Award Number: W81XWH-10-1-0923

TITLE: Lightweight Portable Plasma Medical Device – Plasma Engineering Research Laboratory

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CONTRACTING ORGANIZATION:
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REPORT DATE: October 2013

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:
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### REPORT DOCUMENTATION PAGE

#### Title: Lightweight Portable Plasma Medical Device

**Plasma Engineering Research Laboratory**

**Authors:** Dr. Magesh Thiyagarajan

**Dates Covered:** 27 September 2012 - 26 September 2013

#### Abstract

We have established a state-of-the-art plasma research facility which is capable of conducting various interdisciplinary research projects. We have completed the setup of DC RBD plasma system, 13.56 MHz RF DBD plasma system, laser induced breakdown plasma system, plasma shadowgraphy system and optical emission spectroscopy diagnostics, two color laser interferometry diagnostics. We have completed the design and construction of the portable plasma source. We have made significant progress in testing and characterization and biological testing of the portable plasma source and the research will be continued to make progress.

#### Subject Terms

Plasma, laser, shadowgraphy, bacteria, cancer

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**Security Classification:**

- b. Abstract: U
- c. This Page: U
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INTRODUCTION
The project entitled - Lightweight Portable Plasma Medical Device – Plasma Engineering Research Laboratory has made a momentous advancement on meeting the proposed milestones. The project has two objectives, in which the objective 1 is to develop a plasma research facility and establish a range of experimental plasma systems and diagnostics. The tasks under objective 1 were already completed earlier. The project’s second objective is to develop a prototype of a portable plasma source for biomedical applications such as sterilization, infection treatment and cancer treatment. We have developed a test portable plasma source and tested preliminarily on a range of bacteria and cancer cells and promising results were obtained earlier. We have also designed and constructed another portable non-thermal atmospheric pressure plasma jet based on a dielectric barrier discharge configuration. The plasma and biological testing and characterization are in progress. The PI has established the Plasma Engineering Research Lab (PERL) with a state-of-the-art facilities and equipment obtained through this grant funding as well as several donations from the university and the community. The PI has mentored over 20 undergraduate students and 2 graduate students to perform various research projects. Several of these students have presented their work at various conferences and symposiums and few of those presentations has received best paper awards. The PI has mentored a visiting scientist and 4 postdoctoral research associates. The PI and the research team have published 10 journal articles and currently preparing 5 journal manuscripts which will be submitted in the upcoming weeks. The PERL has received a great visibility in the university campus as well as in the state. A no cost extension was requested and approved to continue and complete the remaining tasks of second objective.
Table 1. The Project Objectives and Tasks

<table>
<thead>
<tr>
<th>Task</th>
<th>Proposed Milestones</th>
<th>Base Line Plan Date</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Setup a direct current - atmospheric - resistive barrier cold plasma system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>2</td>
<td>Setup a 13.56 MHz radio frequency dielectric barrier plasma system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>3</td>
<td>Setup a 900 MHz/2.45 GHz wave plasma system</td>
<td>26 OCT 2011</td>
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</tr>
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<td>4</td>
<td>Setup a laser induced breakdown plasma experimental system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>5</td>
<td>Implement plasma shadowgraphy diagnostics Setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>6</td>
<td>Implement a two color laser interferomtery diagnostics setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>7</td>
<td>Implement a optical emission spectroscopy diagnostics setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
</tbody>
</table>

Objective 1: Establish Plasma Engineering Research Lab

Objective 2: Develop Portable Plasma Source

<table>
<thead>
<tr>
<th>Task</th>
<th>Proposed Milestones</th>
<th>Base Line Plan Date</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Design phase: Design an optimized portable plasma source system</td>
<td>26 OCT 2012</td>
<td>Completed</td>
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<tr>
<td>9</td>
<td>Construction phase: Construct the portable plasma source based on the design analysis and utilizing the existing resources and knowledge gained from objective 1.</td>
<td>26 OCT 2012</td>
<td>Completed</td>
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<tr>
<td>10</td>
<td>Testing and characterization phase: Portable plasma source will be tested and characterized for its operating parameters and plasma parameters.</td>
<td>26 OCT 2014</td>
<td>In Progress</td>
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<td>11</td>
<td>Biological testing: In-vitro biological testing.</td>
<td>26 OCT 2014</td>
<td>In Progress</td>
</tr>
</tbody>
</table>
Progress of Tasks 10 and 11: Testing and characterization phase: Portable plasma source will be tested and characterized for its operating parameters and plasma parameters, and (Task 11) Biological testing: In-vitro biological testing.

I. Inactivation of bacteria in aqueous media in direct and indirect treatment modes

An atmospheric pressure resistive barrier air plasma jet is designed to inactivate bacteria in aqueous media in direct and indirect exposure modes of treatment. The resistive barrier plasma jet is designed to operate at both DC and standard 50-60 Hz low frequency AC power input and the ambient air at 50% humidity level was used as the operating gas. The voltage-current characteristics of the plasma jet were analyzed and the plasma power was measured to be 26 Watt. The plasma jet rotational temperatures ($T_{rot}$) are obtained from the optical emission spectra, from the N2C-B(2+) transitions by matching the experimental spectrum results with the SPECAIR simulation spectra. The reactive oxygen and nitrogen species were measured using optical emission spectroscopy and gas analyzers, for direct and indirect treatment modes. The nitric oxides (NO) were observed to be the predominant long lived reactive nitrogen species produced by the plasma. Three different bacteria including Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative) and Neisseria meningitidis (Gram-negative) were suspended in an aqueous media and treated by the resistive barrier air plasma jet in direct and indirect exposure modes. The results show that a near complete bacterial inactivation was achieved within 120 second for both direct and indirect plasma treatment of S. aureus and E. coli bacteria. Conversely, a partial inactivation of N. meningitidis was observed by 120 second direct plasma exposure and insignificant inactivation was observed for the indirect plasma
exposure treatment. Plasma induced shifts in N. meningitidis gene expression was analyzed using pilC gene expression as a representative gene and the results showed a reduction in the expression of the pilC gene compared to untreated samples suggesting that the observed protection against NO may be regulated by other genes.

A. Experiment

FIG 1. The schematic set up of: (a) the RB air plasma jet, (b) direct and indirect plasma exposure.
The schematic representation of the atmospheric pressure resistive barrier (RB) air plasma jet is shown in Fig. 1(a), together with an image of the air plasma jet. Basically, the RB plasma jet consists of central deionized water cooled cylindrical electrode surrounded by deionized water cooled high density alumina ceramic resistive coating. The central electrode which is approximately 1 cm diameter and 10 cm long is surrounded by a double layer hollow ground electrode separated by an electrode gap space of 2 mm. Discharge streamers are formed between the high-voltage resistive electrode and the ground electrode. With applying enough voltage, the air passing through the RB plasma jet gets ionized and exits through a 2 mm pinhole type opening at the tip of the plasma source generating a stable plasma jet and a continuous flow of active species such as ROS and RNS. The discharge streamers formed in the cylindrical hollow electrode configuration are dynamic with respect to its location between the electrodes, which also assists in preventing localized heating and arc formation. The presented RB plasma jet is developed based on the principles of large volume non-thermal resistive barrier discharge (RBD)\textsuperscript{23-25} and can be used in both direct and indirect plasma exposure modes as shown in Fig. 1(b). Direct exposure of plasma involves, exposure of plasma directly on to a target treatment surface whereas the indirect plasma exposure involves, exposure of only the ROS and RNS generated by the plasma instead of the plasma itself, thus eliminating the effect of any possible UV radiation produced by plasma. For the indirect exposure method the concentrations of the nitric oxides are preserved by reducing the plasma temperature rapidly through a separate external small efficient cooling unit.

The RB plasma jet operates in both DC and low frequency AC, such as the standard operating voltages 120 V/60 Hz and 230 V/50 Hz. The power supply is stepped-up with using a
transformer (6 kV, 30 mA (max)). In addition, the power supply unit also houses transistor-
transistor logic (TTL) and relay controls to select between AC and DC, and power levels and flow
controls. The RB plasma jet uses atmospheric pressure air as the operating gas. A 12 V DC air
compressor is used to force the air through the electrode gap space of the RB plasma jet. Thus,
the plume length, plasma power and ROS including nitric oxides at the tip of the handheld
plasma jet tip are controlled and maintained. The power supply unit also contains a mini 12 V
DC water pump (Geo-Inline) for cooling the electrodes in the handheld plasma probe unit as
well as the optional external cooling unit. The entire RB plasma source is very compact,
portable and light weight. It is constructed to fit within 12 × 10 × 10 inch3 portable metallic case
and the entire power supply and RBS plasma source weighs approx. 20 lbs.

B. Diagnostics

The voltage applied to the high-voltage electrode is measured using a high voltage
probe (Tektronix P6015A) and the discharge current is measured using a current probe
(Tektronix TCP202). The voltage–current waveforms are recorded using a 2 GHz digital
oscilloscope (Tektronix TDS3034C). Accurate measurement of gas temperatures of the RB
plasma jet is a significant experimental challenge. Due to electrical interference using
conventional thermocouple inside the discharge can lead to inaccurate measurements.
Therefore, the optical emission spectroscopy26,27 was employed to measure the rotational gas
temperature \(T_{rot}\) of the plasma jet using a high resolution narrow band (0.004 nm resolution)
monochromator (Acton Research, Model: SP-2750) coupled with a fast gating Andor iStar ICCD
(ANDOR, DH 734). This system has a near-Lorentzian slit function with a half-maximum width of
0.2 nm when the grating density was set to 1200 lines/mm. A high-temperature ceramic fiber-
insulated-wire thermocouple probe capable of measuring temperatures up to 1400 °C was used to measure the temperature of the downstream jet when plasma emission ends after approximately 2.5 cm. The parts per million (ppm) concentration of the ROS and RNS including nitric oxides at different spatial distances from the tip of the plasma jet was measured using two gas sensors namely NOXCANg and Testo 350 M/XL gas analyzers.

C. Bacterial sample preparation and plasma treatment procedure

Bacterial samples of S. aureus (ATCC 259231), E. coli (ATCC 117751), and N. meningitidis serogroup C (ATCC 700532) were obtained from ATCC (Manasses, VA). Bacterial cultures of S. aureus (ATCC 259231) and E. coli (ATCC 117751) were grown overnight on Tryptic Soy agar (BD, Franklin Lakes, NJ) plate overnight at 37°C in 5% CO2. N. meningitidis was grown overnight on GC medium (BD, Franklin Lakes, NJ) plate supplemented with Thayer-Martin supplement I and supplement II (Sigma-Aldrich, St. Louis MO) overnight at 37°C in 5% CO2. Bacteria was collected from overnight cultures using an inoculating loop and placed into 20 ml of Mueller Hinton broth (MHB) (BD, Franklin Lakes, NJ). Bacterial cultures in suspension were adjusted with sterile MHB to an OD at 595 nm (OD595) = 0.2 (Spectronic Genesys 5 Spectrophotometer, Madison, WI) and allowed to incubate (37°C, 5% CO2) till reaching an OD595 = 0.4. N. meningitidis suspensions included agitation by a plate mixer (~100 rpm). After reaching the selected density, 13x100 glass culture tube were filled with 5ml of bacterial suspension and treated to regime of either 0, 30, 60, or 120 seconds of maximum power and flow rate of RB plasma jet in direct or indirect treatment modes by fixing the plasma jet exit above the surface of the media at a distance of approximately 5cm. Control suspension samples pH was monitored before and after 30, 60, and
120 seconds of direct and indirect RB plasma jet treatment using S20 SevenEasy pH meter (Mettler-Toledo, Columbus, OH). Bacterial density was monitored over a period of 24 hours and OD595 readings were recorded. The efficacy of the inactivation was determined by making serial dilutions of untreated and treated samples and plating them on agar plates. Three independent experiments were performed and data were collected for the analysis of post-treatment bacterial growth means over time.

**D. Messenger RNA analysis by quantitative real time-PCR**

Real time polymerase chain reaction (RT-PCR) analysis can provide information about the presence and quantity of a specific gene within a cell. Cells such as bacteria can recognize external stimuli such as NO produced by the RB plasma jet and react accordingly to alter its metabolic processes through gene regulation to respond to the stimuli. Cultures of N. meningitidis were prepared as described above and the untreated and 60 second direct plasma treated samples were incubated at 37°C, 5% CO2. Bacterial RNA was harvested from each sample culture at 2 hour post treatment using a Qiagen RNeasy minikit. Purified mRNA was quantitated via Nanodrop with appropriate standards. cDNA synthesis and quantitative PCR were done using the Superscript III Platinum SYBR Green two-step qRT-PCR kit (Invitrogen) on an Cepheid Smart Cycler. The relative expression of pilC was normalized to that of 16S gene, which served as an internal control. The qRT-PCR experiments were done in triplicate with independently isolated RNA samples.
E. Electrical characterization of the RB plasma jet

The electrical properties of the discharge are obtained using voltage-current characterization. The discharge is initiated by applying a sufficient voltage between electrodes at which the air between electrodes breaks down and results in a self-sustained plasma jet maintained by applied voltage of the power supply. Figure 2 shows the voltage-current waveform of the RB plasma jet. The positive and negative half periods of the applied voltage are not symmetrical due to the existence of only one resistance between the electrodes. It can be seen from Fig. 2 that breakdown of air in atmospheric pressure resistive barrier plasma jet resulting in several current filaments called micro-discharges which are randomly spread in time and space. The
maximum voltage and current values are approximately 20 kV and 3 mA on the positive half period of the applied voltage, correspondingly. Using the voltage–current waveforms, the average power dissipated in the discharge is calculated by integrating the product of the discharge voltage and current over one cycle; according to the following equation (\(T = \) period of the discharge).

\[
W = \frac{1}{T} \int_t^{t+T} I(t)V(t)dt
\]  

(1)

and the average plasma power is measured to be 26 W.

F. Optical emission spectroscopy

The rotational temperature (\(T_{rot}\)) of the RB plasma jet was measured using the optical emission spectroscopy. The rotational temperature and plasma gas temperature can be considered equivalent if there is a small energy gap between the rotational levels compared with that of the vibrational or electronic energy levels and in the presence of thermal equilibrium between the translational and rotational degrees of freedom.29 A local thermodynamic equilibrium (LTE) condition has been assumed and emission spectra of the plasma jet were used for gas temperature determination.

The commonly used technique to estimate rotational temperature is based on finding the best fit between experimental and simulated emission spectra of the second positive system of N2 and considering the plasma gas temperature equivalent with rotational temperature of N2 (Trot).
FIG. 3. Experimental and simulated air plasma emission spectra of N2 SPS for fixed power of 26 watt without cooling unit and at distances: (a) 0.2 cm, (b) 0.8 cm, (c) 1.5 cm, and (d) 2 cm after the nozzle.

In this section, the spectral lines of the nitrogen second positive system (SPS), \((\text{N}_2(C-B)\ (C\ 3\Π\ B\ 3\Π)\ (2+))\), is used to determine the rotational temperature distribution of nitrogen SPS with SPECAIR software. SPECAIR is computer simulation software developed by Laux et. al. on the basis of the Non-Equilibrium Air Radiation code (NEQAIR) by Park. The rotational temperature distribution of the N2 SPS, in the afterglow of the RB plasma jet was determined.
on the basis of simulation of experimental spectra in the range of region 364-383 nm. Fitting of the spectra is carried out with one rotational temperature, and taking into account the instrumental function of the spectrometer. The experimental spectra were compared to the simulated spectra with various rotational temperatures. The rotational temperature for the best fitting between simulated and experimental spectra was determined as the gas temperature. The experimental and simulated spectra of N2 second positive system are presented in Fig. 3.

