterial polymers–a mini review." *Materials Today: Proceedings*, vol. 5. pp. 17156-17161, 2018.

[18] Gabriel, Gregory J., "Infectious disease: Connecting innate immunity to biocidal polymers." *Materials Science and Engineering: R: Reports*, vol. 57. pp. 28-64, 2007.

[19] Hussein, Mohamed A., "Electrochemical corrosion and in vitro bioactivityof nano-grained biomedical Ti-20Nb-13Zr alloy in a simulated body fluid." *Materials*, vol. 11. pp. 26, 2017.

[20] Luo, Shyh-Chyang, "Poly (3, 4-ethylenedioxythiophene) (PEDOT)

nanobiointerfaces: thin, ultrasmooth, and functionalized PEDOT films with in vitro and in vivo biocompatibility." *Langmuir*, vol. 24. pp. 8071-8077, 2008.

[21] Chen, Rui, "Sequential solution polymerization of poly (3, 4-ethylenedioxythiophene) using V2O5 as oxidant for flexible touch sensors." *IScience*, vol. 12. pp. 66-75, 2019.

[22] Kim, Jeonghun, Jungmok You, and Eunkyoung Kim, "Flexible conductive polymer patterns from vapor polymerizable and photo-cross-linkable EDOT." *Macromolecules*, vol. 43. pp. 2322-2327, 2010.

[23] Mantione, Daniele, "Poly (3, 4-ethylenedioxythiophene) (PEDOT)derivatives: Innovative conductive polymers for bioelectronics." *Polymers*, vol. 9. pp. 354, 2017.

[24] Taggart, David K., "Enhanced thermoelectric metrics in ultra-long electrodeposited PEDOT nanowires." *Nano letters*, vol. 11. pp. 125-131, 2010.

[25] DeLongchamp, Dean, and Paula T. Hammond. "Layer-by-layer assembly of PEDOT/polyaniline electrochromic devices." *Advanced Materials*, vol. 13. pp. 1455-1459, 2001.

[26] Long, Yun-Ze, "Recent advances in synthesis, physical properties and applications of conducting polymer nanotubes and nanofibers." *Progress in Polymer Science*, vol. 36. pp. 1415-1442, 2011.

[27] Lövenich, Wilfried, "PEDOT-properties and applications." *Polymer Science Series C*, vol. 56. pp. 135-143, 2014.

[28] Wu, Fan, "A self-assembly method for the fabrication of a three- dimensional (3D) polypyrrole (PPy)/poly (3, 4-ethylenedioxythiophene) (PEDOT) hybrid composite with excellent absorption performance against electro-magnetic pollution." *Journal of Materials Chemistry C*, vol. 4. pp. 82- 88, 2016.

[29] Naarmann, H., and N. Theophilou, "New process for the production of metal-like, stable polyacetylene." *Synthetic Metals*, vol. 22. pp. 1-8, 1987.

[30] Münstedt, H., "Ageing of electrically conducting organic materials." *Polymer*, vol. 29. pp. 296-302, 1988.

[31] Roth, Siegmar, and Maria Filzmoser, “Conducting polymers—thirteen years of polyacetylene doping.” *Advanced Materials,* vol. 2. pp. 356-360, 1990.

[32] Jonas, Friedrich, and L. Schrader, “Conductive modifications of polymers with polypyrroles and polythiophenes.” *Synthetic Metals,* vol. 41. pp. 831-836, 1991.

[33] Gueye, Magatte N., “Progress in understanding structure and transport properties of PEDOT-based materials: A critical review.” *Progress in Materials Science,* vol. 108. pp. 100616, 2020.

[34] Heywang, Gerhard, and Friedrich Jonas, “Poly (alkylenedioxyt hiophene) s-New, Very Stable Conducting Polymers.” *Verlag GmbH & Company KGaA,* vol. 4. pp. 116-118, 1992.

[35] Guerchouche, Kamel, “Conductive polymer based antenna for wireless green sensors applications." *Microelectronic Engineering,* vol.182. pp. 46-52, 2017.

[36] Istif, Emin, “Thiophene-Based Aldehyde Derivatives for Functionalizable and Adhesive Semiconducting Polymers." *ACS Applied Materials & Interfaces,* vol. 12. pp. 8695-8703, 2020.

[37] Jonas, F., and J. T. Morrison, “3, 4-polyethylenedioxythiophene (PEDT): Conductive coatings technical applications and properties.” *Synthetic Metals,* vol. 85. pp. 1397-1398, 1997.

[38] Greczynski, Grzegorz, Th Kugler, and W. R. Salaneck, “Characterization of the PEDOT-PSS system by means of X-ray and ultraviolet photoelectron spectroscopy.” *Thin Solid Films,* vol. 354. pp. 129-135, 1999.

