DEDICATION

To my love, my best friend, my beautiful wife; EDA
ACKNOWLEDGMENTS

I would like to express my sincere and deepest gratitude to my advisors Dr. Gary Friedman and Dr. Alexander Fridman for their invaluable guidance, enduring support, and for constantly educating me throughout my doctoral studies.

I would especially like to express my deep appreciation to Dr. Alexander Gutsol for his guidance and patience, along with opening a whole different way of thinking and perspective into the matters.

I would like to thank the members of my advisory committee including Dr. Wei Sun, Dr. Young Cho, Dr. Adam Fontecchio, and Dr. Alexander Rabinovich for their helpful and constructive comments to improve this thesis. I would also like to thank Dr. Alan Lau for his advice and support over the last four years. I would also like to acknowledge the help of Dr. Andrei Starikovskii, Dr. Victor Vasilets, and Dr. Yuri Mukhin to my work.

I am thankful to all colleagues, co-workers, and friends at DPI and CATE Lab. I would especially like to express my appreciation to Robert Chang, Tanvir Farouk, Gregory Fridman, Shailesh Gangoli, Ondrej Hovorka, and David Staack, for their friendship and for delightful discussions around science, politics, and philosophy. I have been very privileged to have befriended you all.

Finally, I am grateful for the financial support from the Drexel Plasma Institute and the Mechanical Engineering and Mechanics Department.
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Abstract

Uniform Dielectric Barrier Discharge with Nanosecond Pulse Excitation for Biomedical Applications

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For some period of time the use of plasma in medicine has been limited to thermal discharges for cauterization and dissection. The effects of thermal plasma on tissue are entirely related to local heating. Non-thermal plasma, on the other hand, can have many different modes of interaction with tissue. It has been recently demonstrated that direct treatment of smooth surfaces by non-thermal dielectric barrier discharge (DBD) in air is highly effective in killing pathogens. Moreover, DBD can create different sub-lethal and selective effects. These results hold significant promise for medical applications such as sterilization of wound surfaces. However, a typical DBD in air can be highly non-uniform, particularly on topographically non-uniform surfaces such as in most living tissues. This creates significant limitations for use of DBDs in wound care and other biomedical applications. In this thesis, a novel non-thermal plasma system, namely nanosecond-pulsed DBD, has been developed and investigated to address this important limitation. Nanosecond-pulsed DBD is shown to be uniform in air at atmospheric pressure and much more effective in killing bacteria than conventional DBDs, particularly on topographically non-uniform surfaces. Thus, this new plasma system is potentially convenient for in vivo and hospital sterilization cases.
CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 Plasma in Medicine

For some period of time the use of plasma in medicine has been limited to thermal discharges for cauterization and dissection (Vargo 2004, Sumiyama et al. 2006, and Watson et al. 2000). Plasma has been used for electro-surgery where it desiccates tissue by passing electrical current through it (Pollack et al. 2000, Polousky et al. 2000, Lord et al. 1991, Stalder et al. 2005). The argon plasma coagulator (APC) is another early application of plasma for cauterization, tissue devitalization, and removal which also causes local heating and burns due to elevated temperatures (Raiser and Zenker 2006) (Figure 1.1). Some of the surgical applications of the argon plasma coagulator are visceral surgery, skin surgery (Brand et al. 1998), urology, gynecology, brain tumor surgery (Tirakotai et al. 2004), gastroenterology (Ginsberg et al. 2002), breast surgery (Ridings et al. 1998) and bronchological endoscopy (Reichle et al. 2000).

However, the aforementioned thermal plasma interacts with living tissue mainly through temperature and heat. Non-thermal plasma, on the other hand, can have many different modes of interaction where various plasma species can generate different sub-lethal and selective effects (Fridman et al. 2008, Stoffels 2007, Coulombe et al. 2006, Shekhter et al. 2005, Gostev 2008) as demonstrated in recent studies. In non-equilibrium plasmas, electron energies are much higher than the heavy particle (ions and neutral species) energies, resulting in enriched gas phase chemistry without high temperature input through collisions and consecutive dissociation,
excitation, and ionization processes (Kunhardt 2000, Penetrante 1996). Non-equilibrium plasmas, such as Dielectric Barrier Discharge (DBD), are very attractive because of their non-thermal nature. They create new possibilities in biological and medical fields where substances of interest are mostly heat-sensitive such as living tissue, cells, and biomaterials (Laroussi 2009, Stoffels et al. 2008, Yonson et al. 2006, Puac et al. 2006). Some of the recent research subjects of non-thermal plasma applications are 1) inactivation of bacteria on living tissue, 2) accelerated blood coagulation, 3) enhanced cell functions including attachment and proliferation, 4) treatment of malignant tissue, and 5) wound healing (Stoffels 2006, Yildirim et al. 2008, and Kalghatgi et al. 2007).

Systems that employ afterglow from non-thermal plasma for medical treatment and disinfection have been proposed and demonstrated within the last decade (Sladek and Stoffels 2005, and Goree et al. 2006). Non-thermal treatment is possible with thermal plasma if its afterglow is transported and cooled (Shimizu et al. 2008). Although this makes it possible to work with living tissue and heat-sensitive surfaces (Weltmann et al. 2008, Foest et al. 2007), such treatment takes a relatively long time and can’t employ many short-living active plasma species and charges. Direct plasma created right on the tissue, on the other hand, can generate and bring charges and short living active species directly to its surface.
Within the past few years it has been revealed that direct treatment of smooth surfaces and living tissues by non-thermal Dielectric Barrier Discharge (DBD) in air is highly effective in killing pathogens including bacteria and fungi (Fridman 2008a, Birmingham and Hammerstrom 2000, Montie et al. 2000, Laroussi et al. 2002). DBD generates several active species that are quite essential for sterilization and other important biomedical processes. Some of the highly active oxygen-containing species are ozone, atomic oxygen, electronically excited oxygen, and peroxide. In general, oxygen is required to be part of the gas composition to generate the
aforementioned active species and consequently for effective sterilization (Fridman 2008b). It has been demonstrated recently that contact of living tissue with charges from non-thermal atmospheric pressure plasma is the main reason for the observed effects (Fridman et al. 2007 and Deng et al. 2006) and is much more effective for sterilization compared to UV (ultraviolet) or long-living species such as ozone in the plasma afterglow.

1.2 Dielectric Barrier Discharge Plasmas

Dielectric Barrier Discharges (DBDs) are significant among all types of non-thermal plasmas because of their relative simplicity. DBDs offer a unique combination of non-equilibrium and quasi-continuous behavior having high electron mean energy with lower heavy particle (neutral, ion) temperatures. They produce several chemically active species (electrons, radicals, metastables, and ions) with low gas heating (Wagner et al. 2003). Because of these characteristics, DBDs are widely used in gas cleaning (from NOx, SOx, VOC), thin film deposition (Salge 1996, Williamson et al. 2006), ozone production, light sources (excimer UV sources) (Motret et al. 2000), industrial processes of polymer films or fibers to increase wettability and adhesion (Borcia et al. 2003, Massines et al. 1998), and many other technologies (Fridman et al. 2005). In addition, DBDs enable various emerging novel applications in biology and the medical field (Laroussi et al. 2000 and Stoffels et al. 2002). Several interesting medical possibilities have been demonstrated by Fridman et al. in the past few years (Fridman et al. 2006).

DBDs are often applied at atmospheric pressure and in air. There are usually two electrode configurations that have been employed for most of applications: 1)
parallel and 2) concentric configurations (Figure 1.2). Operating principals of DBD are summarized with schematics shown in Figure 1.3 (a through d). In general, when high voltage is applied between two electrodes that are without insulation, an arc (high temperature plasma channel) develops given sufficient time (as short as few milliseconds). A dielectric barrier layer placed in front of at least one of the electrodes (Gibalov and Pietsch 2000) limits the current, avoiding the formation of an arc. Instead, transient non-thermal plasma is generated in the gap. If the applied high voltage remains constant, charge from plasma accumulates on the dielectric surface and reduces the effective field in the discharge gap extinguishing the discharge (Xu and Kushner 1998). In order to sustain the DBD plasma, the applied voltage needs to change over time allowing the charges accumulating on the insulator surfaces to be removed.

