

**İZMİR KATİP ÇELEBİ UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE AND  
ENGINEERING**

**EVALUATION OF EFFECTIVENESS OF COLD ATMOSPHERIC PLASMA  
TREATMENT METHODS FOR PREVENTION OF VENTILATOR-  
ASSOCIATED PNEUMONIA**

**M.Sc. THESIS**

**FATMA İBİŞ**

**Department of Biomedical Technologies**

**Biomedical Technologies Programme**

**Thesis Advisor: Asst. Prof. Dr. Utku Kürşat ERCAN**

**SEPTEMBER 2016**



**İZMİR KATİP ÇELEBİ UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE AND  
ENGINEERING**

**EVALUATION OF EFFECTIVENESS OF COLD ATMOSPHERIC PLASMA  
TREATMENT METHODS FOR PREVENTION OF VENTILATOR-  
ASSOCIATED PNEUMONIA**

**M.Sc. THESIS**

**FATMA İBİŞ  
Y140101005**

**Department of Biomedical Technologies  
Biomedical Technologies Programme**

**Thesis Advisor: Asst. Prof. Dr. Utku Kürşat ERCAN**

**SEPTEMBER 2016**



**İZMİR KATİP ÇELEBİ ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ**

**VENTİLATÖR İLİŞKİLİ PNÖMONİNİN ÖNLENMESİ AMAÇLI  
ATMOSFERİK SOĞUK PLAZMA MUAMELE YÖNTEMLERİNİN  
ETKİNLİĞİNİN DEĞERLENDİRİLMESİ**

**YÜKSEK LİSANS TEZİ**

**FATMA İBİŞ  
Y140101005**

**Biyomedikal Teknolojileri Anabilim Dalı**

**Biyomedikal Teknolojileri Programı**

**Tez Danışmanı: Yrd. Doç. Dr. Utku Kürşat ERCAN**

**EYLÜL 2016**



**Fatma İbiş**, an **M.Sc.** student of **IKCU Graduate School of Science and Technology** student ID **Y140101005**, successfully defended the **thesis** entitled “**EVALUATION OF EFFECTIVENESS OF COLD ATMOSPHERIC PLASMA TREATMENT METHODS FOR PREVENTION VENTILATOR-ASSOCIATED PNEUMONIA**”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

**Thesis Advisor :** **Asst. Prof. Dr. Utku Kürşat ERCAN** .....  
İzmir Katip Çelebi University

**Jury Members :** **Prof. Dr. Bahattin TANYOLAÇ** .....  
Ege University

**Asst. Prof. Dr. Ozan Karaman** .....  
İzmir Katip Çelebi University

**Prof. Dr. Aydın AKAN** .....  
İzmir Katip Çelebi University

**Asst. Prof. Dr. Nermin TOPALOĞLU**.....  
İzmir Katip Çelebi University

**Date of Submission : 19 September 2016**

**Date of Defense : 26 September 2016**



*To my lovely family,*





## **FOREWORD**

I would especially like to thank my advisor, Asst.Prof.Dr. Utku Kürşat Ercan, for his healthy degree of optimism when experiments disappointed and all seemed lost. I would also like to thank him for the warm and friendly atmosphere he garnered in his group; it encouraged sharing of ideas, insightful discussions and a productive work environment.

Next, I would like to thank TÜBİTAK 2211 Graduate Scholarship Programme (TÜBİTAK 2211 Yurt İçi Lisansüstü Burs Programı) for support and aid for my thesis project.

We would like to acknowledge The Scientific and Technology Research Council of Turkey for financial support of this study under grant 114E163.

Additionally, I would like to thank Res.Asst.Eyyüp Yalçın for helping and patient for the viewing of AFM in our university.

Without great friends and family, this endeavor would have concluded before it began. I would like to thank them for believing in me, encouraging me to continue going, and providing distractions from work when they were needed.

I want to thank my parents. It is with their help for all my life that I became who I am today. Thanks for always being there for me, believing in me, and motivating me to set out on my own path. I cannot begin to describe how lucky I feel for having them as my parents. All opportunities and accomplishments I owe to them.

September 2016

Fatma İBİŞ



## TABLE OF CONTENTS

	<u>Page</u>
<b>FOREWORD</b> .....	<b>viii</b>
<b>TABLE OF CONTENTS</b> .....	<b>x</b>
<b>LIST OF FIGURES</b> .....	<b>xv</b>
<b>1. INTRODUCTION</b> .....	<b>1</b>
1.1 Hospital –acquired infections .....	1
1.1.1 Urinary Tract Infections .....	2
1.1.2 Surgical Site Infections .....	2
1.1.3 Ventilator Associated Pneumonia .....	3
1.2 Microorganisms .....	5
1.2.1 Antibiotic-resistant microorganisms .....	5
1.2.2 Fungi.....	6
1.3 Prevention of Infection .....	6
1.3.1 Antiseptics and Antisepsis .....	6
1.3.2 Disinfection .....	7
1.3.3 Sterilization .....	8
1.4 Plasma.....	10
1.4.1 Dielectric Barrier Discharge (DBD) Plasma .....	12
1.4.2 Plasma Chemistry.....	13
1.4.3 Plasma Medicine .....	14
1.4.3.1 Plasma Assisted Blood Coagulation .....	15
1.4.3.2 Plasma Applications for Cancer Therapy.....	15
1.4.3.3 Dental Applications of Cold Atmospheric Plasma .....	16
1.4.3.4 Plasma Applications in Dermatology and Wound Healing .....	16
1.4.3.5 Cold Atmospheric Plasma - Cell Interactions .....	17
1.4.3.6 Plasma sterilization .....	17
1.5 Nebulization .....	18
1.6 Aim of Study .....	18
<b>2. MATERIALS AND METHODS</b> .....	<b>20</b>
2.1 Culvitation of Microorganisms.....	20
2.2 Preperation of Frozen Stock Cultures.....	20
2.3 Preperation of N-Acetyl Cysteine (NAC) Solution.....	21
2.4 Designing and Manufacturing of Electrode and Fluid Holder .....	21
2.4.1 Dielectric Barrier Discharge (DBD) Electrode .....	21
2.4.2 Plasma Jet Electrode.....	22
2.4.3 Fluid Holder .....	23
2.5 Optimization of Plasma Parameters for Appropriate Antimicrobial Effects....	23
2.6 Quantifications Methods for Antimicrobial Effect of Plasma Treatment .....	24
2.6.1 XTT Assay .....	24
2.6.2 Safranine Assay.....	25
2.7 DBD Plasma Treatment of Liquids .....	25
2.8 Antimicrobial Activity of Nebulized Plasma Treated Liquids.....	26
2.9 Evaluation of the Antimicrobial Activity of Plasma Jet Application.....	27

2.10 Optimization of Drip Flow Reactor (DFR) for Formation of Biofilm .....	28
2.11 Tests for Prevention of Formation of Biofilm.....	29
2.12 Antibiofilm Effect of Plasma Treatment .....	31
2.12.1 Antibiofilm Effect of Plasma Nebulized Treated Liquid .....	31
2.12.2 Antibiofilm Effect Air Plasma Jet.....	32
2.13 Microscopic Characterization of Biofilm on Endotracheal Tube Surfaces....	32
<b>3. RESULT.....</b>	<b>34</b>
3.1 Optimization of Plasma Parameters for Appropriate Antimicrobial Effects ...	34
3.2 Evaluation of the Antimicrobial Activity of Plasma Jet Application.....	36
3.3 Biofilm Inactivation Tests with Liquid mediated Plasma Treatment .....	36
3.4 Optimization of Drip Flow Reactor for Formation of Biofilm .....	39
3.5 Tests for Prevention of Formation of Biofilm.....	39
3.6 Biofilm Inactivation Efficacy of Nebulized Plasma Treated Liquids .....	42
3.7 Biofilm Characterization on Endotracheal Tubes Surfaces .....	50
<b>4. DISCUSSION .....</b>	<b>56</b>
<b>5. CONCLUSION.....</b>	<b>60</b>
CURRICULUM VITAE.....	67

## ABBREVIATIONS

<b>HAI</b>	: Hospital acquired infection
<b>HCAI</b>	: Healthcare-associated infection
<b>ICU</b>	: Intensive care unit
<b>MRSA</b>	: Methicillin-resistant <i>Staphylococcus aureus</i>
<b>VAP</b>	: Ventilator associated pneumonia
<b>UTI</b>	: Urinary tract infection
<b>SSI</b>	: Surgical site infection
<b>CFU</b>	: Colony forming unit
<b>VRE</b>	: Vancomycin resistant <i>Enterococcus</i>
<b>UV</b>	: Ultraviolet
<b>EO</b>	: Ethylene oxide
<b>DC</b>	: Direct current
<b>AC</b>	: Alternate current
<b>DBD</b>	: Dielectric barrier discharge
<b>FE-DBD</b>	: Floating electrode dielectric barrier discharge
<b>ROS</b>	: Reactive oxygen species
<b>RNS</b>	: Reactive nitrogen species
<b>APC</b>	: Argon plasma coagulation
<b>RBC</b>	: Red blood cells
<b>PIM</b>	: Powder injection molding
<b>NAC</b>	: N-Acetyl Cysteine
<b>ATCC</b>	: American Type Culture Collection
<b>TSB</b>	: Trypticase soy broth
<b>TSA</b>	: Trypticase soy agar
<b>YPD</b>	: Yeast extract-peptone-dextrose
<b>XTT</b>	: (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)
<b>PBS</b>	: Phosphate buffered saline
<b>rpm</b>	: Revolution per minute
<b>DFR</b>	: Drip flow reactor
<b>SEM</b>	: Scanning Electron Microscopy
<b>AFM</b>	: Atomic Force Microscopy
<b>HDMS</b>	: Hexamethyldisilazane



## LIST OF TABLES

	<u>Page</u>
<b>Table 3.1:</b> Summary of results of antimicrobial effects that were obtained to determine optimum plasma jet parameters.....	36





## LIST OF FIGURES

	<u>Page</u>
<b>Figure 1.1:</b> SSI classification depend on deepness infection .....	3
<b>Figure 1.2:</b> Endotracheal tube and its connections .....	4
<b>Figure 1.3:</b> The patient is connected to ventilator.....	5
<b>Figure 1.4:</b> Resistance of microorganisms against disinfectants. (Prions has resistance to disinfectants because of this it is not above).....	7
<b>Figure 1.5:</b> Cleaning, Disinfection and Sterilization Methods [34].....	9
<b>Figure 1.6:</b> Floating Electrode Dielectric Barrier Discharge (FE-DBD) for direct plasma treatment [39].....	11
<b>Figure 1.7:</b> Plasma Jet for indirect plasma treatment [40]. .....	12
<b>Figure 1.8:</b> DBD Schematic (A) [41]. The treatment view of hand made DBD in our laboratory (B). .....	12
<b>Figure 1.9:</b> Effect of plasma and plasma species on cells.....	14
<b>Figure 1.10:</b> Plasma assisted blood coagulation: (a) Untreated blood, (b) Blood drop after 15 seconds of FE-DBD plasma treatment.....	15
<b>Figure 1.11:</b> Plasma interaction with living tissue .....	17
<b>Figure 2.1:</b> Prepared stock cultures for using in experiments agar (A) and glycerol (B) .....	21
<b>Figure 2.2:</b> In front of custom made copper electrode (A) and the below of electrode (B) the glass on the electrode surface as a dielectric material and copper.....	22
<b>Figure 2.3:</b> In the system for the plasma treatment, power supply, gas tube and electrode were seen (A). The supply connections which were high .....	22
<b>Figure 2.4:</b> The custom made quartz fluid holder .....	23
<b>Figure 2.5:</b> Chemical reaction of XTT [52]. .....	24
<b>Figure 2.6:</b> Samples were read via Multi-mode reader .....	25
<b>Figure 2.7:</b> Direct plasma treatment of suspended organisms culture with 1 mm discharge gap.....	26
<b>Figure 2.8:</b> The application of plasma treated liquid on the suspended organisms.....	27
<b>Figure 2.9:</b> Plasma jet plasma (He/O <sub>2</sub> ) treatment of suspended organisms culture..	28
<b>Figure 2.10:</b> The overview of a drip flow reactor system established for biofilm formation in endotracheal tubes (A), peristaltic pump (B), growing .....	29
<b>Figure 2.11:</b> The inner surface of the endotracheal tube designed to be treated with plasma DBD electrode (A): schematic, (B): side and (C): in front of it. ....	30
<b>Figure 2.12:</b> Air plasma jet treatment on the endotracheal tube .....	32
<b>Figure 3.1:</b> Antimicrobial effect of direct plasma treatment on <i>P. aureginosa</i> (A) <i>A. baumannii</i> (B) and, <i>C. albicans</i> (C) depending on changing frequency. ....	35
<b>Figure 3.2:</b> The antimicrobial effect of the liquid that was treated with plasma during .....	38
<b>Figure 3.3:</b> The amount of biofilm in the drip flow reactor depend maturing time..	39
<b>Figure 3.4:</b> The blocking effect of biofilm formation depends on plasma pre- .....	40
<b>Figure 3.5 :</b> XTT Results following plasma pre-treatment .....	41
<b>Figure 3.6:</b> Results of Safranin Test after Plasma Pre-treatment .....	42
<b>Figure 3.7:</b> Antimicrobial Activity Accord Colony Counting Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested.....	43
<b>Figure 3.8:</b> XTT Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested Organisms .....	45

**Figure 3.9:** Safranin Tests Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested Organisms ..... 47

**Figure 3.10:** Antimicrobial activity of air plasma jet application on biofilm inner the ..... 48

**Figure 3.11:** XTT tests results after the air plasma jet treatment on biofilm was formed on the endotracheal tubes..... 49

**Figure 3.12:** Safranin tests results after the air plasma jet treatment on biofilm was ..... 50

**Figure 3.13:** Scanning Electron Microscopy was used to take views of untreated... 51

**Figure 3.14:** Scanning Electron Microscopy was used to take views of nebulization ..... 52

**Figure 3.15:** Nebulization of plasma treated NAC on endotracheal tube ..... 52

**Figure 3.16:** Representative images of morphological modifications on ..... 53

**Figure 3.17:** Atomic-force microscopy (AFM) was used to take views of non- ..... 54

**Figure 3.18:** Atomic-force microscopy (AFM) was used to take views of..... 55





# EVALUATION OF EFFECTIVENESS OF COLD ATMOSPHERIC PLASMA TREATMENT METHODS FOR PREVENTION VENTILATOR- ASSOCIATED PNEUMONIA

## SUMMARY

Hospital-acquired infections (HAI) (nosocomial infection) are serious health problems that occur when person acquires infection during stay in a hospital for causes other reasons than that infection. Colonization of microorganisms pave the way for deaths, in detail, Ventilator Associated Pneumonia (VAP) is remarkable in terms of mortality accounted for 15% of nosocomial infections. Ventilator associated pneumonia (VAP) is common a disease, especially among patients that are on connected ventilators in intensive care units. It is defined as the pneumonia, in which occurs in at least 48 hours after mechanical ventilation, is provided via endotracheal tubes or tracheostomy. This infection occurs because of assault of microorganisms that settle in endotracheal tubes in respiratory tract and then in lung parenchyma tissue. The most common settling organisms in respiratory tract are *S.aureus*, *P. aureginosa*, *K. pneumonia*, *E.coli* and *Acinetobacter*, *Candida*, *Enterobacter* species. Due to multi drug resistant microorganisms, the current treatments are inadequate and the mortality rate increases. Therefore, plasma can play significant role for the management of ventilator-associated pneumonia. Plasma is ionized gas and defined as the fourth state of matter. The treatments of plasma in health care can ensure quick and pretty effect to protect from resistant microorganism to antibiotics to patients. The purpose of present study is to develop a method of plasma treatment which can be utilized for the control and prevention of ventilatör associated pneumonia. For this concept, we have designed different systems and determined optimum parameters to prevent and eradicate biofilms of *Staphylococcus aureus*, *Acinotabacter baumannii*, *Pseudomonas aureginosa*, and *Candida Albicans* in endotracheal tubes. Our results indicate that, various plasma treatment modalities are capable of prevention and eradication of biofilms grown in endotracheal tubes. Therefore, cold atmospheric pressure plasma treatment methods could be considered as for the management of ventilator associated pneumonia.



## VENTİLATÖR İLİŞKİLİ PNÖMONİNİN ÖNLENMESİ AMAÇLI ATMOSFERİK SOĞUK PLAZMA MUAMELE YÖNTEMLERİNİN ETKİNLİĞİNİN DEĞERLENDİRİLMESİ

### ÖZET

Hastane kökenli enfeksiyonlar (hastane enfeksiyonları) kişinin bu enfeksiyon dışında bir sebepten hastanede yatışı sırasında meydana gelen ciddi bir sağlık problemidir. Mikroorganizmaların kolonizasyonu ölüme yol açıyor, detaylandırırsak, Ventilator İlişkili Pnömoni (VİP) hastane enfeksiyonlarının %15'ini oluşturup ölüm oranı bakımından dikkat çekmektedir. VİP yoğun bakım ünitelerinde ventilatöre bağlı hastalarda görülen yaygın bir hastalıktır. Bu hastalıkta endotrakeal tüpler veya trakeostomi aracılığıyla mekanik ventilasyonun sağlanmasıyla 48 saat içinde pnömoni gelişir. Öncelikle solunum yollarına ardından akciğer parankima dokusuna yerleşen mikroorganizmaların saldırıları nedeniyle bu enfeksiyon meydana gelir. Solunum yollarına en sık olarak yerleşen bakteriler *S.aureus*, *P. aureginosa*, *K. pneumonia*, *E.coli*, *Acinetobacter*, *Candida*, ve *Enterobacter* türleridir.

Antibiyotiklere dirençli organizmalar nedeniyle mevcut tedaviler yetersiz kalmakta ve ölüm oranı artmaktadır. Bu hastalığı çözmek için plazma önemli bir rol oynayabilir. Plazma iyonize gaz olup maddenin dördüncü halidir. Plazma tıp alanında sıcak olmayan plazma olarak adlandırılır ve tıbbi plazma yeni ve gelecek vaat eden bir alandır. Tedavi alanındaki plazma uygulamaları hastaları antibiyotiğe dirençli organizmalardan korumak için hızlı ve güzel etki sağlayabilir. Araştırmanın amacı endotrakeal tüpte oluşan biyofilmi önleyici ve biyofilmi inactive edici plazma uygulamasını geliştirmektir. Biz bu amaç için farklı sistemler tasarladık ve endotrakeal tüpte oluşan *Staphylococcus aureus*, *Acinotabacter baumannii*, *Pseudomonas aeruginosa* ve *Candida albicans* biyofilmini önlemek ve ortadan kaldırmak için uygun plazma parametrelerine karar verdik. Sonuçlarımız çeşitli plazma uygulama yöntemlerinin endotrakeal tüp içinde oluşan biyofilmin önlenmesinde ve yok edilmesinde yeterli olduğunu gösteriyor. Bu nedenle, atmosferik basınçlı soğuk plazma uygulama yöntemlerinin ventilator ilişkili pnömoninin tedavisinin yönetiminde dikkate alınabilir.

## **1. INTRODUCTION**

### **1.1 Hospital –acquired infections**

Infection is a process where an infectious factor can develop and increase number in the tissue of a macro-organism [1]. Hospital-acquired infection (HAI) (nosocomial infection) is a serious health problem that occurs when person acquires infection during stay in a hospital for a cause other than that infection. The patient with HAI does not have this infection or the infection is not in incubation period of HAI before hospitalization. Nosocomial infections can be seen after discharge of patient and it threatens not only patient but also health care staff and the public.[2, 3] Under favor of close adaptation to human system and environmental conditions, organisms that are bacteria, viruses and fungi can cause HAIs [1]. HAIs are the main reasons of deaths and rise morbidity of hospitalized patients. Therefore, HAI is an important issue for patients, healthcare professionals and public health. Longer stay of infected patients in hospital is a significant contributor to healthcare cost [2, 4]. In the United States, almost 4.5 million patients are affected by HAI that causes almost 100,000 mortality/year. Moreover it causes high expense that is nearly from 28 billion dollars to 336 billion dollars per year in excess hospital cost [2, 5-7]. According to The World Health Organization 1.7 million patients are influenced by HAI and annually 37,000 deaths are reported due to HAI in Europe [7]. Hospital acquired infection rate had increased from 0.72% to 0.98% in a period of 1970-2000s due to increased number of hospitalized patients and older age of patients [8]. Eventhough the efforts for prevention of HAI and progress in public health, nosocomial infections continue to develop in patients who stay in hospital and health care professionals [4]. According to publication of the World Health Organisation, the most common HAIs are surgical site, urinary tract and respiratory tract infections. [2, 4]. Development of HAI depends on various factors such as properties of microorganisms, immune system of patient, intrinsic virulence, environment that includes using some materials from hospital and quantity of infective materials [2, 4, 9]. According to National Healthcare Safety Center, during 2009-2010, about 20% of nosocimal infections are

caused by of multidrug resistant organisms (MDROs) [5, 10]. Antibiotic resistances in microorganisms are led by excessive and unnecessary use of antibiotics. Once an organism acquires resistance to a specific antibiotic, then either antibiotic will remain inefficient or higher dose will be needed to eradicate the infection. Methicillin resistant *Staphylococcus aureus* (MRSA) (8.5%), Vancomycin resistant *Enterococci* (VRE) (3%), *Escherichia coli* (2%), Resistant *Enterobacteriaceae*, Multi–drug resistance *Acinetobacter spp.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (ESBL), *Clostridium difficile associated diarrhea (CDAD)* and *Candida albicans* are common MDR organisms responsible for nosocomial infection. They are capable to form biofilms that are sources of contamination and infection in hospitals. Therefore, they are responsible for hospital-acquired pneumonias, wound infections, catheter related bacteremia that is seen frequently in hospitals [4, 5, 10, 11].

### **1.1.1 Urinary Tract Infections**

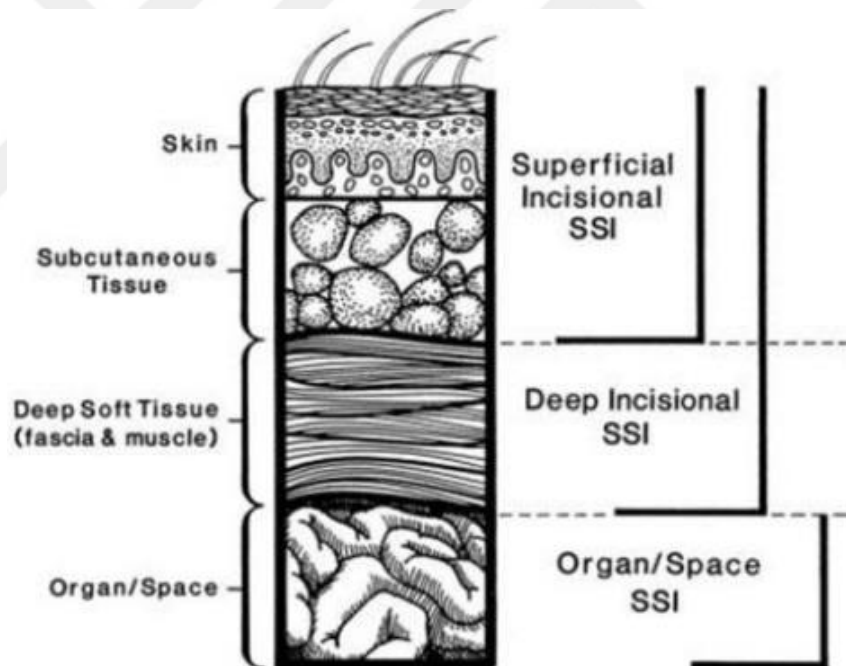
Bacteria are the main reason for causing of Urinary Tract Infection (UTIs) that contain *E. coli*, which is responsible of 80-90% of these diseases, and *S. saprophyticus*. According to reports, UTI accounts for one-third of HAI. Normally, any organisms do not stay in urine. If the bacteria enter urethra, it causes infections that are treated easily in most situations. However, a lower urinary tract infection because of infection may spread in ureters and kidneys. The spreading of bacteria in kidney causes permanent kidney damage. [2, 4, 5, 12]. Urinary tract infections contain; urethritis, cystitis, ureteritis and, pyelonephritis. The prevelange UTIs occur in women and affect bladder and urethra. Cystitis, in other words, infection of the bladder is usually brought on *E. coli* that found in the gastrointestinal tract. Urethritis, put it differently, infection of the urethra can take place if gastrointestinal bacteria expand from anus to urethra [4, 9, 13, 14].

### **1.1.2 Surgical Site Infections**

Surgical site infections (SSIs) may present on surgical wounds that is over or under aponeurosis. SSI is defined as any infection on the site of surgery that develops in 30 days after surgery and increases hospitalization up to 7 to10 days. Depending on the tytem of surgery, SSI can be detected in few days to weeks. Patient's age, gender, type of surgical wound, treatment time, anesthesia types are important factors for the development of SSIs. [1, 9, 15] Contamination of operation room, using non-sterile



equipment, inadequate preparation of patient before operation are main causes for the development of SSIs [4]. SSI can be divided into two types, which are endogenous and exogenous. While endogenous is related to patient floras that are skin, mucous membrane and gastrointestinal tract, exogenous are related to aseptic techniques, insufficient hand cleaning, flora of surgical staff, contaminated equipment, unhygienic environment and ventilation. Aerobic and anerobic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Staphylococcus epidermis*, and *Enterococcus species* from patients' samples and MDR organisms such as Methicillin-resistant *Staphylococcus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE) are common organisms that cause SSIs. Surgical site infections can be categorized as finding either incisional organ or space. Also incisional SSIs are separated related to involving just skin and subcutaneous tissue. Moreover, it seen that at Figure 1.1, deep incisional SSI for more deeply soft tissues of the incision and organ or space SSIs contain organ or space in body [1, 9, 15].