The results indicate the rotational temperature for air discharge at a distance of 0.2 cm from the nozzle is 3050 °C. The \( T_{\text{rot}} \) drops to 2100 °C at 0.8 cm and continue to decrease to 1500 °C at 1.5 cm and to 1050 °C at 2 cm away from the nozzle. The rotational temperature of nitrogen is significantly smaller at 2 cm away from the nozzle than the one of 0.2 cm from the jet exit. This clearly indicates that the nozzle acts as an efficient heat source for the plasma jet. Nitrogen line emissions drop significantly after 2 cm (plasma plume length is 2.5 cm) from the tip of electrode along the axis and beyond which the gas temperature estimation by OES based rotational temperature estimation is challenging. However, the long life active species such as the ROS and RNS produced by the plasma jet can still propagate further in to the surrounding air (NOx concentration was measured to be ~900 ppm at 10 cm away from nozzle) and are important active species particularly for the biomedical applications. Therefore the downstream gas temperatures beyond the plasma emission, which is from 2 cm of the plasma jet exit onwards, were measured by using a high-temperature ceramic fiber-insulated-wire thermocouple probe.
The Fig. 4 shows the axial temperature measurements of the RB plasma jet before attaching the cooling unit. As seen in Fig. 4, the gas temperatures decrease at a much slower rate after approximately 5 cm from the nozzle. The high gas temperatures obtained from N2 rotational temperature is due to excitation of N2 to high rotational/vibrational levels and the excited N2 species are generated in the plasma jet. However, plasma gas temperature drops
down very fast at the end of the jet’s plume. The temperature of the plasma plume at the exit reaches >2500 °C sufficient enough to generate nitric oxides. When the external cooling units were added the gas temperatures were brought close to room temperature at the tip of the handheld plasma source unit.

G. Measurements of long lived active plasma species

RB plasma jet produces wide range of active species such as radicals, electrons, atoms, ions, molecules, ROS and RNS etc. These plasma species contributes to the biological cell interactions and inactivation of the bacteria. The concentrations of the ROS and RNS produced by the plasma jet with and without the cooling unit are measured using gas analyzers. The majority of the short lived ions will recombine before it exits through the cooling unit and at this stage several long lived gas species and other parameters were monitored, including O2, O3, CO, COlow, NO/NO2, NOlow, NOX, CO2(Infrared), SO2, HC, H2S, temperature, pressure, flow, velocity, efficiency, mass, etc. Based on the results we observed that nitric oxides are the predominant long lives species produced by the RB plasma jet and some trace of O3. The nitric oxide formation is a reversible plasma chemical reaction and it can be expressed as

\[ N_2 + O_2 \leftrightarrow 2NO - \Delta E \]  

The decaying concentration of the nitric oxides from the plasma jet can be used as reference for the treatment distance between the biological cells and the plasma source tip. The background NOx concentration are measured using NOxCANg module at various timings and at standard laboratory conditions well before the plasma source was operated and it was measured to be less than 0.5 ppm.
The NOx concentration measurements are carried out at 20 data sets separated by 1 min with 1 second acquisition time at different distances. For consistency in the concentration measurements, three other sampling timings such as 10, 30 and 60 second are tested in addition to 1 second acquisition time. Figure 5 shows the NOx concentration at different distance with different acquisition times. As can be seen, the concentration of NOx species is consistent for various gas acquisition integration times at various distances from plasma jet’s nozzle.

FIG. 5. NOx concentration (ppm) at different distances from plasma jet’s nozzle without the cooling unit.
The NOx concentrations of the plasma jet with and without the cooling unit are measured at various axial distances from the tip and the results are shown in Fig. 6. The measurements show that the NOx concentration peaks at the electrode tip as expected and the NOx concentration was measured to be in the range of 500-660 ppm at 5 cm from the plasma jet exit without the cooling unit. The concentration drops to half of its initial value at ~20 cm and it continues to drop to ~100 ppm at 60 cm and at 100 cm distance from the tip the concentration was very low (<10 ppm). In addition, the Fig. 6 shows the NOx concentrations for...
plasma jet with cooling unit (circles) and it was observed that the NOx concentration after the cooling unit was higher (~950 ppm) compared to that of plasma jet without cooling unit (~615 ppm), this was due to the fact that plasma jet diffuses out after it exits from the nozzle whereas the probe diameter after the cooling unit is very small like a pinhole arrangement with 1 mm diameter opening leading to increased concentration.

NOx is composed of two components such as the mono-nitrogen oxides or nitric oxides (NO) and nitrogen dioxide (NO2). In order to measure the individual concentration levels of NO and NO2 a different diagnostic tool was realized using a gas analyzer (Testo 350 M/XL) capable of identifying and characterizing over several molecular gases including NO and NO2. Table 1 shows the concentrations of NO and NO2 produced by the plasma jet without the cooling unit and the Table 2 shows the concentrations of NO and NO2 produced by the plasma jet with external cooling unit. In both cases, the concentration of NO2 was much lesser compared to that of NO by an order of magnitude or higher.

Table 1. The direct exposure NO and NO2 (ppm) concentrations of the RB plasma jet at various axial distances d (cm) from the jet exit tip. (without the external cooling unit)

<table>
<thead>
<tr>
<th>d</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
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<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
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<tbody>
<tr>
<td>NO</td>
<td>1555.6</td>
<td>631.6</td>
<td>450.8</td>
<td>288</td>
<td>239.7</td>
<td>193</td>
<td>140.2</td>
<td>116</td>
<td>81</td>
<td>90.1</td>
<td>86</td>
<td>54.8</td>
<td>44.3</td>
<td>27.3</td>
<td>10</td>
<td>5.2</td>
</tr>
<tr>
<td>NO2</td>
<td>191.1</td>
<td>57.2</td>
<td>35</td>
<td>21.8</td>
<td>16.7</td>
<td>13</td>
<td>9.3</td>
<td>7.5</td>
<td>5.1</td>
<td>5.4</td>
<td>4.9</td>
<td>3.1</td>
<td>2.4</td>
<td>-</td>
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</tr>
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</table>

20
Table 2. The indirect exposure NO and NO2 (ppm) concentrations of the RB plasma jet at various axial distances d (cm) from the jet exit tip. (with the external cooling unit).

<table>
<thead>
<tr>
<th>d</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
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<tbody>
<tr>
<td>NO</td>
<td>2469.2</td>
<td>1110</td>
<td>651.6</td>
<td>389.3</td>
<td>341.6</td>
<td>200</td>
<td>180</td>
<td>160</td>
<td>90</td>
<td>85</td>
<td>80</td>
<td>49.6</td>
<td>25</td>
<td>12.8</td>
<td>9.5</td>
<td>7.1</td>
</tr>
<tr>
<td>NO2</td>
<td>218</td>
<td>120</td>
<td>79.3</td>
<td>46.4</td>
<td>28.3</td>
<td>25</td>
<td>15.4</td>
<td>13</td>
<td>9.1</td>
<td>7</td>
<td>8</td>
<td>3.9</td>
<td>2</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The ppm concentration of NO is at the preferred level for a wide range of standard biomedical treatment applications. The ppm concentration of NO2 is below the OSHA safety standards. The formation of different active species produced in air plasmas involves very complex plasma chemistries. At such a relatively high gas temperature different nitrogen oxides products are formed from N2 and O2 reactions.31 The O2 concentration affects the production of NOx species air discharges and it has been shown that a threshold value of O2 concentration (5%) is necessary for production of different NOx species.31,32 In such a plasma condition, NO2 will be produced from N2 and O2 in the discharge based on the following reactions.

\[ N_2 \rightarrow 2N \quad \Delta G^* = +911.1 \text{ kJ/mol}^{-1} \quad (3) \]
\[ N + O_2 \rightarrow NO_2 \quad \Delta G^* = -404.3 \text{ kJ/mol}^{-1} \quad (4) \]
\[ N + O_2 \rightarrow NO + O \quad \Delta G^* = -137.3 \text{ kJ/mol}^{-1} \quad (5) \]

Although NO2 production is more favorable than NO production, NO2 decomposes according to following reactions and produces additional NO species.

\[ NO_2 \rightarrow N + O_2 \quad \Delta G^* = +404.3 \text{ kJ/mol}^{-1} \quad (6) \]
\[ NO_2 \rightarrow NO + O \quad \Delta G^* = +267.0 \text{ kJ/mol}^{-1} \quad (7) \]
Thus, considering both process of NO formation; such as from N2 and O2 (according to reactions 3 and 5) and from decomposition of NO2 (reaction 7), results in a higher NO concentration compared to NO2 along the plasma jet (see Table 1-2). Such processes are observed in other thermal plasmas as well.32

**H. Direct versus indirect bacterial inactivation in liquid environment**

Plasma treatments on bacterial cultures of S. aureus, E. coli, and N. meningitidis were done using direct and indirect methods. The demonstration of bacterial inactivation and its quantitative analysis were done by optical density (OD) measurements using UV-VIS spectrometer tested at 595 nm. Considering the OD value proportional with the concentration of the detected bacteria, we determined the inactivation of the bacterial growth. The pH measurements of the plasma treated samples showed similar values as the untreated samples.

The Fig. 7(a) demonstrate that with direct plasma treatment, S. aureus suspensions reached a mean maximum density over a 24 hour period of OD595 = 1.418, OD595 = 1.283, and OD595 = 0.137, with increasing direct treatment of RB plasma jet times of 30s, 60s, and 120s, respectively, while untreated S. aureus bacterial suspensions resulted with a mean maximum density measurement of OD595 = 1.964. The results show that the S. aureus suspended in aqueous media were inactivated with 60s of direct exposure of RB plasma jet. Similarly, E. coli displayed similar trends as S. aureus suspensions as shown in Fig. 7(b).
FIG. 7. Inactivation efficacies of RB plasma jet as a function of direct plasma exposure treatment. Density measurements over 24 hr post treatment on: (a) S. aureus, (b) E. coli, (c) N. meningitidis.
The untreated E. coli suspensions reached a mean maximum density over a 24 hour period of OD595 = 3.305. The OD595 mean maximum density readings over a 24 hour period of E. coli with 30s and 60s of direct treatment of RB plasma jet reached OD595 = 2.77 and OD595 = 1.084, respectively, showing partial inactivation. Whereas, the E. coli suspensions with direct treatment of RB plasma jet for 120s displayed a radically lower OD595 with the mean maximum density reading over a 24 hour period reaching OD595 = 0.259. The inactivation efficacy of the S. aureus and E. coli by the RB plasma jet direct treatment of 120s at 5 cm distance was further corroborated with 1 x 10-6 serial dilution and 100 μl plating of cultures on agar plates as shown in Fig. 8(a) and (b). As shown in Fig 7(c), the direct treatment of RB plasma jet on N. meningitidis suspensions showed a different density pattern compared to E. coli and S. aureus suspensions. The OD595 mean maximum density readings over 24 hour period of N. meningitidis with 30s, 60s, and 120s of direct treatment of RB plasma jet reached OD595 = 1.26, OD595 = 1.32, and OD595 = 0.451, respectively. Untreated suspensions of N. meningitidis displayed a mean maximum density measurement of OD595 = 1.185. The indirect plasma treatment involves exposure of only the long lived active species (ROS and RNS) generated by the plasma instead of the plasma itself, thus excluding the effect of any possible UV radiation produced by plasma. It has been researched that under same treatment condition, a direct plasma treatment is an order of magnitude faster than indirect treatment by plasma active agents such as bombardment of the surface of a microorganism by charged particles in the primary inactivation mechanism.33 In the RB plasma jet indirect exposure method the concentrations of the nitric oxides are preserved by reducing the plasma temperature rapidly through a separate external small efficient cooling unit.
FIG. 8. Bacterial growth represented in terms of CFUs on agar plates for (a) S. aureus, (b) E. coli. (left)-control without plasma treatment, (right)-120s direct RB plasma jet treated.
FIG. 9. Inactivation efficacies of RB plasma jet as a function of indirect plasma exposure treatment. Density measurements over 24 hr post treatment on: (a) S. aureus, (b) E. coli, (c) N. meningitidis.
Utilizing the maximum time of direct exposure of RB plasma jet treatment at which the lowest densities were seen in S. aureus, E. coli, and N. meningitidis suspensions, we found that 120s of indirect exposure of RB plasma jet treatment greatly reduced the densities of S. aureus and E. coli and with mean maximum densities over a 24 hour period reaching OD595 = 0.693 and OD595 = 0.130, respectively; as compared to untreated controls as shown in Fig. 9(a) and (b). As suspected, the 120s of indirect RB plasma jet treatment of N. meningitidis suspensions did not impact the density as determined with the mean maximum density over a 24 hour period reading of OD595 = 1.187, similar to the untreated control as shown in Fig 9(c). Eventually the inactivation of N. meningitidis can be expected since its close relative N. gonnorhea with similar characteristics was effectively inactivated after at least 4 min of plasma treatment.34

**FIG. 10.** Comparative 24-hr post treatment inactivation results for S. aureus, E. coli and N. meningitidis by RB plasma jet exposure in: (a) direct plasma treatment, (b) indirect plasma treatment—modes.
The comparative bacterial growth inactivation results by the direct and indirect RB plasma jet treatments are presented in Fig. 10. Variable differences in the amount of plasma exposure required to inactivate different strains of bacteria has been repeatedly reported but of greater interest is variable resistance to inactivation amongst bacteria of the same species as seen with S. aureus35 and determined in N. gonnorhea36. The identified variability in bacterial resistance to plasma as seen in this study and previously reported studies suggest that some bacteria may be incorporating mechanisms to ameliorate some of the plasma effect. In the use of direct plasma inactivation of bacterial growth, N. meningitidis required greater plasma exposure to allow for its inactivation, while there was no apparent inactivation by indirect plasma treatment. As indicated in Table 1 and 2, NO is a major component of the long lived species produced by the RB plasma jet. N. meningitidis is normally found in the moist tissues of the NO rich environment of the nasal passages.37 Furthermore N. meningitidis is known for its ability to impede host cell defenses against removing the bacteria through inhibition of NO induction cell suicide.38 We can only allude to the incorporation of these mechanisms as a factor that provided tolerance toward indirect plasma treatment consisting of high levels of nitric oxide for N. meningitidis as compared to successful decreases in densities seen in E. coli and S. aureus. Although the results signify that N. meningitidis might be metabolizing NO, further metabolic measurements may be required in order to confirm. Alternately, the ability of plasma to affect bacterial gene expression has been previously described with E. coli treated with direct plasma demonstrating significant changes in gene expression of genes that control the bacterial defense against oxidative stress.39,40 Similarly, a pilC gene expression analysis of N. meningitidis was performed for 60 second direct treatment of RB plasma jet. The pilC gene is
widely known for directing the production of PilC protein that is the primary adhesin allowing for the bacteria to bind to the semi-aqueous mucosal layer inside the nose and throat.41

FIG 11. Quantitative PCR analysis of relative expression of pilC in N. meningitidis untreated and 60 second of RB plasma jet direct treatment.