Although, it has been demonstrated that DBD can be ignited in the form of homogenous plasma at atmospheric pressure in certain gas mixtures, pure nitrogen, and some noble gases (Kanazawa et al. 1988, Yokoyama et al. 1990, Gherardi et al. 2000, Miralai et al. 2000, Massines and Gouda 1998, Rahel et al. 2007), in most cases, DBD (particularly in oxygen-containing gases, e.g. air) results in a multi-streamer mode of operation with formation of microdischarges (Kogelschatz 2003) and subsequent filaments that are visible to the human eye (Figure 1.4). The filaments typically have a diameter on the order of 100 μm (Fridman et al. 2005, Kogelschatz 2002) for discharge gaps that are few millimeters long.
As plasma density in the microdischarges is much higher than in the surrounding space, these microdischarges can be considered as the only active locations of the whole DBD volume, where all of the energy dissipates. Therefore, although average temperature in the discharge volume is small, local temperature around the microdischarge filaments can be relatively high. This temperature non-uniformity can be very important, particularly when the microdischarges in DBD remain in the same position for relatively long periods of time.
Figure 1.3: Schematics of DBD operation

Figure 1.4: Image of a typical DBD in air
Pinning of the microdischarges is more likely to occur on ridges of non-uniform surfaces, like the surfaces of living tissues (Figure 1.5), which may compromise effective treatment. The primary goal of the work reported here, therefore, is to circumvent formation of discharge filaments in DBD and make the discharge more uniform even on relatively non-uniform surfaces.

Figure 1.5: Microdischarges of a conventional dielectric barrier discharge striking on the ridges of the skin (similar to lightning)
1.3 Research Objectives and Approach

This thesis rests on the hypothesis that ultra fast rising external voltage enables generation of uniform plasma that will be effective and convenient for treatment of non-uniform surfaces, e.g. living tissue. The objective of the research is to retain the direct nature of the DBD plasma treatment, and to reduce the sensitivity to topographical non-uniformities of the surface being treated. The scope this research is 1) to develop and investigate non-thermal uniform DBD plasma system, and 2) to demonstrate and assess its efficiency in applications requiring sterilization in air at atmospheric pressure.

1.4 Thesis Outline

Chapter 2 explains the design of the nanosecond-pulsed dielectric barrier discharge device and system for biomedical applications. The chapter starts with the explanation of gas breakdown phenomenon in Dielectric Barrier Discharge and the key facts of the nanosecond-pulsed dielectric barrier discharge system. Chapter 2 also presents the details about the power supply circuit and the high voltage electrodes. Chapter 3 focuses on the thermal, electrical, spectroscopic and uniformity characterization of the novel nanosecond-pulsed system. Chapter 4 examines the sterilization effect of the nanosecond-pulsed DBD plasma and presents a comparison between it and conventional DBD. Finally, Chapter 5 summarizes the research contributions.
2.1 Breakdown of Gas in DBD

It is important to revisit the breakdown mechanism of gas to understand the operation of conventional DBD. When high voltage is first applied to the discharge gap, free electrons gain energy and ionize the background gas by knocking out new (secondary) electrons from heavy particles as they drift to the anode. Multiple avalanches are formed and grow. This process is governed by the Townsend ionization coefficient, $\alpha$, which is a function of the reduced electric field, $E/n$ (where $E$ is the electric field and $n$ is the gas density).

Electron impact ionization dominates during the first phase of the breakdown (Meek and Craggs 1978, Loeb 1960, and Bogdanov et al. 2004). During this phase, many avalanches start. However, not all of them get a chance to develop equally. This is due to the fact that, in the avalanche growth phase (Figure 2.1), usually the charge density in the discharge gap grows non-uniformly. This results in non-uniform growth of the electric field. If the external field grows slower than the non-uniform electric field due to the space charge, it is possible for the field due to the space charge to reach a critical level wherein it becomes comparable to the external field. This is known as the Meek criterion. One of the effects of the high local electric field is that it opposes the external field in some regions suppressing development of avalanches there and enhancing ionization in other places. This effect is illustrated in Figure 2.2. Some avalanches end up being ‘winners’, other become ‘losers’. In fact, the field of
the winning avalanches can be so strong that, in conjunction with the ionizing effects of photons (photoionization), it creates a secondary fast ionization wave called a streamer (Nikandrov et al. 2008, and Gouda and Massines 1999). Typical avalanche-to-streamer transition and streamer propagation are presented in Figure 2.2. The front of this secondary ionization wave actually propagates in the opposite direction.

Figure 2.1: Electron multiplication and avalanche growth (Raizer 1991)

Figure 2.2: Avalanche-to-streamer transition and streamer propagation (Raizer 1991)
When a streamer bridges the gap it forms a channel of weakly ionized plasma. Eventually when the voltage polarity reverses, the residual negative charges from the previous half-cycle contribute to the formation of new avalanches and streamers at (or the vicinity of) the same spot. The outcome of the entire process from first electrons to the streamer formation is called microdischarge. Typical microdischarge parameters in a 1 mm gap in atmospheric-pressure air are presented in Table 2.1.

Table 2.1: Typical microdischarge parameters in a 1-mm gap in atmospheric-pressure air (Fridman et al. 2005, Kogelschatz 2007)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime</td>
<td>1-20 (100) ns</td>
</tr>
<tr>
<td>Filament radius</td>
<td>50 – 100 µm</td>
</tr>
<tr>
<td>Peak current</td>
<td>0.1 A</td>
</tr>
<tr>
<td>Current density</td>
<td>0.1 – 1 kAcm⁻²</td>
</tr>
<tr>
<td>Electron density</td>
<td>$10^{14} – 10^{15}$ cm⁻³</td>
</tr>
<tr>
<td>Electron energy</td>
<td>1 – 10 eV</td>
</tr>
<tr>
<td>Total transported charge</td>
<td>0.1 – 1 nC</td>
</tr>
<tr>
<td>Reduced electric field</td>
<td>$E/n = (1-2)(E/n)_{Paschen}$</td>
</tr>
<tr>
<td>Total dissipated energy</td>
<td>5 µJ</td>
</tr>
<tr>
<td>Gas temperature</td>
<td>~ average, ~ 300 K</td>
</tr>
<tr>
<td>Overheating</td>
<td>5 K</td>
</tr>
</tbody>
</table>

2.2 Key Facts About the Nanosecond-pulsed Dielectric Barrier Discharge System

Uniformity of the plasma could be improved in two ways: 1) increasing uniform pre-ionization of the gas to initiate more avalanches or 2) shortening the voltage rise time (Starikovskaia et al. 2001, Qi et al. 2006) to avoid growth of highly inhomogeneous electric field that promotes growth of some avalanches at the expense
of others. If the number of primary avalanches is high enough before the accumulation of the critical space charge, the discharge is likely to remain uniform even if streamers do occur. The resulting discharge will resemble ‘pulsed avalanche’ regime (Levatter and Lin 1980). In addition, under these conditions, the shape of the electrodes does not affect the location of the avalanches and streamers making the discharge more independent of the topography. A fast rising driving voltage can also shift electron energy distribution function to higher values (Gallagher et al. 1983).

Roughly, the criterion of the uniform discharge development could be formulated with a simple relation:

$$\tau_{\text{rise}} < \frac{d}{v_{\text{d}}}$$

where \(\tau_{\text{rise}}\) represents the excitation pulse rise time, \(d\) the discharge gap length, and \(v_{\text{d}}\) the electron’s drift velocity in the critical electric field (Zatsepin et al. 1998). Let us suppose that the discharge gap is about 1 mm. Let us also take the maximum applied voltage, \(V_{\text{max}}\), for the DBD to be 16 kV. From this, the reduced electric field \((E/n)\) is found to be \(\sim 5.3 \times 10^{-15} \text{ V.cm}^2\) and, on average, the drift velocity \((v_{\text{d}})\) for an electron in air is \(\sim 10^{-7} \text{ cm/sec}\) (tabulated value from Dutton 1975). Summary of the parameters is given in Table 2.2. Accordingly, the time required for an avalanche to travel the inter-electrode distance is \(\sim 10^{-8} \text{ sec} = 10 \text{ ns}\). This time is the characteristic time of build-up of possible local non-uniformities in the electric field within the discharge gap and, therefore, it is the goal of the proposed nanosecond-pulsed DBD to achieve this rise time. The above estimate is consistent with other estimates of \(dV/dt > 1 \text{ kV/ns}\) (Raupassov et al. 2008). Tens of nanoseconds is considerably (at
least 2 orders of magnitude) shorter than rise time of microsecond-pulsed DBDs which have been noted to be non-uniform.