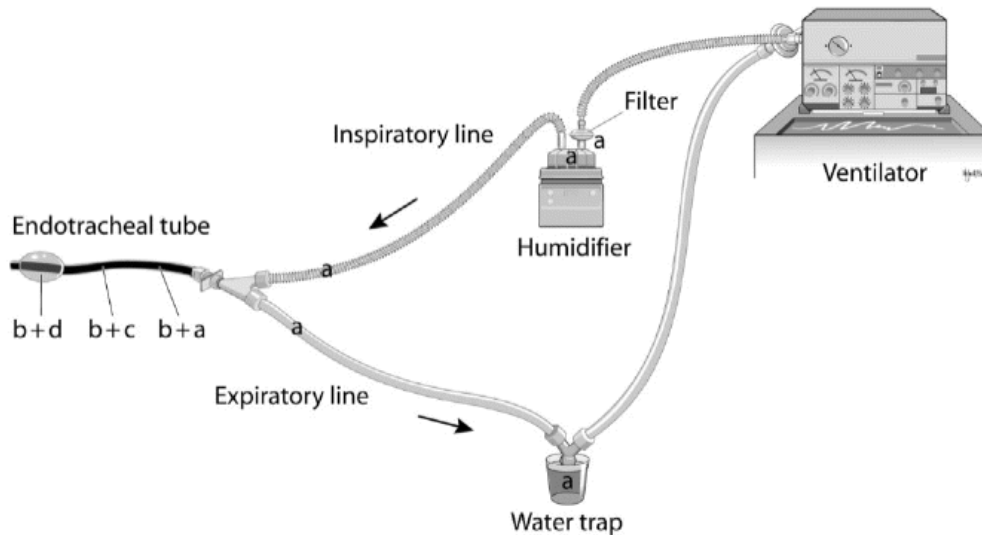


**Figure 1.1:** SSI classification depend on deepness infection

### 1.1.3 Ventilator Associated Pneumonia

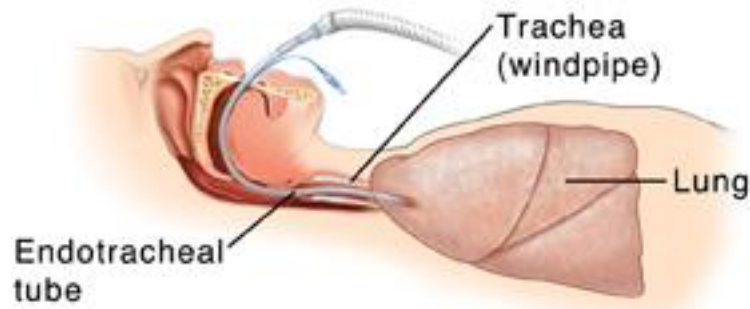
Ventilator associated pneumonia (VAP) is prevalent disease especially among patients that are connected to ventilators in intensive care units. It is defined as the pneumonia that develops in at least 48 hours after mechanical ventilation is provided via endotracheal tubes or tracheostomy. VAP significantly increases mortality rate in

patients who are treated with invasive mechanical ventilation that is need for intubation in intensive care [16].



**Figure 1.2:** Endotracheal tube and its connections

In the process of mechanical ventilation, an endotracheal tube is inserted into trachea, which will direct the air pumped by mechanical ventilator towards lung. During insertion of endotracheal tube and ventilation, the respiratory tract can be damaged. Moreover, the tube is not biocompatible, and serves as a perfect surface for biofilm formation. Also, mechanically ventilated patients are seriously ill and have weaker immune system, which makes them more susceptible to VAP. There are significant mortality in VAP cases accompanied with other diseases. This infection occurs because of assault of microorganisms that settle in endotracheal tubes in respiratory tract and then lung parenchyma tissue. Colonization of microorganisms pave the way for deaths, in detail, VAP includes 15% of HAIs, and these are the most prevalent reasons of deaths because of nosocomial infections having > 75% of mortality rate. Most common settling organisms in respiratory tract are *S.aureus*, *P. aureginosa*, *K. pneumonia*, *E.coli* and *Acinetobacter*, *Candida*, *Enterobacter* species [4, 9, 17].



**Figure 1.3:** The patient is connected to ventilator

Proper disinfection and careful tubing, and using humidifiers should be done to decrease contamination [4]. These methods are insufficient to eradicate organisms that cause ventilator-associated pneumonia.

## 1.2 Microorganisms

Microorganisms were present before plants and animals seemed billions of years ago in the world. Microorganisms are the smallest form of living organisms. Bacteria are classified as gram stain gram (+) and gram (-). Gram-negative bacteria have a thinner peptidoglycan layer than gram-positive bacteria [18, 19]. Later, with gram staining, gram (+) bacteria look purple-violet and gram (-) bacteria seem pink. The same method is red stain safranin, the cell types can be recognized [19, 20]. Gram-negative bacteria examples are *Acinetobacter*, *Escherichia Coli*, *Pseudomonas*, *Enterobacter*, *Salmonella*, *Helicobacter* etc. [21]. The examples of gram-positive bacteria are *Staphylococci* (*Staphylococcus aureus*), *Streptococci*, *Enterococci* etc. [22]. According to size, the distinguishing of bacteria is not possible. However, they can be separated according to shapes that are coccus (*Staphylococcus aureus*), bacillus (*Escherichia coli*), and spirillum (*Treponema pallidum*) [19, 20].

### 1.2.1 Antibiotic-resistant microorganisms

There are serious threats in the hospital environment related to microorganisms. For instance, methicillin-resistant *S.aureus* in other words, multi-resistant strains of *S.aureus* can be transported on the health staff's hands [4]. Multi-drug resistant organisms (MDROs) cause common health problems in the world seriously [23]. In the United States, annually 2-million hospital infections occur and drug-resistant bacteria cause more than half of nosocomial infections. It plays an important role about morbidity

and mortality of patients and economic cost. Numbers that are between \$4 billion-\$30 billion can explain the cost. These microorganisms are not only seen in the patients that are stayed in hospital or discharged from hospital but also seen among patients that are taken some treatments such as indwelling catheters, surgical wounds, and dialysis [24]. Frequently, the arrangement of using antibiotics with limited dose causes insufficient to eliminate MDR pathogens. Because of this reason after effective infection therapy, the patients body are likely to be colonized with MDR pathogens [25]. Multi drug resistant bacteria are such as Methicillin resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus*, *Mycobacterium tuberculosis*, and *Klebsiellapneumoniae*. In order to prevent antibiotic resistance, unnecessary antibiotics should not be used, efficient hygiene must be provided and the infection control procedure must be followed [26].

### **1.2.2 Fungi**

Fungi may present as a single cell or complex multicellular form. They live on the solid or in liquid. They act as a responsible duty of carbon cycles. However, they induce diseases in human such as skin disease that can be ringworm. The fungi are divided three main types that are multicellular filamentous moulds, macroscopic filamentous and single celled microscopic yeast. Yeast is almost as small as red blood cell nearly. Some yeast play important roles in necessary reactions. However, some of yeasts such as *Candida Albicans* induce infection that are related immune system [27, 28] .

## **1.3 Prevention of Infection**

Common methods are used for infection prevention are listed and briefly explained below:

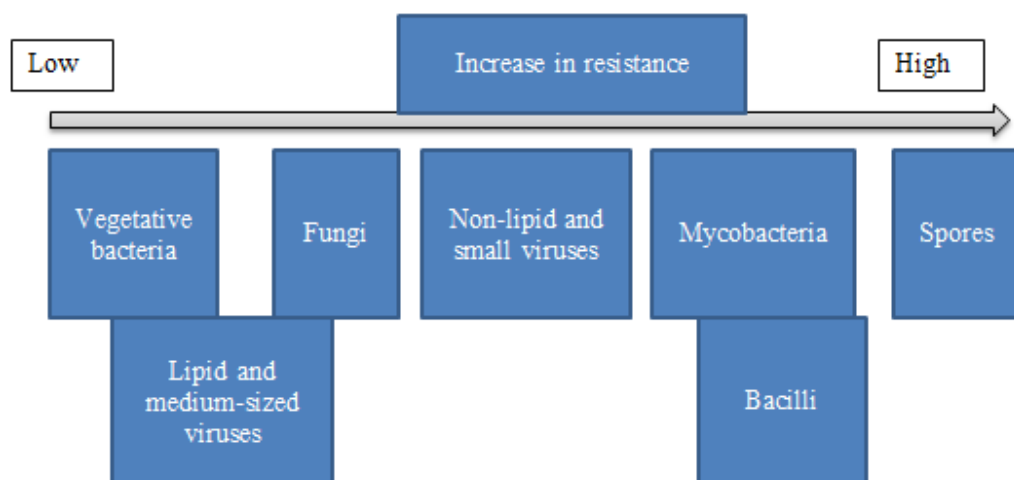
### **1.3.1 Antiseptics and Antisepsis**

Antiseptics are defined as biocidal compounds that harm and hinder the development of microorganisms in or on living structures such as tissue, skin. The skin, the largest organ, constitutes nearly one sixth of human body weight, has enormous of function that are temperature regulation, playing role as a barrier, preventing water loss. So living tissues and skin are more susceptible than hard tissue, it is important to use

right and limited antiseptics, due to their toxicity. There are many formulations and preparations such as surgical scrubs, mouthwashes, antimicrobial hand washes that are utilized as antiseptics because of being biocidal. They provide antiseptic conditions that represent inhibition microorganisms mentioned before; it provides decrease or blocks the hazard of infection. Antiseptic treatments are such as periodic skin hygiene, skin medication before surgical response or wound infection and mucosal membranes treatment. The most common used skin antiseptics and washes are biocides [1, 29, 30].

### 1.3.2 Disinfection

Disinfection is defined as the eradication of microorganisms on living and nonliving environment. Chemical and physical disinfection are two major types of disinfection methods and these methods may not completely eliminate all types of microorganisms. In physical disinfection, physical energy is used for eradication of microorganisms. In chemical disinfection, a chemical substance can kill the living microorganisms via denaturation of the major proteins, enzyme, and genetic material. In the physical disinfection applications, the ratio of killing organisms relies on the dose of physical energy and application time. In chemical disinfection, efficacy relies on killing organisms related to toxicity, quantity of disinfectant and application time. These methods are used to against microbial contamination but are not completely effective to all types of organisms [1, 29, 31]



**Figure 1.4:** Resistance of microorganisms against disinfectants. (Prions has resistance to disinfectants because of this it is not above)

Chemical disinfectants have various chemical groups such as alcohol, aldehyde, chlorine, alkylamine, iodine, phenol, and guanidine, alkaline, peroxide, inorganic, and organic acids that show antimicrobial efficacy. They have different classes related to resistance of microorganisms against disinfectants.

### **1.3.3 Sterilization**

Sterility infers disappearance of living organisms in any form. Sterilization is the process to make sterile tools or anything through killing all forms of living organisms. In the medical field, in order to obtain surgical asepsis, sterilization technique is enhanced for eradicating potential infectious element in environment without harming the sterilized tools. This technique is used for obtaining complete disinfection on hazardous tools used for operation on the patient's body. Moreover it is used for preventive procedure related to infectious diseases [1, 32, 33]. Invasive applications require contact within patients's tissue or mucous membranes and medical tools. This contact could cause infection due of entrance of microorganisms. Any error related to disinfection and sterilization has risk for patients. The sterilization procedure is adjusted according to characterization of surgical items, treatments and patients tissue and mucous membrane [32]. Sterilization techniques are separated two major units as physical and chemical sterilization. In physical sterilization, the process includes using of moist heat (steam), dry heat, and radiation. The steam procedure is a common and safe method depends on existence of steam under pressure. This sterilization process is utilized in controlled vessels such as autoclaves, steam sterilizers, or pressure vessels. Even if this method is reliable, it is not appropriate for temperature or pressure delicate devices or products. Dry-heat or hot air sterilization involves incineration of waste materials. The dry heat sterilization are utilized sterilization ovens and incineration whose temperatures are ranging from 800 to 1,300 °C. The general dry air sterilization is usually applied at 160 °C for 120 minutes, at 170 °C for 60 minutes, at 180 °C for 30 minutes, and at 190 °C for 6 minutes. Even though dry heat sterilization is cheaper and easier than steam process, many temperature sensitive materials or liquid can not be sterilized with this technique. The last common method is the high-energy ionizing radiation method that utilizes X rays and electron beams. Ionizing radiation sterilization is used for sterilization of medical devices, food, wastewater, liquid, solid materials, and pharmaceutical products. Radiation sterilization has limited applicability because

of cost and safety concern. These methods for sterilization and disinfections are seen that in Figure 1.5. There are some technics that are used for special applications, for instance; plasma, pulsed light and supercritical fluids [1, 29, 34].

Moreover, there is a new method for sterilization from United Kingdom. Superoxide water is the mixture of oxidizing species. This product is under trademark STERILOX®. The major products are hypochlorous acid in 144 mg/L of concentration and chlorine radicals. The process takes place at pH 5.0- 6.5 and the potential of oxidation-reduction is higher than 950 mV. During this process, a saline solution is passed over titanium-covered electrodes at a current of about 6 to 9 Amps. Studies have shown that this process effectively causes 5-log reduction of *Mycobacterium tuberculosis*, *M.chelonae*, *HIV*, *MRSA*, *E.coli*, *C.albicans*, *P.aureginosa* in less than 2 minutes. However, antimicrobial efficacy of this process decreases with the existence of organic material and further studies are needed [29, 35, 36].

Cleaning	Disinfection	Sterilization
<b>Manual</b> Cleaning with or without use of brushes, specialized tools	<b>Low Level</b> Kills most vegetative bacteria, some viruses and some fungi.	<b>High Temperature</b> Moist heat/steam Dry heat
<b>Automated Cleaning</b> Ultrasonic Medical Washers	<b>Intermediate Level</b> Kills vegetative bacteria, viruses, fungi and mycobacterium.	<b>Low Temperature</b> Ozone
	<b>High Level</b> Kills all microbial organisms – potential to render device sterile.	<b>Chemical</b> Liquid - Steris Hydrogen peroxide
	<b>Thermal</b> Disinfection via thermal applications under 100°C.	<b>Gas</b> Ethylene Oxide Hydrogen peroxide (gas plasma) - Sterrad
		<b>Radiation</b> -Gamma, E-beam

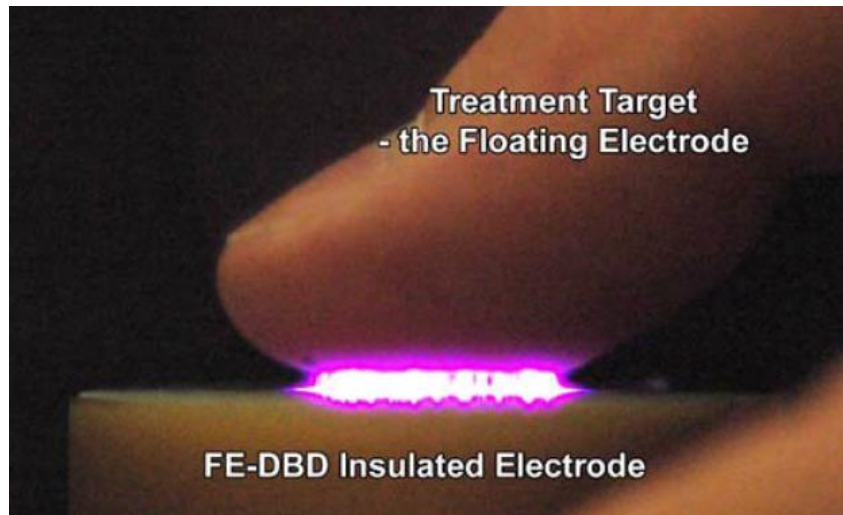
**Figure 1.5:** Cleaning, Disinfection and Sterilization Methods [34].

However, these technics are not enough to eradicate some types of organisms on the surfaces or living tissues.

## 1.4 Plasma

Plasma that is fourth state of matter is an ionized gas and first defined by Irving Langmuir in 1928. He thought that this matter resembles blood plasma because of multicomponent structure and it has free electrons, ionized gas, and reactive species. Plasma constitutes more than 99% of the universe and aurora borealis, lightning sun are examples of natural plasmas. Additionally, plasma can be generated artificially. Plasma is an ionized gas. Ionization is defined as a process that an atom or molecule gains or loses at least one electron. In consequence of ionization the molecule or atom turns into positive or negative form depending on the gain or loss of electron respectively. When the temperature rises, molecules gain energy, it occurs transform plasma as a fourth state after solid, liquid, gas phases. During generation of plasma, electrons take energy much faster than heavier particles with the effect of electrical field. Electrons and ions cause plasma electrically conducting and responsive [37]. According to thermal characteristics, plasma could be generated in various ways. Plasma is categorized as non-thermal, cold or non-equilibrium and thermal, hot or equilibrium plasma according to temperature of free electrons and gas. The electric field helps to generate plasma and electrons gain energy quicker than heavier particles. In non-thermal plasma or nonequilibrium plasma, cooling of ions and neutral molecules are more efficient than energy transference from electrons, the gas remains at low temperature. As for thermal plasma, energy flux transfers from electrons to heavy particles balance and from heavy particles to environment. The temperature of heavy particles and electrons become nearly equal. The plasma temperature is substantial for treating temperature-sensing material [9, 37, 38]. According to treatment method, cold atmospheric plasma can be divided two types as direct and indirect plasma treatment. For direct plasma treatment, the material that is treated such as tissue, skin, cell serve as an electrode, and the discharge is generated between high voltage electrode and the treated material. The example of direct plasma is in Figure 1.6.





**Figure 1.6:** Floating Electrode Dielectric Barrier Discharge (FE-DBD) for direct plasma treatment [39].

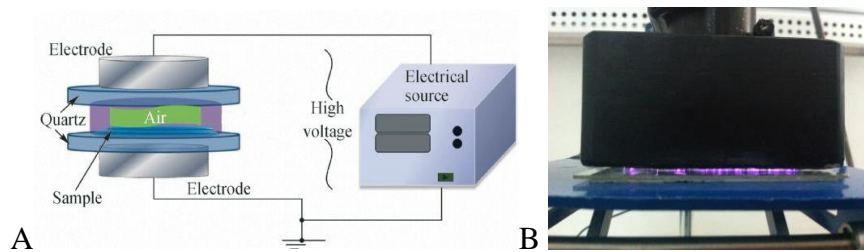
In indirect plasma treatment, the plasma discharge is generated in a region that is not in contact with the treated material and the plasma products are carried with a gas flow. Plasma needles or torches are examples of plasma devices that are utilized in indirect plasma treatment. In addition to direct and indirect plasma treatment also, fluid mediated plasma treatment has been defined. In fluid mediated plasma treatment, firstly, a fluid is treated with direct plasma treatment, which leads chemical modifications in the fluid, and then the treat fluid is transferred to a substance such as bacterial cell. In fluid mediated plasma treatment of fluid is considered as direct and transferring fluid to a substance is considered as indirect plasma treatment. Eventhough it is defined as fluid mediated plasma treatment various materials such as alginate gels metals polymers might be treated in the same way in order to obtained varios plasma mediated effects [38].



**Figure 1.7:** Plasma Jet for indirect plasma treatment [40].

#### 1.4.1 Dielectric Barrier Discharge (DBD) Plasma

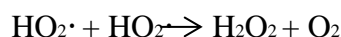
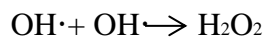
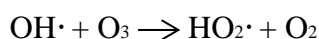
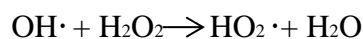
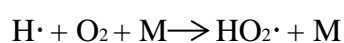
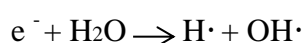
Dielectric Barrier Discharge (DBD) was first defined by Siemens in late 1800s for ozone production. DBD set up consists of one high voltage and one grounding electrode. In the DBD set up at least one of these electrodes should be covered with a dielectric material such as glass, ceramic, quartz. Presence of dielectric material is used to avoid arc formation and limits and it distributes the current over a large area. DBD generates localised shock waves and noise. DBD is largely implemented for ozone product in UV-source. DBD plasma electrode could be set in plane and cylindrical configurations. DBD is one of the most used plasma for medical application due to its flexibility.



**Figure 1.8:** DBD Schematic (A) [41]. The treatment view of hand made DBD in our laboratory (B).

### 1.4.2 Plasma Chemistry

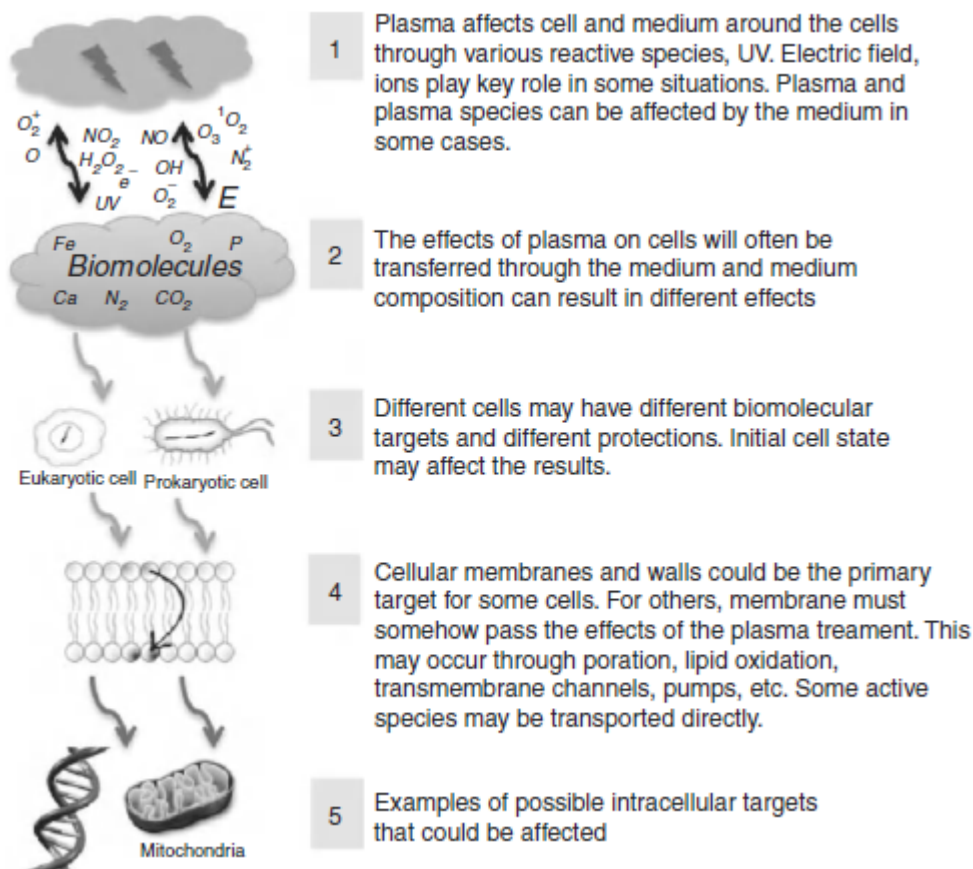
While the electric field is implemented to gas that is in the environment, electrons are accumulated to get higher energy. These electrons collide with gas atoms and molecules. It causes to generation of positively charged ions because of electron lost from ambient gas. The positive ions are essential components related to plasma-chemical processes. Their exothermic reactions do not have any activation energy. It makes their contribution important in plentiful plasma-chemical process. Moreover, for high chemical activity, atom and molecule ions are able to have substantial kinetic energy that decides their additive in reactions. Chemical species formed by plasma treatment. Active plasma is a complex system that is ready to reaction because of high concentrations of charged particles that are electrons and ions, active atoms, radicals and UV-photons. During the plasma generatingh, chemical species act part on the biological influence of cold atmospheric plasma. During formation of plasma reactive oxygen species (ROS), such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), ozone (O<sub>3</sub>), hydroxyl radical (OH), singlet oxygen, superoxide (O<sub>2</sub><sup>-</sup>), and reactive nitrogen species (RNS) such as nitric oxide (NO), nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>). While generating plasma, there is energy transfer that is from electrons to ambient gas as exit and ionization. These processes cause formation of radicals that are H·, OH· and O·. For example, the reactions for obtaining hydrogen peroxide and oxygen molecule can be explained below;



[9, 37].