The results indicate that, 60 seconds of direct plasma treatment has a 4 fold reduction in the relative expression of the pilC gene as compared to the untreated sample as shown in Fig. 11. The values were determined after correcting the gene expression using the internal 16S control. The 16S control gene is a standard functional gene within the bacteria whose expression level is normally constant. The values represent mean ± standard deviations. We can derive from previous reports that demonstrated an increase in the levels of NO in cultures of N. meningitidis results in an increase in expression of genes involved with protection against NO.42 Therefore, the change in gene expression of pilC suggests that other genes involved with protection against NO are also variably affected by direct plasma treatment. The NO
detoxification mechanisms found in the bacterial species used in this study were originally detected as a bacterial defense response against host cell nitrosative stress. The presence of bacterial bio-physical mechanisms that may provide the capabilities to resist specific plasma chemistries as observed with N. meningitidis highlights a research prospect for plasma treatment of infected wounds. The authors have suggested the potential use of RB plasma jet for wound sterilization, although the increasing levels of NO may concern to treating wound tissue. So far, there have been no report of plasma treatment on bacterial infected wounds being negatively impacted in rodent and porcine models or human clinical trials. The RB plasma source can be extended further to personal and small scale treatment of wound infections, medical instrumentation sterilization, along with food and agricultural product sterilization and detoxification.

In conclusion, we have developed a portable, light weight resistive barrier (RB) plasma jet applicable for a range of biomedical applications including bacterial inactivation in aqueous media. The RB plasma jet can be operated at both DC and standard low frequency AC, and also able to function effectively in both direct and indirect plasma exposure configurations based on the type of treatment targets and applications. The characteristics of the RB plasma jet such as the voltage-current curves, plasma power, plasma gas temperature and RNS concentrations were characterized. The voltage-current waveform analysis resulted the plasma power to be 26 W. A high resolution optical emission spectroscopy diagnostic analysis of the N2 second positive band system N2C-B(2+) rotational transitions in the 364-383 nm range showed the gas temperatures equivalent to the rotational (Trot) temperatures to be as 3000 ○C at 0.2 cm from the exit and decayed to 2000 ○C at 0.8 cm and continued to decay to 1500 ○C at 1.5 cm and to
1000 oC at 2 cm and the jet temperature reached room temperature with the addition of external cooling unit for the indirect treatment mode. The parts per million (ppm) concentration of the nitric oxide (NO) at different spatial distances from the tip of the plasma jet were measured to be in the range of 500-660 ppm at 5 cm distance from the electrode and drops to ~100 ppm at 60 cm. The RB plasma jet was effective in inactivating the S. aureus and E. coli in less than 120 seconds determined by measuring the density of bacteria growing in liquid cultures, in both direct and indirect exposure treatment modes. The N. meningitidis appeared to show some resistance to inactivation and required extended plasma exposure as compared to S. aureus and E. coli, especially when the RB plasma jet was used in the indirect mode. The RT-PCR based pilC gene expression analysis was done on 60 second direct plasma treated N. meningitidis samples at which only partial inactivation was achieved. The results revealed that although a significant inactivation was not observed as determined by bacterial growth, the genes that control the ability of bacteria to cause an infection were being inhibited by at least 4 fold differences as compared to untreated bacteria. However, the ability of N. meningitidis to partially resist direct plasma and completely resist indirect plasma treatment suggest that it has incorporated processes that are regulated by genes that are directed by external stimuli such as NO from the RB plasma jet. The findings indicate that N. meningitidis displayed some tolerance to plasma and the long lived reactive nitrogen species, indicating the need for further research toward the specific bacterial inactivation mechanisms of plasma derived reactive nitrogen species.
II. Characterization of a non-thermal atmospheric pressure helium plasma jet

In this project period, we have also applied optical emission spectroscopy diagnostics to investigate the characteristics of a non-thermal atmospheric pressure helium plasma jet. The discharge characteristics in the active and afterglow region of the plasma jet, that are critical for biomedical applications, have been investigated. The voltage-current characteristics of the plasma discharge were analyzed and the average plasma power was measured to be around 18 W. The effect of addition of small fractions of oxygen at 0.1 - 0.5% on the plasma jet characteristics were studied. The addition of oxygen resulted in a decrease in plasma plume length due to the electronegativity property of oxygen. Atomic and molecular lines of selected reactive plasma species that are considered to be useful to induce biochemical reactions such as OH transitions $A^2\Sigma^+(v = 0,1) \rightarrow X^2\Pi(\Delta v = 0)$ at 308 nm and $A^2\Sigma^+(v = 0,1) \rightarrow X^2\Pi(\Delta v = 1)$ at 287 nm, O I transitions $3p^5\text{P} \rightarrow 3s^3\text{S}^0$ at 777.41 nm and $3p^3\text{P} \rightarrow 3s^3\text{S}^0$ at 844.6 nm, $N_2$(C-B) second positive system (SPS) with electronic transition $C^3\Pi_u \rightarrow B^3\Pi_g$ in the range of 300-450 nm and $N_2^+$(B-X) first negative system (FNS) with electronic transition $B^2\Sigma^+_u \rightarrow X^2\Sigma^+_g(\Delta v = 0)$ at 391.4 nm have been studied. The atomic emission lines of helium were identified, including the He I transitions $3p^3\text{P}^0 \rightarrow 2s^3\text{S}$ at 388.8 nm, $3p^1\text{P}^0 \rightarrow 2s^1\text{S}$ at 501.6 nm, $3d^3\text{D} \rightarrow 2p^3\text{P}^0$ at 587.6 nm, $3d^1\text{D} \rightarrow 2p^1\text{P}^0$ at 667.8 nm, $3s^3\text{S}^1 \rightarrow 2p^3\text{P}^0$ at 706.5 nm, $3s^1\text{S}^0 \rightarrow 2p^1\text{P}^0$ at 728.1 nm, and $H_\alpha$ transition $2p-3d$ at 656.3 nm. Using a spectral fitting method the OH radicals at 306-312 nm, the rotational and vibrational temperatures equivalent to gas temperatures of the discharge was measured and the effective non-equilibrium nature of the plasma jet was demonstrated. Our results show that, in the entire active plasma region, the gas temperature remains at $310\pm25$ K and $340\pm25$ K and it increases to $320\pm25$ K and $360\pm25$ K in the afterglow region of the plasma jet for pure helium and helium/oxygen (0.1%) mixture, respectively. Additionally, the vibrational temperatures range
from 2200±100 K and 2500±100 K for pure helium and helium/oxygen (0.1%) mixture, respectively. The plasma jet was tested on heat sensitive polymer films used in biomedical applications such as polyethylene terephthalate (PET) and poly-L-lactide (PLLA) samples continuously for several minutes without causing any physical or thermal damage to the films. The plasma jet produces significant reactive species of interest while the gas temperatures remain very low demonstrating its potential for a range of biomedical applications.

Non-thermal plasma jets that are operated in atmospheric pressure have been proven to be an effective plasma sources because of their feasibility to be used in a range of applications such as sterilization,\textsuperscript{1, 2} surface modification of the polymers\textsuperscript{3-5} environmental and industrial applications\textsuperscript{6} and more recently in medicine.\textsuperscript{7-9} In such plasmas, the high electron temperature enhances the plasma chemistry processes which lead to the generation of highly reactive chemical species e.g. hydroxyl radicals and ozone, while the plasma gas temperature remaining close to the room temperature. Atmospheric pressure plasma jets with lower gas temperatures are suitable for treating temperature sensitive materials without causing damage, and they are also found to be easily adaptable to complex geometries and conventional processes.

Atmospheric pressure plasma jets (APPJs) are capacitively coupled dielectric barrier discharge (DBD) sources typically operated in the radio frequency range, e.g. at 13.56 MHz\textsuperscript{10} or 27.12 MHz\textsuperscript{11} and it can be generated in various electrode arrangements.\textsuperscript{12} In general the APPJs consist of two electrodes in different arrangements. In some studies, a simple nozzle configuration with a dielectric tube surrounded by ground electrode has been used. A pulsed or AC power supply with frequency of several kHz was applied between the electrodes to generate plasma from working gases such as helium, argon and nitrogen.\textsuperscript{6, 13, 14} In most of the APPJ applications, gas temperatures significantly influence the application, thus it needs to be
precisely diagnosed. However, determination of the gas temperature in the DBD plasma jets is a significant experimental challenge due to electrical interferences caused by conventional thermocouples inside the discharge leading to inaccurate measurements. Another problem is that the quartz capillary in such plasma jets is generally narrow. Therefore a convenient way to measure the gas temperature inside or near the discharge volume is to place the thermocouples outside the discharge or measure the temperature after switching off the discharge. Both techniques are inaccurate; the DBD plasma jets are made of quartz, which is an insulating material and it yields a poor heat transfer from the gas to the plasma jet’s wall, therefore, rendering the temperature of quartz capillary wall rather the needed gas temperature. Among different plasma diagnostic methods, optical emission spectroscopy (OES) diagnostics acts as a viable technique for determining discharge parameters.\textsuperscript{6, 15, 16} OES is a non-invasive and non-perturbing plasma diagnostic technique that is easy to use and widely applied for investigation of plasma species and characterization of its gas temperatures. OES enables to identify the reactive plasma species such as radicals, atomic and molecular species and therefore it provides the insight of the plasma chemical processes.\textsuperscript{6, 16} In addition, OES provides valuable information on excited atomic or molecular plasma species, which enables us to determine the rotational and vibrational temperatures of the plasma and thus the non-equilibrium nature of the plasma jet and the plasma gas temperatures.\textsuperscript{6, 16-18}

The plasma jet developed in this work aimed to produce reactive plasma species, such as hydroxyl radicals, excited nitrogen species, singlet oxygen and helium metastable that can be applied for bio-medical applications including inactivation of bacteria, sterilization, and treatment of cancer. This work is aimed on applying optical emission spectroscopy to identify plasma species that are critical for several biomedical applications and determining the plasma
gas temperatures. In addition, the effect of addition of oxygen in the plasma jet characteristics is of focus in this work due to the significance of singlet oxygen radicals in biomedical applications. This work also aimed to produce increased amounts of active species by adding oxygen without significant increase in the plasma jet gas temperature and produce the plasma jet at low temperatures suitable for treating heat sensitive surfaces.

In this paper, the consumed power for plasma generation (plasma power) has been estimated from voltage-current waveform analysis in order to optimize the plasma jet operation. The effects of addition of small fractions of oxygen on the plasma jet are presented. Subsequently, we present the optical emission spectroscopy results of the plasma jet in helium and helium/oxygen mixtures (0.1-0.5% oxygen). The results of our investigation on the plasma jet includes the analysis of optical emission spectra measured in the active region, the region between the electrodes where plasma is formed inside quartz capillary, and also in the jet’s afterglow region where the plasma jet propagates in to the surrounding air. In addition, using a high resolution spectrometer, the OH rotational and vibrational temperatures were determined by fitting the experimental and simulated spectra. The result of this work are useful for a range of atmospheric pressure plasma jet applications, such as surface treatment of polymers, biomedical and environmental applications where OES act as a key diagnostic for investigating the plasma chemical processes.

A. EXPERIMENTAL SETUP

In our investigation, we have designed and constructed an atmospheric pressure dielectric barrier plasma system that can produce a long plasma jet aimed for materials processing and biomedical applications. The schematic of our plasma system is shown in Fig. 1. The plasma jet
was produced in a quartz capillary with inside and outside diameters of 1.3 and 3.0 mm, respectively. A 0.5 mm diameter tungsten rod was used as the central high voltage electrode which is positioned inside the quartz capillary.

FIG. 1. Schematic of the plasma jet system along with V-I and OES diagnostics.

A 10 mm long ring type copper ground electrode positioned outside the quartz capillary at a distance of 40 mm away from the tip of the central high voltage electrode. The primary operating gas for the plasma jet used in this work is Helium and small fractions of oxygen (0.1 - 0.5%) were used as the secondary gas. The gas flow rate was monitored by a two channel power supply/readout (model: MKSPR400) and controlled by two mass flow meters (Model: MKS 1179A). The total gas flow rate was maintained at 3 slm.
A variable sine-wave AC high-voltage power supply ($V_{pp}$: 0-25 kV) operating at 60 kHz was used to generate the plasma jet. The applied voltage waveform was monitored using high voltage probe (Tektronix P6015A) and the current waveform was monitored by current probe (Tektronix TCP202). The current and voltage signals are recorded using digital oscilloscope (Tektronix TDS 3034C). The peak to peak applied voltage value was varied between 6 to 14 kV. The input power was fixed at an optimal value of 18 W for all the experiments.

Optical emission spectra of the plasma jet were measured in order to identify various reactive plasma species in the active and afterglow region of plasma jet and to determine the plasma gas temperatures. The spectra were collected with an optical fiber (diameter of 0.6 mm), placed near to the active zone and the afterglow region of the plasma jet. Two spectrometers of different resolution were used. The optical emission spectra of the discharge in the 200–900 nm region were recorded by broadband Ocean Optics HR2000 spectrometer with a resolution 0.83 nm and the emission of OH radicals in the range of 306-312 nm was recorded with 500 $\mu$s integration time using a 0.08 nm resolution spectrometer system which is a combination of monochromator (Princeton Instruments Acton SP2750) and fast gated CCD (ACTON IStar).

B. Plasma jet length, voltage-current and plasma power measurements

A complete gas breakdown between the electrodes with a formation of bright glow discharge was achieved by applying a high voltage of approximately 6 kV ($V_{pp}$). A long afterglow plasma jet of length up to 40 mm was achieved with a gas flow rate of 3 slm. We were able to vary the plasma jet length by varying the gas flow rates, applied voltage and gas compositions. As shown in the visual view of the plasma jet in Fig. 2 (a) the plasma jet creates a long plume length which propagates in to the surrounding air.
FIG. 2. (a) Visual view of the plasma system. (b) Images of afterglow region of the plasma jet in pure helium and He/O₂ mixtures. Total gas flow rate is 3 slm and plasma power is 18 Watt.

The Fig. 2 (b) shows the photograph of the afterglow region of the plasma jet operating in pure helium at 3 slm and helium/oxygen (0.1% and 0.5% O₂) mixtures. The images were taken by a Sony RX100 digital camera and the exposure time for all of the images was fixed at 1/30 s. The length of plasma plume from the tip of the quartz capillary for pure helium discharge is 25 mm. With 0.1% oxygen added to the Helium gas, the plasma plume length decreased to 17 mm. It was observed that the addition of oxygen in to the plasma jet results in a decreased plasma jet length. Since oxygen is an electronegative molecular gas, the decrease in plasma jet length is believed to be caused by decrease in electron densities and helium metastable densities with the addition of oxygen.
FIG. 3. Voltage-current waveforms of the plasma jet. (a) Discharge in pure Helium and (b) Discharge in He/O\textsubscript{2} mixture (0.1% Oxygen).