Table 2.2: Summary of nanosecond-pulsed DBD parameters

<table>
<thead>
<tr>
<th>ns-DBD</th>
<th>Discharge Gap [mm]</th>
<th>Voltage [kV]</th>
<th>Reduced Electric Field ( (E/n) ) [V.cm(^2)]</th>
<th>Drift Velocity ( (v_d) ) [cm/s]</th>
<th>Time to Bridge the Gap [ns]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>16</td>
<td>( 5.3 \times 10^{-15} )</td>
<td>( 10^{-7} )</td>
<td>10</td>
</tr>
</tbody>
</table>

2.3 Power Supply

A novel non-thermal nanosecond-pulsed DBD has been developed to generate uniform plasma in air at atmospheric pressure. Rather than using expensive and often unreliable semiconductor devices for creating nanosecond pulses (Miles et al. 2001 and Zhukov et al. 2007), a relatively simple double spark gap circuit has been built for the generation of pulses with durations on the order of tens of nanoseconds (Ayan et al. 2008).

The pulse generating circuit has been employed along with a current source to obtain short duration high voltage pulses. The circuit with a double spark gap configuration is shown in Figure 2.3.
The circuit produces repetitive short pulses. Voltage pulse starts when the main spark gap is triggered. When the larger primary (main) spark gap breaks down, charge initially stored in the main capacitor is transferred to the discharge as the voltage across the plasma electrodes rises precipitously. The smaller (secondary) spark gap starts to charge and eventually shorts out the DBD, resulting in a rapid decay of the voltage across the DBD electrodes.

The size of the main spark gap determines the voltage that appears across the discharge electrodes after the spark breakdown. The frequency of voltage pulses is determined by the current source, main capacitor (how fast is the capacitor charged) and is also affected by the peak voltage. The secondary spark gap affects mainly the length of the voltage pulse that is maintained across the DBD electrodes. In the first approximation, rise time of the voltage is independent of spark gaps.
The main spark gap was varied from 15 to 24 mm with at 3 mm intervals, and repetition rates were measured between 250 and 100 Hz, respectively (for various sizes of secondary spark gaps between 2.5 to 4.5 mm). The pulse frequency (repetition) as a function of main spark gap distance is presented in Figure 2.4. Pulse duration is linearly dependent on secondary spark gap length (Figure 2.5). For 2.5 and 4.5 mm gap distances, pulse durations are approximately 15 and 30 ns, respectively. Peak voltage across the DBD is linearly dependant on the main spark gap distance with approximately 1 kV per 1 mm for the above mentioned range (Figure 2.6). As the main gap increases from 10 to 27 mm, peak voltage increases approximately from 10 kV to 27 kV. The rise time of approximately 3 kV/ns is obtained on the front of the voltage pulse. A typical oscillogram of nanosecond-pulsed DBD with ultrafast high voltage pulse is given in Figure 2.7. Additionally, both a single and 10-consecutive (superimposed) voltage signals are presented in Figure 2.8.

![Figure 2.4: Pulse frequency (repetition) versus main spark gap distance for several small spark gap distances (2.5 - 4.5 mm). Frequency values are average of 10 measurements and variance is ±10%](image)
Figure 2.5: Pulse duration (FWHH) versus small spark gap distance for several main spark gap distances (15 - 27 mm). Pulse duration values are average of 10 measurements and variance is ±10%.

Figure 2.6: Peak voltage versus main spark gap distance for several small spark gap distances (2.5 - 4.5 mm). Peak voltage values are average of 10 measurements and variance is ±10%.
Figure 2.7: Oscillogram of typical voltage and current signals
(Main spark gap: 12mm, Secondary spark gap: 3 mm)

Figure 2.8: Superimposed voltage signals versus time
(main spark gap: 15 mm and secondary spark gap: 3mm)
(a) Peak voltage: 15.6 kV, pulse length: 23 ns
(b) 10 voltage signals superimposed
2.4 Electrodes

Two electrodes are required in order to generate DBD: one electrode is powered with high voltage and the other is grounded. There are two electrode configurations with two different types of high voltage electrodes (powered) used. The first configuration is plane-to-plane with flat surface electrode, and the second configuration is sphere-to-plane with spherical electrode. In all cases, the grounded electrode is either flat metal or agar.

2.4.1 Planar electrode

In the first configuration (plane-to-plane) the powered electrode is made out of cylindrical copper and enclosed in Polyetherimide (Ultem®) for insulation (Figure-2.9). The flat surface of the copper cylinder is covered with clear fused quartz (Technical Glass Products, Painesville, OH) as a dielectric barrier. This configuration was employed for characterization and sterilization experiments using with various power densities.

Figure 2.9: Cylindrical electrode cross section
The flat surface electrode is made in two sizes for several different experiments. The larger size cylindrical copper has 25 mm diameter and the thickness of the clear fused quartz is 1 mm. The smaller size electrode cylindrical copper is 10 mm in diameter and covered by 0.66 mm thick quartz.

2.4.2 Test tube electrode

In the second configuration (sphere-to-plane), the high voltage electrode (Figure 2.10) consists of a borosilicate glass (Pyrex®) test tube (cat.# 60825-902, VWR Scientific, San Francisco, CA) with a conductive silver paste (SPI West Chester, PA) filling inside. The thickness of the glass of the test tube is approximately 0.75 mm with a radius of curvature of 5 mm. It should be noted that in some cases, this test tube electrode was positioned to be in contact near its tip with the grounded plane metal electrode.
Figure 2.10: Glass test tube electrode
CHAPTER 3: PLASMA AND DEVICE PHYSICAL CHARACTERIZATION

3.1 Electrical Characterization

3.1.1 Voltage and current

Electrical measurements have been conducted by using a high frequency high voltage probe (#PVM-4, 110 MHz, 1000:1, North Star High Voltage, Marana, AZ) connected in parallel with the discharge and a high frequency current transformer (#CM-10-L, Ion Physics Corporation, 0.1 V/Amp, 45 MHz bandwidth) around the high voltage electrode wire. Electrical schematics with voltage and current probes at positions have been shown previously in Figure 2.3. The probe signals were acquired and recorded using a high speed oscilloscope (500 MHz bandwidth, 5 Gsample/s, TDS5052B Digital Phosphor Oscilloscope, Tektronix, Inc., Richardson, TX). Power dissipation in the discharge was analyzed by measuring instantaneous current and voltage in the gap. Recorded data was processed using customized MATLAB code which integrates the instantaneous power ($V \times I$) over many cycles to determine an average energy per cycle and average power.

3.1.2 Power

Current signal of a typical DBD has many spikes in the oscillograms and they are associated with individual microdischarges. These current spikes are characteristically very short in duration and questions arise regarding the validity of using the measured current to calculate the discharge power (Ayan et al. 2009a). The bandwidth of the current probe and the inverse of the microdischarge duration are
comparable, and some loss of information about the actual discharge current may occur. For this reason, the electrical measurements for average power were verified with custom made calorimetric setup (Figure 3.1). Calorimeter is composed of a peristaltic pump (model # 3386, Control Company, TX), two mercury thermometers (model # 112C, -1 - 51°C, 1/10°C div., Palmer Instruments, Inc., NC), a copper chamber and an insulation casing. Water is pumped at a controllable flow rate through the copper tube that surrounds the chamber and encloses the DBD electrodes. One thermometer is placed upstream to the chamber to measure the inlet temperature of the water. A second thermometer is located downstream from the chamber to measure the outlet temperature of the water. The system is insulated to ensure that the only heat loss is attributed to the flowing water. The insulation is minimum 10 cm thick around the system.