### 1.4.3 Plasma Medicine

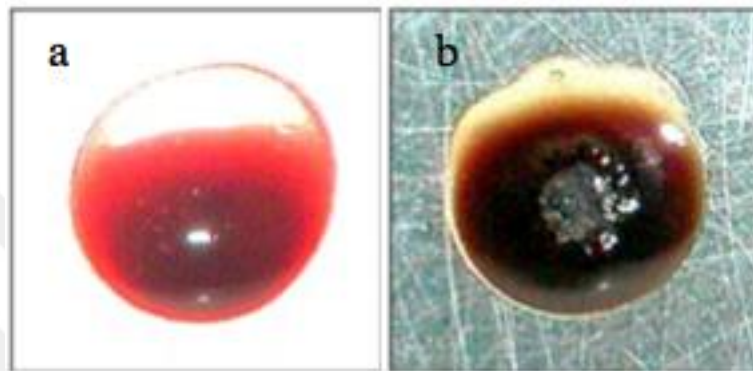
Plasma medicine is new and up-and-coming area. Thermal plasmas can be used for cauterization, tissue removal, cutting tissue, sterilization of medical tools in health areas. In addition, treatments of plasma health care can ensure quick and pretty influential according to protecting resistant microorganism to antibiotics, and hospital infections. It can be supplied the entering of reactive species from plasma to cell wall and cytoplasm, result of this stage cells affect [9, 38, 42]. Plasma medicine is come across some healthy treatments that are used for improving of wound healing and treatment of skin, cardiovascular, and digestive system. During these treatments, some reactions take place; these are shown in Figure 1.9. Moreover, dental treatment is applied by plasma nowadays. Plasma holds promise for cure of cancer. The bleeding controlling can be ensured by plasma applications. As well as treatment, plasma can improve biomaterial quality and it can provide tissue analysis, diagnosing of diseases. Cold atmospheric plasma is also purposive creating new drugs in pharmacological area [38, 43].



**Figure 1.9:** Effect of plasma and plasma species on cells

### 1.4.3.1 Plasma Assisted Blood Coagulation

Blood coagulation is a critical subject regard to wound healing in the medical area. It is a natural response of the human body to provide homeostasis, because of vessel damage. Friedman et al. confirmed to expedite blood coagulation with floating electrode-dielectric barrier discharge plasma (FE-DBD) by activating some of coagulation proteins and by rising  $\text{Ca}^{2+}$  ion concentration. These proteins provide rapid fibrinogen gathering.



**Figure 1.10:** Plasma assisted blood coagulation: (a) Untreated blood, (b) Blood drop after 15 seconds of FE-DBD plasma treatment

The tests are demonstrated that the cold plasma treatment can trigger natural coagulation processes in Figure 1.10. The plasma can change the ionic strength of the solution and pH [9, 38].

### 1.4.3.2 Plasma Applications for Cancer Therapy

Cancer is called as a disease which in some cells have rised metabolism and avoid the processes of natural regulated growth and death for different causes. They can pile up in the body than other normal cells. According to current studies, the non-thermal plasma treatment could play a role in cancer therapy. Fridman *et al*, 2013, have researched that melanoma cells go through necrosis at specific plasma treatment doses that were 15 s at  $1.4 \text{ W/cm}^2$  and apoptosis at lower plasma doses 5 s at  $0.8 \text{ W/cm}^2$ . Additionally, Vandamme et al. have reported antitumor activity of cold atmospheric DBD plasma on U87MG malignant glioma and HCT-116 colorectal carcinoma. According to results, accumulation of cancer cells in S phase, which is indicator of decayed of cancer cell proliferation and generation of DNA damage guided to apoptosis and multiphase cell cycle arrested due by the reason of plasma generation of ROS. Kim et al. have submitted a new way to cancer treatment by non-

thermal plasma treatment. They have demonstrated that cultured TC-1 lung carcinoma cells are more vulnerable to non-thermal Helium microplasma jet treatment than cultured fibroblasts and undergo apoptosis. According to forementioned information, non-thermal plasma treatment could be a new method for cancer therapy [9, 38].

#### **1.4.3.3 Dental Applications of Cold Atmospheric Plasma**

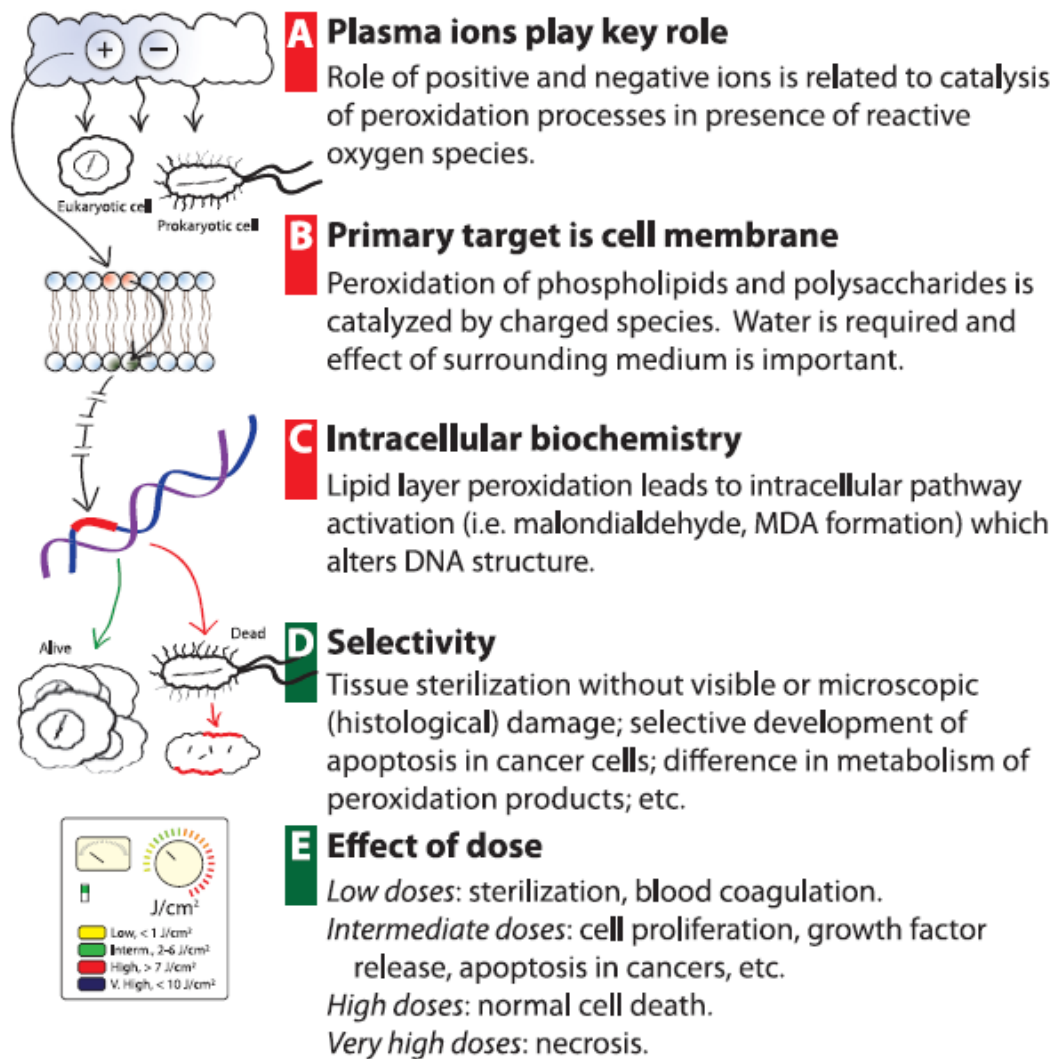
There are three major applications in dentistry. First one is related to killing of bacteria is subjected teeth surfaces and inner tooth root canals. Also, it provides some anti-inflammatory activity. Secondly, the plasma treatment enhances adhesion and incorporation of materials like included in tooth fillings. The last one is whitening of the tooth enamel [9, 38]. There were two practices about plasma and dentistry in our plasma medicine laboratory. Firstly the using atmospheric low temperature plasma activated hydrogen peroxide and deionized water on dental bleaching. Also, the second one was to evaluate the bleaching efficacy and colour stability of non-equilibrium plasma source activated agents . For this purpose two hundred bovine enamel-dentin samples were used by treating 40% hydrogen peroxide gel and plasma treatments during specific time. After these experiments, we obtained that plasma treatment could provide more effective and significant bleaching safely.

#### **1.4.3.4 Plasma Applications in Dermatology and Wound Healing**

Non-thermal plasma could be promoted wound healing by decreasing the microbial colonization and direct influence on skin cells. For this context, argon plasma jet (MicroPlaSter®) was used as an indirect treatment for wound healing firstly. The plasma treatment is efficient to give acceleration wound healing by decreasing number of bacteria such as *Klebsiella oxytoca* and *Enterobacter cloacae*. Food and Drug Administration (FDA) has accepted the plasma skin regeneration technology. This thermal nitrogen plasma jet was used for some clinical application such as superficial skin lesions, wrinkles [9, 44]. Also Emmert et al. has submitted that the DBD plasma treatment for 1 min/day during 30 days. They have obtained significant reduction of *S.aureus* colonization on the skin with decreased itching, as a result the healing of atopic eczema has improved [45].

### 1.4.3.5 Cold Atmospheric Plasma - Cell Interactions

Plasma provides not only physics effect but also it provides therapeutic effect. It induces biological reply in cells and living tissue. With this influence, it can be thought that interaction mechanism relies on method of plasma producing and the accepting of producing plasma by organisms. In order to obtain wished effect on living structure, reactive species, electrons, and ions have important roles. These interaction have been showed that in Figure 1.11. Moreover, the quicker bacteria inactivation on tissue is provided by direct plasma [43].



**Figure 1.11:** Plasma interaction with living tissue

### 1.4.3.6 Plasma sterilization

Plasma sterilization depends on an assembly of factors like Ozone, UV photons and reactive species. The generating of plasma is easy and cheap via atmospheric plasma. Moreover it provides safe sterilization comparing toxic chemical substances. The

results of experiments show that various cold plasma treatments that are Dielectric Barrier Discharge, plasma jet, gliding arc discharges wipe out of organism that can be in planktonic or biofilm version [7, 9, 46]. Plasma sterilization can be utilized various areas that are living tissue, the disinfection of large-scaled of air and water streams, inactivation of hazardous pathogens in food industry, the finishing dangerous bleeding without harming tissue [38].

### **1.5 Nebulization**

There are different ways that are decided by doctors for delivering medication related to illness to human bodies. A nebulizer works by linking tubes with a compressing air source. This medical tool is adjusted the oxygen and air are blasted to the liquid deposit rapidly and inhaled to patient. This transition will occur aerosol that include the medication needed for patient [47]. Nebulization is a treatment of medical concept by inhalation. These treatments are effective for respiratory tract problems that are pneumonia, respiratory congestion, asthma bronchospasms etc. Practically nebulizer is used for infants and small children. Nebulizer using that is inhaled treatment is more effective than intravenous treatment because of reaching directly infected lung tissue. Antibiotics can be used to heal against bacterial infections via nebulizer even if they do not prevent from lung infections enough. [48-50]. Nebulized antibiotics are used for treating respiratory infections almost 70 years. It has important advantages that are related decreasing side effects. Although nebulized antibiotics seem to be the best way for ventilator-associated pneumonia, it has many obstacles for healing of this disease [51]. For this concept, we consider that plasma medicine is a new approach for finding a solution for VAP.

### **1.6 Aim of Study**

Plasma medicine is a new and rapidly developing field that aims to develop new therapeutic solutions. The cold atmospheric plasma is used in different medical areas that are mentioned before. The plasma medicine treatments have significant antimicrobial effects on bacteria, fungi, viruses and prions. Ventilator-associated pneumonia is an important healthcare problem that increases morbidity and mortality. It develops as a result of biofilm formation in endotracheal tube. The purpose of this study is to develop a method of plasma treatment which can be



preventing the formation of biofilm and inactivation of biofilm that is formed in the endotracheal tubes with the conducting of Cold Atmospheric Plasma.



## **2. MATERIALS AND METHODS**

### **2.1 Cultivation of Microorganisms**

*Staphylococcus aureus* (ATCC 25923), *Acinetobacter baumannii* clinical isolate, *Pseudomonas aeruginosa* (ATCC 27853), and *Candida Albicans* (ATCC 90028) strains were supplied from Department of Microbiology, Medical Faculty at Ege University on agar plates. One colony of each pathogen was collected from agar plates and grown overnight at 37 °C and 200 revolution per minute (rpm) in shaking incubator. Bacteria were cultivated in Trypticase Soy Broth Medium (TSB) (Sigma-Aldrich-22092-500G) and *C.albicans* was cultivated in yeast extract-peptone dextrose (YPD) (Sigma-Aldrich-Y1375-250G). After overnight cultivation, 100 µl of grown cultures were transferred and spread over agar plates. Bacteria stock cultures plates were prepared with Trypticase Soy Agar (TSA) (Sigma-Aldrich-22091) and yeast stock culture plate was prepared using YPD agar (Sigma-Aldrich-Y1500-250G) and the plates were stored at 4<sup>0</sup>C in the fridge. Whenever a new culture was needed one colony was collected and grown overnight in shaker incubator. Each stock plate was refreshed in every 2 months.

### **2.2 Preperation of Frozen Stock Cultures**

Frozen glycerol stock cultures were prepared with overnight grown cultures. 850 µl of overnight grown culture was mixed with 150 µl of sterile glycerol in a cryotube using aseptic technique in Class II Biological safety cabinet, homogenized by pipetting and stored -20 °C for one night. Then cryotubes were transferred to freezer and stored at -80 °C. Glycerol stock cultures were refreshed every 6 months. Whenever needed, one cryotube was thawed and plated over agar plate to prepare a fresh agar stock culture. It has shown that in Figure 2.1.



**Figure 2.1:** Prepared stock cultures for using in experiments agar (A) and glycerol (B)

### 2.3 Preparation of N-Acetyl Cysteine (NAC) Solution

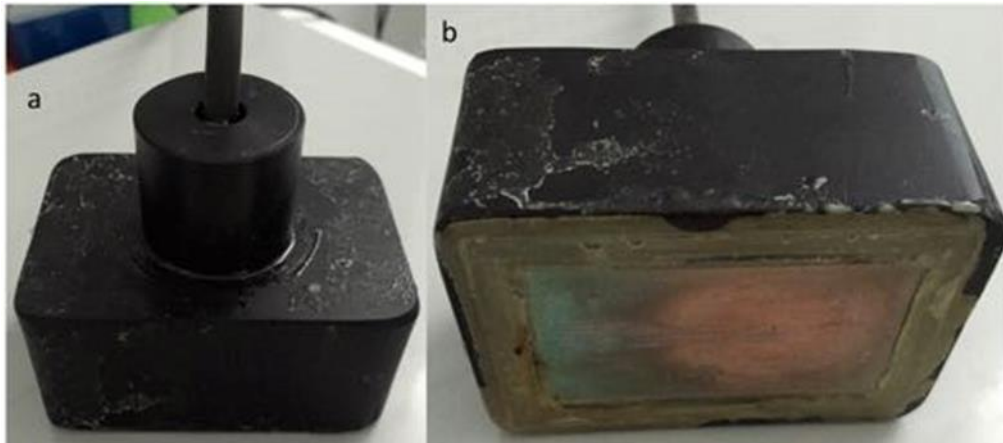
In all experiments, a final 5 mM concentration of working N-Acetyl cysteine (NAC) (Sigma- Aldrich) solution was used. NAC powder was weighted and dissolved in 1X sterile phosphate buffered solution (PBS) to reach 100 mM concentration that is stored at  $-20^{\circ}\text{C}$  as NAC stock solution. Whenever needed NAC stock solution was appropriately diluted in 1X PBS solution in order to reach 5 mM concentration.

### 2.4 Designing and Manufacturing of Electrode and Fluid Holder

In order to generate non-thermal plasma, custom-made electrodes were used in our experiments.

#### 2.4.1 Dielectric Barrier Discharge (DBD) Electrode

In order to generate non-thermal DBD plasma, a custom-made electrode was used. A copper plate with 10 mm thickness and 38 x 64 mm dimensions was embedded into insulator polyethylene housing. The surface of the electrode was covered with a glass slide with 50 x 75 mm dimensions and 1 mm thickness and a high voltage electrical cable was soldered to copper plate to connect the electrode to plasma power supply. It is showed that below in Figure 2.2.



**Figure 2.2:** In front of custom made copper electrode (A) and the below of electrode (B) the glass on the electrode surface as a dielectric material and copper surface beneath of the glass were seeded.

#### 2.4.2 Plasma Jet Electrode

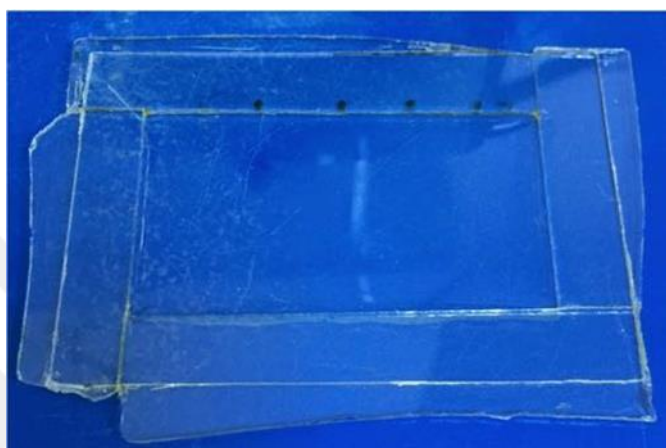
Plasma jet electrode for the plasma jet treatment was constructed using a hollow cylindrical copper. A glass pasteur pipette with a was glued in to inner side of cylindrical cooper electrode which serves as high voltage electrode. Then a copper wire was concentrically assembled to system, which serves as grounding electrode. Then a silicone tube was connected to electrode system in order to provide required gas flow. Hand made plasma jet electrode is shown that in Figure 2.3.



**Figure 2.3:** In the system for the plasma treatment, power supply, gas tube and electrode were seen (A). The supply connections which were high voltage, grounding, and gas stream (B).

### 2.4.3 Fluid Holder

A glass fluid holder was constructed to keep liquid during plasma treatment. The custom-made glass fluid holder was made using a glass slide (the base part) with 50 x 75 mm sizes. Appropriate parts from another glass slide were cut using a glass cutter. Those parts, which serve as to walls of the fluid holder, were glued the base glass slide to create a reservoir. The fluid holder has inner reservoir dimensions of 57 x 30 mm with a liquid column height of 1 mm below.



**Figure 2.4:** The custom made quartz fluid holder

### 2.5 Optimization of Plasma Parameters for Appropriate Antimicrobial Effects

A series of experiments have been carried out in order to determine the optimum antimicrobial effect. The power supply was operated at 31.4 kV of peak to peak voltage, 1.5 kHz of frequency in order to obtain significant eradications of microorganisms. Working concentrations of organisms were determined using spectrophotometer. Serial dilutions of overnight grown culture were transferred into spectrophotometer cuvette and absorbances were taken 0.2 absorbance at 600 nm represents  $10^8$  CFU/ ml for microorganism. Once the  $10^8$  CFU/ ml of concentration determined further dilutions were made when needed.

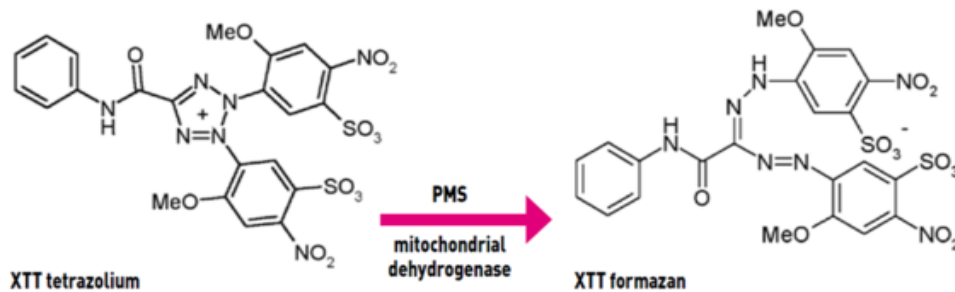
1 ml of  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$  CFU/ml of cultures were taken into fluid holder and direct DBD plasma treatment was carried out at a fixed 1 mm of discharge gap at different voltage and frequency settings.

## 2.6 Quantifications Methods for Antimicrobial Effect of Plasma Treatment

XTT and safranin assays were used to detect the effect of plasma treatment.

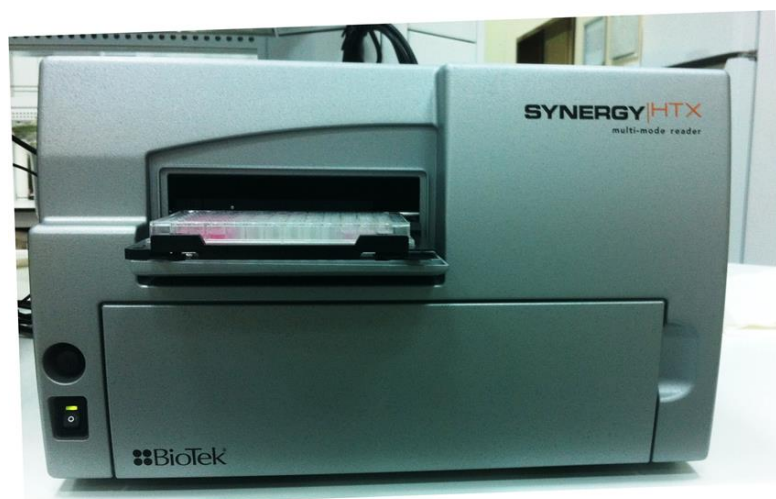
### 2.6.1 XTT Assay

XTT (2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) assay is a measurement method depends on the respiratory metabolic activity of living cells. It is a tetrazolium salt that is depending upon the modification of the yellow tetrazolium salt XTT to occur an orange formazan dye because of metabolic activity of cells [9].



**Figure 2.5:** Chemical reaction of XTT [52].

XTT and menadione solutions with final concentrations of 0.5 mg/mL and 50  $\mu$ M were prepared respectively with 1X PBS, 50  $\mu$ l of XTT and 1  $\mu$ l of menadione was added into 1 ml of each sample that were tested and incubated at 37  $^{\circ}$ C. After the incubation, 200  $\mu$ l of sample was collected from each endotracheal tubes and transferred into 96 well plate. Then absorbance of each sample was taken using a multiwell plate reader at 492 nm. It is below in Figure 2.6.



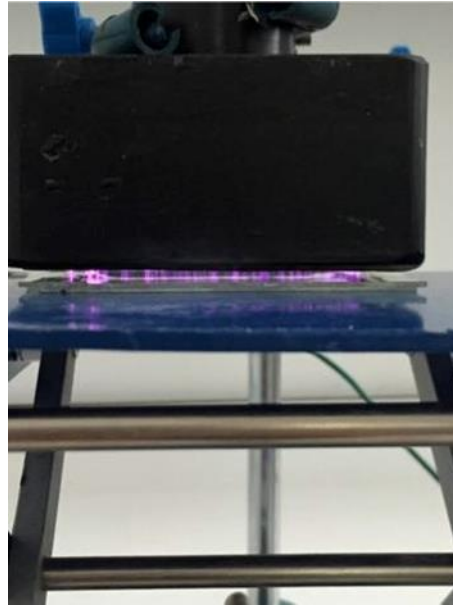
**Figure 2.6:** Samples were read via Multi-mode reader

### **2.6.2 Safranin Assay**

Safranin test was used to detect the amount of extracellular polymeric substance that provides attachment of cells to each cells and surface during biofilm formation. Biofilms were grown and treated with various plasma treatment methods. Then, tubes were washed using 1X PBS to remove non-adherent cells. Afterwards, 2 ml 1% safranin was added on the endotracheal tubes and held for 15 minutes. Then the samples were washed with 1X PBS to remove excess safranin from the surface of endotracheal tubes and samples held for 30 min to let the samples dry completely [53]. Once the samples were dried, 2 ml of 30% acetic acid was added to each sample to dissolve safranin that stained EPS to be quantified. 200 ul of dissolved safranin was transferred into 96-well plate and absorbance of each well was measured at 550 nm.

### **2.7 DBD Plasma Treatment of Liquids**

At each treatment, 1 ml of deionized water, PBS solution and NAC solution were treated at a time. A micro second pulsed AC power supply was used to generate atmospheric cold plasma for given voltage frequency. For the liquid mediated plasma treatment 1 ml deionized water, 1 ml 1X PBS and 1 ml 5 mM NAC solutions were transferred to fluid holder separately and treated with DBD plasma for particular time points. During treatment of liquids, the discharge gap was fixed to 1 mm. It shows that in Figure 2.7.



**Figure 2.7:** Direct plasma treatment of suspended organisms culture with 1 mm discharge gap.

### **2.8 Antimicrobial Activity of Nebulized Plasma Treated Liquids**

1 ml of  $10^7$  CFU/ml *S.aureus*, *P.aureginosa*, *A.baumannii* and *C.albicans* were placed six well plate and the palte was transferred in to biological safety cabin to let the samples dry. After samples were fully dried, 2 ml of plasma treated liquid was transferred into nebulizer and nebulized over organisms. Total nebulization time of 2 ml liquid was measured as about 7 minutes. 3% hydrogen peroxide was used as a negative control. The treatment image is shown that in Figure 2.8



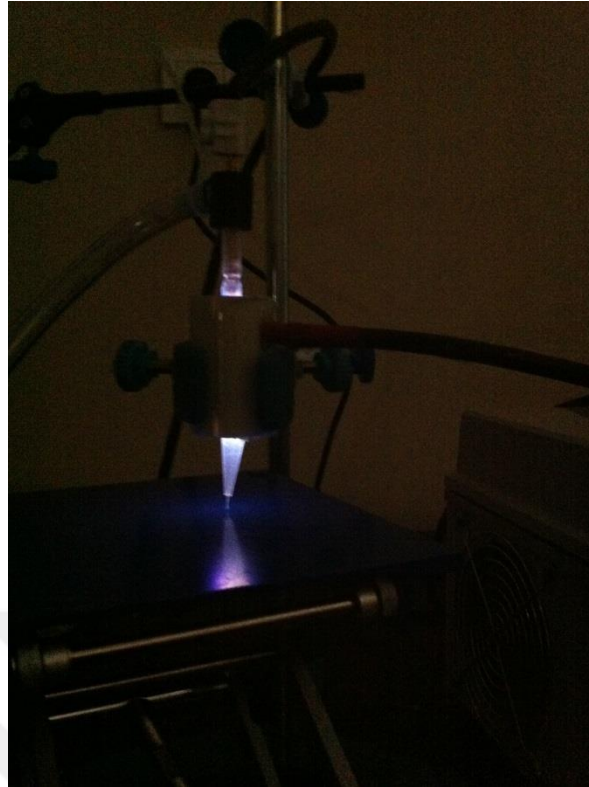


**Figure 2.8:** The application of plasma treated liquid on the suspense organisms

After the nebulization process, samples were held for 10 minutes for interaction between organisms and treated liquid and then 1 ml of 1X PBS was added on bacteria and yeast for resuspension. Then serial dilutions were done appropriately and 100 µl of each sample were plated on nutrient agar plates and plates were incubated at 37°C for 24 hours. After incubation, surviving colonies were counted to determine antimicrobial activity. Each experiment was repeated 3 times.