The voltage and current waveforms were obtained for various applied voltages ranging from 6 to 14 kV ($V_{pp}$) and gas compositions of He and He + O\textsubscript{2} (0.1 - 0.5% O\textsubscript{2}). A typical example of voltage-current waveform of the plasma discharge in He and He + 0.1 % O\textsubscript{2} is shown in Fig. 3, in which the applied voltage and gas flow rate was set at 12 kV ($V_{pp}$) and 3 slm respectively. The DBD plasma jet generates a very uniform discharge in pure helium gas and only two small current peaks exist on the current waveform during each half period of the applied voltage. Both peaks are smooth and the maximum current is $\sim$10 mA and the duration of the current peaks are approximately 0.8 $\mu$s. A clear change in the voltage-current waveform was observed when oxygen was added to the plasma jet. Addition of the oxygen to the plasma jet results in a small increase of the current peak intensity to $\sim$12 mA and decrease of the current peaks duration to 0.5 $\mu$s. The results indicate that the voltage slightly decreases with increase of the current peaks. The consumed power for plasma generation is calculated by integrating the product of the discharge voltage and current over one cycle; according to the following equation ($T = $ period of the discharge)$^{19}$
\[ W = \frac{1}{T} \int_T^{T+T} I(t)V(t)dt \]  
(1)

and the average plasma power is measured to be 18 W.

C. Plasma species identification using optical emission spectroscopy

Optical emission spectroscopy was used to study the plasma jet in He and He/O\textsubscript{2} gas mixtures in terms of identifying the reactive plasma species produced by the plasma jet as well as determining the plasma gas temperatures. The emission spectra were measured at two locations, first in between the high voltage and ground electrodes (active region) and the second was at 5 mm away from capillary exit tip and into the plasma jet’s plume (afterglow region). Fig. 4 (a) and (b) shows the time resolved optical emission spectra in the range of 200 - 900 nm measured in the active region for pure helium and helium/0.1% oxygen mixture respectively.

The emission spectra in the active region of the plasma jet is composed of OH transition \( A^2\Sigma^+(\nu = 0,1) \rightarrow X^2\Pi(\Delta\nu = 0) \) at 308 nm, and OH transition \( A^2\Sigma^+(\nu = 0,1) \rightarrow X^2\Pi(\Delta\nu = 1) \) at 287 nm; He I transition \( 3p^3P^0 \rightarrow 2s^1S \) at 388.8 nm, He I transition \( 3p^1P^0 \rightarrow 2s^1S \) at 501.6 nm, He I transition \( 3d^3D \rightarrow 2p^3P^0 \) at 587.6 nm, He I transition \( 3d^1D \rightarrow 2p^1P^0 \) at 667.8 nm, He I transition \( 3s^3S^1 \rightarrow 2p^3P^0 \) at 706.5 nm, and He I transition \( 3s^1S^0 \rightarrow 2p^1P^0 \) at 728.1 nm; H\textsubscript{a} transition 2p-3d at 656.3 nm, O I transition \( 3p^5P \rightarrow 3s^5S^0 \) at 777.41 nm, O I transition \( 3p^3P \rightarrow 3s^3S^0 \) at 844.6 nm and spectral band of the N\textsubscript{2} transition \( C^3\Pi_u \rightarrow B^3\Pi_g(\Delta\nu = 0) \) at 337.1 nm. Discharge in pure helium shows a higher intensity of OH radicals compared to the plasma with He/0.1% O\textsubscript{2} gas mixture at 308 nm. The presence of OH and N\textsubscript{2} emission lines in the active region of the discharge is due to the presence of residual H\textsubscript{2}O and N\textsubscript{2} impurities in helium gas. Addition of small amount of oxygen (0.1%) to plasma jet results in an increase in the intensity of singlet oxygen lines at
777.41 nm and 844.6 nm, while the intensity of the other emission lines has decreased. Oxygen is an electronegative gas, hence, addition of oxygen to the plasma produces $O^-$ and $O^{2-}$ ions by electron attachment mechanisms which results in a decrease of electron density and consequently decrease in the intensity of the other lines at a constant power.\(^6\)

FIG. 4. Optical emission spectra of the active zone region of plasma jet. (a) Discharge in pure helium. (b) Discharge in helium/oxygen mixture (0.1%). Total gas flow rate is 3 slm and plasma power is fixed at 18 W.

The emission spectra of the afterglow region of the plasma jet in pure helium and helium/oxygen mixtures are shown in Fig. 5 (a) and (b), respectively. The optical emission
spectra of the plasma jet in the afterglow region are significantly different from the emission spectra measured in the active region as shown in Fig. 4 (a) and (b). The emission spectra in the afterglow region of the plasma jet where plasma propagates into the surrounding air is composed of molecular nitrogen \( \text{N}_2 \) second positive system (SPS) with electronic transition \( C^3\Pi_u \rightarrow B^3\Pi_g \) in the range of 300-450 nm. \( \text{N}_2^+ \) first negative system (FNS), has a spectral band at 391.4 nm with electronic transition \( B^2\Sigma_g^+ \rightarrow X^2\Sigma_g^+ (\Delta \nu = 0) \), which is clearly visible in the measured emission spectra. Other emission lines belong to OH emission lines at 308 nm, transition \( A^2\Sigma^+(\nu = 0,1) \rightarrow X^2\Pi(\Delta \nu = 0) \) and OH transition \( A^2\Sigma^+(\nu = 0,1) \rightarrow X^2\Pi(\Delta \nu = 1) \) at 287 nm, and the spectral lines of helium atoms including, He I transition \( 3p^3P^0 \rightarrow 2s^3S \) at 388.8 nm, He I transition \( 3p^1P^0 \rightarrow 2s^1S \) at 501.6 nm, He I transition \( 3d^3D \rightarrow 2p^3P^0 \) at 587.6 nm, He I transition \( 3d^1D \rightarrow 2p^1P^0 \) at 667.8 nm, He I transition \( 3s^3S^1 \rightarrow 2p^3P^0 \) at 706.5 nm, He I transition \( 3s^1S^0 \rightarrow 2p^1P^0 \) at 728.1 nm, and H\(_\alpha\) transition 2p-3d at 656.3 nm, O I transition \( 3p^5P \rightarrow 3s^5S^0 \) at 777.41 nm and O I transition \( 3p^3P \rightarrow 3s^3S^0 \) at 844.6 nm. Strong \( \text{N}_2 \) emission lines are commonly observed in atmospheric pressure non-equilibrium air discharges. The identified excited nitrogen molecules are produced from electron impact reactions in the plasma plume as it propagates into the ambient air. In Fig. 5 (b) the emission spectrum of He/O\(_2\) (0.1%) mixture is very similar to pure helium discharge in the case of identified species. The intensity of molecular nitrogen emission bands is less intense compared to atomic lines when a small amount of oxygen (0.1%) is added to the plasma jet. In addition the intensity of atomic lines such as He I transition \( 3d^1D \rightarrow 2p^1P^0 \) at 667.8 nm, He I transition \( 3s^3S^1 \rightarrow 2p^3P^0 \) at 706.5 nm, He I transition \( 3s^1S^0 \rightarrow 2p^1P^0 \) at 728.1 nm and O I transition \( 3p^5P \rightarrow 3s^5S^0 \) at 777.41 nm is also decreased. The corresponding emission lines are identified using the NIST atomic spectra database\(^{20}\) and Lofthus et. al.\(^{21}\)
FIG. 5. Optical emission spectra of the afterglow region of plasma jet. (a) Discharge in pure helium. (b) Discharge in helium/oxygen mixture (0.1%). Total gas flow rate is 3 slm and plasma power is fixed at 18 W. Emission spectra are recorded at 5 mm after the edge of capillary.

The identified reactive plasma species such as the singlet oxygen, hydroxyl radicals, and neutral nitrogen molecules generated by the plasma jet are measured with the OES diagnostics and these reactive plasma species can play a significant role in biomedical applications
especially operating at the atmospheric pressure.\textsuperscript{7, 22} Similar significant generation of reactive oxygen species such as singlet oxygen O and O\textsubscript{3} were observed with He/O\textsubscript{2} gas mixture used in a plasma jet which resulted in enhanced efficacy of bacterial inactivation.\textsuperscript{23} It is believed that the oxygen species in the discharge contributes to the sterilization process due to its strong oxidative effect on the outer structure of the cells.\textsuperscript{7, 22} The OH radicals generated by the plasma plays a significant role through chemically attacking the outer structure of the cells and the production of the nitrogen containing group adds to the lethality of the process.\textsuperscript{24}

**D. Rotational and vibrational temperature measurements**

The emission spectra of OH (A-X) radicals are widely used for plasma diagnostics, since they enable us to determine the rotational ($T_{rot}$) and vibrational temperature ($T_{vib}$) by fitting the experimental spectra on to a simulated spectra.\textsuperscript{25} For all spectral simulations, a LIFBASE software\textsuperscript{26} has been used. The emission intensity of the OH radicals is a function of the concentration of excited hydroxyl species, rotational and vibrational energy distributions, and the volume of plasma being analyzed. The OH rotational temperature in the active zone and afterglow region of the DBD plasma jet have been determined based on the simulation of emission spectra in the range of 306-312 nm with transition $A^{2}\Sigma^{+}(\nu = 0) \rightarrow X^{2}\Pi(\Delta\nu = 0)$. A spectral emission line can be characterized as a function of intensity, wavelength, profile and its width as expressed by\textsuperscript{27}

$$I_{n,v,J}^{\ell',\ell,J'} = N_{n,v,J} A_{n,v,J}^{\ell',\ell,J'} h\nu_{n,v,J}^{\ell',\ell,J'}$$

where $n$, $\nu$ and $J$ represents quantum numbers of electronic, vibrational and rotational levels respectively.
FIG. 6. Experimental and simulated spectra of OH radicals. (a) Discharge in Pure He and in active zone region (between HV and ground electrodes). (b) Discharge in He/O₂ mixture (0.1%) and in active zone region (between HV and ground electrodes). (c) Discharge in Pure He and in the afterglow region. (d) Discharge in He/O₂ mixture (0.1%) and in the afterglow region. Plasma power was fixed at 18 Watt.

The single prime (′) signifies the upper level magnitudes and a double prime (″) signifies the lower level magnitudes. \( N_{u',J'J} \) is the density of the upper level of the molecule, \( v \) is the frequency of the transition and \( A_{u',J',J}^{v,u,J} \) is Einstein’s transition probability of emission. The equation (2) can be modified as
\[ I^{\nu', \nu', J' \rightarrow J \rightarrow n', \nu', J} = C n', \nu' \nu', J' \rightarrow J \rightarrow n', \nu', J} \frac{S_{JJ}}{Q_{kT}(T_n)} \exp\left(-\frac{\hbar c k}{h k}(\nu_n/\nu_m)\right) \frac{G_m}{Q_{rot}(T_m)} \exp\left(-\frac{\hbar c k}{h k}(\nu_m/\nu_m)\right) \]  

(3)

where \( C \) is a constant, \( h \) is the Planck’s constant, \( k \) is the Boltzmann constant, \( c \) is the velocity of light and \( N_n \) is the density per volume of the molecules in electronic state of \( n' \). The line strengths \( S_{JJ} \) are calculated from\(^2\) and \( S_{\nu\nu} \) are the band strengths. Furthermore, considering the non-Gaussian experimental line shape, a contribution of the Lorentzian function is taken into account. In this case, best fitted line shape was a mixture of a Voight and a Lorentzian profile.

The line intensity as function of wavelength (\( \lambda \)) can be expressed as:

\[
I(\lambda) = I^{\nu', \nu', J' \rightarrow J \rightarrow n', \nu', J} \left[ 1 - M \exp\left( -\frac{\lambda - \lambda^{\nu', \nu', J' \rightarrow J \rightarrow n', \nu', J}}{FWHM} \right)^2 \right] + M \frac{1}{4(\lambda - \lambda^{\nu', \nu', J' \rightarrow J \rightarrow n', \nu', J})^2 + 1} \]  

(4)

where, \( \lambda^{\nu', \nu', J' \rightarrow J \rightarrow n', \nu', J} \) is the wavelength of the transition \( n', \nu', J' \rightarrow n'', \nu'', J'' \) and \( M \) is a fraction of the Lorentzian contribution to the Voight profile of the instrumental function. The rotational \( (T_{rot}) \) and vibrational temperatures \( (T_{vib}) \) of the plasma jet were estimated by matching the experimental spectra on to the LIFBASE software simulated spectra.\(^2\) In order to make the comparison, the intensity of both simulated and experimental spectra are normalized based on the (0-0) band and when a good agreement between experimental and simulated spectra were achieved, the corresponding OH rotational and vibrational temperatures have been deduced. The results of best fitting between experimental and simulated spectra for OH emission in the active zone of the plasma jet for He and He + 0.1% O\(_2\) are shown in Fig. 6 (a) and (b). Similarly the results of best OH spectral fitting in the afterglow region of the plasma jet for He and He + 0.1% O\(_2\) are shown in Fig. 6 (c) and (d). Based on the fitting method, the OH rotational temperatures in the active zone region of the plasma jet was measured to be 310 ± 25 K for helium only and
340 ± 25 K for helium with 0.1% oxygen gas mixture. Whereas the measured rotational temperature in the afterglow region at 5 mm away from the capillary edge has increased to 320 ± 25 K for helium only and 360 ± 25 K for helium with 0.1% oxygen gas mixture. In addition, the vibrational temperatures of the plasma jet was measured to be 2200 ± 100 K for helium only and 2500 ± 100 K for helium with 0.1% oxygen gas mixture. Our results show that the OH rotational temperatures of the afterglow region of the plasma jet are slightly higher than the active zone region. The non-equilibrium characteristic of the plasma is resulting from the significant difference in the vibrational and rotational temperatures. Based on the results, the higher vibrational temperatures compared to rotational temperature ($T_{\text{vib}} > T_{\text{rot}}$) demonstrate that, the plasma jet produced is in a non-equilibrium condition. In atmospheric pressure plasmas the gas temperature is equal to the rotational temperature due to the higher collisional rates, demonstrating the very low gas temperature of the plasma jet and its suitability for biomedical applications and surface treatment of bio-polymers. The plasma jet was tested on heat sensitive polymer films used in biomedical applications such as polyethylene terephthalate (PET) and poly-L-lactide (PLLA) continuously for several minutes without causing any physical or thermal damage on the film surfaces.