When the plasma ignites, dissipated energy in the chamber is taken away by copper chamber and copper tube surrounding the chamber and transferred to the running water. Temperature measurements from both thermometers are recorded every minute throughout the experiments. Since the heat transfer to the water is a rather slow process, it took typically more than 100 minutes to reach the steady-state conditions in most cases. After reaching the steady state, the heat transferred from the discharge (thus the average power dissipation in the discharge gap) can be calculated by using flow rate, water specific heat capacity, and a constant water temperature difference between inlet and outlet:

\[ \dot{Q} = \dot{m} C_p \Delta T \]
where $\dot{Q}$ is heat, $\dot{m}$ is the mass flow rate, $C_p$ is the specific heat capacity of water, and $\Delta T$ is the temperature difference between inlet and outlet ports.

![Figure 3.1: Schematic of calorimeter setup](image)

Additionally, a curve with double exponential term is fit to the experimental data to ensure that the converged value is accurate. The double exponential term curve is as follows:

$$\Delta T = c_1 + c_2(1 - e^{-c_3 t}) + c_4(1 - e^{-c_5 t})$$
Here $t$ is time, and $c_1$, $c_2$, $c_3$, $c_4$, and $c_5$ are arbitrary coefficients. A sample set of data with curve fitting is given in Figure 3.2. It is found that the electrical and calorimetric power measurements agree to within less than 10% indicating that the bandwidth of the current transformer is at least sufficient for these measurements (Table 2.1).

Figure 3.2: Experimental data and curve fitting for $\Delta T$.
Electrical power measurement: $5.02 \pm 0.44$ W (average of 10 measurements)
Table 3.1 Comparison between calorimetric and electrical power measurements

<table>
<thead>
<tr>
<th>Parameter [unit]</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow [ml/min]</td>
<td>16.55</td>
</tr>
<tr>
<td>Specific heat capacity (@ 25 °C) [J/g.°C]</td>
<td>4.1855</td>
</tr>
<tr>
<td>ΔT (fit) [°C]</td>
<td>4.0613</td>
</tr>
<tr>
<td>Power (calorimetry) [W]</td>
<td>4.689</td>
</tr>
<tr>
<td>Power (electrical) [W]</td>
<td>5.02</td>
</tr>
<tr>
<td>Difference % 6.6</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Characterization of the Uniformity of the Plasma

3.2.1 Side view imaging

Along with nanosecond-pulsed DBD, a few other DBD systems are also assessed visually for uniformity analysis. Initially, side view pictures of discharges are taken by a digital camera (Nikon D70). Side view images of two conventional type DBDs with plane-to-plane configuration are given in Figure 3.3 with 25 mm diameter active area. In Figure 3.3, filaments are clearly visible for both types in the 1 mm discharge gap between the high voltage electrode (top) and the grounded electrode (bottom). Detailed short time exposure images of filaments are captured with a CCD camera on the spectrometer (Figure 3.4). A concave mirror with 5 cm focal length is used to focus the light emitted from the plasma onto the slit of the spectrometer. In order to facilitate spatially resolved measurements, the plasma image is magnified approximately five times. Figures 3.5 and 3.6 illustrate the real and contrast enhanced images of the filaments (microdischarges) of conventional
discharges created between two test tube electrodes (cross section lines on Figure 3.5 will be explained in the next chapter). In this electrode configuration (Kozlov et al. 2001, Kozlov and Wagner 2007), the filaments are localize at the opposing tips of the electrodes and do not laterally wander as they would in the case of planar electrodes. Arrows on Figure 3.6 identify the location of the electrodes (Figure 3.6 images are contrast enhanced for clarity).

Figure 3.3: Images of two discharges with plane-to-plane configuration: (a) Sinusoidal waveform, (b) Microsecond-pulsed waveform

Figure 3.4: Spectroscopic measurement setup for single filament
Figure 3.5: Locations of 21 cross sections on the images of two types of DBDs with two test tube electrodes (1 mm gap). Exposure time of sinusoidal DBD is 50 msec (600 cycles) and microsecond-pulsed DBD is 1 s (1000 cycles).

Figure 3.6: Contrast enhanced images of two types of DBDs with two test tube electrodes (1 mm gap). Exposure time of sinusoidal DBD is 50 msec (600 cycles) and microsecond-pulsed DBD is 1 s (1000 cycles).
On the other hand, nanosecond-pulsed DBD with glass test tube electrode is shown in Figure 3.7. This discharge typically appears dim. Nevertheless, it can be seen in Figure 3.7(a-b) that plasma is spread all over the spherical tip of the electrode. Figure 3.7a is taken in the presence of background light and Figure 3.7b is taken in a dark room. Both images are taken under the same conditions, i.e., repetition rate is approximately 190 Hz and exposure time of the photography is 0.62 s. Table 3.2 provides a comparison of the size of the filament and voltage rise rates for different dielectric barrier discharge systems.

Figure 3.7: Side view of nanosecond-pulsed DBD between test tube electrode and ground metal electrode (a) with background light and (b) in a complete dark room for the same exposure time (bottom halves of the images are due to reflection from the ground plate electrode surface)
Table 3.2: Size of the filament versus voltage rise rates for different dielectric barrier discharge systems

<table>
<thead>
<tr>
<th>Plasma system</th>
<th>Voltage rise</th>
<th>Filament size</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusoidal</td>
<td>0.5 V/ns</td>
<td>~0.18 mm</td>
<td>Fig. 3.6</td>
</tr>
<tr>
<td>Microsecond-pulsed</td>
<td>20 V/ns</td>
<td>~0.1 mm</td>
<td>Fig. 3.6</td>
</tr>
<tr>
<td>Nanosecond-pulsed</td>
<td>3000 V/ns</td>
<td>-</td>
<td>Fig 3.7 &amp; Fig. 3.9</td>
</tr>
<tr>
<td></td>
<td>(3kV/ns)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Side view images of the nanosecond-pulsed DBD show that it is “filament-free” and much more uniform than the conventional DBDs (microsecond-pulsed and sinusoidal). Here, it should be highlighted that in Figure 3.3 the inter-electrode distance is a constant 1 mm in the entire plasma gap. On the other hand, it can be seen in Figure 3.7 that the plasma gap varies from 0 mm to approximately 4 mm for nanosecond-pulsed DBD with a test tube electrode. Despite the large gap range, nanosecond-pulsed DBD presents a uniform glow-like appearance, whereas despite the constant 1 mm gap, conventional discharges exhibit a filamentary non-uniform structure.

3.2.2 Lichtenberg figures

Although long exposure imaging is helpful to demonstrate distinction between nanosecond-pulsed DBD and the conventional DBDs, a new experimental setup is designed and built for qualitative yet more rigorous uniformity analysis. Qualitative
uniformity measurements of the various discharges have previously been done by simply exposing a commercial photo film to the plasma. If conventional plasma is generated on a dielectric surface, microdischarges create a "branching" form as soon as they strike on the surface. Optical emission from the plasma can be registered if the surface is a photo film. Images on the photo film provide information about the size and the number of the microdischarges, and they are referred to as Lichtenberg figures (Chirokov et al. 2004). A schematic of the setup is presented in Figure 3.8. The photo film is placed between the insulated test tube electrode and the grounded metal electrode. A roll-to-roll setup driven by an electric motor is engaged to advance the photo film with a rate of about 1 m/s, where pulses of DBD plasma were being ignited. The speed of the photo film is selected so that Lichtenberg figures of each single pulse (cycle) of DBDs plasma could be resolved. Lichtenberg figures are developed on black & white and color photo films. Black & white photo films possess two different sensitivities, i.e. ISO100 and ISO3200. The experiment is carried out in a dark room.

