A Study on Atmospheric Pressure Plasma Jet for Hospital Acquired Bacterial Inactivation by Different Working Gases with Varying Flow Rate and Exposure Time.

by

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Thank you.

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LIST OF ABBREVIATIONS

A.baumannii	*Acinetobacter baumannii*
AC	Alternating current
APPJ	Atmospheric pressure plasma jet
Ar	Argon
BA	Blood agar
DBD	Dielectric barrier discharge
DNA	Deoxyribonucleic acid
E.coli	*Escherichia coli*
EtO	Ethylene oxide
HAIs	Hospital-acquired infections
He	Helium
LTE	Local Thermodynamic Equilibrium
MH	Mueller- Hilton
MRSA	*Methicillin-resistant Staphylococcus aureus*
N_2	Nitrogen
NB	Nutrient broth
NO	Nitrogen oxide
OES	Optical Emission spectroscopy
OH	Hydroxyl
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
T_e	Electron temperature
T_o	Heavy particles temperature
UV	Ultraviolet

Penyahaktifan Bakteria Penyebab Penyakit Jangkitan Hospital dengan Pelbagai Jenis Gas dan Kadar Aliran Tekanan Atmospheric Plasma Jet

ABSTRAK

Plasma Jet Tekanan Atmosfera (APPJ) merupakan gas terion yang dihasilkan dalam tekanan atmosfera pada suhu bilik dengan menyalurkan gas ke dalam satu tiub kecil dan seterusnya dialirkan voltan yang tinggi pada kawasan tersebut. Plasma sejuk ini mempunyai pelbagai kelebihan yang boleh diaplikasikan dalam pelbagai bidang termasuk bidang perubatan dan industri steril. Kajian ini memfokuskan kepada aplikasi plasma dalam bidang steril, terutama APPJ dalam bidang steril. Objektif kajian ini adalah bagi membuktikan kebolehan plasma untuk menyahaktifkan bakteria, untuk mengkaji keberkesanan APPJ terhadap pelbagai jenis microorganism, gas, kadar aliran gas dan masa didedahkan kepada plasma, mengkaji spektrum pancaran optik daripada APPJ dan objektif yang terakhir adalah untuk melihat bakteria selepas pendedahan plasma dengan menggunakan mikroskop kuasa tinggi. Kajian ini menggunakan APPJ yang telah dihasilkan sendiri bagi menyahaktif *Acinetobacter baumannii* (*A.baumannii)* dan *Methicillin-resistant Staphylococcus aureus* (*MRSA)* dengan gas yang berbeza iaitu He, He/N_2 dan Ar dengan pelbagai kadar aliran gas dengan julat 300ml/ min sehingga 1000ml/min dalam masa 9, 12,15 dan 18 saat pendedahan APPJ. Selepas itu, sampel yang didedahkan kepada APPJ dipindahkan ke permukaan agar yang baru dan dieramkan selama 24 jam pada suhu 37°C untuk melihat pertumbuhan bakteria. Bakteria *A.baumannii* dan *MRSA* berjaya dinyahaktifkan dengan APPJ. Plasma He mempunyai kelebihan menyahaktifkan bakteria berbanding gas yang lain. Bakteria *A.baumannii* dan *MRSA* dinyahaktif sepenuhnya dalam masa 9 dan 12 saat pada 1000 ml/min. Plasma Ar berjaya mencapai peratusan yang minimum, 0% pada 9 saat. Walaubagaimanapun, kadar penyahaktifan bakteria tidak konsisten terutama untuk bakteria *MRSA.* Bagi plasma He/N_2, pula memerlukan 12 saat untuk mencapai kadar 0% penyahaktifan bakteria bagi kedua-dua jenis bakteria pada 1000ml/min. Kadar aliran gas yang dikaji bermula daripada 300ml/min sehingga keputusan menunjukkan kadar optimum untuk penyahaktifan bakteria. Kadar aliran yang optimum untuk menyahaktifkan bakteria adalah 1000ml/min. Kehadiran spesies zarah, terutamanya spesies nitrogen dan oksigen dalam plasma adalah salah satu faktor yang menyahaktifkan bakteria. Bentuk dan diameter bakteria berkurang selepas pendedahan APPJ. Bakteria juga tidak dalam kelompok selepas pendedahan dan rosak kerana bentuk bakteria telah berubah. Kesimpulanya, APPJ boleh digunakan untuk menyahaktifkan bakteria disebabkan oleh kehadiran spesies reaktif dalam plasma yang menyumbang kepada kerosakan sel-sel.