Optical emission spectroscopy diagnosis was successfully employed for characterizing the atmospheric pressure non-thermal helium plasma jet and the effects of addition of small fractions of oxygen at 0.1 - 0.5% on the characteristics of the plasma jet were investigated. The addition of oxygen resulted in a decrease in plasma plume length due to the electronegativity property of oxygen. The voltage-current characteristics of the plasma discharge were analyzed and the average plasma power was measured to be around 18 W. The reactive species produced by the plasma jet which are critical to a range of biomedical applications. Using optical emission
spectroscopy diagnostics the identification of radicals and excited species produced in the active zone and afterglow region of the plasma jet have been carried out. It was observed that, the most intensive spectral lines belong to the OH emission lines transition $A^2\Sigma^+(v = 0,1) \rightarrow X^2\Pi(\Delta v = 0)$ at 308 nm and $A^2\Sigma^+(v = 0,1) \rightarrow X^2\Pi(\Delta v = 1)$ at 287 nm, the spectral lines of the helium atoms including He I transition $3p^3P^0 \rightarrow 2s^3S$ at 388.8 nm, He I transition $3p^1P^0 \rightarrow 2s^1S$ at 501.6 nm, He I transition $3d^3D \rightarrow 2p^3P^0$ at 587.6 nm, He I transition $3d^1D \rightarrow 2p^1P^0$ at 667.8 nm, He I transition $3s^3S \rightarrow 2p^3P^0$ at 706.5 nm, He I transition $3s^1S \rightarrow 2p^1P^0$ at 728.1 nm, and Hα transition $2p-3d$ at 656.3 nm, O I transition $3p^5P \rightarrow 3s^5S$ at 777.41 nm and O I transition $3p^3P \rightarrow 3s^3S$ at 844.6 nm. Additionally, in the afterglow region of the plasma jets strong emission of the molecular nitrogen $N_2$(C-B) second positive system (SPS) with electronic transition $C^3\Pi_u \rightarrow B^3\Pi_g$ in the range of 300-450 nm and $N_2^+$(B-X), first negative system (FNS) spectral band with electronic transition $B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+(\Delta v = 0)$ at 391.4 nm were observed. Also, the spectra produced in the active zone and afterglow region in the atmospheric air, showed a significant variation in the emission spectra and spectral line intensities. The active zone emission spectra were dominated by OH and helium line intensities whereas the afterglow region emission spectra were dominated by a range of $N_2$ lines intensities. A high resolution OES was applied to determine the rotational and vibrational temperature of OH radicals transition $A^2\Sigma^+(v = 0) \rightarrow X^2\Pi(\Delta v = 0)$ in the range of 306-312 nm. The OH rotational temperature equivalent to plasma gas temperature was measured to be 310 ± 25 K and 340 ± 25 K in the active region and it increased to 320 ± 25 K and 360 ± 25 K in the afterglow region in the atmospheric air for pure helium and helium/oxygen (0.1%) mixture, respectively. The vibrational temperatures of the OH radicals estimated to be 2200 ± 100 K and 2500 ± 100 K, for pure helium and helium/oxygen (0.1%) mixture, respectively. The difference between the vibrational and rotational temperature represents the non-equilibrium
level of the discharge. The results indicate that the OH rotational temperatures of the afterglow region of the plasma jet are slightly higher than the active zone region, yet the temperatures are very low and suitable for treating heat sensitive materials such as bio-polymers. The plasma jet was tested on heat sensitive polymer films that are used in biomedical applications such as polyethylene terephthalate (PET) and poly-L-lactide (PLLA) films continuously for several minutes without causing any physical or thermal damage on the film surfaces. In conclusion, the OES technique was used to characterize an atmospheric pressure plasma jet in helium and helium/oxygen discharge. The plasma jet generates the significant reactive species at low gas temperatures which promises its great potential for a wide range of biomedical applications.

III. Characterization of a non-thermal helium and helium/oxygen gas mixture plasma jet.

In this project period, we have also applied optical emission spectroscopy diagnostics to investigate the characteristics of a non-thermal atmospheric pressure helium and helium/oxygen gas mixture plasma jet. In this work, an atmospheric pressure low temperature plasma pencil generated in helium and helium/oxygen gas mixtures was characterized in detail for its discharge characteristics, plasma power, reactive plasma species produced, plasma density and plasma temperatures using electrical and optical emission spectroscopy (OES) diagnostics. A visible plasma plume length of approximately 33 mm was generated using a 60 kHz AC HV power supply. The OES results showed the most intensive plasma emission lines of OH transition $A^2 \Sigma^+ (v = 0,1) \rightarrow X^2 \Pi (\Delta v = 0)$ at 308 nm and OH transition $A^2 \Sigma^+ (v = 0,1) \rightarrow X^2 \Pi (\Delta v = 1)$ at 287 nm, O I transition 3p$^5$P$\rightarrow$3s$^5$S$^0$ at 777.41 nm, O I transition 3p$^3$P$\rightarrow$3s$^3$S$^0$ at 844.6 nm and N$_2$(C-B) second positive system (SPS) with electronic transition $C^3\Pi_u \rightarrow B^3\Pi_g$ in the range of 300-450 nm. The effects of controlled oxygen content on the plasma pencil and on various helium
plasma emissions and electrical properties are studied. A spatial distribution of reactive plasma species produced by the plasma pencil is presented. The electron density of plasma jet was estimated to be in the order of \(2.1 \times 10^{14}\) cm\(^{-3}\) using Stark broadening line profile of the hydrogen alpha emission. The temperatures of afterglow plasma pencil were evaluated using OH rotational temperatures and the results demonstrate temperatures of 316 K and 362 K for pure He and He/O\(_2\) gas mixtures (0.1% O\(_2\)), respectively. Preliminary results on inactivation of \textit{S. pneumoniae} on a solid surface and in liquid suspension were carried out using the plasma pencil for biomedical applications.

Atmospheric pressure non-thermal plasma sources operating over a range of power levels has been used for industrial and materials processing applications,\(^1\)-\(^4\) and increasingly applied in biomedical applications.\(^2\), \(^3\), \(^5\), \(^6\) Several atmospheric pressure plasma sources have been developed recently, including the plasma pencil that produces stable plasma at room temperatures applied for bacterial inactivation,\(^7\) non-destructive sub-mm radio frequency plasma needle applied for surface treatment of biomaterials,\(^8\) one atmosphere uniform glow discharge plasma,\(^9\) dielectric barrier discharge based atmospheric pressure plasma jet,\(^10\) resistive barrier plasma discharge,\(^11\)-\(^13\) and microwave powered atmospheric pressure plasma jets.\(^14\) In general, the majority of the plasma sources operate with a flow of noble gasses like helium, argon and/or their mixtures with other gases such as air, nitrogen, oxygen and water vapor.\(^14\) Depending on the specific application requirements, a plasma source has to be developed and the characteristics of the plasma need to be diagnosed. In this work, we have constructed an atmospheric pressure plasma pencil in our laboratory based on kHz driven dielectric barrier discharge suitable for biomedical applications. A similar plasma source was investigated previously by the author.\(^15\)
however it was carried out in argon and argon/water vapor mixture as a source for polymer surface treatment application. The main goal of this current work was to produce a low-temperature atmospheric pressure plasma generating higher concentration of selective reactive plasma species required for biomedical applications, e.g. OH radicals, singlet oxygen, RNS and ROS.

In this work, we describe the characteristics of an atmospheric pressure plasma pencil in helium and helium/oxygen mixture propagating in to open air. The plasma pencil is capable of working in different gas mixtures, e.g. argon, helium and their mixture with oxygen and water vapors. The different modes of operation and its low gas temperatures along with its efficient production of active species will potentially allow for a wide range of applications. The electrical characterization was performed based on voltage-current characterization of the plasma pencil operated in helium and He/O₂ gas mixtures. The plasma power was estimated from voltage-current waveforms and the input power was kept constant while varying the applied voltage. Subsequently, optical emission spectroscopy was utilized for investigation of reactive species in plasma and determination of OH radical rotational temperature.

The size and preciseness of the plasma pencil would allow it for a range of biomedical applications that involves localized treatments. One such area of interest is pediatric otorhinolaryngology, specifically the treatment of middle ear and paranasal infections in infants and children. *Streptococcus pneumoniae* (the pneumococcus) is one the primary bacteria that causes such infections in the different locations of the respiratory tract of humans, including acute otitis media (middle ear infection), sinusitis (paranasal infection), bronchitis and pneumonia (lung infections). Of the infections caused by the pneumococcus, otitis media (OM) is one of the highest prevalent pediatric diseases worldwide. OM is the most frequently
diagnosed illnesses in children under 15 years of age and is the primary reason for frequent
physician visits.\textsuperscript{18} Traditional treatment of pneumococcal infections in the middle ear and sinuses
has consisted of a series of antibiotics; in fact, OM infections are considered the major reason for
antibiotic use in children in developed countries.\textsuperscript{19} Due to the rise in acquired bacterial resistance
to antibiotics, there is a pressing need for alternative treatments to both reduce the antibiotic use
in children and to combat bacterial infections from already resistant bacteria. To identify the
usefulness of the plasma pencil for biomedical applications, we assessed plasma pencil treatment
on pneumococcal cultures grown on solid blood agar plates and within liquid media.

\textbf{A. Plasma pencil and diagnostics}

In this investigation an atmospheric pressure plasma pencil has been developed. The plasma
was generated in a quartz capillary with inside and outside diameters of 1.3 and 3.0 mm,
respectively. Figure 1 shows a schematic of our atmospheric pressure plasma pencil system and
diagnostics.

The plasma pencil consists of two electrodes, separated by air gap and a dielectric quartz
capillary tube. The high voltage electrode is a 0.5 mm diameter tungsten rod placed inside the
quartz capillary connected to a 25 kV, 60 kHz in-house constructed high voltage power supply.
The ground electrode is a copper ring of 10 mm long placed around the capillary tube and axially
positioned at 40 mm away from the high voltage electrode tip. The gas flow was monitored by a
two channel power supply/readout (model: MKSPR400) and controlled by two mass flow meters
(Model: MKS 1179A).
Plasma is generated inside the quartz capillary and in between the electrodes excited by a 60 kHz sinusoidal wave voltage. The applied voltage was varied from 4 kV to 8 kV peak to peak. The applied voltage waveforms were monitored using a high voltage probe (Tektronix P6015A) and the current waveforms were monitored by a current probe (Tektronix TCP202). The current and voltage signals were recorded using a digital oscilloscope (Tektronix TDS 3034C). High
purity He with main impurity of H₂O present in the helium cylinder at a range of 0.5 ppm were used for generating plasma at flow rates ranging from 1 slm to 7 slm (slm: standard liters per minute). In our experiments, admixtures of oxygen at varied percentages from 0.1 to 1% of the primary He operating gas were employed by changing of the gas flow rates. Two types of optical diagnostics were used for characterization of the plasma pencil. The time and space resolved spectroscopy measurements of the plasma emission was carried out using a broadband spectrometer (Ocean Optics HR2000+ES) in the range of 200-900 nm with 0.83 nm resolution. The emission of OH radicals in the range of 300-350 nm (transition $A^2\Sigma^+ (v = 0) \rightarrow X^2\Pi (v = 0)$) was recorded with 500 µs integration time using a 0.08 nm resolution spectrometer system made up of a monochromator (Princeton Instruments Acton SP2750) and a CCD (ACTON IStar). The plasma emissions were collected in a direction perpendicular to the plasma pencil axis and at 1 mm increments in axial direction using a collimating lens. The plasma emission was transmitted to the spectrometer via optical fiber. The spectrometer was equipped with double grating turrets, one with 1200 grooves mm⁻¹ grating and the other 600 grooves mm⁻¹ grating.

B. Bacterial sample preparation and plasma treatment procedure

*S. pneumoniae* strain TIGR4 was grown in Todd-Hewitt broth with 0.3% yeast extract and cells were collected during logarithmic growth $\text{OD}_{620\text{nm}} = 0.5$ for solid surface cultures and $\text{OD}_{620\text{nm}} = 0.4$ for liquid media cultures. The bacterial concentration for $\text{OD}_{620} = 0.5$ was $10^7 - 10^8 \text{ CFU ml}^{-1}$ (CFU = colony forming units) and for $\text{OD}_{620} = 0.4$ was $10^6 - 10^7 \text{ CFU ml}^{-1}$. For treatments of solid surfaces, 100µl ($4 \times 10^6 \text{ CFU}$) of bacterial samples were inoculated onto the center of a 50 mm diameter Petri dish (plate). A disposable 35 mm wide Drigalski spatula was placed with one end at the inner edge of the Petri dish and the other end over the inoculated
sample. The plate was rotated at least 10 times to allow for spread of bacterial sample over the entire agar surface, allowing a pneumococcal density of approximately $2.04 \times 10^3$ CFU mm$^{-2}$ on the blood agar plate. For treatment of bacteria on the agar surface, the plasma pencil was fixed at distance of 10 mm between the plasma-pencil nozzle and agar surface. The cultures were exposed to 60s and 120s of either He or He/0.5% O$_2$. After treatment with plasma, the cultures were grown overnight at 37°C in 5% CO$_2$. The treated area was assessed in comparison to the untreated surrounding agar surface for zones of inactivation. The areas of the zones of inactivation were measured to quantify the amount of pneumococcal inactivation.

For treatment of the pneumococcus in liquid cultures, the bacteria suspension was prepared as described above to OD$_{620} = 0.4$. From the prepared bacterial suspension, 200 μl was transferred to a 96 well micro-well plate. The plasma pencil was fixed at distance of 3 mm between the plasma-pencil nozzle and the liquid media surface. The pneumococcal suspensions were exposed to 60s, 120s, and 300s of either He or He/0.5% O$_2$. After treatment, serial dilutions were performed and 10 μl of sample was placed onto a blood agar plate to grow overnight at 37 °C in 5% CO$_2$. After overnight growth, the efficacy of the inactivation was determined by taking CFU counts of the untreated and treated samples.

C. Electrical characterization

The electrical characterization experiments were performed on the atmospheric pressure plasma pencil with helium and oxygen admixtures. Initially, the helium gas was injected into the quartz capillary at a flow rate of 1 slm and the breakdown discharge occurred between the two electrodes and inside the quartz capillary occurred at a minimum input voltage of 3.5 kV$_{p-p}$. However, the discharge was unstable inside the quartz capillary at this minimum input voltage of 3.5 kV$_{p-p}$ and therefore plasma did not exit from the capillary in to surrounding air resulting an
absence of a prominent afterglow region. By increasing the input voltage to 4 kV$_{p-p}$, the plasma pencil successfully produced an afterglow plasma in to the open air and a long plasma plume length was formed. In order to diagnose the electrical properties of the plasma pencil, the voltage and current waveforms were measured during the discharges at 4, 4.5, 5, 5.5 and 6 kV$_{p-p}$ and the effects of gas flow rates and oxygen admixture percentages in the plasma discharge were investigated. Figure 2 shows the voltage and current waveforms for the plasma pencil for pure helium and helium-oxygen mixture (0.5% O$_2$ in the discharge) at a total gas flow rate of 4 slm.

The plasma pencil generates a very uniform discharge in pure helium gas and only two small current peaks exist on the current waveform during each half period of the applied voltage. Both peaks are smooth, the maximum current is 10.2 mA, and the duration of the current peaks are approximately 1 $\mu$s. A clear change in the voltage-current waveform was observed when oxygen was added to the plasma pencil. Addition of the oxygen to the plasma pencil results in a small increase of the current peak intensity to 10.8 mA and decrease of the current peaks duration to 0.8 $\mu$s. The results indicate that the voltage slightly decreases with increase of the current peaks.

Using the voltage-current waveforms, the average power (W) of the discharge was calculated according to the following equation, where T is the period of the discharge:\textsuperscript{20, 21}

\[
W = \frac{1}{T} \int_{t}^{t+T} I(t)V(t)dt.
\]  \hspace{1cm} (1)
FIG 2. Voltage and current waveforms as a function of time for (a) Applied voltage waveform (b) Current waveform for pure He and (c) Current waveform for He/O₂ mixture (0.5%), applied voltage is 6 kVₚ₋ₚ and the total gas flow rate is 4 slm.