[39] Jönsson, S. K. M., “The effects of solvents on the morphology and sheet resistance in poly (3, 4-ethylenedioxythiophene)–polystyrenesulfonic acid (PEDOT–PSS) films.” *Synthetic metals,* vol. 139. pp. 1-10, 2003.

[40] Lapitan Jr, Lorico DS, Bernard John V. Tongol, and Shueh-Lin Yau. “In situ scanning tunneling microscopy imaging of electropolymerized poly (3, 4-ethylenedioxythiophene) on an iodine-modified Au (1 1 1) single crystal electrode.” *Electrochimica acta,* vol. 62. pp. 433-440, 2012.

[41] Ugur, Asli, “Low-dimensional conduction mechanisms in highly conductive and transparent conjugated polymers.” *Advanced Materials,* vol. 27. pp. 4604-4610,

2015.

[42] Kirchmeyer, Stephan, and Knud Reuter, "Scientific importance, properties and growing applications of poly (3, 4-ethylenedioxythiophene)." *Journal of Materials Chemistry*, vol. 15. pp. 2077-2088, 2005.

[43] Keiper, Adam, "The nanotechnology revolution." *The New Atlantis*, vol. 2. pp. 17-34, 2003.

[44] Nasrollahzadeh, Mahmoud, "An introduction to nanotechnology." *Interface science and technology*. Vol. 28. pp. 1-27, 2019.

[45] Terstappen, Georg C., "Strategies for delivering therapeutics across the blood–brain barrier, "Nature *Reviews Drug Discovery*, vol. 20. pp 362-383, 2021.

[46] Firdhouse, M. Jannathul, and P. Lalitha, "Biocidal potential of biosynthesized silver nanoparticles against fungal threats." *Journal of Nanostructure in Chemistry*, vol. 5. pp. 25-33, 2015.

[47] Sharma, Sneha, Subrata Jain, and G. Tripathi, "Toxicological perspectives of fish with special reference to silver nanoparticles." *Journal of Experimental Zoology, India*, vol. 22. pp. 667-692, 2019.

[48] Yun'an Qing, Lin Cheng, "Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies." *International journal of nanomedicine*, vol. 13. pp. 3311, 2018.

[49] Bruna, Tamara, "Silver nanoparticles and their antibacterial applications." *International Journal of Molecular Sciences*, vol. 22. pp. 7202, 2021.

[50] Krutyakov, Yu A., "Synthesis and properties of silver nanoparticles: advances and prospects." *Russian Chemical Reviews*, vol. 77. pp. 233, 2008.

[51] Ahamed, Maqusood, Mohamad S. AlSalhi, and M. K. J. Siddiqui, "Silver nanoparticle applications and human health." *Clinica chimica acta*, vol. 411.pp. 1841-1848, 2010.

[52] Siddiqi, Khwaja Salahuddin, Azamal Husen, and Rifaqat AK Rao, "A review on

biosynthesis of silver nanoparticles and their biocidal properties." *Journal of nanobiotechnology*, vol. 16. pp. 1-28, 2018.

[53] Marambio-Jones, Catalina, and Eric MV Hoek, "A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment." *Journal of nanoparticle research*, vol. 12. pp. 1531-1551, 2010.

[54] Dos Santos, Carolina Alves, "Silver nanoparticles: therapeutical uses, toxicity, and safety issues." *Journal of pharmaceutical sciences*, vol. 103. pp. 1931-1944, 2014.

[55] Das, Subhamoy, and Aaron B. Baker, "Biomaterials and nanotherapeutics for enhancing skin wound healing." *Frontiers in bioengineering and biotechnology*, vol. 4. pp. 82, 2016.

[56] Alexander, J. Wesley, "History of the medical use of silver." *Surgical infections*, vol. 10. pp. 289-292, 2009.

[57] Alarcon, Emilio I., May Griffith, and Klas I. Udekwu, "Silver nanoparticle applications." *Springer International Publishing*, vol. 10. pp. 93-125, 2015.

[58] Costerton, J. W., and K. J. Cheng, "The role of the bacterial cell envelope in antibiotic resistance." *Journal of Antimicrobial Chemotherapy*, vol. 1. pp. 363-377, 1975.

[59] Beveridge, Terry J., "Structures of gram-negative cell walls and their derived membrane vesicles." *Journal of bacteriology*, vol. 181. pp. 4725-4733, 1999.

[60] Delcour, Anne H., "Outer membrane permeability and antibiotic resistance." *Biochimica ET Biophysica Acta (BBA)-Proteins and Proteomics*, vol. 1794. pp. 808-816, 2009.