Inactivation of Bacteria Causing Hospital Acquired Infection by Varying Atmospheric Pressure Plasma Jet Working Gas and Flow Rate.

ABSTRACT

Atmospheric Pressure Plasma Jet (APPJ) is ionized gas that is generated at atmospheric pressure and at room temperature, by allowing the gas to flow in a small tube with the higher voltage supplied to the area. Plasma offers many advantages that makes it suitable in various applications, including medicine and sterilization. This research focuses on a cold plasma application, namely APPJ, in the sterilization field. The objective of this study is to establish the plasma for the inactivation of bacteria, to study the microbial inactivation effects of APPJ variables in term of the types of microbes, the types of gases, the various gas flow rates, and the exposure times, to study the optical emission spectrum of the APPJ, and to observe the bacteria after exposure to plasma by high power microscope. In this study, a self-developed APPJ was used to inactivate *Acinetobacter baumannii* (*A.baumannii)* and *Methicillin-resistant Staphylococcus aureus* (*MRSA)* by using different gases, namely He, He/N_2, and Ar at various gas flow rates in the range of 300ml/min to 1000ml/min, for 9, 12, 15, and 18 seconds of exposure. After that, the treated samples were transferred to a new agar surface and incubated for 24 hours at 37◦C to observe bacterial growth. The *A.baumannii* and the *MRSA* were successfully inactivated by the APPJ. The He plasma inactivated the bacteria more effectively than the other gases. The *A.baumannii* and the *MRSA* bacteria were fully inactivated after 9 and 12 seconds, respectively, at the flow rate of 1000ml/min. Ar plasma achieved 0% survival at 9s exposure, however the inactivation of bacteria were not consistent especially for *MRSA.* He/N_2 required 12 seconds exposure to achieve 0% survival for both bacteria at 1000ml/min. The gas flow rate in the experiment started from 300ml/min until results showed the optimum flow rate for inactivating bacteria. Optimum flow rate to inactivate the bacteria is 1000ml/min. The presence of the particle species, especially nitrogen and oxygen species in plasma is one of the factors that helped to inactivate the bacteria. The shapes of the bacteria were altered, and the diameters were reduced, after the exposure. Additionally, the bacteria did not form clusters after the exposure, and were damaged due to the shapes of the bacteria are changing. In conclusion, the APPJ can be used to inactivate the bacteria, due to the presence of reactive species in the plasma that contribute to the cell damage.

CHAPTER 1

INTRODUCTION

1.1 Introduction

A plasma is a collection of free charged particles moving in random directions that is, on the average, electrically neutral (Lieberman, & Lichtenberg, 2005). Plasma can be produced by applying high voltage to a gas flowing in a chamber or a tube. There are two types of plasma, namely hot plasma and cold plasma (Morent & De Geyter 2011; Miyamoto, 2011; Von Keudell et, al., 2010; Shintani, Sakudo, Burke & McDonnell, 2010; Fridman, 2008; Nehra, Kumar, & Dwivedi 2008; Tendero, Tixier, Tristant, Desmaison, & Leprince, 2006; Gaunt, Beggs, & Georghiou, 2006; Fridman & Kennedy, 2004; Bittencourt, 2004; Chapman, 1980).

For this research, cold plasma is used, based on its characteristic, namely, low temperature, safety, without chemical usage and simple operation. Studies on cold plasma have previously been conducted in a vacuum chamber, but currently, atmospheric plasma has attracted greater interest among researchers. This is due to atmospheric plasma's ease of operation and reduced costs compared to those of conventional plasma that produced in a vacuum (Morent & De Geyter 2011; Shintani, Sakudo, Burke & McDonnell, 2010; Nehra, Kumar, & Dwivedi, 2008).