FIG 3. Plasma powers as a function of applied voltage and oxygen percentage. (a) Effect of gas flow rate and applied voltage on plasma power (b) effect of oxygen percentage in the discharge on plasma power, at a total gas flow rate of 4 slm.
In Fig. 3.a, power dissipated in the discharge is presented as a function of the applied voltage for different operating gas flow rates used. The minimum power of the plasma pencil is observed to be about 8.8 W for pure Helium plasma at an applied voltage (peak-to-peak) of 4 kV and a gas flow rate of 1 slm. The applied voltage was increased up to 6 kV, which is the breakdown point of the quartz capillary, resulting in an increase of power up to 20.4 W for discharge in pure helium at a gas flow rate of 4 slm. The plasma power as a function of oxygen percentage in the plasma pencil is presented in Fig. 3.b. Basically, the power dissipation in pure helium discharge is higher, which is explained by broader current peaks. It is necessary to mention that the addition of oxygen to gas flow results in a decrease of the input power from 20.4 W (in case of pure helium) to 17.8 W and 15.8 W for discharges containing 0.1% and 1% of oxygen, respectively. The decrease in plasma power with addition of oxygen is due to contraction of the current peaks at higher amounts of oxygen in gas mixture that results in decrease of the integral determining power dissipation in the discharge.

D. Visualization of the plasma pencil in helium and helium/oxygen admixtures

The plasma pencil can be successfully launched in pure helium discharge with application of voltage at values higher than 3 kV (peak to peak). Addition of oxygen admixture to the feed gases required higher threshold voltages of up to 5 kV, especially at a higher concentration of oxygen (1% oxygen). The plasma pencil generated from a complete breakdown of entire inter-electrode gap (active zone) with formation of a long bright afterglow propagating in to open air and its plume length depends on the composition of the feed gas, applied voltage and flow rates of the operating gas. Figure 4.a displays color photographs of the plasma pencil (without Teflon cover) generated at 6 kV and in pure helium for different flow rates in the range of 1-7 slm and
FIG 4.b displays the images of the plasma pencil in He/O\textsubscript{2} gas mixture from 0.1-1\% of Oxygen in discharge.

![Image of plasma pencil](image)

FIG 4. (color) Photographs of the plasma pencil. (a) Images of plasma pencil in pure He as a function of gas flow rate. (b) Images of plasma pencil in He/O\textsubscript{2} mixtures for different oxygen percentage in discharge. Plasma power is fixed at 18 Watt.

In addition, we observed that the plasma pencil’s plume length greatly depends on the gas flow rate and it shows very small noticeable dependence with changes in applied voltage. Next, the plasma plume length was measured using the plasma pencil images. Figures 5.a and 5.b show
the plasma plume lengths as a function of helium gas flow rates and oxygen percentages in the discharge, respectively. As can be seen for discharges in pure helium (Fig. 5.a), the plume length at lowest flow rate of 0.5 slm is about 10 mm and sharply increases to the maximum value of 33 mm at a flow rate of 5 slm. The plasma pencil’s plume length at gas flow rate of 7 slm is about 26 mm. Fig. 5.b shows that the addition of the oxygen to the plasma pencil results in a sharp decrease of the pencil’s plume length. Plasma pencil’s plume length in pure helium discharge at 4 slm is 29.8 mm, while the plume length decreases with addition of oxygen to 20.12 mm and 5.1 mm, at discharges contain 0.1% and 1% of oxygen, respectively. The reason for decrease of the plume length is that oxygen is an electronegative molecular gas. The addition of oxygen to the plasma pencil results in electron attachment reactions with oxygen molecules; thus, the electron density in the discharge, and consequently the helium metastable concentrations in the discharge decreases resulting in a decrease of the plasma pencil’s plume length.

FIG 5. Plasma pencil’s plume length as a function of (a) He gas flow rate ranges from 0.5-7 slm and (b) Oxygen percentage in discharge from 0.1-1% with a fixed total gas flow rate of 4 slm.
E. Optical emission spectroscopy and plasma pencil species identification

Optical emission spectroscopy is considered to be a suitable tool in characterizing the plasma properties in terms of the identification of the reactive plasma species, plasma gas temperature evaluation and the relative emission intensities of the various species in different conditions.\textsuperscript{22-26} In the non-thermal plasma pencil at atmospheric pressure, the mean electron energy is significantly higher than the energy of neutral plasma species.\textsuperscript{27} The reaction between high energy electrons with helium, water vapor, nitrogen and oxygen at atmospheric pressure will induce the molecules to be dissociated, excited and ionized for production of different radicals and active species. In this section, the optical emission spectroscopy was used to study the plasma pencil in He and He/O\textsubscript{2} gas mixture and the reactive plasma species were identified. The axial and space resolved emission spectra in the range of 200-900 nm were recorded and analyzed. Axial emission spectrum of the plasma pencil allowed determination of the main reactive plasma species produced in the plasma pencil. In Fig. 6, the results of spectroscopic measurements with 0.83 nm resolution of the plasma discharge in He and He/O\textsubscript{2} mixtures (0.5\%) are presented. The emission spectra shown in Fig. 6.a, were recorded in the middle of the active zone region (the distance between the HV and ground electrode) of the plasma pencil. The emission spectra shown in Fig. 6.b, were recorded at 5 mm from the edge of the capillary (afterglow region).

The emission spectra are mainly composed of the spectral line of the He atoms. The most intensive emission lines correspond to He I transition $3p^3\text{P}^0\rightarrow 2s^3\text{S}$ at 388.8 nm, He I transition $3p^1\text{P}^0\rightarrow 2s^1\text{S}$ at 501.6 nm, He I transition $3d^3\text{D}\rightarrow 2p^3\text{P}^0$ at 587.6 nm, He I transition $3d^1\text{D}\rightarrow 2p^1\text{P}^0$ at 667.8 nm, He I transition $3s^3\text{S}^1\rightarrow 2p^3\text{P}^0$ at 706.5 nm, and He I transition $3s^1\text{S}^0\rightarrow 2p^1\text{P}^0$ at 728.1 nm.
FIG 6. Axial spectrums of the plasma pencil in He and He/O₂ mixtures (0.5%), (a) emission spectra of the active zone region and (b) emission spectra of the afterglow region of the discharge measured at 5 mm from the edge of capillary. The total gas flow rate is 4 slm and plasma power is fixed at 18 W.

In addition to He lines, the other identified reactive plasma species include intensive emission lines belonging to OH transition $A^2Σ^+ \rightarrow X^2Π$, $Δν = 0$, at 308 nm, and OH transition $A^2Σ^+ \rightarrow X^2Π$, $Δν = 1$, at 287 nm, the spectral band of the N₂ transition $C^3Π_u \rightarrow B^3Π_g$, $Δν = 0$, at 337.1 nm, H₂ transition 2p-3d at 656.3 nm, O I transition 3p³P→3s³S⁰ at 777.41 nm and O I transition 3p³P→3s³S⁰ at 844.6 nm. The OH radicals in the active zone region of the plasma are formed from water vapor impurities present in the helium gas cylinder.²⁸ The corresponding emission lines are identified based on the NIST atomic spectra database²⁹ and Lofthus et. al.³⁰

In order to investigate the effect of O₂ on the emission intensities of the reactive plasma species, different concentrations of the O₂ were added to the plasma pencil. Next, the emission spectra of the plasma with different O₂% in the discharge were collected and the emission lines
of OH at 308 nm (transition $A^2\Sigma^+ \rightarrow X^2\Pi$, $\Delta v = 0$), N$_2$ at 337 nm (transition $C^3\Pi_u \rightarrow B^3\Pi_g$), H$_\alpha$ at 656 nm (transition 2p $\rightarrow$ 3d), He at 706 nm (transition 1s3s $^3S$ - 1s2p $^3P^0$) and O I at 777 nm (transition 2s$^2$2p$^3(^4S\,^0)$3s $\rightarrow$ 2s$^2$2p$^3(^4S\,^0)$3p) were chosen for space resolved analysis of the emission spectroscopy. The results of spatial distribution of the active species intensities show that the emission spectrum of He/O$_2$ (0.5 %) mixture is very similar to pure helium discharge when comparing identified species. Moreover, it is clearly recognizable that the addition of oxygen to the plasma pencil results in a decrease of the emission lines intensities. The possible reasons for decrease of the emission line intensities are decreases of the plasma power with addition of the oxygen in the discharge and quenching of OH radicals by water vapor present within the helium gas cylinder or in open air.\textsuperscript{15, 31} Similar observation has been reported previously by others on plasma containing oxygen or other electronegative molecular gases.\textsuperscript{15, 21, 32}

As can be seen in Fig. 7, the discharge in pure helium shows a higher intensity of OH radicals compared to the plasma pencil that contains O$_2$ gas mixture for both cases (the active zone and afterglow region) of the plasma pencil. In the active zone of the plasma, the most intensive emission line belongs to O I at 777 nm, while in the afterglow region of the plasma pencil, N$_2$ emission line at 337 nm is the most intensive emission line in the emission spectra. Furthermore, addition of higher amount of oxygen to the plasma results in a decrease of these emission lines in the discharge.
FIG. 7. Normalized intensity of the selected reactive species as a function of the oxygen concentration in the discharge with a total gas flow rate of 4 slm. (a) in the active zone region of the plasma pencil and (b) in the afterglow region of the plasma pencil at 3 mm after the edge of capillary.

Oxygen is an electronegative gas, hence, when oxygen is added to the plasma pencil, the added oxygen captures significant free electrons and produces $\text{O}^{-}$ and $\text{O}^{2-}$ ions. Thus, the electron attachment by oxygen results in a decrease of the electron density and electron mean energy.\textsuperscript{33-35} The OH radicals are mainly produced by electron-water vapor collision process according to the following reaction, and therefore the decrease of the electron density leads to decrease of the intensities of OH and other atomic emission lines at a constant power.\textsuperscript{28, 33, 34}

$$e + \text{H}_2\text{O} \rightarrow e + \text{H} + \text{OH} \quad (2)$$

The reason for increase of O I intensity in the active zone region of the plasma pencil (see Fig. 7.a) is that when oxygen is added to the pure helium plasma pencil, the added oxygen can be dissociated according to the following reactions and results in a formation of singlet oxygen (O) and O (\textsuperscript{1D}).

$$e + \text{O}_2 \rightarrow \text{O}^{-} + \text{O} \quad (3)$$

$$e + \text{O}_2 \rightarrow e + \text{O} + \text{O} \textsuperscript{(1D)} \quad (4)$$
\[ e + O_2 \rightarrow 2e + O^+ + O \]  \hspace{1cm} (5)

In addition, in the afterglow region of the discharge when plasma pencil propagates in the open air, the OH radicals can be produced by the interaction of O (\( ^1D \)) and H\(_2\)O according to the following reaction:\(^{35}\)

\[ O (^1D) + H_2O \rightarrow 2 \text{OH} \]  \hspace{1cm} (6)

On the other hand, the singlet oxygen and ozone molecules generated in the discharge react with the produced OH radicals according to the following reactions and thus, the OH radicals intensity in the plasma pencil decreases with addition of oxygen percentage to the pure helium plasma pencil.\(^{35}\)

\[ \text{OH} + O \rightarrow H + O_2 \]  \hspace{1cm} (7)

\[ \text{OH} + O_3 \rightarrow \text{HO}_2 + O_2 \]  \hspace{1cm} (8)

F. Space resolved optical emission spectroscopy of the plasma pencil

The spatial distribution characteristics of chemically active species are very important in developing the plasma pencil kinetic model and understanding the mechanism of biomedical applications of the plasma pencil. Here, we present the spatially resolved spectra of the afterglow region of the plasma pencil.

The spatially resolved optical emission spectra of the afterglow region were collected for pure helium and helium/oxygen mixture (0.5%), with resolution of 2.5 mm. Next, the intensities of the intensive reactive plasma species were chosen for deeper analysis of the space resolved emission spectroscopy.
FIG. 8. The spatial distribution of the reactive species emission intensities as a function of the distance from the edge of capillary. (a) Pure He (b) He/O\textsubscript{2} (0.5%) 

As can be seen in Fig. 8, the emission intensity of the OH radicals (\(A^2\Sigma^+ \rightarrow X^2\Pi_\Delta\nu = 0\)) and N\textsubscript{2} (C\textsuperscript{3} \Pi_u \rightarrow B\textsuperscript{3} \Pi_g), H\textsubscript{a} and O I and helium vary with distance from the edge of quartz capillary. It can be seen from Fig. 8.a, that the emission intensity of helium, O I and N\textsubscript{2} (C\textsuperscript{3} \Pi_u \rightarrow B\textsuperscript{3} \Pi_g) rises initially up to 7 mm from the edge of capillary and then decreases, while the emission intensity of the OH (\(A^2\Sigma^+ \rightarrow X^2\Pi_\Delta\nu = 0\)) and H\textsubscript{a} decreases with increase of distance from the capillary. The emission intensity of N\textsubscript{2} at 10 mm is almost 8-10 times larger than the intensity at the edge of the capillary. Moreover, the emission intensity of N\textsubscript{2} reaches a maximum value after OH radicals. The maximum intensity of N\textsubscript{2} is located at 10 mm while the OH radicals’ maximum intensity is located at 7 mm. This behavior can be explained by the fact that H\textsubscript{2}O dissociation is initiated by electron induced processes inside the discharge and in the short distance after the edge of capillary, where the electron exhibit required the energy.\textsuperscript{15} In the case of the plasma pencil in helium/oxygen mixture (0.5%), the intensity decay of the helium in the plasma plume has an exponential decay, initially increasing up to 10 mm from the edge of
capillary and after that again decreasing (FIG 8.b). The most intensive emission line belong to O I, which is located inside the quartz capillary. The N₂ emission intensity reaches to its maximum outside the capillary and at 5 mm distance from the edge of capillary. The identified excited nitrogen molecules are produced from electron impact reactions in the plasma plume as it propagates into the ambient air. The main reason for the decrease of the emission intensities of the reactive plasma species with the distance from the edge of capillary is the decrease of the high energy electron density and mean energy. Outside the capillary the increase of the N₂ (SPS) emission could be a result of energy transfer from metastables and decreasing of the OH emission by increasing N₂ in the plasma pencil and lower H₂O density in the surrounding air compared to nitrogen.

The identified reactive plasma species such as the singlet oxygen, hydroxyl radicals, and neutral nitrogen molecules detected in the plasma pencil can play a significant role in biomedical applications especially operating at the atmospheric pressure. Similar significant generation of reactive oxygen species such as singlet oxygen (O) and O₃ were observed with He/O₂ gas mixture used in a plasma pencil which can result in enhanced efficacy of bacterial inactivation. It is believed that the oxygen species in the discharge contributes to the sterilization process due to its strong oxidative effect on the outer structure of the cells, while the OH radicals generated by the plasma plays a significant role through chemically attacking the outer structure of the cells and the production of the nitrogen containing group adds to the lethality of the process.
G. OH rotational temperature determination using Boltzmann plot method

The plasma gas temperature has been derived from OH rotational temperatures. The OH rotational temperature is usually considered to be equal to the plasma gas temperature considering the low rotational level of the OH radicals OH (A-X) and small rotational quantum numbers $J$.\textsuperscript{15, 40-42} The Boltzmann plot of the rotational population of the OH radicals has been widely used for this purpose.\textsuperscript{15, 40, 43, 44} Figure 9 shows the high resolution emission spectra of the OH radicals in pure helium and helium oxygen (0.5%), respectively. Using the OH emission spectra and assuming that the OH rotational states are Boltzmann distributed according to the following equation

$$ I_{JJ'} \approx h\nu_{JJ'}(2J + 1)A_{JJ'} \exp \left( -\frac{B_v h\nu_{JJ'}(J + 1)}{k_BT_{rot}} \right), \quad (9) $$

where $\nu_{JJ'}$ is the frequency of transition from rotational level $J'$ to $J''$, $A_{JJ'}$ is the corresponding transition probability, $B_v$ is the rotational constant, $k_B$ is the Boltzmann constant and $h$ is the Planck constant. The rotational energy $E(cm^{-1})$ equals to $B_v J(J + 1)$. The energy and probability data of OH transitions were taken from literature.\textsuperscript{45, 46} The corresponding OH rotational temperature is equal with gas temperature when the rotational population distribution is in equilibrium with heavy species translational temperature in the discharge. In atmospheric pressure plasma, this assumption is valid on atmospheric pressure plasmas when high collisional reactions occur. A detailed description of the used method can be found in Sarani. et al. 2010.\textsuperscript{15}
FIG. 9. High resolution OH emission spectra. (a) Pure He. (b) He/O$_2$ (0.5%). Plasma power is 18 W and total gas flow rate is 4 slm.