**2.9 Evaluation of the Antimicrobial Activity of Plasma Jet Application**

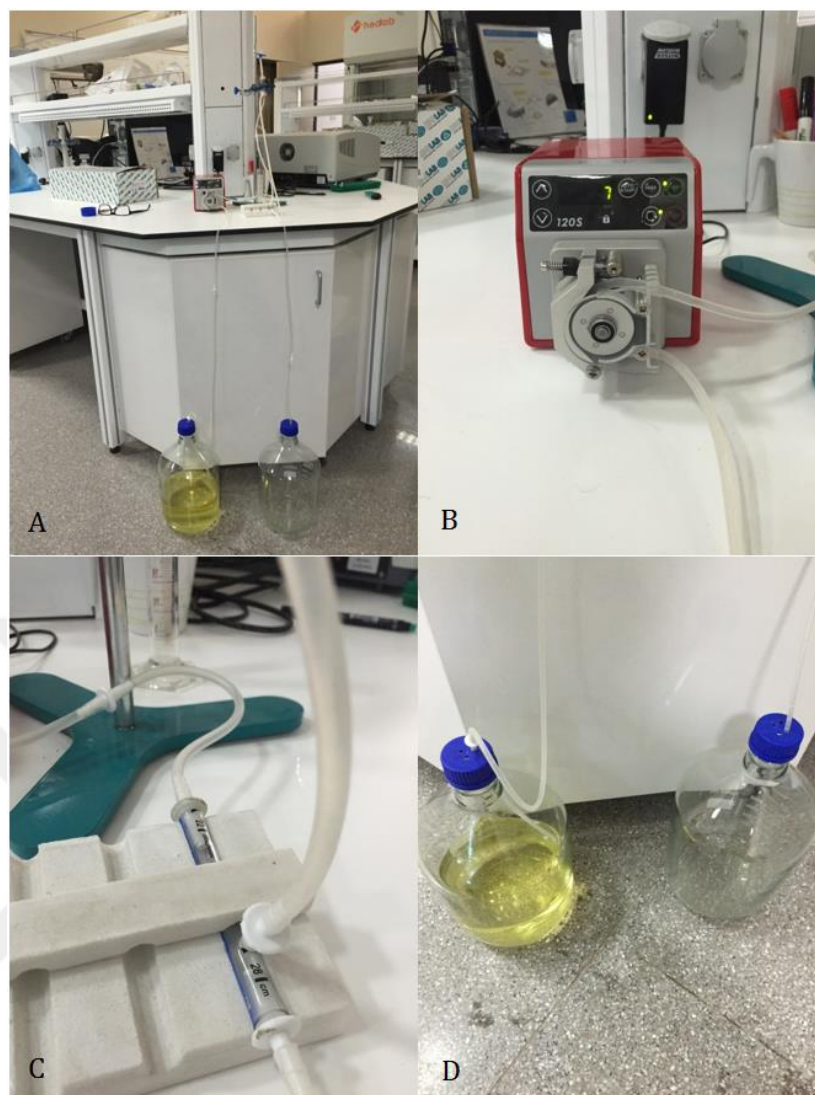
Antimicrobial effects of air, He/O<sub>2</sub> (99.5%: 0.5%) and N<sub>2</sub>/O<sub>2</sub> (90%: 10%) plasma jets, were tested on 1 ml, 10<sup>7</sup> CFU/ml of *S.aureus*, *P.aureginosa*, *A.baumannii* and *C.albicans* at various frequency settings, treatment time and gas flow volume. In plasma jet optimisitaion study, 2, 4, and 6 standart liters per minute of gas flow, 500 Hz, 1000 Hz, 1.5 kHz of frequency were tested at a constant voltage of 31.4 kV at 5, 15 and 60 seconds of treatment time points. Plasma jet treatment is shown that in Figure 2.9.



**Figure 2.9:** Plasma jet plasma (He/O<sub>2</sub>) treatment of suspended organisms culture.

### **2.10 Optimization of Drip Flow Reactor (DFR) for Formation of Biofilm**

A drip flow reactor system is useful to grow low shear biofilm at an interface of air and liquid. These reactors are adaptable to different conditions. They are appropriate for laboratory working. The significant of biofilm biomass that is needed can be obtained [54]. In our study, DFR was utilized in order to grow biofilm in endotracheal tubes similar to clinical conditions. 5 cm pieces of endotracheal tube were cut and connected to reactor system, which is composed of a closed system of tubing and a peristaltic pump as shown in Figure 2.10. In the system of the container contained fresh medium and the other container was used as waste collection. An overnight organism culture was grown in shaker incubator and biofilm medium was prepared as described previously.



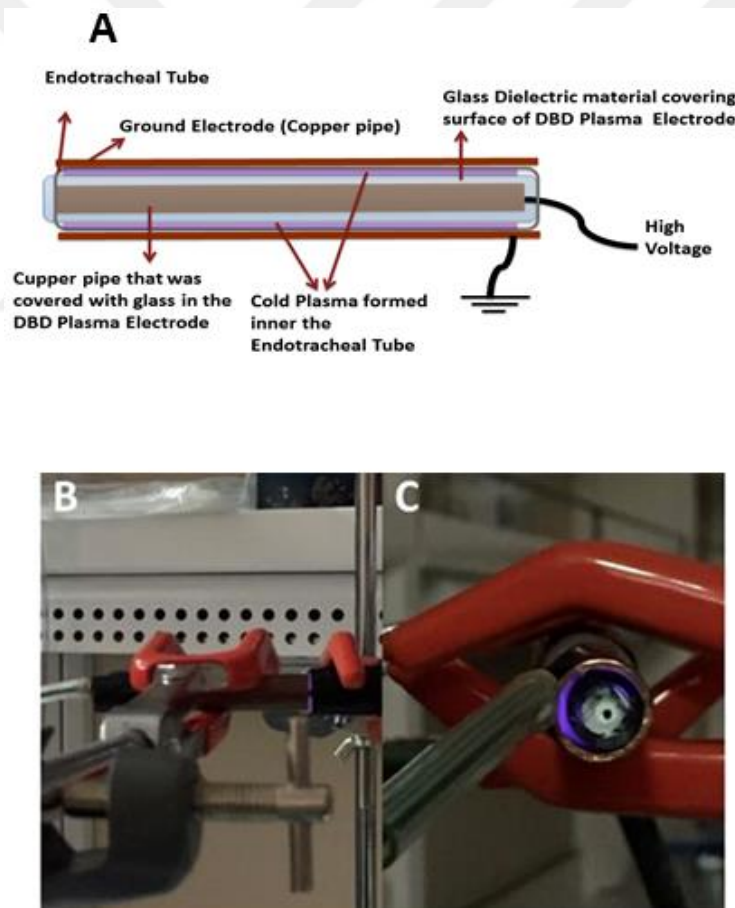
**Figure 2.10:** The overview of a drip flow reactor system established for biofilm formation in endotracheal tubes (A), peristaltic pump (B), growing biofilm in endotracheal tubes (C), the views of feeding container that provided fresh broth and waste container where collected waste (D).

The biofilm medium was circulated in the system until the whole system was filled with biofilm culture and the pump was stopped and held for 6 hours in order to allow growth of bacteria. After 6 hours of growth of bacteria fresh medium was circulated in the system for 18, 42 and 66 hours in order to reach a total 1, 2, and 3 days of maturation duration. After completion of maturation period, colony-counting test was carried out to determine biofilm growth.

### 2.11 Tests for Prevention of Formation of Biofilm

As previously reported in the literature, materials other than fluids may gain antimicrobial activity when treated with plasma. Therefore, we have tested if biofilm

formation could be prevented on the surface of plasma treated endotracheal tube. A cylindrical DBD plasma electrode was designed to treat inner side of endotracheal tube. A copper wire was inserted into a glass tube and sealed using silicone to build up high voltage electrode which could be inserted into endotracheal tube. Then the endotracheal tube with the high voltage electrode inside, was placed inside a copper tube which serves as grounding electrode. The defined setup provides plasma treatment the inner surface of endotracheal tube. Inner side of endotracheal tube was treated at 1.5 kHz frequency and 31.4 kV voltage for 15 seconds, 1 and 2 minutes. This system is shown that in Figure 2.11. After plasma treatment for formation of biofilm, endotracheal tubes were connected to drip flow reactor. When the biofilm formation was completed, in the other words, one day later, endotracheal tubes were separated from drip flow reactor and colony-counting assay was performed.



**Figure 2.11:** The inner surface of the endotracheal tube designed to be treated with plasma DBD electrode (A): schematic, (B): side and (C): in front of it.

## **2.12 Antibiofilm Effect of Plasma Treatment**

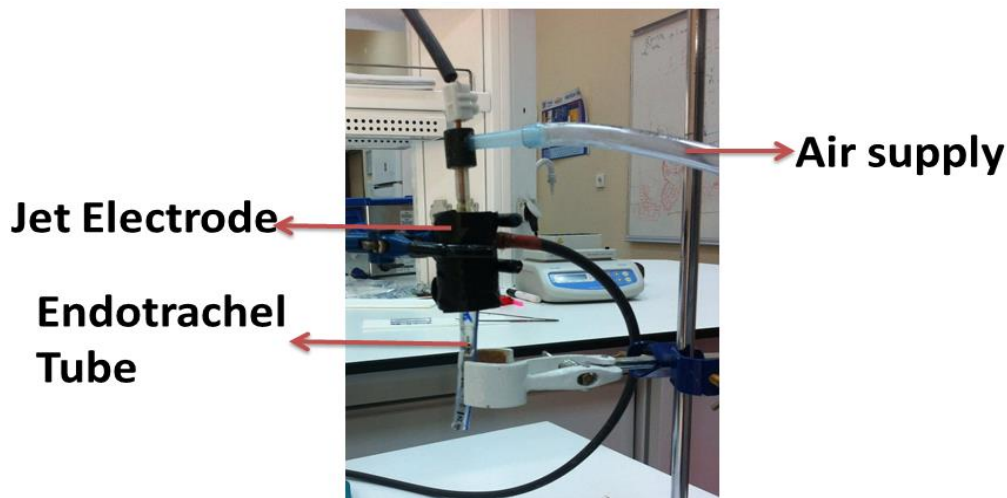
Antibiofilm effects of plasma treatment were tested on biofilm forms of mentioned organisms and methods that are followed for different plasma treatment techniques are given below.

### **2.12.1 Antibiofilm Effect of Plasma Nebulized Treated Liquid**

Antibiofilm effect of nebulized plasma-treated liquid was tested on biofilm forms of *Staphylococcus aureus* (ATCC 25923), *Acinetobacter baumannii* Clinical Isolated Stream, *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 90028) strains. Deionized Water, PBS and NAC solutions were treated by plasma as explained previously. At a time, 1 ml of each liquid was treated and 4 ml of total liquid was treated for each test. Biofilm was grown inside 5 cm long segments of endotracheal tube. We have cut 3 pieces of each tubes, the length was nearly 1 cm. Every piece was put in 2 ml PBS in the TUBE falcon in order to remove biofilm, they were stayed 5 minutes and 35 °C in the ultrasonic water bath sonicator and they were vortexed for homogenization. After this step 10 µl liquid were taken in the falcon and added ependorf, and 3 dilutions that was 1:10 were completed, after these serial dilution, petri plates were spreaded with 100 µl solution that had organisms and stayed in the incubator for one night, at 37 °C. Moreover, safranin and XTT tests were also carried for the characterization of biofilm. For the positive control no plasma treatment was used. Also, only gas flow without plasma discharge was applied in order to assure that gas flow does not inactivate biofilm physically. Before the nebulization, biofilm liquid that was stayed in the endotracheal was emptied and washed for removing non-adherent biofilm in the tube. Plasma treated liquids were transferred into nebulizer separately and the nebulizer was connected to endotracheal tube and plasma treated liquids were nebulized through the tube. The nebulization process was continued 20 minutes for each tube. After this step, almost 20 minutes were waited to complete interaction between nebulized plasma-treated liquids and biofilm. Untreated samples were used as positive control. Moreover, untreated liquids were also nebulized through endotracheal tubes to ensure that flow of nebulized fluid does not cause antibiofilm effect. After nebulization of plasma treated liquids, colony-counting assay was carried out as previously described.

### 2.12.2 Antibiofilm Effect Air Plasma Jet

Optimization experiments have shown that air plasma jet shows superior antimicrobial effect over He and N<sub>2</sub> plasma jets. Therefore, air plasma jet was selected for further antibiofilm experiments. Similar to nebulization experiments biofilms of different organisms were grown in endotracheal tubes as described previously. The jet electrode was connected to endotracheal tube and air plasma jet was generated at 31.4 kV, 1.5 kHz with 4 slm of gas flow. The jet treatment was applied for 1 minute; it is shown in Figure 2.12.



**Figure 2.12:** Air plasma jet treatment on the endotracheal tube

Before the treatment, biofilm was stayed in the endotracheal tube was emptied and washed for removing non-adherent biofilm in the tube and after the plasma treatment was done as described previous. For the positive control no plasma treatment was used. Also, only gas flow with out plasma discharge was applied in order to assure that gas flow does not inactivate biofilm physically.

### 2.13 Microscopic Characterization of Biofilm on Endotracheal Tube Surfaces

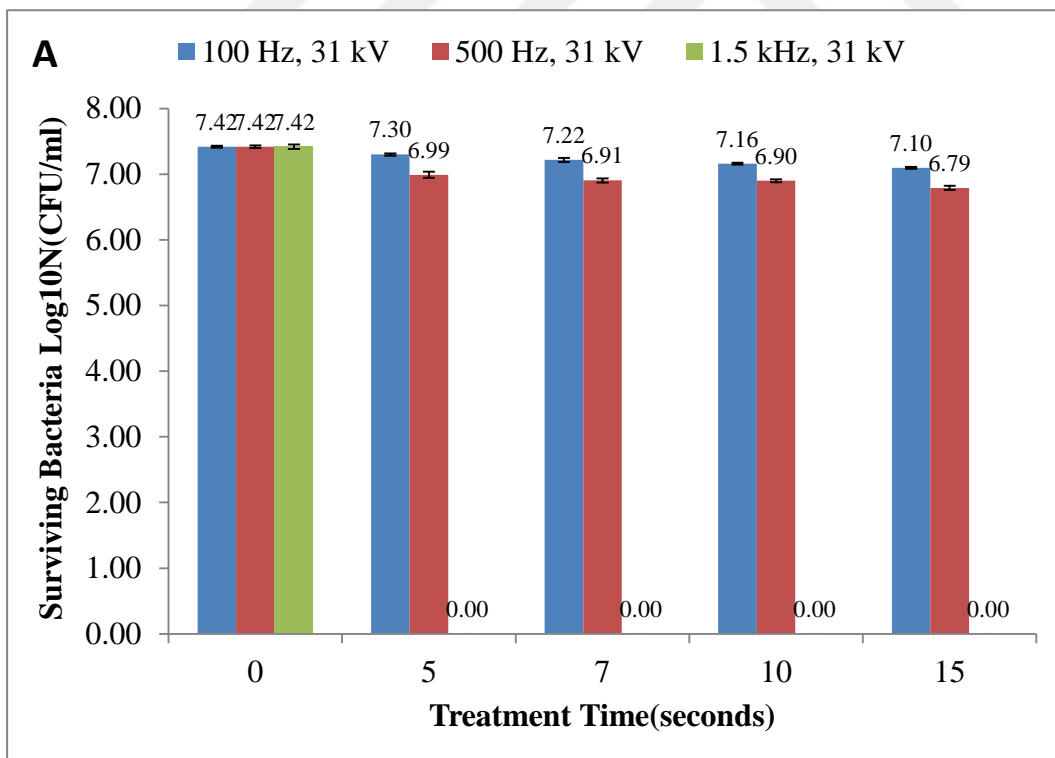
Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) were used to visiulize and characterize biofilm and antibiofilm efficacies of various plasma treaments inner surface of the endotracheal tubes. Before each microscopy, biofilms were grown on endotracheal tube surfaces and treated with various plasma treatment methods as described previously. For SEM imaging after plasma treatment, endotracheal tubes pieces were chemically fixed. For the fixation process, samples

were held in 2.5% glutaraldehyde for 2 hours, washed, then transferred into, 1% osmium tetroxide and washed again. Then samples were dipped in to serial ethanol baths with increasing concentration of ethanol (35%, 50%, 70%, 85%, 95%, and 100%) to remove deionized water. Then samples were held in hexamethyldisilazane (HDMS), for 45 minutes. Then all samples were kept at the room temperature during 12 hours to let them dry. Fixed samples were coated with gold just before the SEM imaging. SEM imaging was carried out at 7 kV of voltage (JEOL JSM-6060, Tokio, Japan). SEM imaging was done for all organisms and all types of plasma treatment that we mentioned before. For AFM imaging, biofilms were grown and various plasma treatments were applied in a similar manner as done for SEM imaging. After plasma treatment, samples held at room temperature in order to remove excess liquid. AFM images were collected using via AFM (Nanosurf Flex, Liestal, Switzerland) at non-contact mode and 164 kHz frequency.

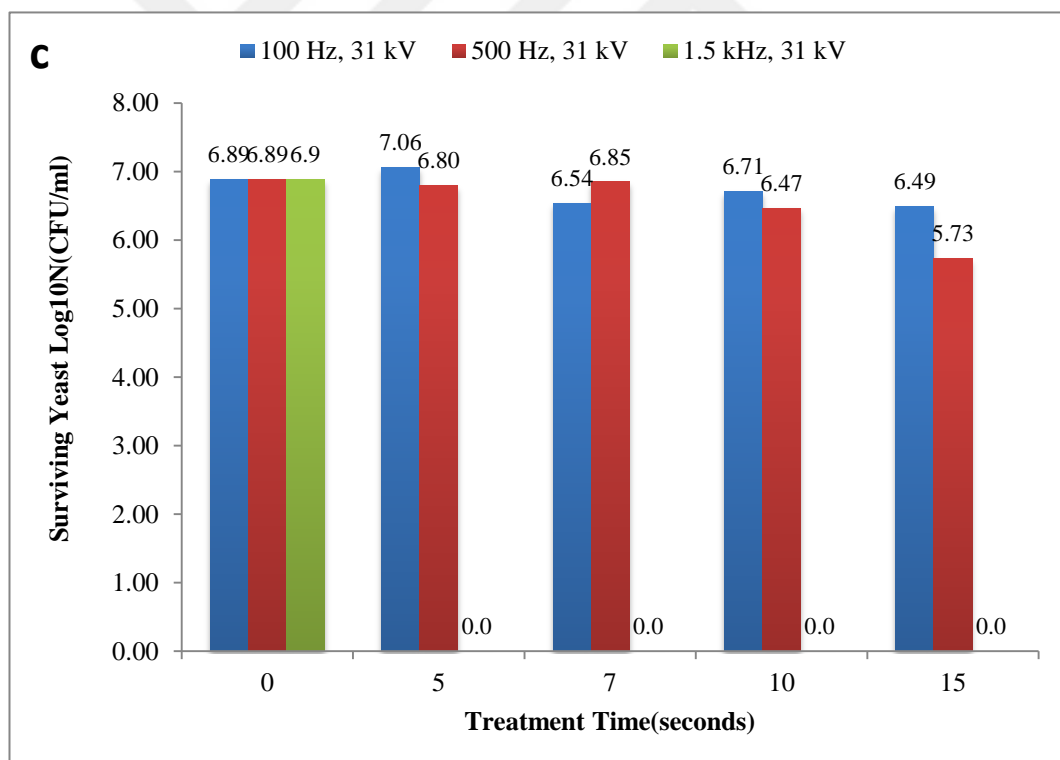
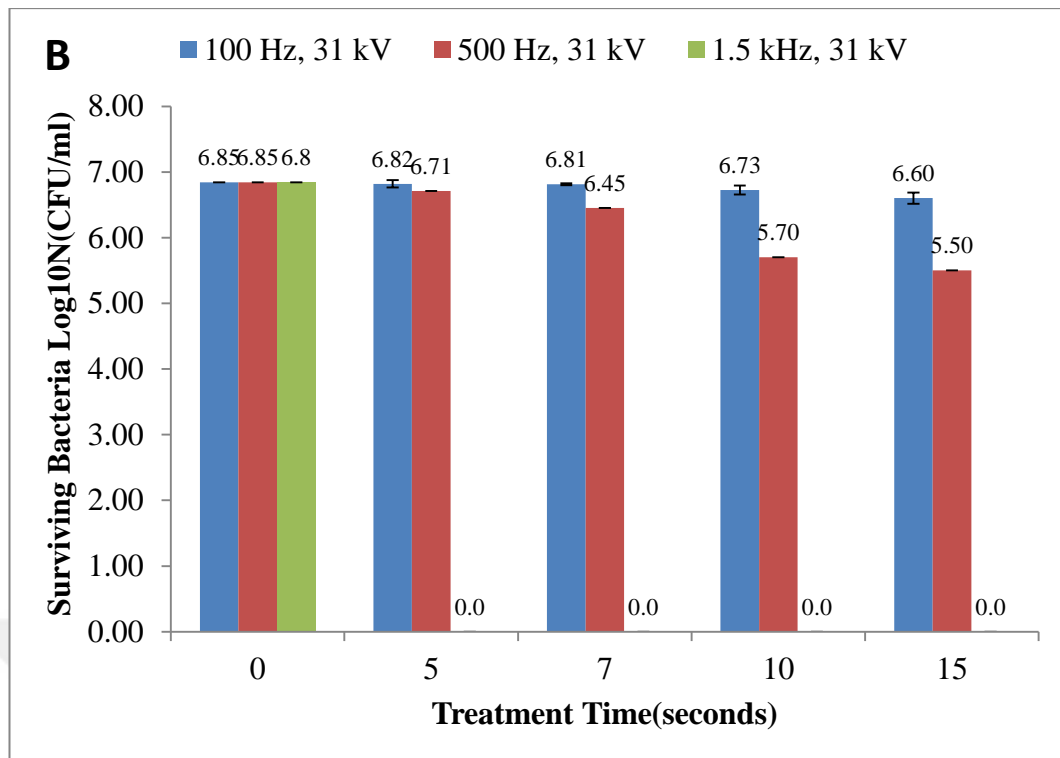
### 3. RESULT

#### 3.1 Optimization of Plasma Parameters for Appropriate Antimicrobial Effects

In the optimization experiments different voltage values ranging from 31kV to 31kV and different frequencies ranging from 100Hz to 1,5kHz were tested at 5, 7, 10 and 15 seconds of treatment durations for all organisms (*P. aeruginosa*, *A. baumannii*, *C. albicans*, and *S.aureus*). Our results have shown that direct treatment of organisms at 31 kV of peak to peak voltage and 1.5 kHz of frequency settings have led statistically significant ( $p < 0.05$ ) 7 log of reduction for all treatment durations as shown in figure 3.1. Therefore 31 kV of peak-to-peak voltage and 1.5 kHz of frequency were determined as optimum operating conditions of plasma power supply.







**Figure 3.1:** Antimicrobial effect of direct plasma treatment on *P. aureginosa* (A) *A.baumannii* (B) and, *C.albicans* (C) depending on changing frequency.