Atmospheric plasma working gas can interact with other gases in the environment. This can affect surface changes, surface modification and microorganism

inactivation. Current research in plasma sterilization is primarily conducted in medical field. The clinical application deals with the human health. The major risk factor in the medical field is infection with pathogenic microorganism, which are capable of causing diseases in humans. Sterilization is especially important in the clinical application, which involves directly contact with the human body, and patients at an elevated risk of infection. Proper sterilization is important, to prevent infection in both patients and healthcare workers.

Most sterilization methods involving atmospheric plasma studies use direct plasma methods, such as dielectric barrier discharge. The most common microorganisms used for plasma sterilization are *Escherichia coli (E-coli)*, *Pseudomonas Aeruginosa*, and *Staphylococcus Aureus*. Researchers have used different gases such as helium, argon, nitrogen and others. Some of them have also mixed two different gases together, such as helium and nitrogen, argon and oxygen, argon and nitrogen, and others, at flow rates ranging from 1 l/min to 10 l/min (Kuwahata, Yamaguchi, Ohyama, & Ito, 2015; Kolb et al., 2012; Tipa, Boekema, Middelkoop, & Kroesen, 2012; Joshi et al., 2010; Sun, Qiu, Nie, & Wang, 2007; Lee, Paek, Ju, & Lee, 2006). The effects of plasma on microorganisms, in terms of how the plasma process inactivates the microorganisms and how exactly the bacteria inactivate are still being researched.

Previous researchers used high gas flow rates to inactivate the bacterial species most commonly used in this type of research, namely, *E-coli* and *Staphylococcus aureus* (Kuwahata et al., 2015; Kolb et al., 2012; Tipa, Boekema, Middelkoop, & Kroesen 2012; Joshi et al., 2010; Sun, Qiu, Nie, & Wang, 2007; Lee, Paek, Ju, & Lee, 2006). They required time exposures ranging from 5 seconds to 250 seconds to inactivate the bacteria, and the operation time depends on the gas flow rate. The

consumption of gas at high gas flow rates increases the operation costs (Kuwahata et al., 2015; Kolb et al., 2012; Tipa, Boekema, Middelkoop, & Kroesen 2012; Joshi et al., 2010; Sun, Qiu, Nie, & Wang, 2007; Lee, Paek, Ju, & Lee, 2006).

In this study, an atmospheric pressure plasma jet is used to inactivate bacteria at the optimum gas flow rate and operation time. This research focused on the inactivation of multi-drug resistance bacteria by using a self-developed atmospheric pressure plasma jet.

The different working gases, such as helium, nitrogen, and argon were used for this research, and their effects on inactivated bacteria were compared. The reactive species in plasma were analysed by using the spectrometer to identify the particle species in the plasma light and how they affect bacteria inactivation. At the end of this research, the optimization of bacteria inactivation was carried out by determining the best working gas and the minimum gas flow with the shortest operation time.

1.2 Problem Statement

Bacteria, or any microorganism in hospitals, are responsible for hospital acquired infections (HAIs) (Farrell, 2011; Joshi et al., 2010; Aly, Al-Mousa, & Al Asar, 2008). *Methicillin-resistant Staphylococcus aureus (MRSA)* and *Acinetobacter baumannii (A.baumannii)* are two of the multi-drug resistant bacteria species that contributed to the HAI problem (Joshi et al., 2010; Dancer, 2009; Aly, Al-Mousa, & Al Asar, 2008). They pose especially high risks to patients with low immunity, such as patients in the Intensive Care Unit (ICU) (Aly, Al-Mousa, & Al Asar, 2008). Generally, the patients are reliant on medical equipment, especially during surgery and intensive

treatment. In that case, proper decontamination is very important, in order to keep the instruments clean and safe to use.

Microorganisms, such as bacteria can be inactivated by using current sterilization and disinfection methods such as the autoclave, heating or thermal, ethylene oxide (EtO), hydrogen peroxide gas, liquid formulated peracetic acid systems, radiation, and detergents (Maisch et al, 2012; Nagatsu, 2011; Shintani & McDonnell 2011a; Miao & Yun, 2011; Kostov et al, 2010; Yamaguchi, 2001). Unfortunately, there are limitations with these methods. Some methods, such as heating or thermal are not suitable for heat-sensitive materials, such as polymers, which are commonly used as materials to make medical devices (Sakudo, 2013; Moreau, Orange, & Feuilloley, 2008). Another method is autoclaving. One problem with this method is that it is time-consuming. Also, the high temperature of the autoclave, which is around 121°C, causes the degradation of the thermo-labile materials and implants (Sakudo, 2013; Kawamura et al., 2012; Klämpfl et al., 2012).