FIG. 10. Boltzmann plot of the OH ($A^2\Sigma - X^2\Pi$) transition in plasma of (a) Discharge in pure He and in active zone region (between HV and ground electrodes). (b) Discharge in He/O$_2$ mixture (0.5%) and in active zone region (between HV and ground electrodes). (c) Discharge in pure He and in the afterglow region. (d) Discharge in He/O$_2$ mixture (0.5%) and in the afterglow region. Plasma power was fixed at 18 W.
In Figure 10, we presented $\ln(I_\lambda/(2J+1)A_{jj})$ for transition $OH(A^2\Sigma - X^2\Pi)$ as a function of levels energy for $J<13$. This plot describing distribution of $OH(A^2\Sigma)$ radicals among rotational levels for pure helium and helium/oxygen mixture (0.5%) at discharge generated at fixed power of 18 W. Based on the Boltzmann plot method, the OH rotational temperature in the active zone region of the plasma pencil was measured to be 309.52 K and 366.48 K, for pure helium and helium/oxygen gas mixture (0.5% oxygen), respectively. Whereas the measured rotational temperature in the afterglow region at 5 mm away from the capillary edge was increased to 315.66 K and 362 K, for pure helium and helium/oxygen gas mixture (0.5% oxygen), respectively. As can be seen, the addition of the oxygen to the plasma pencil results in an increase of the OH rotational temperature. The excited OH radicals characterized by low rotational temperature are produced by electron impact excitation of ground state OH according to flowing reaction.\(^15\)

$$OH(X^2\Pi) + e \rightarrow OH(A^2\Sigma)^+ + e, \quad (E_{\text{threshold}} = 4\,eV), \quad (10)$$

**H. Electron density calculation**

The analysis of the stark broadened line profiles is a well-established technique for plasma diagnostics. Plasma electron density can be obtained from some of their characteristics such as their full width at half maximum (FWHM).\(^{47-49}\) One of the most common methods for determination of the electron density is using the hydrogen Balmer full width at half maximum.\(^{47}\)

At atmospheric pressure, the profile of atomic spectral lines emitted from the plasma can be approximated by a Voigt function. In this work, the line shape of the hydrogen alpha transition is determined by Lorentzian and Gaussian broadening mechanisms that results in a Voigt function.\(^{41}\) The Lorentzian part of the profile correspond to the contribution of the Stark and Van
der Waals collisional broadening that have their origin in the interaction between the emitting particles and the particles around them. The Gaussian part of the profile corresponds to the contribution of the Doppler and instrumental broadening. The Doppler broadening is due to the thermal movement of the emitted particle and instrumental broadening caused by measurement devices. The broadening due to the natural lifetime is too small and is not taken into account in the present work.

For atmospheric pressure plasmas, low gas temperature condition, and electron densities around $10^{14}$ cm$^{-3}$, the Van der Waals broadening has to be taken into account. The van der Waals broadening is caused by the neutral perturbers and its FWHM can be estimated as

$$\Delta \lambda_{dW} = 8.18 \times 10^{-26} \lambda_0^2 (\bar{R}^2) \frac{2}{T_{\text{gas}}} \frac{3}{N} \sum_i \left( \frac{a_i^2 \chi_i}{\mu_i} \right)$$

(11)

where $\lambda_0$ is the wavelength in nm, $\mu$ is the reduced mass in atomic mass unit, $N$ is the neutral particle density in cm$^{-3}$, $i$ is the helium gas and $\chi$ is the fraction of the perturber. The value of the polarizability of the $\alpha$ perturber for helium gas is $2.05 \times 10^{-25}$ cm$^3$. After considering the fine structure and calculating the value for atomic lines, the FWHM can be estimated as

$$\Delta \lambda_{dW} (nm) = \frac{C}{T^{10}}$$

(12)

where $C$ is a constant for Van der Waals broadening and in the case of the hydrogen alpha line in helium gas has the value of 2.42. Using the above equation the Van der Waals broadening was estimated as 0.0393 nm.

The Doppler broadening and Resonance broadening are not taken into account in the present work because their values are negligible due to the low gas temperature and atmospheric
pressure conditions. In this work, the instrumental slit function is well approximated by a Gaussian profile due to the fact that monochromator slits with equal width were used. The shape and the broadening caused by instrumental were determined by using a Mercury-Argon pen lamp and considering the emission line at 435 nm. The instrumental profile resulted after using our experimental device very well fitted with a Gaussian function with an instrumental width of 0.17 nm. The convolution of the above Lorentzian and Gaussian line shapes results in a so-called Voigt shape with FWHM, $\Delta \lambda_V$ of $^{52}$

$$\Delta \lambda_V \approx \sqrt{\frac{\Delta \lambda_L}{2} + \Delta \lambda_G^2 + \frac{\Delta \lambda_L}{2}}$$ (13)

The Lorentzian full width at half maximum (FWHM) is the sum of the Lorentzian FWHMs, while the Gaussian FWHM is the square root of the sum of the squared Gaussian FWHMs.$^{41}$ In order to determine the electron density, the measured line of the H$\alpha$ is fitted with a Voigt function with FWHM of 0.22 nm. Figure 11 shows the measured line broadening of H$\alpha$ line for the helium plasma fitted with a Voigt function along with the instrumental broadening of the line at 435 nm of the Mercury-Argon pen lamp fitted with Gaussian function.

FIG 11. An example of the Voigt fit of the H$\alpha$ line for pure helium plasma along with the instrumental broadening of the Mercury-Argon pen lamp fitted with Gaussian function.
The Lorentzian part of the FWHM is used to estimate the Stark broadening which results in a $\Delta \lambda_s$ of 0.014 nm. Using Eq. (14) for Stark broadening of the H$_{\alpha}$,\textsuperscript{51} the electron density in the afterglow region of the plasma pencil was estimated as $2.1 \times 10^{14}$ cm$^{-3}$.

$$\Delta \lambda_s = 1.78 \left( \frac{n_e}{10^{23} m^{-3}} \right)^{\frac{2}{3}}$$ \hfill (14)

I. Plasma pencil treatment of \textit{S. pneumoniae} on blood agar plates and in liquid media

The plasma treatment of the pneumococcus on the solid blood agar plates caused inactivation within the treated surface area. A time-dependent and input gas-dependent response was observed in the pneumococcal inactivation on the solid blood agar surface as demonstrated in Figure 12. A mean inactivation of $4.0 \times 10^4$ CFU and $1.3 \times 10^5$ CFU was observed after treatment for 60s with He plasma and He/0.1% O$_2$, respectively. By increasing the treatment time to 120s, a mean inactivation of $5.2 \times 10^5$ CFU and $1.0 \times 10^6$ CFU was identified for He and He/0.1% O$_2$ plasma, respectively. It was interesting that a mere 0.1% addition of oxygen gas was able to double the inactivation rate of the plasma treatment. In a similar study using \textit{Streptococcus mutans}, a close relative to the pneumococcus, investigators identified an increase in the inactivation by injection of O$_2$ into the He plasma.\textsuperscript{53} It has been known for some time now that oxygen radicals play a role in bactericidal activity.\textsuperscript{54} This has further been demonstrated with several other plasma systems.\textsuperscript{7, 55, 56} Another aspect of this research identified that doubling of the treatment time allowed for the formation of widespread inactivation zones. The respiratory tract is covered with a mucous membrane that secretes mucus to lubricate and protect the membrane.\textsuperscript{57}
FIG 12. Plasma treated area (indicated by white circles) on agar plate inoculated with *S. pneumoniae*. (a) discharge in pure He and (b) discharge in He/O$_2$ (0.1%).

During pneumococcal infection, fluid buildup occurs around the mucous membranes due to increased swelling. To identify the effectiveness of the plasma pencil for inactivation of *S. pneumoniae* in liquid, we treated pneumococcal suspensions in liquid media. As was identified with treatment of solid surfaces, we observed a time-dependent and input gas dependent response to plasma treatment. Pneumococcal suspensions in liquid media treated with He plasma for 60s, 120s, and 300s did not show a significant inactivation as compared to untreated samples. However, the addition of 0.1% O$_2$ to the admixture allowed for a 40% inactivation from $1.8 \times 10^7$ CFU ml$^{-1}$ to $7.1 \times 10^6$ CFU ml$^{-1}$ ± SEM $2.9 \times 10^6$ of the pneumococcal suspension after 300s of treatment. We have designed, constructed and investigated a low temperature plasma pencil driven by a kHz AC power supply. The electrical and optical characteristics of the plasma pencil in helium and helium/oxygen gas mixture have been investigated using electrical characterization and optical emission spectroscopy diagnostic techniques. The effects of oxygen at various concentrations on the helium plasma pencil were investigated. The plasma power was
estimated to be in the range of 8-20 W for different applied voltages and gas mixtures. Identification of the different radicals and excited species produced in the active zone and afterglow region of the plasma pencil have been carried out. The emission spectroscopy analysis revealed that the most intensive emission lines belong to OH transition $A^2 \Sigma^+ (\nu = 0,1) \rightarrow X^2 \Pi (\Delta \nu = 0)$ at 308 nm and OH transition $A^2 \Sigma^+ (\nu = 0,1) \rightarrow X^2 \Pi (\Delta \nu = 1)$ at 287 nm, O I transition 3p$^5$P$\rightarrow$3s$^5$S$^0$ at 777.41 nm, O I transition 3p$^3$P$\rightarrow$3s$^3$S$^0$ at 844.6 nm and N$_2$(C-B) second positive system (SPS) with electronic transition $C^3\Pi_u \rightarrow B^3\Pi_g$ in the range of 300-450 nm. Also, different atomic emission lines include He I transition 3p$^3$P$^0$$\rightarrow$2s$^1$S at 388.8 nm, He I transition 3p$^1$P$^0$$\rightarrow$2s$^1$S at 501.6 nm, He I transition 3d$^3$D$\rightarrow$2p$^3$P$^0$ at 587.6 nm, He I transition 3d$^1$D$\rightarrow$2p$^1$P$^0$ at 667.8 nm, He I transition 3s$^3$S$^1$$\rightarrow$2p$^3$P$^0$ at 706.5 nm, He I transition 3s$^1$S$^0$$\rightarrow$2p$^1$P$^0$ at 728.1 nm and H$_\alpha$ transition 2p-3d at 656.3 nm have been identified. The effect of adding oxygen on electrical and spectral properties of the plasma pencil has been investigated. Addition of oxygen to the plasma pencil resulted in a decrease in plasma power and therefore decreased the electron density and also the intensity of emission lines. Plasma density has been estimated from Stark broadening of the hydrogen line at 656 nm and is in order of $2.1 \times 10^{14}$ cm$^{-3}$. The plasma gas temperature was evaluated using OH rotational temperature. The OH rotational temperature for pure He plasma is 315.66 K and is 362 K for pure helium and helium/oxygen gas mixture (0.5% oxygen), respectively. The plasma pencil generates significant amounts of reactive species at low gas temperatures which promises its great potential for a range of biomedical applications. The preliminary work with inactivation of $S. \ pneumoniae$ on solid surface and liquid suspension using the plasma pencil suggests this as a potential tool for biomedical applications. Further studies on the biological side such as the effects on inactivation on mucosal tissue need to be assessed.
KEY RESEARCH ACCOMPLISHMENTS

The project has met the project deadlines for majority of the proposed tasks under the Objective 1 and 2 during this period year. The status and remarks of the project milestones are given below.

Table 2. Status and remarks of the project milestones

<table>
<thead>
<tr>
<th>Task</th>
<th>Proposed Milestones</th>
<th>Base Line Plan Date</th>
<th>Status/Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Objective 1: Establish Plasma Engineering Research Lab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Setup a direct current - atmospheric - resistive barrier cold plasma system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>2</td>
<td>Setup a 13.56 MHz radio frequency dielectric barrier plasma system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>3</td>
<td>Setup a 900 MHz/2.45 GHz wave plasma system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>4</td>
<td>Setup a laser induced breakdown plasma experimental system</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>5</td>
<td>Implement plasma shadowgraphy diagnostics Setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>6</td>
<td>Implement a two color laser interferometry diagnostics setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>7</td>
<td>Implement a optical emission spectroscopy diagnostics setup</td>
<td>26 OCT 2011</td>
<td>Completed</td>
</tr>
<tr>
<td>II. Objective 2: Develop Portable Plasma Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Design phase: Design an optimized portable plasma source system</td>
<td>26 OCT 2012</td>
<td>Completed</td>
</tr>
<tr>
<td>9</td>
<td>Construction phase: Construct the portable plasma source based on the design analysis and utilizing the existing resources and knowledge gained from objective 1.</td>
<td>26 OCT 2012</td>
<td>Completed</td>
</tr>
<tr>
<td>10</td>
<td>Testing and characterization phase: Portable plasma source will be tested and characterized for its operating parameters and plasma parameters.</td>
<td>26 OCT 2014</td>
<td>In Progress</td>
</tr>
<tr>
<td>11</td>
<td>Biological testing: In-vitro biological testing.</td>
<td>26 OCT 2014</td>
<td>In Progress</td>
</tr>
</tbody>
</table>
REPORTABLE OUTCOMES

A. MENTORING

The PI is dedicated to mentor students of diversity and underrepresented minority groups.

The PI has mentored the following scientists in the research
Dr. Zhen Ma
Dr. Kenneth Williamson
Dr. Abdollah Sarani
Dr. Cosmina Nicula
Xavier Gonzales

The PI has mentored the following Graduate Students
Heather Anderson
Anudeep Reddy Kandi
Jennifer Chancellor

The PI has mentored the following Undergraduate Students
Eduardo Valdez
Francisco Rodriguez
Bokang Yang
Thurman Walling
James Shames
Isaac Colmenero
Kim Pham
Megan Norfolk
Jennifer Anderson
Amanda Whitmill
B. CONFERENCE PUBLICATIONS


105. Valerie Ferdin, Abdollah Sarani and Magesh Thiyagarajan. Surface Modification of Polyethylene Terephthalate by Atmospheric Pressure Non-thermal Plasma Jet. (College Station, TX: 9th Annual LSAMP Conference (Best Paper Award), 2013).