### 3.2 Evaluation of the Antimicrobial Activity of Plasma Jet Application

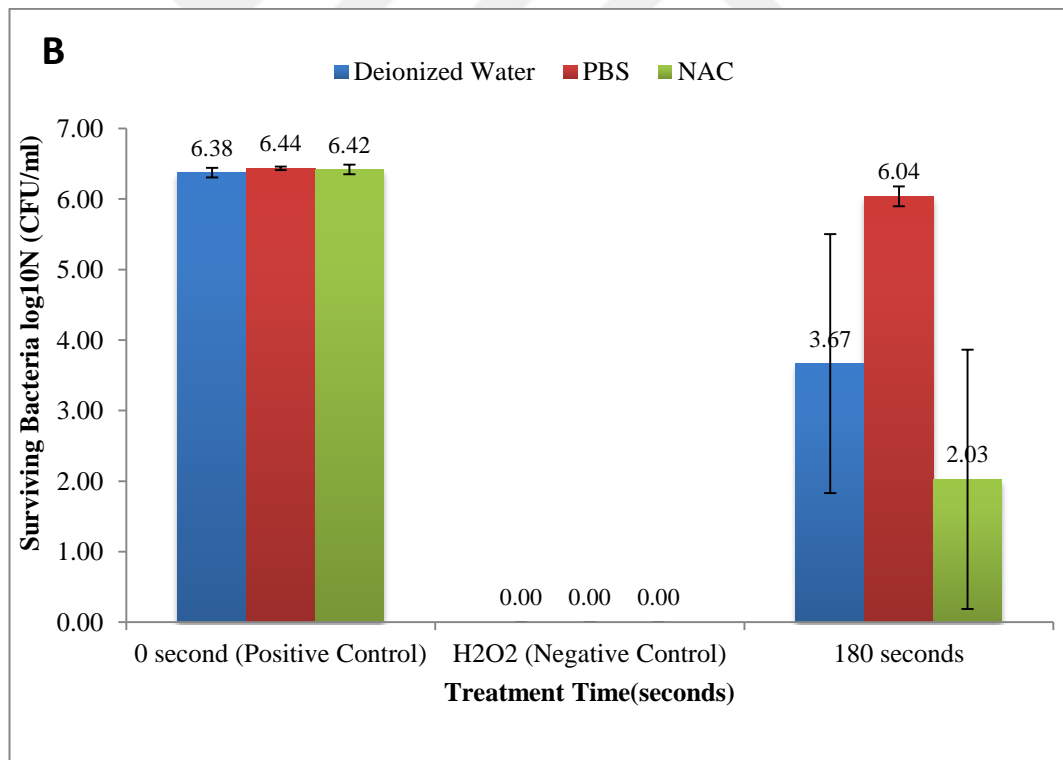
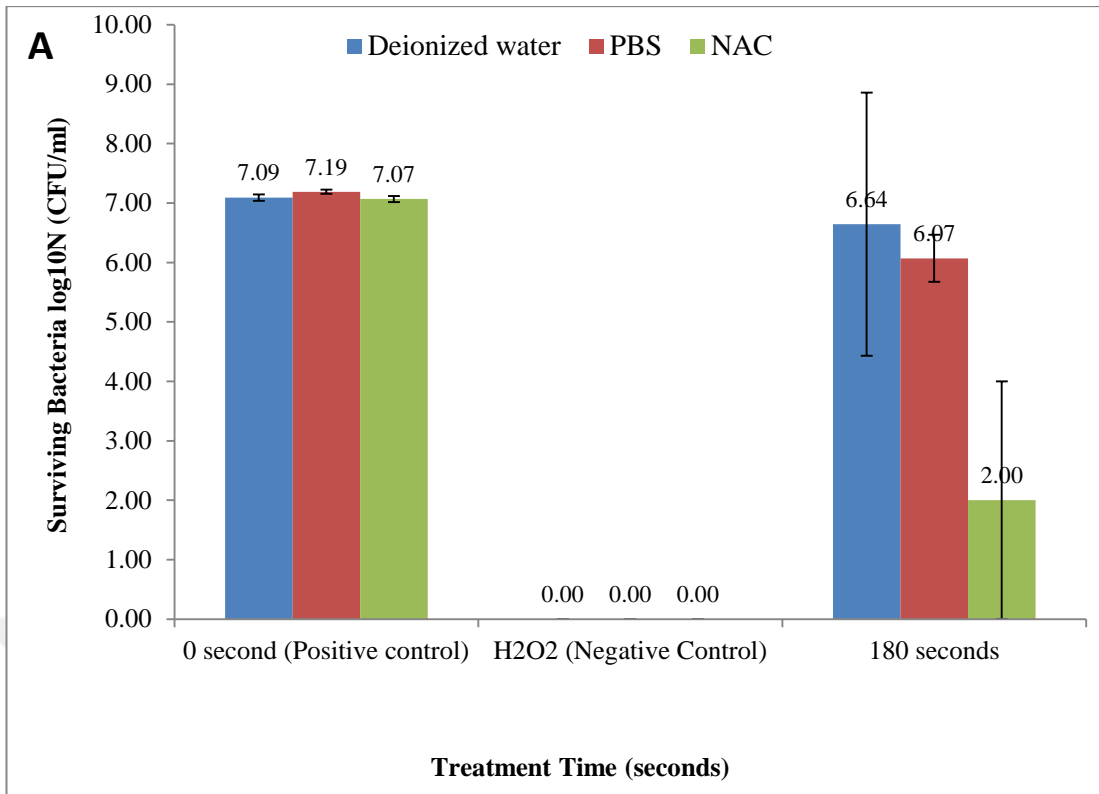
Similar to optimization of direct treatment experiments we attempted to determine optimum conditions for plasma jet treatment at various voltage and frequency settings as explained previously. In jet experiments we also have tested various gas types with various flow rates that are mentioned previously. As summarized in 3.1, air plasma jet that was operated at 31 kV of voltage, with 4 slm of flow rate lead complete (7 log) inactivation of all organisms after 60 seconds of treatment duration.

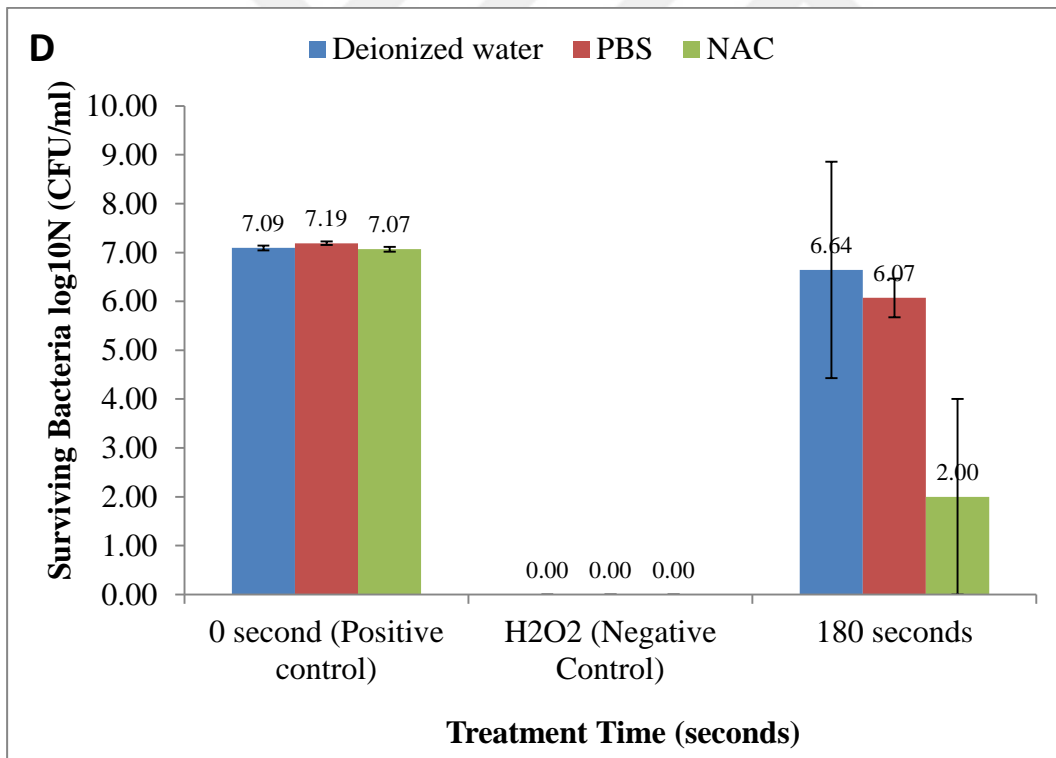
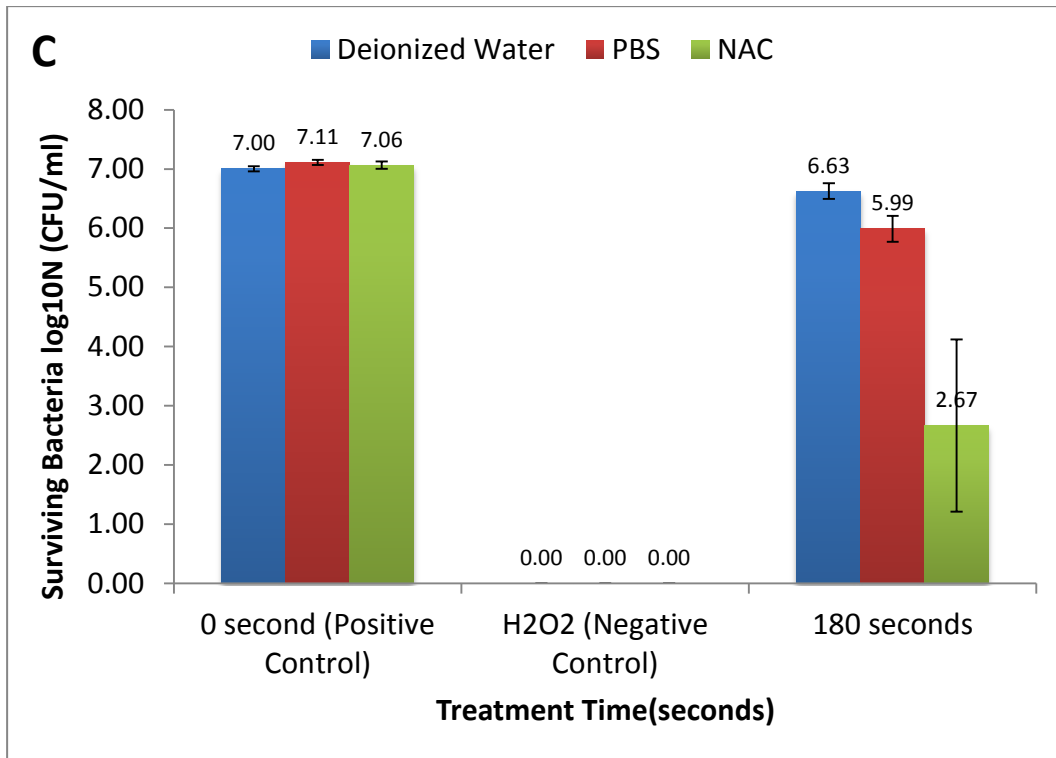
**Table 3.1:** Summary of results of antimicrobial effects that were obtained to determine optimum plasma jet parameters.

Combination of Gas	Assessment of Antimicrobial Activity
Dry Air	According result of dry air plasma treatment with below parameters, 4 slm gas flow rate, 60 seconds plasma treatment, 500 Hz, 31.4 kV and 1,5 kHz, 31.4 kV For all organisms, exact microbial inactivation (7 log) that was statistically significant ( $p < 0.05$ ) was obtained.
N <sub>2</sub> /O <sub>2</sub> (%90:%10)	Nitrogen-oxygen plasma jet treatment could not be seen any antimicrobial effects beside all parameters were tried.
He/O <sub>2</sub> (%99.5:%0.5)	Helium plasma jet treatment with all parameters that were tried was not seen antimicrobial effects.

### 3.3 Biofilm Inactivation Tests with Liquid mediated Plasma Treatment

Tests of liquid mediated plasma treatment show that only specific parameters that were 1.5 kHz and 31 kV could be obtained antimicrobial effect although different parameters were tried. According these results, 3-minute plasma treated NAC solution has efficient antimicrobial properties. Firstly, we detected  $10^8$  CFU/ml concentration of all organisms with spectrophotometer and we studied not only  $10^7$  CFU/ml but also  $10^6$  CFU/ml and  $10^5$  CFU/ml. Complete microbial inhibition was observed with initial  $10^6$  CFU/ml and  $10^5$  CFU/ml concentrations (Figure 3.2).

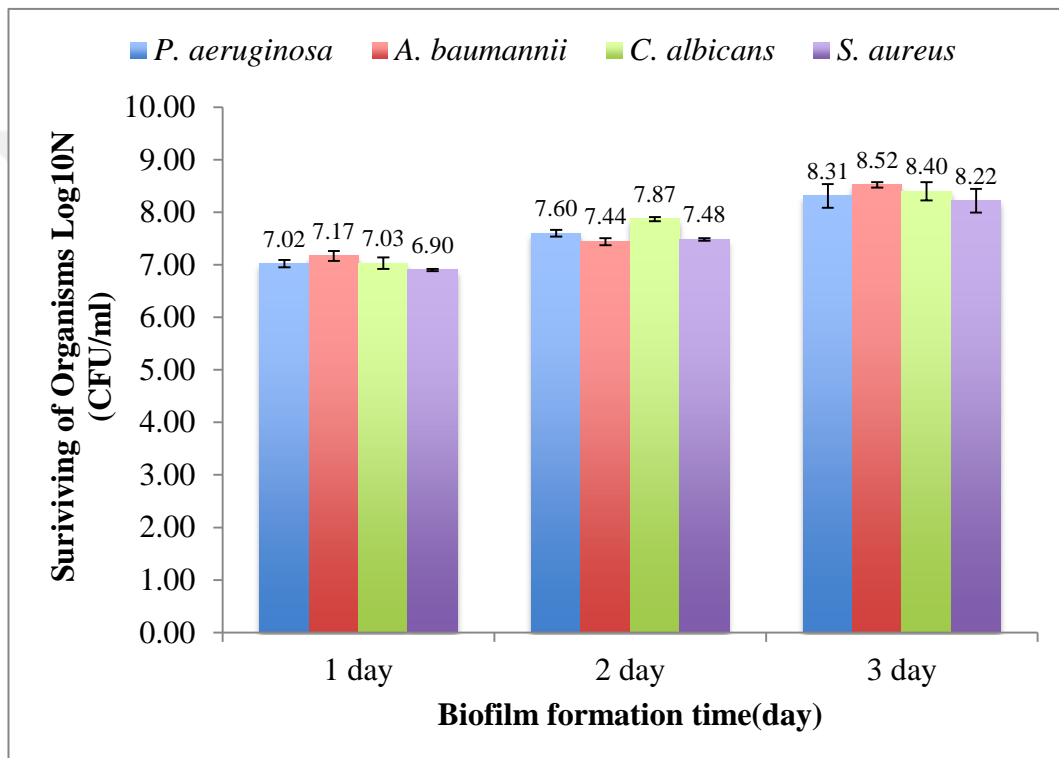




**Figure 3.2:** The antimicrobial effect of the liquid that was treated with plasma during 3 minutes at 1.5 kHz frequency and 31 kV voltage on *P. aeruginosa* (A), *A. baumannii* (B) *C. albicans* (C) and *S. aureus* (D) (\*: p<0,05).

### 3.4 Optimization of Drip Flow Reactor for Formation of Biofilm

We have determined required biofilm maturation duration in order to achieve about  $10^7$  CFU/ml of bacterial load on the 1 cm segments of endotracheal tubes. Therefore 5 cm long pieces of endotracheal tubes were connected to drip flow reactor system and were incubated for 1, 2 and 3 days. Our results have revealed that, a total 24 hours of biofilm maturation leads to  $10^7$  CFU/ml of bacterial load on the inner surface of endotracheal tube. Therefore in all biofilm experiments biofilm maturation duration was determined as 1 day.

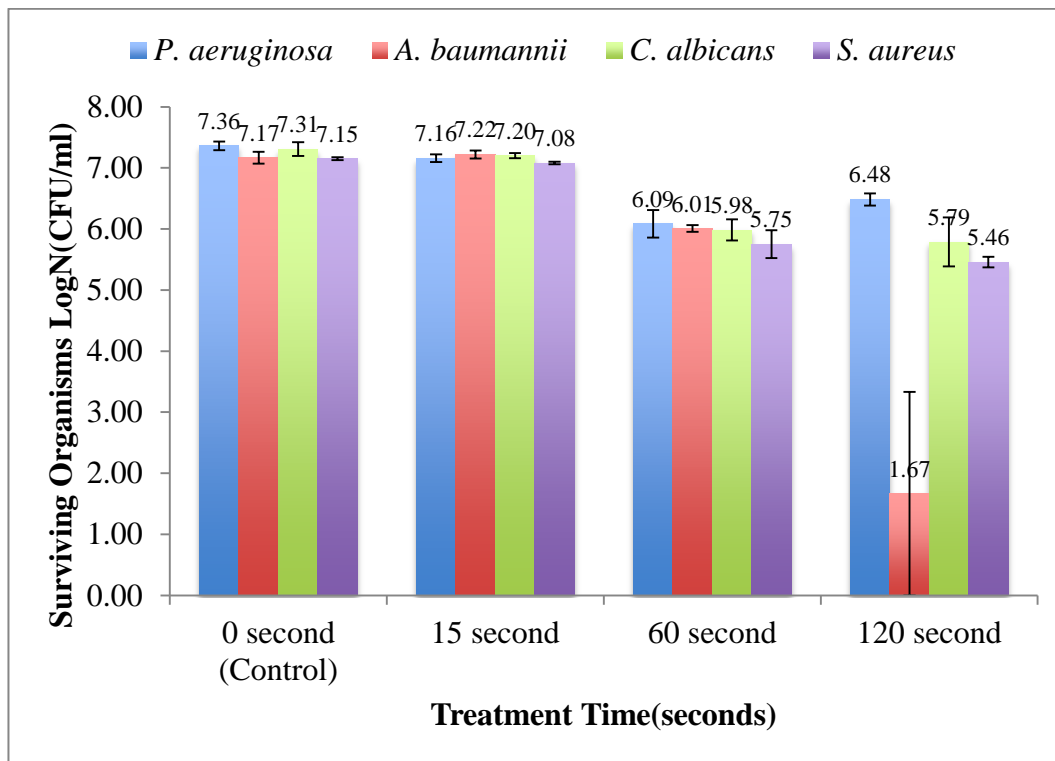


**Figure 3.3:** The amount of biofilm in the drip flow reactor depend maturing time.

### 3.5 Tests for Prevention of Formation of Biofilm

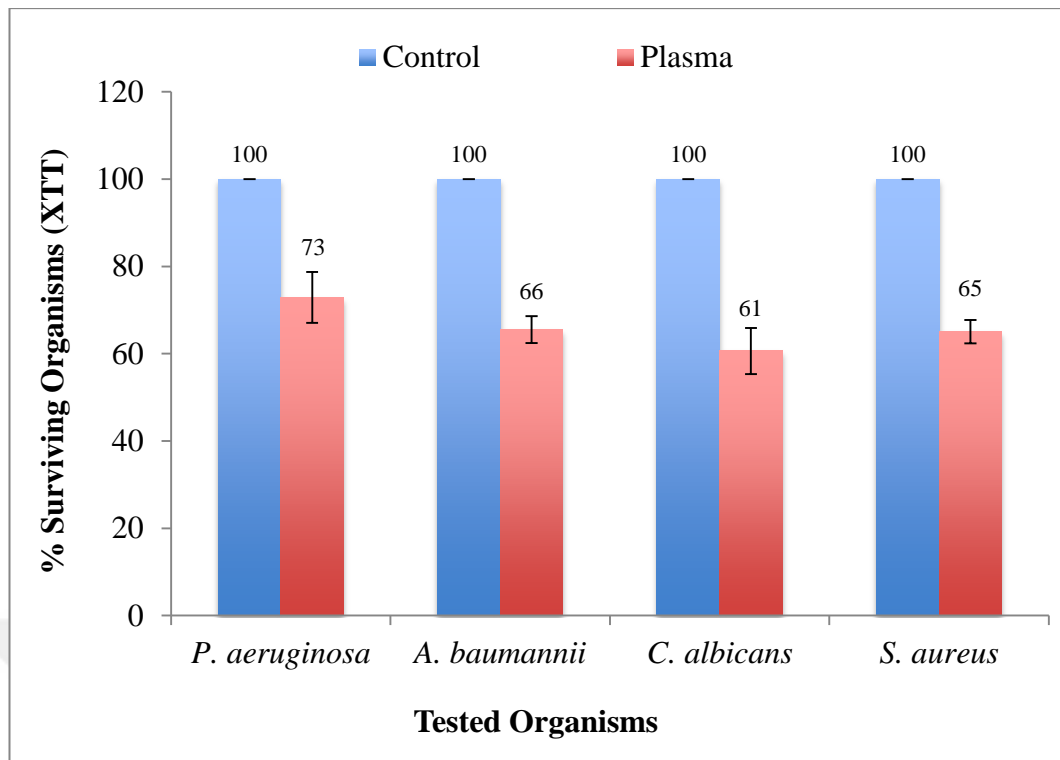
In order to evaluate biofilm prevention efficacy of direct plasma treatment, inner surface of endotracheal tube surfaces were firstly treated and then biofilms were matured. As shown in Figure 3.4. 60 seconds of pre-treatment of endotracheal tubes lead about 1-log prevention of biofilm. Increasing the pre-treatment duration from 60 seconds to 120 seconds improved biofilm prevention only for *A. baumannii*.

Pre-treatment of endotracheal tubes with air-jet plasma has not led any biofilm prevention according to colony counting assay. Therefore, XTT and safranin assays were not carried out for air plasma jet pretreatment experiments.



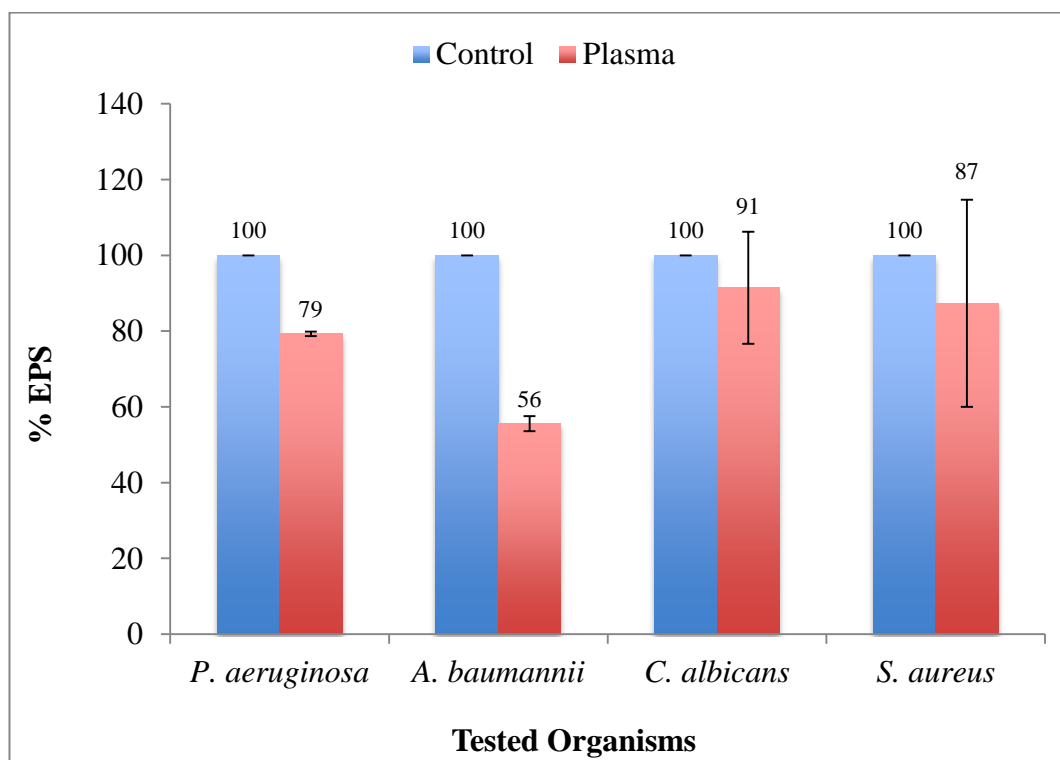
**Figure 3.4:** The blocking effect of biofilm formation depends on plasma pre-treatment time

Colony counting assay results for biofilm prevention were also validated with XTT and safranin assays. In XTT assay, biofilm viability results were expressed as a percentage. Inactivation was detected to depend on microorganism types, for instance, the value was 30% for *P.aureginosa* and for the others microorganism were nearly 35-40%. Except *A.baumannii*, XTT results of other microorganisms' strains were parallel by comparison with colony counting. Although we expected to lower percentage of viability, we obtained results like others organisms viability results (Figure 3.5).



**Figure 3.5 :** XTT Results following plasma pre-treatment

According to result of safranin assay, amount of EPS was decreased significantly with plasma pretreatment application for *P.aureginosa* and *A.baumannii* biofilms. On the other side treatments of *C. albicans* and *S.aureus* were not seen efficient reductions (Figure 3.6).