To analyze the distinction in uniformity more profoundly, Lichtenberg figures of the nanosecond-pulsed DBD are compared with those of a conventional discharge, i.e. microsecond-pulsed DBD. For microsecond-pulsed DBD, charge patterns are obtained for 10 kV peak voltage pulses with a maximum 10 kV/μs rise with 2-5μs pulse width. In the case of nanosecond-pulsed DBD, voltage rise and pulse duration are approximately 3 kV/ns and 20 ns, respectively. Thus, both voltage rise and pulse duration are at least two orders of magnitude longer for the microsecond-pulsed DBD compared to its nanosecond DBD counterpart. Microsecond-pulsed DBD is operated
at a 120 Hz repetition rate, its lowest repetition rate, in order to facilitate capture of consecutive pulses.

Both plasma systems are operated with the same test tube electrode. The photo film is situated between the grounded plane metal electrode and the test tube electrode near its tip.

![Figure 3.8: Schematic of experimental setup to acquire the Lichtenberg figures on photo film](image)

As evident from the Figure 3.9, Lichtenberg figures show significant differences between the two discharges. The nanosecond-pulsed discharge displays a round pattern that is approximately equivalent in size to the diameter of the high voltage electrode without any bright spot or irregular pattern distribution. The contact point of the electrode appears as a dark point at the center of Figure 3.9a. Rays-type
pattern at the edge of the spot appears likely due to the secondary surface discharge being driven by the lateral component of electric field. Figure 3.9(a-b) also verifies that nanosecond-pulsed DBD ignites uniformly over a relatively large range of electrode gap distances (0 - 4 mm). It is worth noting that 0 – 4 mm inter-electrode gap range is due to the curvature of the glass covered high voltage electrode as it is in contact near its tip with the grounded plane metal electrode. On the other hand, discharge patterns of microsecond-pulsed DBD in Figure 3.9(c-d) clearly shows the filamentary structure (microdischarges) when used with the same electrode under the same conditions.
Figure 3.9: Lichtenberg figures of two different DBD systems on the emulsion of the photo films: (a) nanosecond-pulsed DBD - b&w, (b) nanosecond-pulsed DBD - color, (c) microsecond-pulsed DBD - b&w, (d) microsecond-pulsed DBD - color
3.3 Thermal Characterization of the Plasma

3.3.1 Optical emission spectroscopy

Optical emission spectroscopy (OES) is employed to measure the vibrational and rotational temperatures of the DBDs in the plasma volume using 375.4 nm and 380.4 nm vibrational lines and rotational structure at this region of the second positive system of molecular nitrogen, N$_2$ (C$^3\Pi$-B$^3\Pi$). The experimental spectrum is compared to the simulated spectrum with $T_{\text{vib}}$ and $T_{\text{rot}}$ determined by the best fit (minimum RMSE) between the modeled and experimental spectrum detailed in the literature (Yalin et al. 2002, Laux et al. 2003, Packan et al. 2003, Staack et al. 2006, and Staack et al. 2007).

Prior to the investigation of nanosecond-pulsed DBD, in order to set a benchmark for the rest of the study, two conventional non-uniform DBD systems are characterized. The features of the two power supplies that have been employed to generate plasma are as follows: 1) Microsecond-pulsed DBD system can generate up to 35 kV peak-to-peak voltage with 120 Hz to 1 kHz repetition rate, and 1.5 to 5 $\mu$s pulse width at the half maximum of the voltage pulse, 2) Sinusoidal DBD system can generate up to 35 kV peak-to-peak voltage with 12 kHz frequency. Typical waveforms of two power supplies are given in Figure 3.10 where peak-to-peak voltage is 17 kV for sinusoidal and 18 kV for microsecond-pulsed systems. Multiple current peaks in the oscillograms (Figure 3.10) reflect a characteristic non-uniform DBD discharge.
Figure 3.10: Oscillograms of (a) sinusoidal and (b) microsecond-pulsed DBDs

Optical emission spectroscopy show that the rotational temperatures are between 340 and 400 K for both types of DBD. For DBD plasmas, rotational
temperature is essentially equal to the translational (gas) temperature (Nozaki et al. 2001, Nozaki et al. 2002). Here, one important point should be clarified. To be more precise, there can be 2 different temperatures defined for conventional DBDs: 1) temperature of the microdischarges (maximum temperature) and 2) average temperature of discharge gap (taking the entire gap into account). OES measures the maximum temperature, namely temperature of the microdischarge. Since rotational temperature (gas temperature) has been obtained via spectroscopy, and the lifetime of the upper state of $N_2$ is very short (Chelouah et al. 1994) compared to the shortest voltage pulse duration (microsecond-pulsed DBD), it means that the collected light and measured temperature correspond to the plasma state during the time of the microdischarge.

The rotational and vibrational temperatures of the two conventional DBDs are measured at different power levels. Power densities are calculated based on the power input and the size of the active area of the electrode. Given in Figure 3.11 & 3.12 are the averages of 3 measurements under the same conditions and the error bars shown representing the deviation of those three measurements.

As might be expected, Figure 3.11 shows a near linear increase in rotational (gas) temperature with average power density. Moreover, the two distinct type of voltage waveforms seem to fit to the same slope (although no overlap is seen within their respective operating ranges due to power supply limitations). This demonstrates that the power-temperature relation is largely independent of voltage waveform shape for the power range studied here. Thus, although sterilization effects may be different
for different discharges, i.e., dependant on several physical and chemical factors, it appears that gas temperature depends only on average power.

In Figure 3.12, vibrational temperatures are plotted for different average power densities. Even though, it looks like there exists a trend of a decrement in vibrational temperature as the power increases, this is not really clear because of the relatively low sensitivity of the calculation method for the vibrational temperature, and therefore all values can be considered to be roughly the same (3300 – 3400 K). Nonetheless, a slight increase in vibrational temperature accompanying a decrease rotational temperature has been observed before and is not unexpected as the rates of vibrational to translational energy transfer decrease with decreasing translational temperature (Staack et al. 2006).

Figure 3.11: Rotational temperature as a function of average power density for the sinusoidal DBD and the microsecond-pulsed DBD
Figure 3.12: Vibrational temperatures as a function of average power density for the sinusoidal DBD and the microsecond-pulsed DBD

In addition to the volume discharge temperatures, spatial temperature distribution along a single filament has also been measured in order to investigate the effect of energy dissipation in the volume onto near-surface locations. A double glass test tube electrode configuration with a 1 mm distance between electrodes is used to obtain a single filament. Temperatures for the two different types of DBD filaments have been measured over 20 equally divided intervals (at 21 cross sections) along the axial direction of single filaments shown in Figure 3.5. Spectra at each cross section have been acquired simultaneously and temperature calculations have been conducted on each spectrum for different locations (of the axial position). A sample of set of spectra at various locations along the filament is presented in Figure 3.13 (discrete).
Also Figure 3.14 illustrates spatial intensity for the wavelength range of interest (continuous).

The radial thickness of the microsecond-pulsed DBD is clearly seen to be thinner than that of the sinusoidal DBD. This may be due to the fact that the volumetric ‘memory’ effect (Eliasson et al. 1987) is stronger for sinusoidal DBD which has a much higher current pulsing frequency, and therefore each streamer has better marked “road” that is formed by the diffuse decay of the previous streamer.

![Figure 3.13: Spectra at various locations along the filament](image)

Figure 3.13: Spectra at various locations along the filament
(cross sections : 1, 3, 5, ….17, 19, 21)
The aforementioned results of temperature measurements along the axis of single filaments are plotted in Figure 3.15. These measurements do not reveal any significant differences in rotational and vibrational temperatures along the discharge channel (except possibly very near the electrodes). Rotational temperature distributions are uniform along filament for both types of discharges, thus power appears to dissipate more or less evenly.

Along the central axis, emission intensity (for the region of interest of the spectrum) from the single filament is minimal in the middle and increases towards the electrode surfaces, roughly doubling for both types of discharges. However, as seen in Figure 3.15, an increase in the emission intensity does not necessarily imply that the temperature is higher. Also, a slight increase in the light intensity at the electrodes might be due to inevitable reflection from the glass test tube surfaces. In summary,
Figure 3.15(a-b) indicates that the rotational and vibrational temperatures can be considered constant along the channel. In general, vibrational temperatures are one order of magnitude higher than rotational temperatures and this indicates the non-equilibrium nature of the discharge.