Other methods are suitable for heat-sensitive materials, but they also have their limitations. For example, the radiation method is very expensive and radiation can harm the operator, while the EtO is corrosive chemical and is not environmentally friendly. Moreover, the process is very complicated and time consuming (Kawamura et al., 2012).

In this research, a new method for sterilization, namely cold plasma treatment, is being developed to overcome the problems above. It has the advantages of a low processing temperature, no chemicals being used, and a short processing time (Sakudo, 2013; Ni et al., 2013; Ehlbeck, et.al., 2011; Stoffels, Sakiyama, & Graves, 2008;

Laroussi & Leipold, 2004; Laroussi, Mendis, & Rosenberg 2003). Plasma is suitable for heat-sensitive materials because its temperature is below 40°C, as reported by Klämpfl et al.(2012). Indeed, studies have shown that plasma has the capacity to inactivate microorganisms and does so effectively (Nishida, Liu, Fan, Iwasaki, & Ou, 2013; Ni et al, 2013; Maisch et al., 2012; Klämpfl et al., 2012; Ehlbeck, et.al 2011; Kostov et al, 2010; Stoffels et al., 2008; Laroussi & Leipold, 2004; Laroussi et al., 2003).

However, in the inactivation bacteria by APPJ, the researchers used the higher gas flow rate and longer exposure time for the inactivation of the bacteria (Kuwahata et al., 2015; Kolb et al., 2012; Tipa et al., 2012; Joshi et al 2010; Sun et al., 2007; Lee et al., 2006; Laroussi et al., 1999). In this research, the optimum gas flow rate and time exposure are determined in order to reduce gas and exposure time for the inactivation bacteria by self-developed APPJ.

1.3 Objective

This research focuses on the inactivation of bacteria by using an atmospheric pressure plasma jet. The objectives of this study are as follows:

1. To study the microbial inactivation effects of APPJ variables, in term of:

    a. Types of microbes

        i. The microbes used in this research are bacteria, namely *Acinetobacter baumannii (A.baumannii)* and *Methicillin-resistant Staphylococcus aureus (MRSA).*

b. Types of gas

i. There are many types of working gas that can be used to generate the plasma and for this research, different gases were used to inactivate the bacteria. The gases used were helium, helium mixed with nitrogen, and argon.

c. Various gas flow rates

i. Gas was flowing into the plasma system at rates in the range from 300ml/min to 1000ml/min.

d. Exposure time.

i. These samples were exposed to the APPJ for time periods of 9s, 12s, 15s, and 18s.

2. To study the optical emission spectrum of the APPJ.

1.4 Scope of Project

Plasma technology has been chosen for this project due to no chemicals being used, shorter operation time, simple operation, and low operating temperature, compared to those of other methods. The scope of this project is to focus on the inactivation of bacteria by using an atmospheric pressure plasma jet (APPJ). The atmospheric pressure plasma jet in this study is self-developed by using a quartz glass tube and copper electrodes. The copper electrode is wrapped around the quartz glass tube with specific gaps that will be discussed in detail in chapter 3.

There are two types of bacteria samples used in this research, namely gram positive and gram negative. The gram positive bacteria used in this study are the *A.baumannii* and the gram negative ones are the *MRSA.* For this study, bacteria cultures were prepared by staff of Hospital Tuanku Fauziah (HTF) staff. The bacteria samples were prepared by using the serial dilution method, then spread onto the agar surfaces. After that, the samples were incubated for 24 hours at 37°C.

After being incubated, the bacteria samples were exposed to the APPJs using different gases, gas flow rates and exposure times. The gases used on these samples were helium, helium mixed with nitrogen, and argon. The gas flow rates for each type of gas ranged from 300ml/min to 1000ml/min.