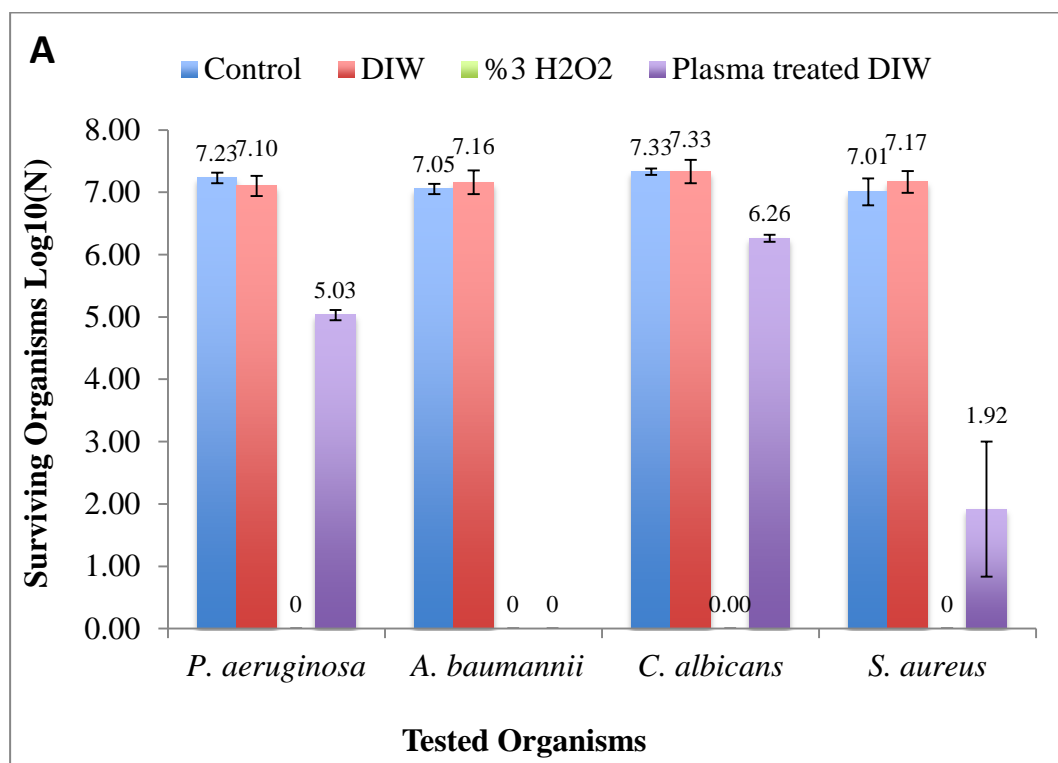


**Figure 3.6:** Results of Safranin Test after Plasma Pre-treatment

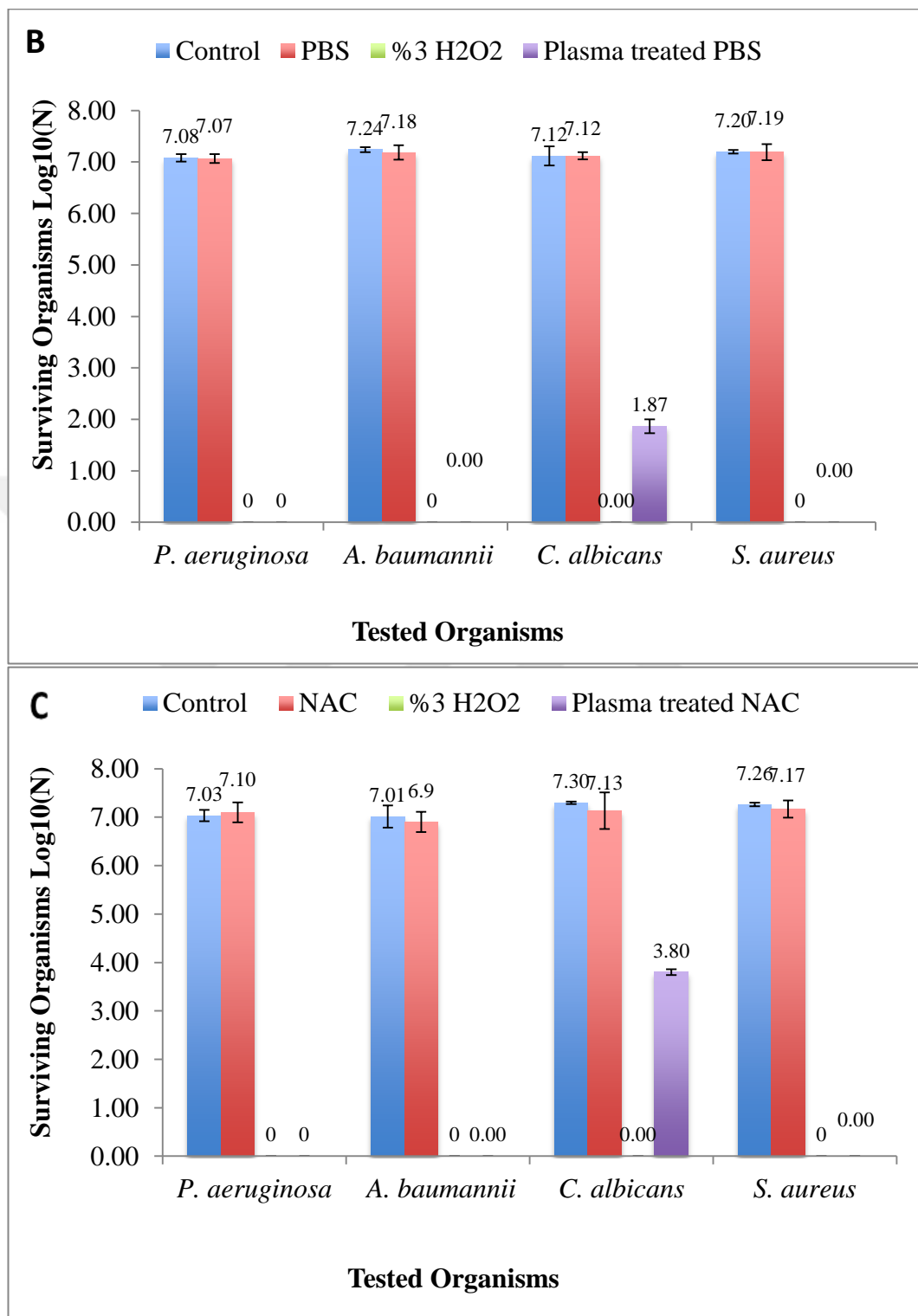
Taken together, it can be concluded that plasma pretreatment could be a unique method for the prevention of ventilator-associated pneumonia.

### 3.6 Biofilm Inactivation Efficacy of Nebulized Plasma Treated Liquids

Biofilm inactivation efficacies of nebulized plasma treated Deionized Water, PBS and NAC solution were evaluated with colony counting assay. Moreover, XTT and safranin assays were used to validate antibiofilm activity of nebulized plasma treated liquids. Colony counting assay result have showed that plasma treated water led 2 log reduction of *P. aureginosa* biofilm is 2 log inactivation, *A.baumannii* is 7 log, *C.albicans* is 1 log and *S.aureus* is 5 log inactivation (Figure 3.7-A). Secondly plasma treated PBS solution results show that there are 7 log inactivation for *P.auregina*, 7 log inactivation for *A.baumannii*, 5.5 log inactivation for *C.albicans* and 7 log inactivation for *S.aureus* (Figure 3.7-B). Third step was plasma treated NAC solution, we obtained significant results this part, there are 7 log inactivation for *P.aureginosa*, *A.baumannii* and *S.aureus*, for the last results related to *C.albicans*, it was observed 3.5 log inactivation (Figure 3.7-C).



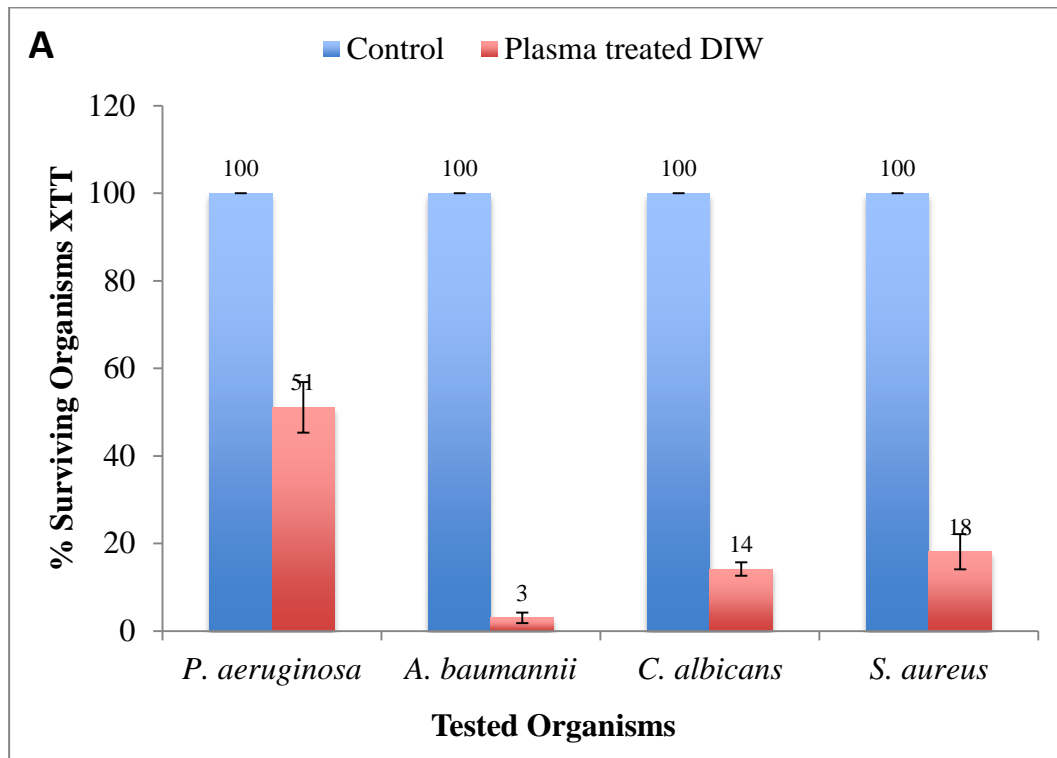


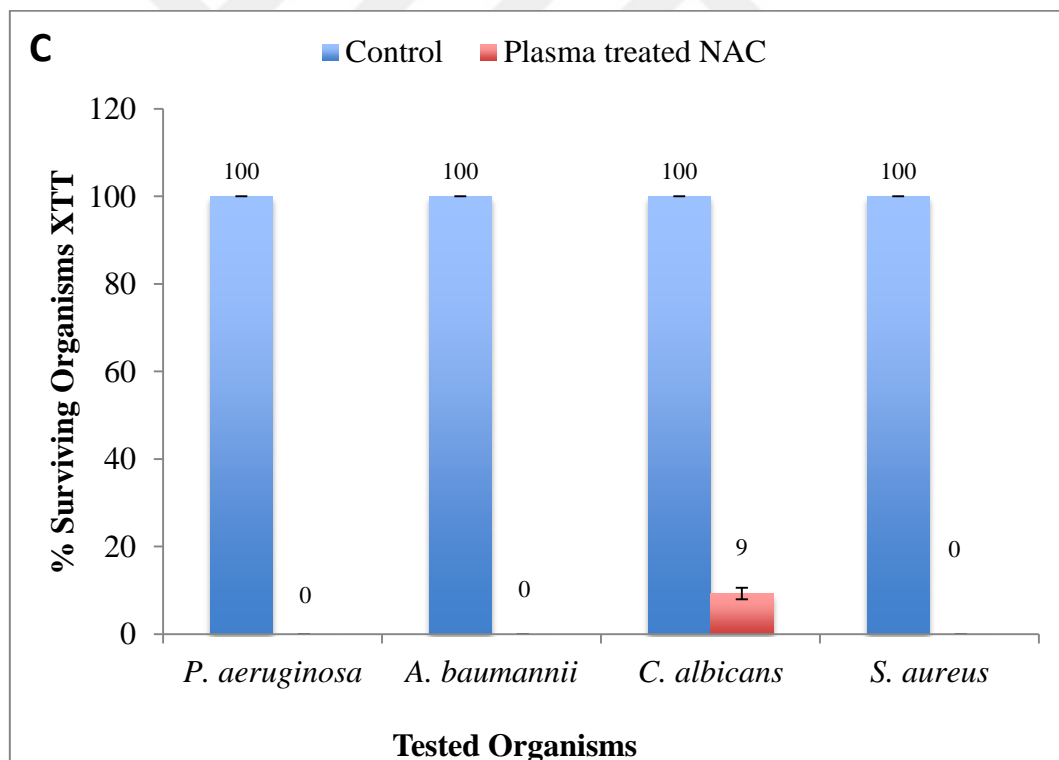
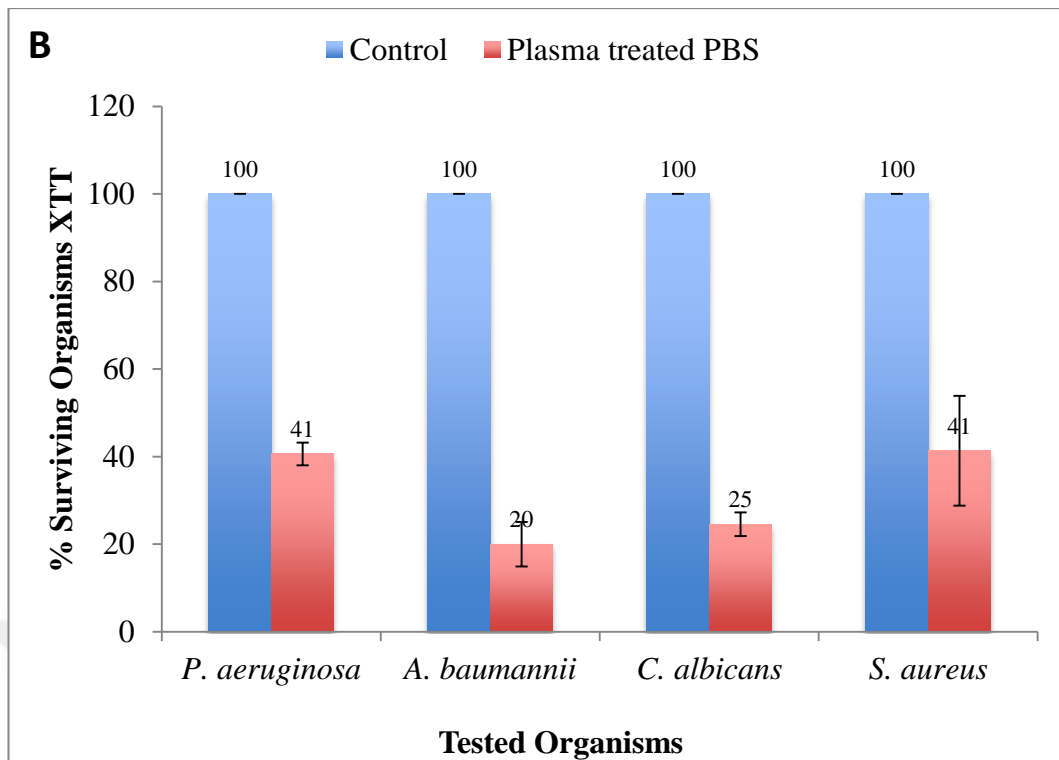


**Figure 3.7:** Antimicrobial Activity Accord Colony Counting Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested Organisms

XTT test results were consistent with findings from the colony-counting assay carried out for the nublization of plasma treated NAC solution to nebulization of

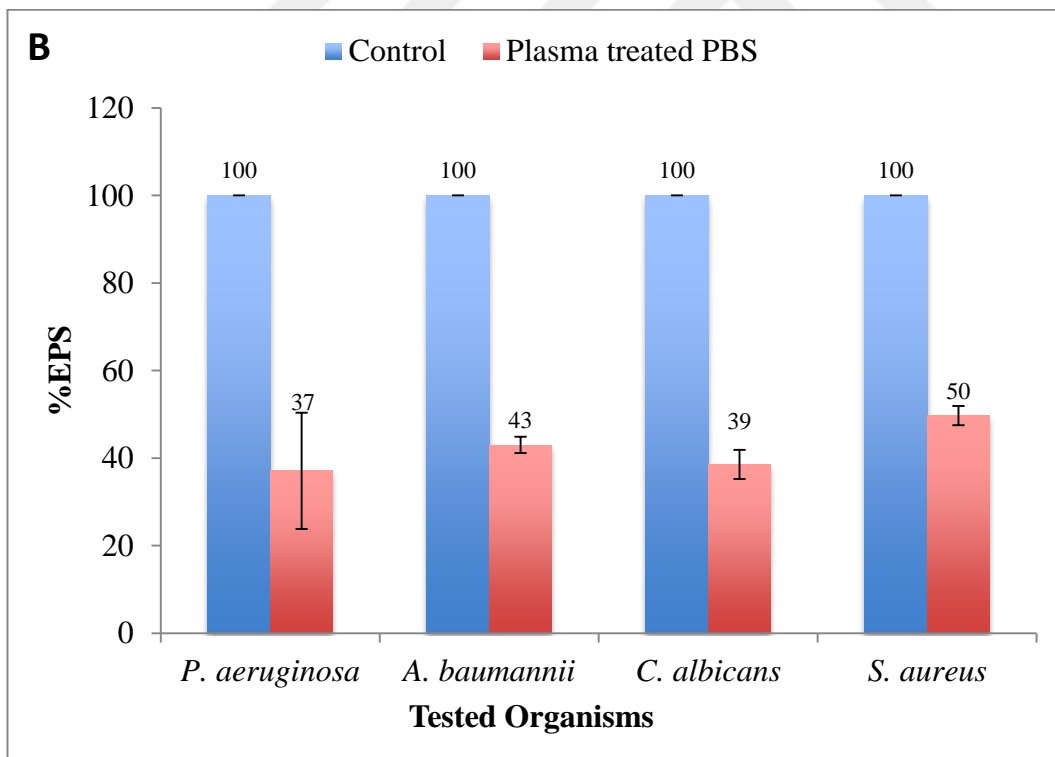
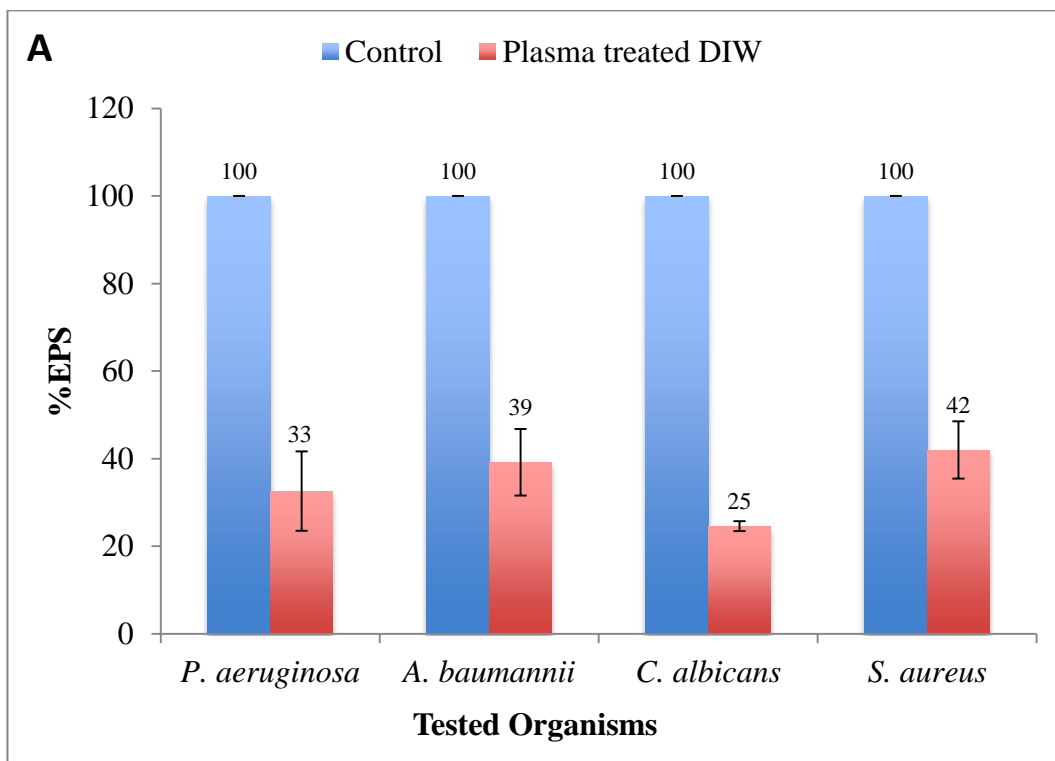
plasma treated colony-counting test of NAC solution show parallelism with XTT test of NAC. Moreover, *P.aureginosa*, *A.baumannii*, and *S.aureus* have inactivation completely and *C.albicans* has just 9% value when compared with the control group that is 100% (Figure 3.8). As per XTT result, after the nebulization with plasma treated DIW and PBS were observed reduction of viability significantly.

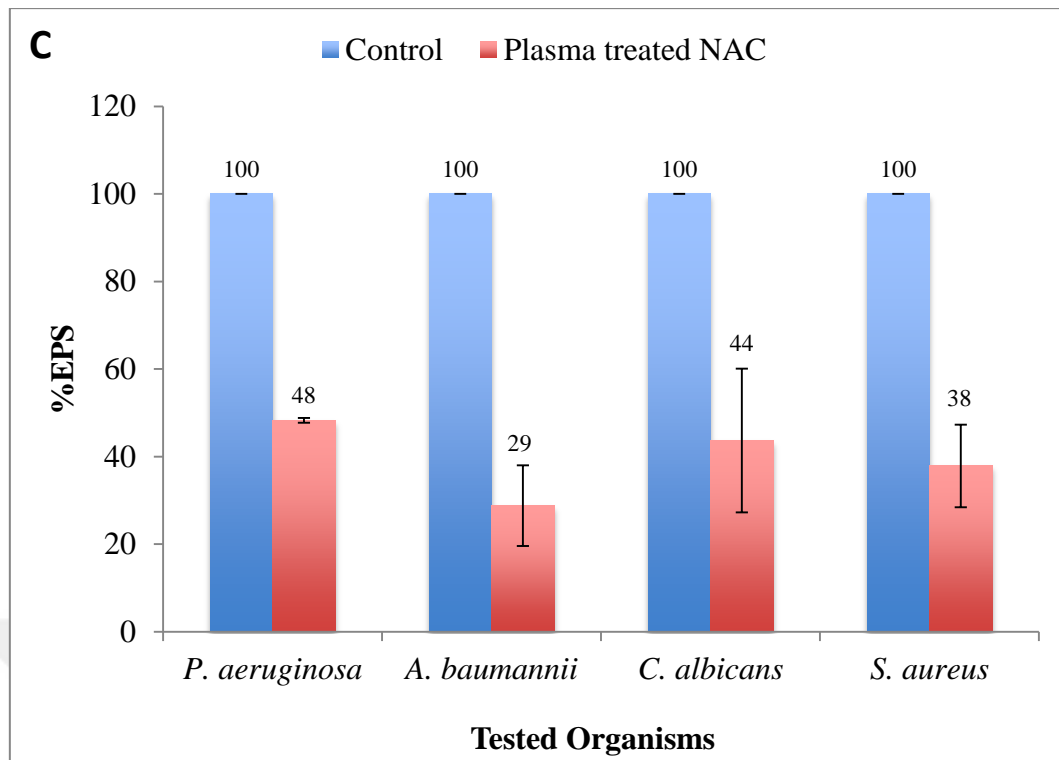




**Figure 3.8:** XTT Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested Organisms

As to safranin test results showed decreased significantly consequence of nebulization of plasma treated liquid on biofilms (Figure 3.9).



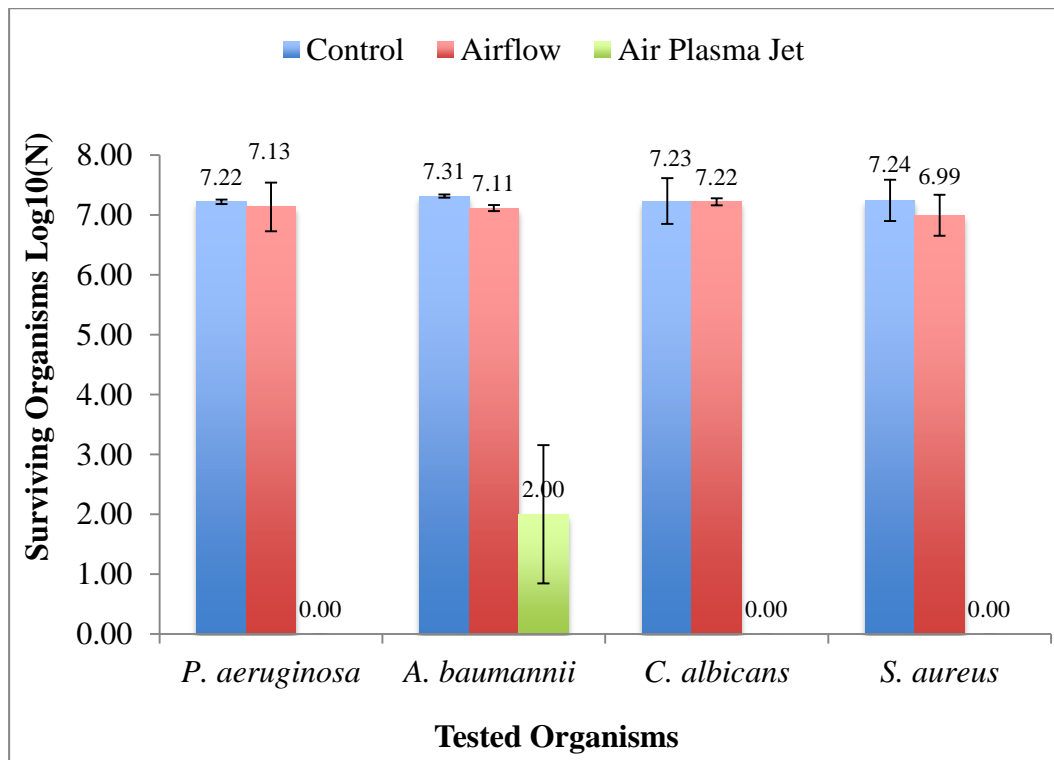


**Figure 3.9:** Safranine Tests Results of Nebulisation Plasma treated (A) DIW, (B) PBS, and (C) NAC Solutions on the Tested Organisms

According to safranine results, it was observed significant reduction of EPS depend on liquid types that were nebulized. With respect to the data that were obtained as results of study, in different groups were observed minimum 50% reduction of EPS. These results were changed according types of liquid. For example, plasma treated deionized water was provided 75% reduction of *C.albicans* biofilm with nebulization. With plasma treated NAC solution nebulization 70% reduction of *A.baumannii* biofilm was obtained. According to reduction of EPS layer depending on nebulization of each liquid, we can say that significant values were obtained and plasma treated liquid can disrupt structure of biofilm. Even there was not exact inactivation, the inactivation system can be improved and provided exact inactivation via different arrangement. The assessment of antimicrobial effect of plasma treated liquid on biofilm that was formed on endotracheal tubes via colony counting, XTT, and safranine tests; we could reach more effective results with NAC solution.