Figure 3.15: (a) Rotational and (b) Vibrational temperature distributions along the microdischarges.
A different setup is used to measure temperature of the nanosecond-pulsed DBD. A fiber-optic bundle (Princeton Instruments-Acton, 10 fibers – 200 μm core) is utilized to acquire the optical emission from the discharge and to transmit it to the spectrometer (Acton Research SpectraPro 500i with Roper Scientific model 7430 CCD camera or Princeton Instruments – Acton Research, TriVista TR555 spectrometer system with PIMAX digital ICCD camera, Trenton, NJ) and spectra are digitally acquired with approximately 0.6 nm resolution (Figure 3.16). The temperature of the camera is set at -25°C in all experiments.

![Figure 3.16: Spectroscopic measurement setup for DBD](image)

The background noise obtained for the same exposure time is subtracted from the discharge emission spectrum prior to the temperature estimation. Low pressure mercury lamp is used to determine the slit function and calibrate the spectrometer. The ambient room temperature is 22°C throughout the spectroscopic measurements.
Curve fitting of model spectra (Laux 2002) to experimental data is carried out. The second positive system spectrum of nanosecond-pulsed DBD and best fit simulated spectrum are given in Figure 3.17. Rotational and vibrational temperatures are measured to be $313.5 \pm 7.5$ K and $3360 \pm 50$ K, respectively, for the power range that has been used for the experiments explained below. These ‘temperatures’ describe the relative population of different vibrational and rotational levels of the $C^3$ state of molecular nitrogen. Rotational distribution of the $C^3$ state under the pulsed excitation at high overvoltage reflects the rotational population of the ground state and gives information regarding the temperature of the gas. Vibrational spectra of the second positive reflect the interplay between the population by electron impact from different lower states and depopulation due to the collisional quenching and radiative processes. Thus the vibrational distribution is an indicator of the electron’s temperature in the discharge region. Thus, it is demonstrated that the nanosecond-pulsed DBD does not heat the gas but provides strong excitation of the gas due to the high energy of the electrons. Figure 3.18 shows nanosecond-pulsed DBD igniting on a finger without causing any damage or even discomfort.
Figure 3.17: Spectra of nanosecond-pulsed DBD for spectroscopic temperature measurement. Rotational temperature: $313.5 \pm 7.5$ K and vibrational temperature: $3360 \pm 50$ K. (Model spectrum: Laux 2002, Staack et al. 2006)

Figure 3.18: Nanosecond-pulsed DBD igniting on finger (exposure time: 1 s)
3.3.2 Surface temperature

In addition to the spectroscopic temperature measurements, surface temperature on the grounded electrode is measured in the presence of the discharge using a reversible liquid crystal temperature indicator (model 4002B, Accuracy: ± 1°C, LCR Hallcrest L.L.C., IL). A sheet of the temperature indicator is placed over the grounded copper electrode and acts as the secondary electrode in the discharge (Goree et al. 2006). The room temperature is 22°C during the surface temperature measurements. Surface temperature due to plasma is measured at 25°C, while the temperature of the ground electrode surface without the discharge is also measured to be 22°C.

3.4 Summary of Key Results and Conclusions

Several characterizations have been conducted to explore the feasibility of using nanosecond-pulsed DBD for living tissue treatment. Electrical measurements for average power are verified with a custom-made calorimeter.

Two conventional DBD systems have been analyzed spectroscopically and single filament measurements show that there is no significant change in gas temperature along the channel.

Volume discharge measurements demonstrate that microdischarges within the volume for different types of discharges have the same gas temperature for the same power. A power-temperature relation is found to be somewhat linear and largely independent of voltage waveform shape.
Nanosecond-pulsed DBD and other DBD systems are assessed visually for uniformity analysis. Side view long exposure images of the nanosecond-pulsed DBD show that it is “filament-free” and much more uniform than the conventional DBDs. Some other qualitative experiments with photo films verify that nanosecond-pulsed DBD is completely uniform.

Also it is found that with spherical electrode nanosecond-pulsed DBD can ignite and be sustained over a wide range of inter-electrode gap.

Optical emission spectroscopy measurements reveal that nanosecond-pulsed DBD is almost at room temperature with 313.5 ± 7.5 K and 3360 ± 50 K for rotational and vibrational temperatures, respectively. Surface temperature increases of 3°C are observed after 5 minutes of plasma exposure with typical power density.
4.1 Qualitative Demonstration of Sterilization

Nanosecond-pulsed DBD has been tested for demonstration of sterilization by treating bacteria culture on agar. Skin flora bacteria, a mix of *Staphylococcus*, *Streptococcus*, and yeast, are transferred onto a blood agar plate (Trypticase Soy Agar with 5% Sheep Blood; Cardinal Health, Dublin, OH) for a sterilization demonstration (Fridman et al. 2006). Figure 4.1 shows the image of an agar surface covered with skin flora (dark red area covering most of the surface) being sterilized (light red area) with nanosecond-pulsed DBD treatment for 15 s. This result does show both the sterilization ability of the discharge as well as its efficiency. Treatment with power as low as a few tens of mW for 15 s, with an average power density of approximately 1 mW/mm² (discharge diameter equal to electrode diameter) can sterilize. This power density is one order of magnitude lower than typical conventional DBD power densities. For the same duration, complete sterilization can be attained with nanosecond-pulsed DBD at a significantly lower power density.

4.2 Qualitative Comparison of Conventional and Nanosecond-pulsed Dielectric Barrier Discharge on Topographically Non-uniform Surfaces

In addition to a typical sterilization experiment, effectiveness of the DBD excited by nanosecond rise and fall time voltage pulses has been tested for inactivation of the bacteria located within indentations on surfaces (Ayan et al.
It is vital for the discharge to produce uniform plasma independent of the uniformity of the bacteria covering surface that serves as one of the DBD electrodes, so the discharge can sterilize irregular surfaces completely.

Figure 4.1: Agar with skin flora treated by nanosecond-pulsed DBD ($V_{\text{max}}$: 20 kV, Repetition rate: 190 Hz)

The agar used for bacteria culture in this study is made out of a broth (Difco Brain Heart Infusion Agar powder, Fisher Scientific, PA) mixture that solidifies shortly after it is poured in the petri dish. In general, two different sorts of agar plates are fashioned and used for topographically non-uniform (uneven) surface sterilization experiments. The first type is characterized by a flat planar surface which is used in
studies of non-uniform surfaces by placing meshes over them, as well as for control samples.

The second type of agar plates which are specifically prepared to have non-uniform surfaces (with ridges and indentations) on the agar to mimic the real case for living skin tissue. The agar ridges are molded by placing a polymer form at the bottom of the dish. Then, agar is poured on top of the mold and left to dry. After the agar is solidified, it is removed and inverted and placed into the new (final) dish. Different steps of patterned agar preparation are presented in Figure 4.2.

Figure 4.2: Patterned agar preparation
*Escherichia coli* bacteria (*E. coli* K12 strain, Ward's Natural Science, Rochester, NY) are plated onto the surface of agar with concentrations of up to $10^8 – 10^9$ CFUs/ml (colony forming units/ml). The suspension volume (water and bacteria) is enough to completely cover the surface of the agar. Concentrations were measured and calculated on flat agar plates with a dilution assay which is a common technique in microbiology (Singleton and Sainsbury 2002, Silverthorn et al. 2004). The dilution assay protocol is presented in Figure 4.3.