[61] De Jong, Wim H., and Paul JA Borm, "Drug delivery and nanoparticles: applications and hazards." *International journal of nanomedicine*, vol. 3. pp.133, 2008.

[62] Vardanyan, Zaruhi, "Effects of various heavy metal nanoparticles on Enterococcus hirae and Escherichia coli growth and proton-coupled membrane trans-port." *Journal of nanobiotechnology*, vol. 13. pp. 1-9, 2015.

[63] Khan, Mujeeb, "Antibacterial properties of silver nanoparticles synthesized using Pulicaria glutinosa plant extract as a green bioreductant." *International Journal of Nanomedicine,* vol. 9. pp. 3551, 2014.

[64] Greulich, Philip, "Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics." *Molecular systems biology*, vol. 11. pp. 796, 2015.

[65] Kim, Jun Sung, "Antimicrobial effects of silver nanoparticles." *Nanomedicine: Nanotechnology, biology and medicine,* vol. 3. pp. 95-101, 2007.

[66] Seil, Justin T., and Thomas J. Webster, "Antimicrobial applications of nanotechnology: methods and literature." *International journal of nanomedicine,* vol. 7. pp. 2767, 2012.

[67] Beyth, Nurit, "Alternative antimicrobial approach: nano-antimicrobial materials." *Evidence-based complementary and alternative medicine,* vol. 2015. pp. 1-16, 2015.

[68] Lakshmi Prasanna, V., and Rajagopalan Vijayaraghavan, "Insight into the mechanism of antibacterial activity of ZnO: surface defects mediated reactive oxygenspecies even in the dark." *Langmuir,* vol. 31. pp. 9155-9162, 2015.

[69] Lu, Zhong, "Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria." *Journal of Materials Science: Materials in Medicine,* vol. 24. pp. 1465-1471, 2013.

[70] Sehmi, Sandeep K., "Antibacterial surfaces with nanoparticle incorporation for prevention of hospital-acquired infections." PhD. thesis, UCL (University College London), London, 2016.

[71] Brown, Stephanie, John P. Santa Maria Jr, and Suzanne Walker, "Wall teichoic acids of gram-positive bacteria." *Annual review of microbiology*, vol. 67. pp. 313-336, 2013.

[72] Peleg, Anton Y., and David C. Hooper, "Hospital-acquired infections due to gram-negative bacteria." *New England Journal of Medicine*, vol. 362. pp. 1804-1813, 2010.

[73] Sandine, William E, "Roles of Lactobacillus in the intestinal tract." *Journal of Food Protection*, vol. 42. pp. 259-262, 1979.

[74] Subramanian, Keerthana, “Exploring the antibacterial effects of polymersomes embedded with silver nanoparticles and doxorubicin.” M.S. thesis, Northeastern University, United States, 2017.

[75] Ventola, C. Lee, “The antibiotic resistance crisis: part 1: causes and threats.” *Pharmacy and therapeutics*, vol. 40. pp. 277, 2015.

[76] Liu, Yaling, “The shape of things to come: importance of design in nanotechnology for drug delivery.” *Therapeutic delivery*, vol. 3. pp. 181-194, 2012.

[77] Sehmi, Sandeep K., “Potent antibacterial activity of copper embedded into silicone and polyurethane.” *ACS applied materials and interfaces*, vol. 7. pp. 22807-22813, 2015.

[78] Ali, Moazzam, and Markus Winterer, “ZnO nanocrystals: surprisingly ‘alive’.” *Chemistry of Materials*, vol. 22. pp. 85-91, 2009.

[79] Ozkan, Ekrem, Elaine Allan, and Ivan P. Parkin, “The antibacterial properties of light-activated polydimethylsiloxane containing crystal violet.” *RSC Advances*, vol. 4. pp. 51711-51715, 2014.

[80] Noimark, Sacha, “Photobactericidal polymers; the incorporation of crystalviolet and nanogold into medical grade silicone.” *RSC Advances*, vol. 3. pp. 18383-18394, 2013.

[81] Zhang, Bohan, “Preparation of Nanoparticles by Green Synthesis and a Study on their Antibacterial and Anticancer Properties.” M.S. thesis, Northeastern University, United States, 2019.

[82] Dever, Laura A., and Terence S. Dermody, “Mechanisms of bacterial resistance to antibiotics.” *Archives of internal medicine*, vol. 151. pp. 886-895, 1991.

[83] Noimark, Sacha, “Incorporation of methylene blue and nanogold into polyvinyl chloride catheters; a new approach for light-activated disinfection of surfaces.” *Journal of Materials Chemistry*, vol. 22. pp. 15388-15396, 2012.