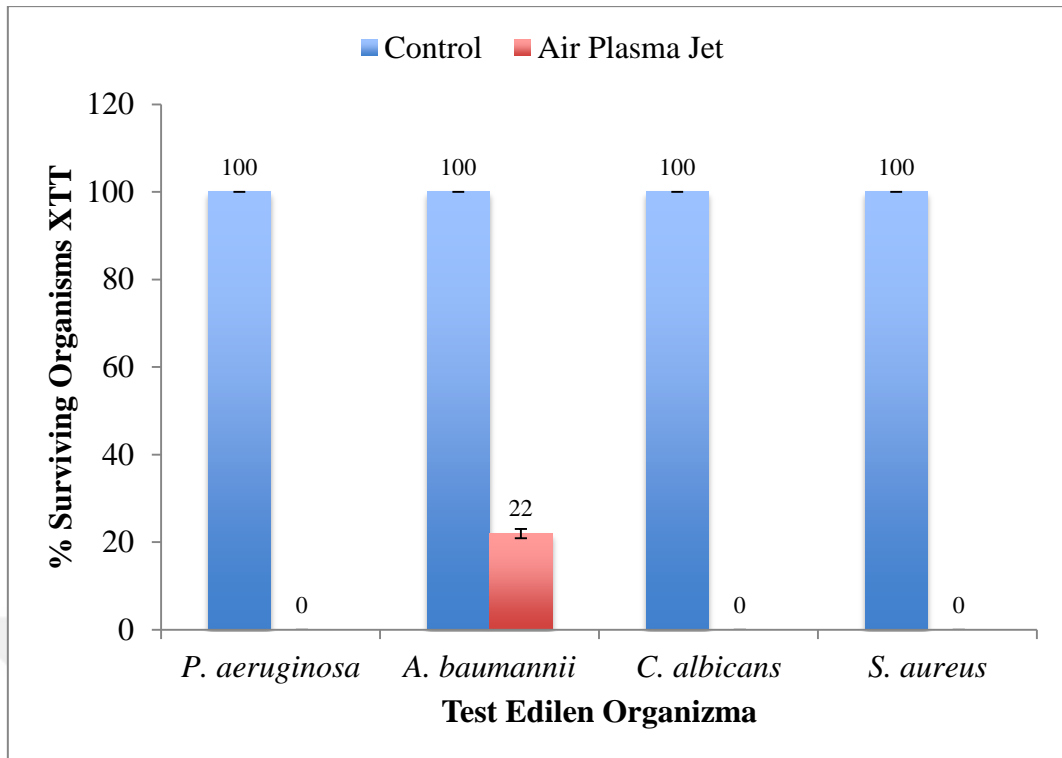
Additionally we studied with air jet because it was the most effective jet compared among helium and nitrogen jet. Aforementioned biofilm formation was made in the endotracheal tubes and then plasma treatment was completed on the tubes with particular parameters, which are 31 kV, 1.5 kHz and 4-slm airflow rate during one

minute. After these studies colony counting, XTT, and safranin tests were implemented and examined results. According to colony counting results except *A.baumannii*, other organisms showed complete inactivation. *A.baumannii* was shown that 5 log inactivation (Figure 3.10).



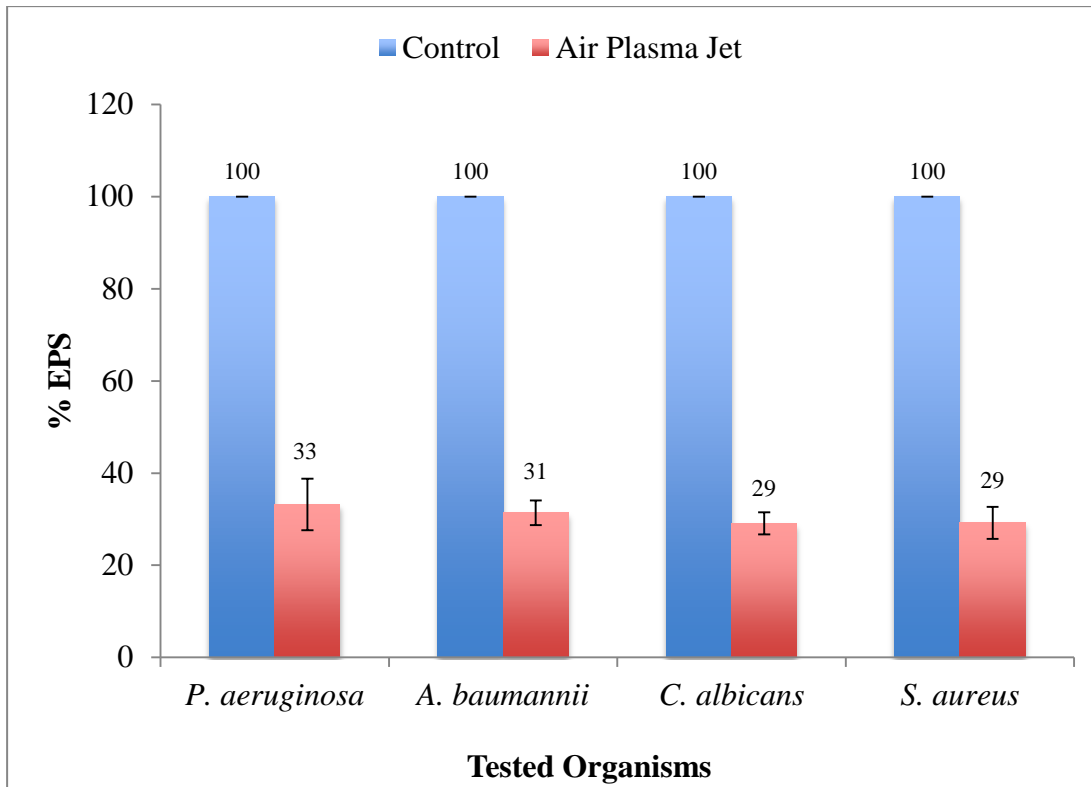
**Figure 3.10:** Antimicrobial activity of air plasma jet application on biofilm inner the endotracheal tubes

After the colony counting tests, XTT test was made to evaluate metabolic activity of organisms. We obtained parallel results that are obtained with colony counting assay. While About 80% inactivation was determined for *A.baumannii* while, other organisms had complete inactivation according to XTT assay (Figure 3.11).



**Figure 3.11:** XTT tests results after the air plasma jet treatment on biofilm was formed on the endotracheal tubes

Additionally, safranin tests were completed after the plasma treatment on the biofilm that was formed on the endotracheal tubes. According to test, all organisms had 70% reduction of EPS layer (Figure 3.12).

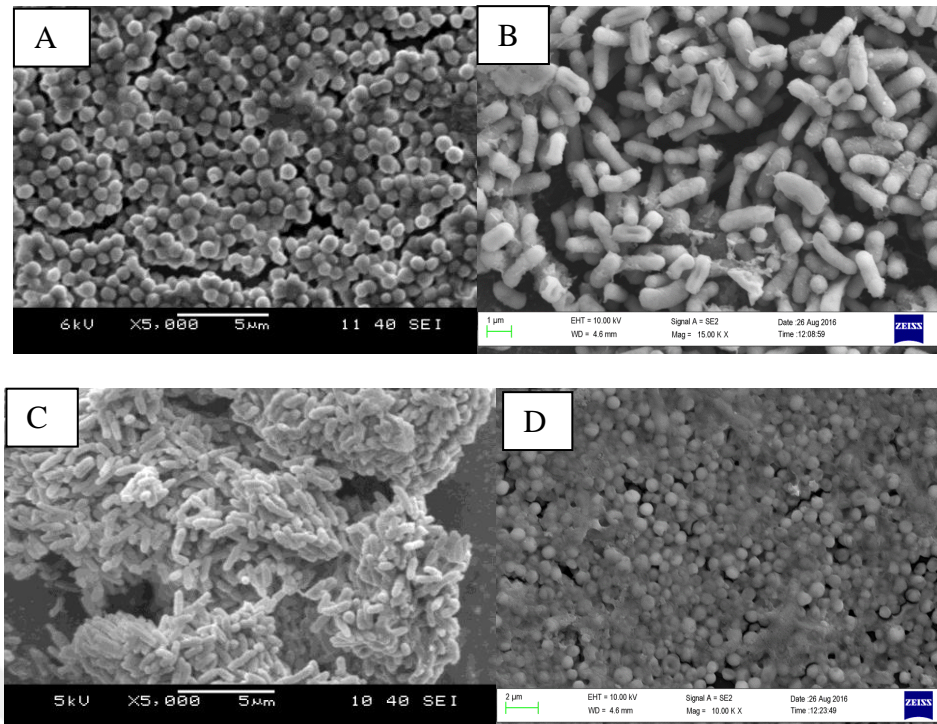


**Figure 3.12:** Safranin tests results after the air plasma jet treatment on biofilm was formed on the endotracheal tubes

### 3.7 Biofilm Characterization on Endotracheal Tubes Surfaces

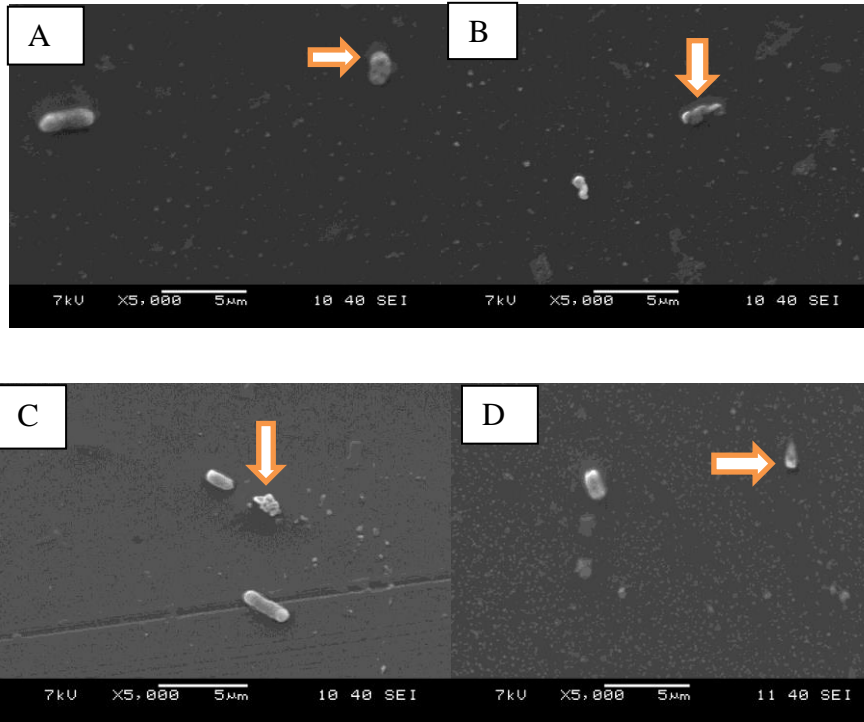
Viewing of samples in the SEM and AFM were carried out with sensitively. Before SEM and AFM analysis, biofilm formation was done for each microorganism that are *S.aureus*, *A.baumannii*, *P.aureginosa* and *C.albicans* on endotracheal tube surfaces. Biofilms have grown during one day for 2 groups, which plasma untreated samples (control) and nebulization of plasma treated NAC solution for each microorganisms in study. After one-day biofilms of *S.aureus*, *A.baumannii*, *P.aureginosa* and *C.albicans* cover the endotracheal tube surfaces in figure 3.13. Microorganisms have grown enormously and they have formed EPS structure. Biofilm has not had any defect, they have presented healthy.





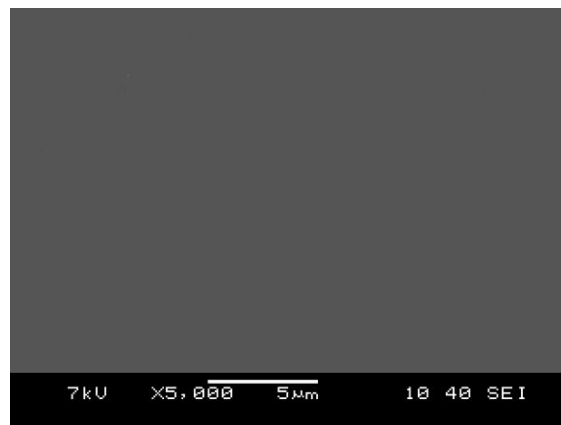
**Figure 3.13:** Scanning Electron Microscopy was used to take views of untreated plasma of *S.aureus* (A), *A.baumannii*(B), *P.aureginosa*(C) and *C.albicans* (D)

After nebulization of plasma treated NAC solution on biofilms that were *S.aureus*, *A.baumannii*, *P.aureginosa* and *C.albicans* on endotracheal tubes have shown that Figure 3.14. In contrast plasma treated samples have leaky and damages, the white arrows show these damaged microorganisms. Numbers of microorganisms have decreased exactly. These results have proved to colony counting results, because we obtained inactivation exactly. In addition because of plasma microorganisms interaction, the microorganism did not adhere the surface.



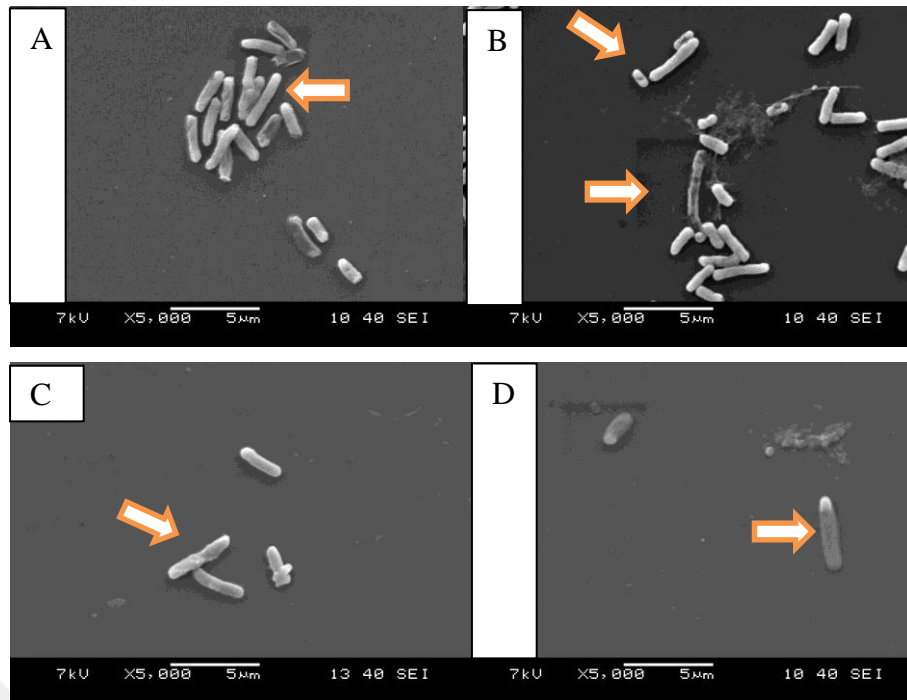
**Figure 3.14:** Scanning Electron Microscopy was used to take views of nebulization of plasma-treated NAC on *S.aureus* (A), *A.baumannii* (B), *P.aereginosa* (C) and *C.albicans* (D)

The plasma did not cause abnormalities on the endotracheal surfaces it can be seen in Figure 3.15. For this sample the nebulization of plasma treated NAC solution was done.



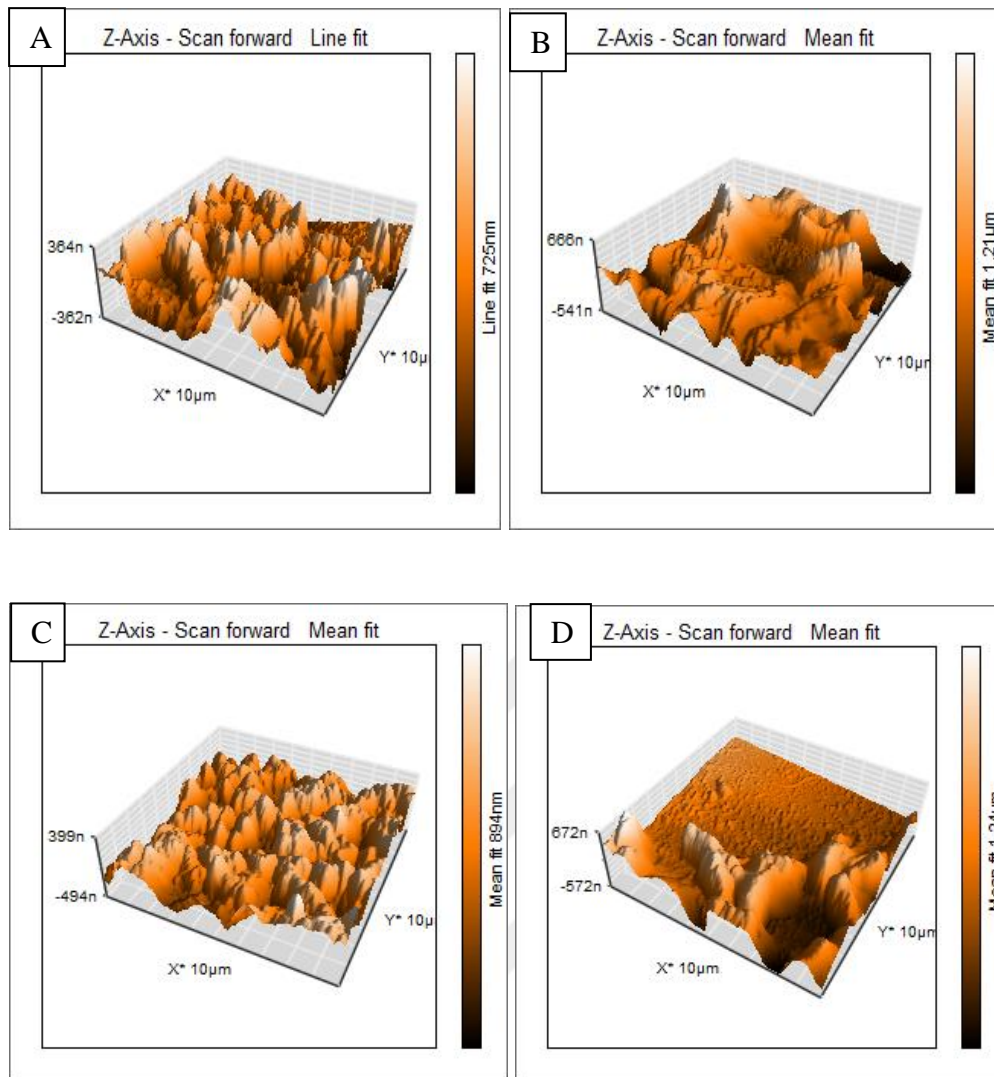
**Figure 3.15:** Nebulization of plasma treated NAC on endotracheal tube

After plasma treatment, we have observed certain morphological changes for some samples in Figure 3.16 below. The arrows show that variance.



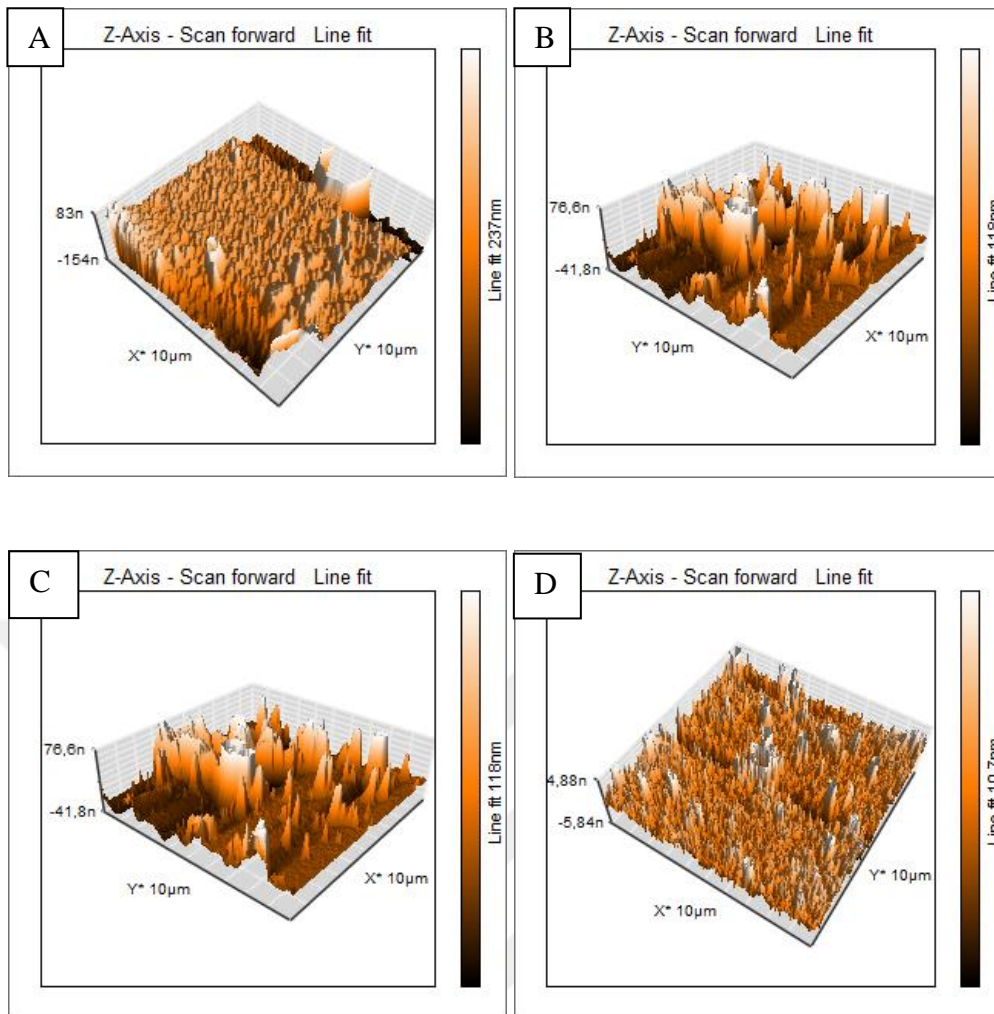
**Figure 3.16:** Representative images of morphological modifications on microorganisms; *P.aureginosa* (nebulisation with plasma treated PBS)(A), *A.baumannii* (air plasma jet treatment)(B), *P.aureginosa* (nebulisation with plasma treated DIW)(C) and *C.albicans* (nebulisation with plasma treated NAC)(D)

After biofilm formation we have obtained almost 1000 nanometer thickness for *S.aureus* and *P.aureginosa* and over 1000 nanometer for *A.baumannii* and *C.albicans*. They are seen that in Figure 3.17.



**Figure 3.17:** Atomic-force microscopy (AFM) was used to take views of non-thermal plasma-treated *S.aureus* (A), *A.baumannii*(B), *P.aereginosa*(C) and *C.albicans* (D)

The results that were obtained from AFM imaging show parallelism with results of colony counting. For this concept, all plasma treated samples have less layer of biofilm comparing untreated samples. According to results of images, nebulized plasma treated solutions have significant results in respect to thickness of biofilms for all organisms among samples. In detail, after the nebulized plasma treated NAC solution on microorganisms, the samples have remarkable views that were *S.aureus*, *A.baumannii* and *P.aereginosa* had complete inactivation. For these microorganism and solution, AFM results have been observed more reduction in thickness of biofilm



**Figure 3.18:** Atomic-force microscopy (AFM) was used to take views of nebulization of plasma-treated NAC on *S.aureus* (A), *A.baumannii* (B), *P.aureginosa* (C) and *C.albicans* (D)

#### 4. DISCUSSION

Non-thermal plasma treatment has antimicrobial effect and is more notable by scientist. UV photons, reactive oxygen and nitrogen species are provided microbial inactivation and they damage microorganisms during plasma discharge [55]. In addition to antimicrobial effect of plasma, also plasma treated materials may gain stronge antimicrobial activity. Poor *et al.* has shown that plasma treated calcium alginate gels have gain antimicrobial activity that has persisted up to 4 weeks. [56]. Moreover, İbiş *et al* has shown that non-thermal plasma treatment of implant materials prevent biofilm formation up to 50%. Their study has proved that non-thermal plasma treatment of UHMWPE, Ti6Al4V, 304 SS and 316L SS materials can eliminate biofilm and damage extracellular polymeric substance [57]. Yorsaeng *et al* has reported that the plasma treated natural rubber latex film from medical gloves have antibacterial activity on *S. aureus* and *E.coli* and better mechanical properties comparing non-plasma treated gloves [58]. In the present study, we have treated PVC endotracheal tubes were directly treated using DBD plasma in order to evaluate if endotracheal tubes may gain antimicrobial activity similar to previously reported studies. As depicted in Figure 3.4, 15 seconds of plasma treatment of endotracheal tubes does not show any antimicrobial activity. However, after 60 seconds of plasma treatment of endotracheal tubes about 1-log biofilm prevention was observed. When plasma treatment time was increased to 120 seconds, biofilm prevention effect was significantly inproved for only *A. baumannii*. Also XTT and safranine assays carried out after pre-treatment of endotracheal tubes have shown that microbial metabolic activity and EPS content were not decreased significantly which reflects possible viable but nonculturable state (VBNC) of organism. Further experiments such as, chemical and surface characterization of PVC, should be carried out to better understand biofilm prevention efficacies of plasma-treated endotracheal tubes.