---

**Figure 4.3: Protocol for dilution assay**

1. **Thaw the bacteria stock (original concentration)**
   
   $[\text{Bacteria}] = a \times 10^b$

2. **Prepare serial dilutions from original concentration (1:10)**
   
   $a \times 10^{(b-1)}, a \times 10^{(b-2)}, a \times 10^{(b-3)}, a \times 10^{(b-4)}, \ldots$

3. **Seed each dilutions on agar plates ($n=4$)**
   
   *Incubate 12 hours*

4. **Identify the countable plates of serial of diluted concentration**
   
   • Count Colony Forming Unit (CFU) on the plates
   • Determine average CFU count

5. **Determine the original concentration**

   EX: if $i^{th}$ dilution has $n$ CFU then, original concentration $= n \times 10^i$
Before plasma treatment, bacteria seeded plates are left to dry in the laminar flow hood (Labconco, Class-II, Kansas City, MO) for 1 hr along with the control plates. After plasma treatment, treated and untreated (control) samples are cultured for 12 hrs in the incubator (VWR, Sheldon Manuf. Inc., Model#1545, OR) at 37 °C. Sterilization results are evaluated by visually examining the resulting colonies after incubation. Samples are also observed up to a 48 hrs incubation period in which delayed recovery is not found in the plasma treated regions.

The sterilization effectiveness of nanosecond-pulsed DBD on non-uniform surface is proved by comparing with conventional DBD. For nanosecond-pulsed DBD applying 20 ns long, 16 kV pulses with approximately 3kV/ns rise/fall times and for microsecond-pulsed DBD applying 1.5 μs long, 10 kV pulses with 20V/ns rise time both are produced at 120 Hz to maintain the comparable power. In all experiments, power density for the active area of the electrode is maintained at 100 mW/cm² (approximately 75 mW for 1 cm electrode diameter). Parameters for both systems are summarized in Table 4.1.

Table 4.1: Summary of nanosecond- and microsecond-pulsed DBD parameters

<table>
<thead>
<tr>
<th></th>
<th>Pulse Length [ns]</th>
<th>Voltage [kV]</th>
<th>Front [V/ns]</th>
<th>Frequency [Hz]</th>
<th>Power [mW/cm²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns-DBD</td>
<td>20</td>
<td>16</td>
<td>3000</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>μs-DBD</td>
<td>1500</td>
<td>10</td>
<td>20</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>
The first part of the non-uniform surface sterilization experiments is done by placing a stainless steel mesh over the flat surface agar as shown in Figure 4.4. In this case, a metal mesh simulates the effects of ridges and indentations; i.e. surface non-uniformities. The opening between the wires of the mesh is 1.5 mm, the wires are 0.25 mm in diameter, and total thickness of the mesh is 0.5 mm. The gap between the bottom of the high voltage electrode and the top of the mesh is 1 mm.

A typical sterilization result comparing both nanosecond-pulsed and microsecond-pulsed DBD systems is shown in Figure 4.5. Light color areas in this figure are the locations on the agar that are completely sterilized by plasma treatment and the rest of the agar with a darker color is \textit{E.coli} covered. On the left-hand side of Figure 4.5, sterilization is achieved at the ‘valleys’ with a slightly larger diameter than the active area of the electrode in 30 s. In contrast, conventional DBD affects a significantly smaller area (on the right-hand side of Figure 4.5). The concentration of sterilization effect to the middle part for microsecond-pulsed DBD can be due to possible occurrence of surface discharges following the extinction of the microdischarges. These preliminary results from the sterilization experiments clearly indicate that the nanosecond-pulsed discharge is much more effective and faster than the conventional microsecond DBD in killing \textit{E.coli} bacteria.
Figure 4.4: Illustration of mesh on top of the agar to mimic the indentations
(10 mm diameter electrode with 0.66 mm quartz)
In the second group of experiments, the sterilization effect of novel DBD system has been tested and also compared with that of a conventional DBD system. For this experiment, the agar is patterned with a 3-level recess with 2 mm width and 0.33 mm depth at every stage. Dimensions are shown on a schematic in Figure 4.6.
It should be also noted that prior to these experiments, uniform distribution of bacteria on the patterned surfaces are verified by seeding low enough concentrations ($10^3 – 10^4$ CFU/ml) to get a countable number of colonies on the surface (Figure 4.7).

Figure 4.6: Schematic of 3-level recess patterned agar

Figure 4.7: Uniform distribution of bacteria on patterned surface
A two-piece spacer with 0.5 mm thickness is placed between the high voltage electrode and the top surface of the agar. In Figure 4.8, the side view of electrode on patterned agar surface (Figure 4.8a), and the typical appearance of a conventional microsecond-pulsed DBD (Figure 4.8b) and a nanosecond-pulsed DBD (Figure 4.8c) are given.

Figure 4.8: Side view of (a) high voltage electrode in light room with no plasma, (b) conventional microsecond DBD and (c) nanosecond-pulsed DBD on the patterned agar surface (three steps with 0.33 mm height and 2 mm width). Pictures of discharges have been taken in a dark room with 0.5 s exposure time at 120 Hz repetition (for both systems)
As it can be easily seen from Figure 4.8b, the conventional microsecond-pulsed DBD produces microdischarges (filaments) that are often terminated on the top of asperities and irregularities (mostly at smaller gaps or corner of the surface features) whereas the nanosecond-pulsed DBD exhibits a rather diffuse structure in Figure 4.8c. Although the latter is also slightly more intense on the corners, it is fully covering the entire gap.

The exclusive features of nanosecond-pulsed DBD can also be easily seen in Figure 4.9 with the distinction in sterilization performance. This figure shows a top view of a treated 3-level recess patterned agar that is given in Figure 4.8 previously. In the case of nanosecond-pulsed DBD, all three steps are uniformly treated and sterilized (Figure 4.9a) whereas in the case of conventional microsecond-pulsed DBD, only partial sterilization is achieved in the vicinity of the edge of the first step where the discharge gap is minimal (Figure 4.9b).
Figure 4.9: 3-level recessed agar surface treated with (a) nanosecond-pulsed DBD and (b) microsecond-pulsed DBD. Width of each step (distance between the horizontal lines) is approximately 2 mm. For both plates treatment time: 30 s., concentration: $10^8$ CFU/ml
4.3 Quantitative Comparison of Conventional and Nanosecond-pulsed Dielectric Barrier Discharge on Topographically Non-uniform Surfaces

Sterilization efficacies of nanosecond-pulsed DBD and conventional DBD have also been compared quantitatively. *E.coli* suspensions with different concentrations are seeded on the patterned agar plates. Four different patterns are used to assess the sterilization as a function of surface pattern depth. The preparation method of patterned agar is described in the previous chapter. Drawings of the patterns with dimensions are given in Figure 4.10.

![Figure 4.10: Four different agar patterns with different depth (dimensions in mm)](image)

Figure 4.11 shows the typical difference between uniform nanosecond-pulsed DBD and conventional DBD. Arrows on each pictures show the lowest level of the pattern (Type 2). When nanosecond-pulsed DBD is applied, all surfaces at different levels are sterilized. In contrast, microsecond DBD sterilizes only the highest level
while it can partially affect the bacteria at the bottom of the valleys and cannot fully sterilize.

Figure 4.11: Sterilization effect of two systems on valleys (dashed circles indicate the active area of the electrode)

In this experiment, an electrode with 10 mm diameter active area is used. After plasma treatment, bacteria quantification is done by counting the colony forming units on the treated surface within the 10 mm diameter circle. Initial seeding concentrations are determined by a standard dilution assay method (Figure 4.3). Following the plasma treatment, agar plates are placed into the incubator for 12 hrs.
Bacteria colonies are then counted (after-treatment). At some higher seeding concentrations, plasma treatment resulted in partial sterilization wherein it is not possible to quantify the CFUs after treatment. In those cases, quantification is carried on with the next lower dilution. In every case, the highest countable seeding concentration is taken into account and the CFU log reduction is calculated from the difference between initial seeding and post-treatment counts. The protocol for determining the log reduction is given in Figure 4.12.
Figure 4.12: Flow chart for determining the log reduction in bacteria population after plasma treatment on patterned agar

1. **Prepare serial dilutions with known concentration**
2. Seed on **patterned** agar plates (concentration = \( n_{\text{initial}} \))
   - Dry plates for 1 hour
   - Conduct the plasma treatment
3. Incubate 12 hours
4. Identify the countable plates
   - **If plate is NOT countable**
   - **If plate is countable**
5. Divide the treated into four sub-areas
   - Count Colony Forming Unit (CFU) on the treated area
6. Determine average surviving \( n_{\text{final}} \) CFU count
7. Determine the reduction in bacteria
   - Ex: log reduction = \( \log (n_{\text{initial}} - n_{\text{final}}) \)
Results for log reduction of bacteria colonies after a 30 s treatment are presented in Figure 4.13. As expected, data shows that the sterilization effect diminishes as the pattern depth increases. In addition, in most cases nanosecond-pulsed DBD is found to be inactivating more than 1 order of magnitude more bacteria than with conventional DBD. Also, the *E.coli* CFU log reduction on the Type 2 pattern as a function of plasma treatment time is presented in Figure 4.14.