The effectiveness of APPJ at inactivating the bacteria was observed from the growth of the bacteria on the agar surface. The treated samples were subcultured to new agar plates for the observation of the bacteria. The samples on new agar plates were cultured for 24 hours at 37°C. The effects of plasma inactivation on bacteria samples were observed in terms of bacterial growth. If the bacteria were still growing, then the bacteria were still active. The structure of the bacteria was observed before and after exposure, to monitor the effects of the APPJ process. The structure was analysed using a high power microscope. Then, the results were compared between controls and treated samples. The control samples are defined as samples that are not exposed to the plasma while the treated samples are the sample exposed to plasma.

Next, the reactive species on plasma were measured using an optical emission spectrometer. At the end of the study, the optimum parameters to inactivate the bacteria in short time were determined.

1.5 Thesis organization

This thesis contains five chapters. The first chapter introduces the research by defining plasma, how plasma is formed, and the applications of plasma. The introduction is followed by the problem statements, as well as the objectives and the scope of this project.

The second chapter is the literature review. This chapter presents the information and the facts from previous research on the subject, as well as theories, and methodologies relevant to this project. It begins with the introduction of plasma and the different types of plasma, followed by an introduction to different types of microorganisms. This chapter also describes the capacity of plasma to inactivate bacteria and to affect their structure.

The third chapter presents a detailed description of the research methodology used in this study. This chapter first explains the bacteria preparation, protocol, starting with the serial dilution method, which is then followed by the plasma treatment. Various parameters must be taken into consideration during the treatment of bacteria with plasma, namely the specific gas used, the gas flow rate, and the exposure time. Then, the growth and the structure of the treated samples are observed by using the subculture method. The bacterial structure is visualized by using a high power microscope. Finally, the radical species in the plasma plume are identified with the aid of an optical emission spectrometer.

Then, the results obtained in the experiment are presented in the fourth chapter. Finally, the correlation between the various parameters of the experiment and the plasma inactivation of the bacteria is discussed.

Chapter five summarizes the research and finalizes the study's conclusions. Recommendations, suggested improvements, and future developments in plasma jet research are also included.

CHAPTER 2

LITERATURE REVIEW

This chapter summarizes prior studies done on the subject, as well as the procedures or methods used, in terms of sample preparation, and also, the results obtained from those studies.

2.1 Introduction

Hospital-acquired infections (HAIs) are one of the problems in a hospital environment and they pose the greatest risk to patients who have a low immunity, such as the patients in the Intensive Care Units (ICUs) (Joshi et al., 2010). Microorganisms such as bacteria, viruses, fungi, or parasites, can cause HAIs. These microbes can be found on, or inside, the patient's body (e.g. skin), the environment or the hospital equipment. Patients can also contract an HAI from health care workers or other patients (Rizzo, 2006).

Patients are usually in contact with medical devices or surgical instruments. Medical instrument in direct contact with tissues or parts of the patient's body are considered critical item. This device should be sterilized properly to prevent infection (Rutala, 2008).

Proper disinfection and sterilization are very important, to prevent HAIs. Current sterilization methods include autoclaving, hot water, dry heat (oven), radiation,

liquid germicides, and toxic chemicals (Miao & Yun, 2011; Nagatsu, 2011; Kostov et al., 2010; Von Keudell et al. 2010). However, thermal, chemical, and radiation sterilization methods are not suitable for heat-sensitive materials. Additionally, radiation and toxic chemicals raise significant safety concerns (Moreau et al., 2000). Polymer are a type of heat-sensitive materials commonly used in medical devices and are easily damaged by thermal treatment (Nagatsu, 2011; Moreau et al.,2008). Table 2.1 lists the common methods that have been used to sterilize medical equipment (Rutala, 2009; Patel, 2003).

These problems can be overcome by using atmospheric plasma. Plasma operates at low temperatures, is safe, requires short operation times, and does not leave behind any toxic chemicals after processing (Nagatsu, 2011; Choi et. al. 2006; Moisan et al., 2001; Moreau et al., 2000). Plasma has been applied in various industries such as packaging, surface modification (such as etching and deposition), semiconductor technology, optical device and solar cell fabrication, textile, food sanitation, medical industry (e.g. Sterilization method, disinfection, decontamination and cleaning) (Nagatsu, 2011; Miao & Yun, 2011; Kostov et al., 2010; Stoffel et al., 2008).