Similarly, various liquids such as PBS, water, NAC, saline solution gain antimicrobial activity after plasma treatment. Ercan *et al* plasma treated NAC solution acquires strong antimicrobial activity on planktonic and biofilm forms of bacteria and fungus when treated with air DBD plasma and the antimicrobial effect remains up to 2 years [2, 55]. Antimicrobial activity of plasma treated NAC solution was attributed to ROS and more dominantly to RNS generated during plasma formation.

Moreover, Oehmigen *et al.* reported that 0.9% NaCl solution acquires antimicrobial activity upon treatment with a surface DBD plasma [59]. Similarly Shainsky *et al.* have demonstrated that plasma treated water was converted to a strong antimicrobial solution after DBD air plasma treatment [60].

In our study, we have observed significant antimicrobial activity of nebulized plasma treated water, PBS and NAC solution over biofilm forms of tested microorganisms. However, nebulized plasma treated NAC solution has shown superior antimicrobial activity compared to nebulized plasma-treated PBS and water according to results of colony counting, XTT and safranin assays. Superior antimicrobial activity of NAC solution over plasma treated water and PBS is consistent with previously published literature. Previously Ercan *et al.* have compared antimicrobial activities of DBD air plasma treated water, PBS and NAC solution and found that plasma treated NAC solution have shown stronger antimicrobial activity compared to plasma treated water and PBS [2].

It is commonly reported that pH of plasma treated liquids decreases as consequence of plasma treatment. Several authors have reported such a reduction in pH, and the acidification of solutions, but the exact mechanism is still not fully understood [61-63]. It is anticipated that a drop in pH could be due in part to generation of nitric acid via interaction of RNS, ROS, and hydrolysis of water that gives rise to proton and hydroxyl radical.

As depicted in Figures 3.7, 3.8 and 3.9, nebulization of plasma treated PBS and NAC solution have led complete inactivation of all bacterial strains and about 5 and 3-log reduction of *C. albicans* respectively according to colony counting assay results. However, XTT results have shown metabolic activity of organisms that were exposed to nebulized plasma treated PBS. Moreover, tested organisms were exposed

to nebulized plasma treated NAC solution metabolic activity was completely terminated for bacterial strains, and a residual metabolic activity was measured for *C. albicans*. Persistent metabolic activity of organisms after exposure of nebulized plasma treated PBS was attributed to viable but nonculturable (VBNC) state of microorganisms. VBNC state is a common consequence of harsh environmental conditions and stress factors including, extreme osmotic conditions, extreme temperatures, nutrient starvation. In VBNC, state microorganisms can sustain viability but cannot duplicate. During VBNC, state organisms exhibit various modifications such as, change in morphology, alterations in structure and composition of cell membrane and cell wall, reduced metabolic activity. Once the stress factor is removed organisms may continue to duplicate [64]. Previously, oxidative stress was reported as an inducer of VBNC state [26, 27]. As described previously, cold atmospheric plasma treatment and plasma treated liquids are strong sources of oxidative stress. Moreover, Cooper *et al.* have reported that air DBD plasma treatment induces VBNC state in *Bacillus stratosphericus*.

As depicted in the Figure 3.16, our SEM imaging results have revealed morphological modifications in organisms that are exposed to nebulized-plasma treated liquids that are consistent with previous reports.

In the present study we have attempted to mimic conditions and biofilm formation in endotracheal tubes in intensive care unit conditions. However, to evaluate antibiofilm activity of various plasma treatment methods, first biofilms were formed in endotracheal tubes and then air plasma jet and nebulized plasma treated liquids were exposed to biofilms. However, in the clinical settings, initial bacterial load for the induction of biofilm formation is lesser than the bioburden that was used in the present study. Also, for the experimental part, biofilms were formed in endotracheal tube in 24 hours. In clinical practice biofilm formation with 7-log bacterial load may take days. Therefore, bacterial growth and inactivations should also be considered. Synchronized biofilm formation and nebulization of plasma treated liquids should be considered. Such model will assist better understand required plasma exposure frequency, amount of nebulized plasma treated liquids, and duration of nebulization and air plasma jet treatment for control and prevention of VAP.



In other words, continuous biofilm formation process and exposure of various plasma treatment methods during biofilm formation should be further studied to better understand efficacy of various cold plasma treatment methods for the control of VAP.



## 5. CONCLUSION

Antimicrobial activities of various plasma treatment methods such as direct, indirect and fluid mediated plasma treatment are widely reported. However, studies regarding antimicrobial activity of these plasma treatment methods have not implemented to particular applications such as eradication of biofilm inside endotracheal tube, as explained in the present study.

In the present study we have investigated antibiofilm capabilities of air jet plasma, nebulized plasma treated liquids and pre-treatment of endotracheal tube surface for the prevention and control of VAP purpose.

In conclusion our results have revealed that nebulized plasma treated liquids show strong antimicrobial activity on biofilm forms of *S.aureus*, *A.baumannii*, *P.aureginosa* and *C.albicans*, which are common pathogens for the development of VAP, grown in endotracheal tubes. In detail, nebulized plasma treated NAC solution have shown superior antimicrobial activity over water and PBS. Nebulized-plasma treated NAC solution leads complete inactivation on biofilm forms of *S.aureus*, *A.baumannii*, *P.aureginosa* and about 4-log inactivation on biofilm form of *C. albicans*. Also XTT and safranin assays have shown consistent results with colony counting assay that supports our findings regarding antimicrobial activity of nebulized plasma treated NAC solution. Similarly, air plasma jet, leads complete microbial inactivation on biofilm forms of *S.aureus*, *P.aureginosa* and *C.albicans* and leads up to 5 log reduction on the biofilm form of *A.baumannii*. Additionally, pre-treatment of endotracheal tubes was not capable of completely preventing formation of biofilm forms of all tested organisms in the endotracheal tube and biofilm prevention efficacy remained around 1-log. Moreover, SEM and AFM imaging results have indicated eradication of biofilm forms of all tested pathogens in

the endotracheal tube upon application of nebulized plasma treated liquids and air jet plasma treatment.

Best of our knowledge the present thesis is the first study reporting the antimicrobial activity of nebulized-plasma treated liquids. Also for the first time this proof of concept study has shown plasma treatment methods for the eradication of biofilm in hollow tube.



## REFERENCES

- [1] **Z. Filetoth**, *Hospital Acquired Infection*: John Wiley & Sons, 2008.
- [2] **U. K. Ercan, H. Wang, H. Ji, G. Fridman, A. D. Brooks, and S. G. Joshi**, "Nonequilibrium Plasma- Activated Antimicrobial Solutions are Broad-Spectrum and Retain their Efficacies for Extended Period of Time," *Plasma processes and polymers*, vol. 10, pp. 544-555, 2013.
- [3] **M. A. H. Darla M Goeres, Nicholas A Beck, Kelli Buckingham-Meyer, Jackie D Hilyard, and L. A. L. Linda R Loetterle, Diane K Walker & Philip S Stewart**, "A method for growing a biofilm under low shear at the air liquid interface using the drip flow biofilm reactor."
- [4] **G. Ducel, J. Fabry, and L. Nicolle**, "Prevention of hospital acquired infections: a practical guide," *Prevention of hospital acquired infections: a practical guide*, 2002.
- [5] **M. M. Pettigrew, J. K. Johnson, and A. D. Harris**, "The human microbiota: novel targets for hospital-acquired infections and antibiotic resistance," *Annals of epidemiology*, vol. 26, pp. 342-347, 2016.
- [6] **C. Zaph**, "Which species are in your feces?," *The Journal of clinical investigation*, vol. 120, pp. 4182-4185, 2010.
- [7] **N. O'Connor, O. Cahill, S. Daniels, S. Galvin, and H. Humphreys**, "Cold atmospheric pressure plasma and decontamination. Can it contribute to preventing hospital-acquired infections?," *Journal of Hospital Infection*, vol. 88, pp. 59-65, 2014.
- [8] **P. W. Stone, E. Larson, and L. N. Kavar**, "A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000," *American journal of infection control*, vol. 30, pp. 145-152, 2002.
- [9] **U. K. E. thesis**, "Mechanisms Underlying Antimicrobial Efficacies of Non-Thermal Dielectric Barrier Discharge (DBD) Plasma-Treated N-Acetyl Cysteine (NAC) Solution" Doctorate, Drexel University, 2013.
- [10] **D. M. Sievert, P. Ricks, J. R. Edwards, A. Schneider, J. Patel, A. Srinivasan, et al.**, "Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010," *Infection Control & Hospital Epidemiology*, vol. 34, pp. 1-14, 2013.

- [11] **C. Geffers and P. Gastmeier**, "Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System)," *Dtsch Arztebl Int*, vol. 108, pp. 87-93, 2011.
- [12] **J. Redder, R. Leth, and J. K. Møller**, "Analysing risk factors for urinary tract infection based on automated monitoring of hospital-acquired infection," *Journal of Hospital Infection*, vol. 92, pp. 397-400, 2016.
- [13] **M. E. Sobieszczyk**. (n.d.). *Urinary Tract Infection*. Available: <http://www.columbia.edu/itc/hs/medical/pathophys/id/2009/utiNotes.pdf>
- [14] **M. C. Staff**. (n.d.). *Diseases and Conditions* *Urinary tract infection (UTI)*. Available: <http://www.mayoclinic.org/diseases-conditions/urinary-tract-infection/basics/causes/con-20037892>
- [15] **C. f. D. C. a. Prevention**. (2016). *Surgical Site Infection (SSI) Prevention* Available: <http://www.cdc.gov/nhsn/PDFs/pscmanual/9pscscscurrent.pdf>
- [16] **A. Gürgün, E. P. Korkmaz, F. Bacakoğlu, O. Başoğlu, N. Dirican, Ş. Aydemir, et al.**, "[The role of endotracheal aspiration in the diagnosis of ventilator associated pneumonia]," *Tuberkuloz ve toraks*, vol. 61, pp. 288-294, 2012.
- [17] **S. Amanullah**, "Ventilator-Associated Pneumonia Overview of Nosocomial Pneumonias," ed: Medscape, 2012.
- [18] **M. T. Madigan, J. M. Martinko, and J. Parker**, *Brock biology of microorganisms* vol. 514: prentice hall Upper Saddle River, NJ, 1997.
- [19] **C. Omer and J. Gibbs**, "Protein prenylation in eukaryotic microorganisms: genetics, biology and biochemistry," *Molecular microbiology*, vol. 11, pp. 219-225, 1994.
- [20] **N. W. Schaad**, *Laboratory guide for identification of plant pathogenic bacteria*: APS press, 1988.
- [21] **N. A. Saunders and M. A. Lee**, *Real-time PCR: advanced technologies and applications*: Horizon Scientific Press, 2013.
- [22] **J. R. Tagg, A. S. Dajani, and L. W. Wannamaker**, "Bacteriocins of gram-positive bacteria," *Bacteriological reviews*, vol. 40, p. 722, 1976.
- [23] **N. Dirican, A. T. Oz, H. Pullukcu, S. Aydemir, and F. Bacakoğlu**, "The Effect of Fluoroquinolone Use in the Respiratory Intensive Care Unit on the Development of Hospital-Acquired MRSA Infection and Its Prognosis," *Mikrobiyol Bul*, vol. 48, pp. 28-39, 2014.
- [24] **T. P. Lodise and P. S. McKinnon**, "Burden of Methicillin- Resistant *Staphylococcus aureus*: Focus on Clinical and Economic Outcomes," *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, vol. 27, pp. 1001-1012, 2007.
- [25] **A. L. Panlilio, D. H. Culver, R. P. Gaynes, S. Banerjee, T. S. Henderson, J. S. Tolson, et al.**, "Methicillin-resistant *Staphylococcus aureus* in US hospitals, 1975–1991," *Infection Control & Hospital Epidemiology*, vol. 13, pp. 582-586, 1992.
- [26] **I. Arana, A. Muela, J. Iriberry, L. Egea, and I. Barcina**, "Role of hydrogen peroxide in loss of culturability mediated by visible light in *Escherichia coli* in a freshwater ecosystem," *Applied and environmental microbiology*, vol. 58, pp. 3903-3907, 1992.
- [27] **M. Gourmelon, J. Cillard, and M. Pommepuy**, "Visible light damage to *Escherichia coli* in seawater: oxidative stress hypothesis," *Journal of Applied Bacteriology*, vol. 77, pp. 105-112, 1994.

- [28] **R. Weller, R. Price, A. Ormerod, N. Benjamin, and C. Leifert**, "Antimicrobial effect of acidified nitrite on dermatophyte fungi, *Candida* and bacterial skin pathogens," *Journal of Applied Microbiology*, vol. 90, pp. 648-652, 2001.
- [29] **G. E. McDonnell**, *Antisepsis, disinfection, and sterilization*: American Society of Microbiology, 2007.
- [30] **W. G. Kreyling, M. Semmler-Behnke, and Q. Chaudhry**, "A complementary definition of nanomaterial," *Nano Today*, vol. 5, pp. 165-168, 2010.
- [31] **G. Sykes**, "Disinfection and sterilization," *Disinfection and sterilization*, 1958.
- [32] **W. A. Rutala and D. J. Weber**, "Disinfection and sterilization in health care facilities: what clinicians need to know," *Clinical infectious diseases*, vol. 39, pp. 702-709, 2004.
- [33] **T. G. Klämpfl, G. Isbary, T. Shimizu, Y.-F. Li, J. L. Zimmermann, W. Stolz, et al.**, "Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest," *Applied and environmental microbiology*, vol. 78, pp. 5077-5082, 2012.
- [34] **P. BioLabs**. (n.d.). *Cleaning, Disinfection, and Sterilization Validations of Reusable Medical Devices*. Available: <http://www.slideshare.net/pacificbiolabs/cleaning-disinfection-and-sterilization-validations-of-reusable-medical-devices>
- [35] **W. A. Rutala and D. J. Weber**, "New disinfection and sterilization methods," *Emerging infectious diseases*, vol. 7, p. 348, 2001.
- [36] **J. Selkon**, "Super-oxidized water, preparation and use thereof as sterilizers and medicaments," ed: Google Patents, 2002.
- [37] **A. Fridman**, *Plasma chemistry*: Cambridge university press, 2008.
- [38] **G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, and A. Fridman**, "Applied Plasma Medicine," *Plasma Processes and Polymers*, vol. 5, pp. 503-533, 2008.
- [39] **G. Fridman, M. Peddinghaus, M. Balasubramanian, H. Ayan, A. Fridman, A. Gutsol, et al.**, "Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air," *Plasma Chemistry and Plasma Processing*, vol. 26, pp. 425-442, 2006.
- [40] <http://io9.gizmodo.com/5899149/now-you-can-replace-your-antibacterial-soap-with-a-plasma-jet>, "Now you can replace your antibacterial soap with a plasma jet," 2016 2016.
- [41] **Z. W. Yan LI(), Yong WANG**. (2016). *Ni/MgO catalyst prepared via dielectric-barrier discharge plasma with improved catalytic performance for carbon dioxide reforming of methane*.
- [42] **M. G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. Van Dijk, et al.**, "Plasma medicine: an introductory review," *New Journal of Physics*, vol. 11, p. 115012, 2009.
- [43] **D. Dobrynin, G. Fridman, G. Friedman, and A. Fridman**, "Physical and biological mechanisms of direct plasma interaction with living tissue," *New Journal of Physics*, vol. 11, p. 115020, 2009.
- [44] **M. A. Bogle, K. A. Arndt, and J. S. Dover**, "Evaluation of plasma skin regeneration technology in low-energy full-facial rejuvenation," *Archives of dermatology*, vol. 143, pp. 168-174, 2007.

- [45] **S. Emmert, F. Brehmer, H. Hänßle, A. Helmke, N. Mertens, R. Ahmed, et al.**, "Atmospheric pressure plasma in dermatology: Ulcus treatment and much more," *Clinical Plasma Medicine*, vol. 1, pp. 24-29, 2013.
- [46] **K. Gibson, C. Haas, S. Güçeri, and A. Fridman**, *Plasma assisted decontamination of biological and chemical agents*: Springer Science & Business Media, 2011.
- [47] **T. Manfredi**. (n.d.). *Benefits of a Nebulizer*. Available: <http://www.healthguidance.org/entry/15786/1/Benefits-of-a-Nebulizer.html>
- [48] **B. W. Romero**. (2016). *Nebulization Therapy*. Available: <http://nursingcrib.com/demo-checklist/nebulization-therapy/>
- [49] **WebMd**. (2016). *Nebulizers: Home and Portable Nebulizers for Asthma Treatment*. Available: <http://www.webmd.com/asthma/guide/home-nebulizer-therapy>
- [50] **K. Price**. (2016). *What Is Nebulizer Therapy?* Available: <http://www.livestrong.com/article/252388-what-is-nebulizer-therapy/>
- [51] **F. G. Zampieri, A. P. Nassar Jr, D. Gusmao-Flores, L. U. Taniguchi, A. Torres, and O. T. Ranzani**, "Nebulized antibiotics for ventilator-associated pneumonia: a systematic review and meta-analysis," *Critical Care*, vol. 19, p. 1, 2015.
- [52] **A. GmbH**. (n.d.). *No. 12 Cell Proliferation Assay XTT*. Available: <https://www.applichem.com/en/literature/applications/no-12-cell-proliferation-assay-xtt/>
- [53] **C. Lembke, A. Podbielski, C. Hidalgo-Grass, L. Jonas, E. Hanski, and B. Kreikemeyer**, "Characterization of biofilm formation by clinically relevant serotypes of group A streptococci," *Applied and environmental microbiology*, vol. 72, pp. 2864-2875, 2006.
- [54] **K. Schwartz, R. Stephenson, M. Hernandez, N. Jambang, and B. R. Boles**, "The use of drip flow and rotating disk reactors for Staphylococcus aureus biofilm analysis," *JoVE (Journal of Visualized Experiments)*, pp. e2470-e2470, 2010.
- [55] **U. K. Ercan, J. Smith, H.-F. Ji, A. D. Brooks, and S. G. Joshi**, "Chemical Changes in Nonthermal Plasma-Treated N-Acetylcysteine (NAC) Solution and Their Contribution to Bacterial Inactivation," *Scientific reports*, vol. 6, 2016.
- [56] **A. E. Poor, U. K. Ercan, A. Yost, A. D. Brooks, and S. G. Joshi**, "Control of multi-drug-resistant pathogens with non-thermal-plasma-treated alginate wound dressing," *Surgical infections*, vol. 15, pp. 233-243, 2014.
- [57] **F. İbiş, H. Oflaz, and U. K. Ercan**, "Biofilm Inactivation and Prevention On Common Implant Material Surfaces by Non-Thermal DBD Plasma Treatment," *Plasma Medicine*, 2016.
- [58] **S. Yorsaeng, O. Pornsunthorntawe, and R. Rujiravanit**, "Preparation and characterization of chitosan-coated DBD plasma-treated natural rubber latex medical surgical gloves with antibacterial activities," *Plasma Chemistry and Plasma Processing*, vol. 32, pp. 1275-1292, 2012.
- [59] **K. Oehmigen, J. Winter, M. Hähnel, C. Wilke, R. Brandenburg, K. D. Weltmann, et al.**, "Estimation of possible mechanisms of Escherichia coli inactivation by plasma treated sodium chloride solution," *Plasma Processes and Polymers*, vol. 8, pp. 904-913, 2011.

- [60] **N. Shainsky, D. Dobrynin, U. Ercan, S. G. Joshi, H. Ji, A. Brooks, et al.**, "Retraction: Plasma Acid: Water Treated by Dielectric Barrier Discharge," *Plasma processes and Polymers*, vol. 9, 2012.
- [61] **Y. Xu, Y. Tian, R. Ma, Q. Liu, and J. Zhang**, "Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*," *Food chemistry*, vol. 197, pp. 436-444, 2016.
- [62] **M. J. Traylor, M. J. Pavlovich, S. Karim, P. Hait, Y. Sakiyama, D. S. Clark, et al.**, "Long-term antibacterial efficacy of air plasma-activated water," *Journal of Physics D: Applied Physics*, vol. 44, p. 472001, 2011.
- [63] **K. Oehmigen, M. Hähnel, R. Brandenburg, C. Wilke, K. D. Weltmann, and T. Von Woedtke**, "The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids," *Plasma Processes and Polymers*, vol. 7, pp. 250-257, 2010.
- [64] **X. Su, L. Guo, L. Ding, K. Qu, and C. Shen**, "Induction of Viable but Nonculturable State in *Rhodococcus* and Transcriptome Analysis Using RNA-seq," *PloS one*, vol. 11, p. e0147593, 2016.





## CURRICULUM VITAE



**Name Surname:** Fatma İBİŞ

**Place and Date of Birth:** İzmir, 02.02.1990

**Address:** Bayraklı/ İZMİR

**E-Mail:** fatmaibiss@gmail.com

**B.Sc.:** Ege University/ Bioengineering 2014

### **Professional Experience and Rewards:**

The scholarship for Ph.D in United Kingdom from NEWTON FUND (2016-2019)

The scholarship for Ph.D in Foreign Countries, from TÜBİTAK (Scientific and technological research council of Turkey) (2016-2018)

The Scholarship for Master Thesis about Priority Areas, from TÜBİTAK (Scientific and technological research council of Turkey) (2015-2016)

### **List of Journal Papers**

-İbiş, F., Oflaz, H., Ercan, U., K., (2015) Biofilm Inactivation and Prevention On Common Implant Material Surfaces by Non-Thermal DBD Plasma Treatment, Plasma Medicine, In press

-Çelik, B., Çapar, İ., D., Ercan, U., K., İbiş, F., Erdilek, N., (2015) Bleaching Efficacy of Atmospheric Low-Temperature Power Source, Journal of Dental Research, In Press

### **Publications and Patents:**

-İbiş, F., Pekbağrıyanık T., Ulu, M., Enhoş, Ş., Ercan, U., K., (2016) Comparative Assessment of Antimicrobial Efficacy of Non-Thermal Plasma and Er:YAG Laser on Titanium Discs for Nonsurgical Treatment of Peri-implantitis, 6<sup>th</sup> International Conference on Plasma Medicine (ICPM-6), September 4-9, 2016, Bratislava, Slovakia

-N. Arık, A. İnan, **F. İbis**, E. A. Demirci, U. K. Ercan, O. Karaman, N. Horzum (2016) Effects of Cold Plasma Treatment on Electrospun Nanofibers, Electrospun, May 2016, Italy

-Adımcı, P., **İbis F.**, Ercan, U., K. Bağış B., (2016) Effect of Non-Thermal Plasma Treatment on the Bond Strength of Resin Cements to Different CAD/CAM Blocks, TDA's 22nd International Congress of Dentistry, March 2016, Erzurum, Turkey

-Adımcı, P., **İbis F.**, Ercan, U., K. Bağış B., (2016) Effect of Non-Thermal Plasma Treatment on Wettability of Different CAD/CAM Materials, TDA's 22nd International Congress of Dentistry, March 2016, Erzurum, Turkey

-Çelik, B., Çapar, İ., D., **İbis, F.**, Ercan, U., K., , Erdilek, N., (2016), Can Deionized Water Substitute Bleaching Agents by Utilizing Non-Thermal DBD Plasma for Non-vital Tooth Bleaching Purpose? , COST TD 1208 3rd Annual Meeting “Electrical Discharged with Liquids for Future Applications” 13th-17th March 2016, Kocaeli, Turkey

-**İbis, F.**, Oflaz, H., Ercan, U., K., (2015) Biofilm Inactivation and Prevention On Common Implant Material Surfaces by Non-Thermal DBD Plasma Treatment, Joint Conference of COST ACTIONS TD1208 “Electrical discharges with liquids for future applications” & MP1101 Biomedical Applications of Atmospheric Pressure Plasma Technology, 13-16 September 2015, Bertinoro, Italy

-Çölgeçen, Ö., Ercan, U., K., Altuntaş, M., **İbis, F.**, The effects of Non-thermal Plasma Application of Surface Wettability of Zirconia Ceramics, 22nd International Scientific Congress of Turkish Prosthodontics and Implantology Association, 12-15 November 2015, Antalya, Turkey

## **PUBLICATIONS/PRESENTATIONS ON THE THESIS**

-**İbis, F.**, Ercan, U., K., Antimicrobial Activity of Nebulized Plasma Treated Solutions, COST TD 1208 3rd Annual Meeting “Electrical Discharged with Liquids for Future Applications” 13th-17th March 2016, Kocaeli, Turkey