![Figure 4.13: CFU Log reductions on different patterns with 30 s plasma treatment](image)

In the literature, there exists a widely used kinetics measurement parameter to characterize the sterilization efficacy in a broader perspective. The parameter is often referred to as a D-value and is essentially equal to the time required to reduce the
original concentration by 1 log (Laroussi 2005). In Figure 4.14, the shortest treatment time is 5 s reductions in bacteria population are already more than 1 log for both DBD systems. Therefore, these results set the D-value at a few seconds which is a relatively shorter time compared to the other studies (Laroussi et al. 1999 and Herrmann et al. 1999).

![Figure 4.14: CFU log reduction on Type 2 pattern (max depth: 0.78 mm) as a function of plasma treatment time. Statistics are collected by dividing the treated area into four equal sections (quarter circle area) and performing a count in each area](image)

4.4 On the Mechanism of Sterilization

Plasma is a chemically active medium including thermal, electric, radiative energy forms and they interact with background gas and substances being exposed.
There are several factors in the plasma that can cause inactivation of bacteria (Stoffels 2007, Gaunt et al. 2006, Lerouge et al. 2000); namely heat, UV radiation, ozone, and charged particles (electrons and positive and negative ions).

In order to help understand the basic mechanism of sterilization with nanosecond-pulsed DBD, some experiments are designed and implemented. In the first experiment, plasma treatment applied on the bacteria seeded surface in the presence of air flow in the single recess (channel) agar. A schematic of the single recess pattern is shown in Figure 4.15. A diagram of the system is presented in Figure 4.16. Air was supplied from a tank and flow rate regulated with a digital flow controller (Omega Engineering, Inc., Model#: FMA-2620A & FMA-2607A). Additionally, a top view of the single step recessed agar that is used for this experiment is given in Figure 4.17. The depth and the width of the recess are 1 mm and 10 mm, respectively. In the first case (Figure 4.17a), the surface of the agar plates are treated for 30 s in a regular fashion. In the second and third cases (Figure 4.17b and Figure 4.17c), air is blown through the gap between the quartz and the bottom of the recess with 0.3 and 3 SLPM flow, yielding 0.5 m/s and 5 m/s air velocity in this cross section, respectively.
In terms of sterilization, the results are similar to each other except for slight size differences in the size of the sterilized area. However there is no qualitative difference between the air flow and no flow cases, i.e. there is no partial sterilization due to the plasma afterglow downstream of the flow (to the right hand side of the figure). The lack of major differences in the figures indicates that the sterilization
occurs through direct contact with the plasma. The sterilizing agents cannot be blown or translated away from the electrode location with air flow.

Additionally, another experiment is conducted to assess the effect of UV in nanosecond-pulsed DBD. A VUV (vacuum UV) grade MgF\textsubscript{2} window (Crystran Limited, UK) has been placed on the \textit{E.coli} seeded flat agar surface. The area covered by the MgF\textsubscript{2} window receives only UV and the rest of the treated area is exposed to whole plasma products. As presented in Figure 4.18, the area covered (exposed to only UV) is not sterilized whereas the rest of the treated area is sterilized. This result suggests that UV, by itself, is not the major bactericidal factor in the plasma.
Figure 4.17: 30 s treatment with nanosecond-pulsed DBD (a) without air flow, (b) with 0.3 SLPM air flow, (c) with 3 SLPM air flow on single level recessed agar. Arrows indicate the direction of the flow. (Outer and inner circles represent quartz and electrode active area, respectively)
Taking the last two experiments together in to consideration: 1) the afterglow of the plasma contains only neutral atoms, radicals and molecules, some of which are in an excited state (Deng et al. 2006 and Moisan et al. 2002), and most of the charged particles remain in the plasma region (lifetime of charged particles at atmospheric pressures is very short) and 2) UV is not playing a major role in those experiments. The conclusion drawn from these results is that sterilization by nanosecond-pulsed DBD is mostly due to the charged particles.
4.5 Summary of Key Results and Conclusions

Sterilization capability of nanosecond-pulsed DBD has been first demonstrated on plain agar with skin flora. Then sterilization effectiveness of nanosecond-pulsed DBD on topographically non-uniform surfaces has been tested and proven with a series of experiments.

Sterilization efficacy of nanosecond-pulsed DBD and conventional DBD have also been compared quantitatively where nanosecond-pulsed DBD is found to be inactivating 1 to 1.5 orders of magnitude more bacteria than with conventional DBD.

As it is shown in the previous chapter, the temperature of nanosecond-pulsed DBD is measured to be at room temperature level. Therefore, ‘temperature of the plasma’ cannot be the factor that causes sterilization. Additionally, experiments in this chapter on mechanism of the sterilization rules out ozone and UV, and concluded that charged particles are the most important factors in the plasma.
CHAPTER 5: SUMMARY AND CONCLUSIONS

5.1 Summary of the Research and Conclusions

A novel uniform non-thermal plasma system was developed in this work for live tissue sterilization and other medical applications. Experiments on non-uniform surfaces using meshes and patterned agar revealed that the nanosecond-pulsed DBD can penetrate valleys (indentations between ridges), whereas the microsecond-pulsed DBD fails to do so as well. The nanosecond-pulsed DBD with short rise time and high overvoltage is insensitive to the surface non-uniformities of the agar and does not require uniform discharge gaps as it can ignite and be sustained over a wide gap range. Thus, DBDs with nanosecond rise times are potentially more convenient for in vivo and real sterilization cases with non-uniform profile surfaces.

It should be emphasized that the technique employed to generate a few tens of nanosecond long pulses is relatively simple and inexpensive. This method may be easily used for a variety of applications.

The uniformity of this nanosecond-pulsed DBD is proven qualitatively with a new technique for such high frequency discharge, using high speed photosensitive film exposure. Lichtenberg figures of nanosecond-pulsed DBD show clearly that few tens of nanosecond pulse duration avoids streamer formation and generates uniform discharge in atmospheric pressure air.

The ability of the nanosecond-pulsed discharge to sterilize has been demonstrated, quantified, and compared with that of a conventional discharge. This novel DBD is proven to be much more effective in killing bacteria on surfaces than with conventional DBD. Experiments that have been conducted in order to
understand the basic mechanism of sterilization by comparing direct and indirect plasma effects indicate that charges (electrons and ions) play a major role in sterilization with nanosecond-pulsed DBD.

5.2 Research Contributions

The main research contribution in this work has been developing a novel dielectric barrier discharge system that enables uniform plasma treatment for living tissue sterilization in atmospheric pressure air. This system can also be used in other biological studies and non-uniform surface treatment applications. The thesis research and activities will help to develop knowledge and novel solutions in the Plasma Medicine field.

The specific contributions of this research are summarized as follows.

- Development of a uniform dielectric barrier discharge system with nanosecond pulse excitation for the first time in air and at atmospheric pressure.

- Demonstration of uniform plasma on topographically non-uniform surfaces for the first time in air at atmospheric pressure.

- Demonstration and quantification of sterilization on non-uniform surfaces with nanosecond-pulsed DBD treatment. The nanosecond plasma works significantly better than conventional DBD on non-uniform surfaces. This makes it a promising tool for medicine.
• Confirmation that the mechanism of sterilization in nanosecond DBD is associated with charges.

• Elimination of microdischarges, therefore offering a safer treatment without possible local heating.


48. Levatter, J.I., and Lin, S.-C., 1980. Necessary conditions for the homogeneous formation of pulsed avalanche discharges at high gas pressures. J. Appl. Phys. 51(1)


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