[84] Roberts, Mariyn C. "Resistance to tetracycline, macrolide-lincosamide-streptogramin, trimethoprim, and sulfonamide drug classes." *Molecular biotechnology*, vol. 20. pp. 261-283, 2002.

[85] Gangell, Catherine, "Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis." *Clinical infectious diseases*, vol. 53. pp. 425-432, 2011.

[86] Wong, Craig S., "Risk factors for the hemolytic uremic syndrome in children infected with Escherichia coli O157: H7: a multivariable analysis." *Clinical Infectious Diseases*, vol. 55. pp. 33-41, Sep. 2011.

[87] Yasmin, Farhana, "Drug resistant pathogens isolated from wound infection." B.S. thesis, Brac University, Bangladesh, 2021.

[88] Lowy, Franklin D., "Staphylococcus aureus infections." *New England journal of medicine*, vol. 339. pp. 520-532, 1998.

[89] Rasigade, Jean-Philippe, and François Vandenesch, "Staphylococcus aureus: a pathogen with still unresolved issues." *Infection, Genetics and Evolution*, vol. 21. pp. 510-514, 2014.

[90] Simone, Charles B., "Photodynamic therapy for the treatment of non-smallcell lung cancer." *Journal of thoracic disease*, vol. 4. pp. 63, 2012.

[91] Ormond, Alexandra B., and Harold S. Freeman, "Dye sensitizers for photodynamic therapy." *Materials*, vol. 6. pp. 817-840, 2013.

[92] Dougherty, Thomas J., "Photodynamic therapy (PDT) of malignant tumors." *Critical reviews in oncology/hematology*, vol. 2. pp. 83-116, 1984.

[93] Castano, Ana P., Tatiana N. Demidova, and Michael R. Hamblin, "Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization." *Photodiagnosis and photodynamic therapy*, vol. 1. pp. 279-293, 2004.

[94] Rajesh, S., "Antimicrobial photodynamic therapy: An overview." *Journal of Indian Society of Periodontology*, vol. 15. pp. 323, 2011.

[95] Maillard, Jean-Yves, "Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems." *Therapeutics and clinical risk management*, vol. 1. pp. 307, 2005.

[96] Revelas, Angela, "Healthcare–associated infections: A public health problem." *Nigerian medical journal: journal of the Nigeria Medical Association*, vol. 53.

pp. 59, 2012.

[97] Davies, Julian, and Dorothy Davies, "Origins and evolution of antibiotic resistance." *Microbiology and molecular biology reviews*, vol. 74. pp. 417-433, 2010.

[98] Chang, Li-Chi, "One-pot synthesis of poly (3, 4-ethylenedioxythiophene)-Pt nanoparticle composite and its application to electrochemical H 2 O 2 sensor." *Nanoscale research letters*, vol. 7. pp. 1-8, 2012.

[99] Ilieva, M., "Effect of deep UV laser treatment on electroless silver precipitation in supported poly-3, 4-ethylenedioxythiophene layers." *Journal of Optoelectronics and Advanced Materials*, vol. 11. pp. 1444, 2009.

[100] Park, James T., and Jack L. Strominger, "Mode of action of penicillin: biochemical basis for the mechanism of action of penicillin and for its selective toxicity." *Science*, vol. 125. pp. 99-101, 1957.

[101] Salton, M. R. J., "Studies of the bacterial cell wall: IV. The composition of the cell walls of some gram-positive and gram-negative bacteria. " *Biochimica et biophysica acta*, vol. 10. pp. 512-523, 1953.

[102] Alhumaid, Saad, "Antimicrobial susceptibility of gram-positive and gram-negative bacteria: a 5-year retrospective analysis at a multi-hospital healthcare system in Saudi Arabia." *Annals of clinical microbiology and antimicrobials*, vol. 20. pp. 1-18, 2021.

[103] Raza, Muhammad Akram, "Size-and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes." *Nanomaterials*, vol. 6. pp. 74, 2016.

[104] Sedighi, Ali, Majid Montazer, and Saeedeh Mazinani, "Fabrication of electrically conductive superparamagnetic fabric with microwave attenuation, antibacterial properties and UV protection using PEDOT/magnetite nanoparticles. " *Materials & Design*, vol. 160. pp. 34-47, 2018.

[105] Kumar, A. Madhan, "PEDOT/FHA nanocomposite coatings on newly developed Ti-Nb-Zr implants: Biocompatibility and surface protection against corrosion and bacterial infections." *Materials Science and Engineering: C*, vol. 98. pp. 482-495, 2019.

[106] Noimark, Sacha, Elaine Allan, and Ivan P. Parkin, “Light-activated antimicrobial surfaces with enhanced efficacy induced by a dark-activated mechanism.” *Chemical Science*, vol. 5. pp. 2216-2223, 2014.