104. Eduardo Valdes, Abdollah Sarani and Magesh Thiyagarajan. Surface modification of Polyurethane sheets using an atmospheric pressure RF plasma jet in helium/oxygen gas mixture. (College Station, TX: 9th Annual LSAMP Conference (Best Paper Award), 2013).


92. Heather Anderson, Magesh Thiyagarajan, Xavier Gonzales. In Vitro Cold Plasma Treatment Inducing Apoptosis in Acute Myeloid Leukemia Cancer Cells. (Corpus Christi, TX: TAMUCC ELITE Graduate Symposium (Best Paper Award), 2012).

91. Hoang Thi Pham, Magesh Thiyagarajan, Guadalupe Vidal. Sterilization of Pseudomonas aeruginosa with non thermal plasma and the effect of non-thermal plasma on bacterial growth. (Corpus Christi, TX: TAMUCC Honors Symposium, 2012).


83. Jennifer Ausland, Guadalupe Vidal, Kim Pham, Magesh Thiyagarajan. Effective non-thermal plasma deactivation of Bacillus cereus and Salmonella Typhimurium in chicken...
poultry, eggs, tomato and papaya. (Galveston, TX: 10th Annual Pathways Research Conference, 2012).

82. Heather Anderson, Megan Norfolk, Magesh Thiyagarajan, Xavier Gonzales. *In Vitro Cold Plasma Treatment Inducing Apoptosis in Acute Myeloid Leukemia Cancer Cells.* (Galveston, TX: 10th Annual Pathways Research Conference, 2012).


C. JOURNAL MANUSCRIPTS AND BOOKS PUBLISHED


Conclusion
The PI and the research team have completed majority of the proposed tasks on or before the deadline and the tasks that are in progress will be completed in the upcoming year. The research outcomes are demonstrated. The research team has been taking initiatives for the future research work on animal testing and is aiming to request additional funding from funding agencies.
APPENDIX A – PI Resume

Magesh Thiyagarajan, Ph.D.
Assistant Professor of Engineering
Director of Plasma Engineering Research Lab (PERL)
School of Engineering & Computing Sciences, College of Science and Engineering
Texas A&M University – Corpus Christi
6300 Ocean Drive, EN 222D, Corpus Christi, TX 78412, USA
Phone: 361-825-2144, Fax: 361-825-3056, Email: Magesh@tamucc.edu
http://sci.tamucc.edu/~magesh

Academic Preparation

- PhD, Electrical Engineering (Applied Plasma Physics), University of Wisconsin - Madison, 2008 (Work completed December 2007)
- BE, Electrical and Electronics Engineering, University of Madras - India, 2001. (University Gold Medalist)

Research Expertise

Plasmas, atmospheric pressure plasmas, air plasmas, non-thermal (cold) plasmas, laser induced plasmas, resistive barrier discharges, optical diagnostics, laser shadowgraphy, laser interferometry, optical emission spectroscopy, laser induced RF coupled plasmas, direct current plasmas, ball lightning, plasma medicine, plasmas in applied microbiology, plasmas in biomedical applications, plasma induced cancer treatment.

Employment and Professional Experience

Assistant Professor of Engineering - Director of Plasma Engineering Research, Texas A&M University - Corpus Christi. (July 2009 - Present).

- Direct Plasma Engineering & Science Research Projects
- Mentor interdisciplinary students to carry out research
- Lead the research group on portable plasma - biomedical device for combat care application
- Lead the research group on bacterial deactivation through cold plasma
- Lead the research group on treatment of cancer cells through cold plasma
- Teach engineering course in the undergraduate engineering program
- Key Lead on Plasma Medicine and Plasma Sterilization of Medical Devices.
- Task leader and key contributor for large scale plasma waste gasification project.
- Extensive engineering, R&D and process control in optical data storage project.
- Technology, market research, fetched government proposals for a total of $1.6 Million.

Research Fellow, University of Wisconsin – Madison, WI. (June 2004 - December 2007).
- Laser and RF Plasmas for Industrial and Military Applications
- The principal focus of my research is to design, implement, diagnose and characterize the laser induced plasmas for advanced industrial and military application.
- Designed and built experimental setups to characterize high power 10 MW UV laser focused plasma in the presence of dielectric window materials for micro machining.
- Collaborated with MIT and Texas Tech University research groups for optimized results.

Teaching Assistant, University of Wisconsin – Madison, WI. (August 2004 - June 2006).
- Taught Electric Circuits Laboratory Course – for four semesters with class strength of 30.
- Received the Honorable mention for exceptional teaching performance during 2004 – 05.

- Designed and developed a novel plasma stealth antenna prototype for military applications.

- Developed a 25 kV high-voltage experimental plasma ball lightning system

Research Assistant, University of Tennessee – Knoxville, TN. (August 2002 - May 2004).
- Developed an effective biological plasma decontamination system- Patent Pending.
- Presented research results to funding sources and fetched $2 Million for further research.
- Designed a commercial model of the plasma decontamination unit.

- Designed Analog and Digital IC circuit design using TTL and LSI chips.
- Team Leader: Designed and implemented a Transistor Curve Tracer project.

Professional and Honor Society Memberships

- Nuclear and Plasma Sciences Society
- American Physical Society
- Institute of Electrical and Electronics Engineers (IEEE)
- American Society of Mechanical Engineers (ASME)
- NASA Texas Space Grant Consortium
- Louis Stokes Alliances for Minority Participation (LSAMP)
- McNair Scholars Program
- Sigma Xi
- American Society For Microbiology
- Tau Beta Pi – Engineering Honor Society
- Eta Kappa Nu – Electrical Engineering Honor Society
- MIT Entrepreneur Club
- Toastmasters International Club

Licensures, Certifications & Trainings

- Six-Sigma Green Belt Certification, General Electric Company (GE).
- COMSOL Multiphysics Simulation Training, COMSOL.
- TRIZ (Innovative way for new ideas), General Electric Company (GE).
- Hiring the Right People, General Electric Company (GE).
- Entrepreneurial Business Program Certification, School of Business, UW-Madison.
- Biological Defense Safety Program and Technical Safety, TAMUCC.
- Laser Safety Certification, Laser Institute of America, TAMUCC.
- Professional Ethics, IEEE Corpus Christi Section.
- CITI Collaborative Institutional Training Initiative –
  o Responsible Conduct of Research, TAMUCC.
  o Working with Institutional Animal Care and Use Committee, TAMUS HSC.
  o Working with Mice in Research Settings, TAMUS HSC.
  o Reducing Pain and Distress in Laboratory Mice and Rats, TAMUS HSC.
Research and Scholarly Activities

Funded Research Grants

1. Magesh Thiyagarajan (Principal), "Lightweight Portable Plasma Medical Device," Sponsored by Department of Defense - Telemedicine and Advanced Technology Research Center - USAMRAA, $700,000.00. (September 2010 - September 2014).


3. Magesh Thiyagarajan (Principal), "Enhanced graduate and undergraduate student learning through enhanced research infrastructure and integrated interdisciplinary research practices,” Sponsored by Title V Program, U.S. Department of Education through Texas A&M University-Corpus Christi, $141,198.38. (September 2013 - August 2014).

4. Magesh Thiyagarajan (Principal), "Novel Cold Plasma Therapy for Treatment of Cutaneous Squamous Cell Carcinoma (Skin Cancer)," Sponsored by Texas Research Development Fund (TRDF) Post-Doctoral Support Grant, $25,000.00. (September 2012 - August 2013).


8. Magesh Thiyagarajan (Principal), "Cold Plasma & NOx Induced Apoptosis Research on Various Human Cell Structures Aimed for Skin Cancer Treatment," Sponsored by Texas Research Development Fund (TRDF), $25,000.00. (September 2010 - September 2012).


10. Waldbeser, Lillian S (Principal), Magesh Thiyagarajan (Co-Principal), "The Effect of Non-thermal Plasma on Human Leukemia and Lymphoma Cells," Sponsored by University Research Enhancement Grant, $9,000.00. (September 2010 - August 2011).

12. Magesh Thiyagarajan (Principal), Mark Olson (Principal), "Dynamic Light Scattering for Nanotechnology Research and Education," Sponsored by Higher Education Fund (HEF), State, $60,000.00. (2011 - 2012).

13. Magesh Thiyagarajan (Principal), Integrating Sustainability into Courses: Principles and Tools to Expand Your Educative Capacity, National Science Foundation, NSF grant DUE, $1000.00 0716599. (October 2010)


Submitted Research Proposals


17. Magesh Thiyagarajan (Co-Principal), Dugan Um (Principal), “Research Experience for Undergraduates Site: Taking Ethical Considerations into MEMS (Micro Electro Mechanical System) Research” Sponsored by National Science Foundation (NSF), $453,790.00. (2013).


19. Magesh Thiyagarajan (Principal), “Young Faculty Award (YFA) – Laser Plasma Induced Functional Inorganic Nanofluids with Unprecedented Specificity and Reactivity” Sponsored by DARPA, $273,368. (2012)


22. Magesh Thiyagarajan (Principal), LD Chen (Co-Principal), Mehrube Mehrubeoglu (Co-Principal), Paul Zimba (Co-Principal), Mark Olson (Co-Principal), “MRI: Acquisition of a transmission electron microscope (TEM).” Sponsored by National Science Foundation (NSF), $1,048,248.00. (2012).


26. **Magesh Thiyagarajan** (Principal), Chen, Lea-Der (Co-Principal), Tintera, George Dunkin (Co-Principal), Balasubramanyam, Mirley (Co-Principal), "Collaborative Research: Improving Student Reflection and Metacognitive Thinking: A Texas Collaborative for Faculty Development (IMRT)," Sponsored by National Science Foundation (NSF), $64,706.00. (2011).

27. Waldbeser, Lillian (Principal), **Magesh Thiyagarajan** (Co-Principal), Mehrubeoglu, Ruby (Co-Principal), Jones, Harlan (Co-Principal), "Skin Cancer Treatment and Monitoring by Nonthermal Plasma-hyperspectral Imaging," Sponsored by DHHS - National Institutes of Health, $452,371.00. (2011).


30. **Magesh Thiyagarajan** (Co-Principal), Tintera, George Dunkin (Co-Principal), Fernandez, John D (Co-Principal), Balasubramanyam, Mirley (Co-Principal), "Collaborative Research: Improving Student Reflection and Metacognitive Thinking: A Texas Collaborative for Faculty Development," NSF – CCLI Phase 2, Federal, $59,932.00. (2010).

31. **Magesh Thiyagarajan** (Co-Principal), Um, Dugan (Principal), Karayaka, Hayrettin (Co-PI), Simionescu, Petru-Aurelian (Co-PI), "CNS - CISE - Research Experiences for Undergraduates Sites (Computer Sci. & Engg)," NSF - REU, $328,665.00. (2010).

32. **Magesh Thiyagarajan** (Co-Principal), Waldbeser, Lillian (Principal), Mehrubeoglu, Ruby (Co-Principal), "Induction of program cell death of melanocytes and other skin cancer cells by ionized plasma," CPRIT-Cancer Prevention and Research Institute of Texas, $1,063,429.00. (2010)

33. **Magesh Thiyagarajan** (Co-Principal), Katsman, Alex (Principal), "Universal Atmospheric Contaminant Scrubber for Submersibles", Source: NAVY - Small Business Innovative Research (SBIR). $ 100,000, (2009)


Publications

Peer-Reviewed Books


Peer-Reviewed & Indexed Book Chapter Series


Peer-Reviewed Journal Publications (ISI Journals)


10. **Magesh Thiyagarajan**, Heather Anderson†, Xavier F. Gonzales‡. “Induction of apoptosis in human myeloid leukemia cells by remote exposure of resistive barrier cold plasma.” *Biotechnology and Bioengineering*. (Accepted for publication in the next issue in 2013)


**Manuscripts Currently Under Peer-Review**

Postdoc$, Graduate Student#, Undergraduate Student*


Publications – Professional Conference Proceedings & Abstracts


4. Xavier Gonzales, Guadalupe Vidal-Martínez, Jennifer Ausland, Kim Hoang Pham, **Magesh Thiyagarajan**. Air Plasma Jet Induced Bacterial Inactivation in Dry, Aqueous and Food Environments. (San Francisco, California: 40th IEEE International Conference on Plasma Science (ICOPS), 2013).


16. Eduardo Valdes\textsuperscript{a}, \textbf{Magesh Thiyagarajan}, Abdollah Sarani\textsuperscript{a}. \textit{Surface modification of planar Polyurethane using an atmospheric pressure RF plasma jet in helium/oxygen gas mixture.} (Seattle, WA: SACNAS National Conference \textbf{(Best Paper Award)}, 2012).

17. \textbf{Magesh Thiyagarajan}, Guadalupe Vidal-Martínez\textsuperscript{a}, Alison Doyoungan\textsuperscript{a}, Hoang Pham\textsuperscript{a}. \textit{Cold plasma inactivation of Escherichia coli on agar plates as an alternative sterilization method and its effect on bacterial growth.} (San Francisco, CA: 112th ASM - American Society for Microbiology Conference, 2012)

18. Guadalupe Vidal-Martínez\textsuperscript{a}, Hoang Pham\textsuperscript{a}, \textbf{Magesh Thiyagarajan}. \textit{The effect of cold plasma on Staphylococcus aureus and Pseudomonas aeruginosa growth and its potential use as an alternative method for infection treatment.} (San Francisco, CA: 112th ASM - American Society for Microbiology Conference, 2012)


21. \textbf{Magesh Thiyagarajan}, Guadalupe Vidal\textsuperscript{b}, Hoang Pham\textsuperscript{a} and Jennifer Ausland\textsuperscript{a}. \textit{Effect of Non-thermal Plasma Exposure on Regrowth Potential of Foodborne and Nosocomial Pathogens.} (Edinburgh, Scotland: 39th IEEE International Conference on Plasma Science (ICOPS), 2012).


**Publications – Symposium Presentations and Abstracts**

$Postdoc, #Graduate Student, *Undergraduate Student

1. Valerie Ferdin*, Abdollah Sarani$ and **Magesh Thiyagarajan**. *Surface Modification of Polyethylene Terephthalate by Atmospheric Pressure Non-thermal Plasma Jet*. (College Station, TX: 9th Annual LSAMP Conference ([Best Paper Award](#)), 2013).

2. Eduardo Valdes*, Abdollah Sarani$ and **Magesh Thiyagarajan**. *Surface modification of Polyurethane sheets using an atmospheric pressure RF plasma jet in helium/oxygen gas mixture*. (College Station, TX: 9th Annual LSAMP Conference ([Best Paper Award](#)), 2013).


10. Samantha Valdez*, Heather Anderson†, Xavier Gonzales‡, Magesh Thiyagarajan. Application of Non-thermal Plasma on Acute Myeloid Leukemia Cells to Induce Apoptosis. (College Station, TX: 9th Annual LSAMP Conference 2013).


12. Heather Anderson†, Magesh Thiyagarajan, Xavier Gonzales‡. In Vitro Cold Plasma Treatment Inducing Apoptosis in Acute Myeloid Leukemia Cancer Cells. (Corpus Christi, TX: TAMUCC ELITE Graduate Symposium (Best Paper Award), 2012).


15. Heather Anderson†, Megan Norfolk*, Magesh Thiyagarajan, Xavier Gonzales‡. In Vitro Cold Plasma Treatment Inducing Apoptosis in Acute Myeloid Leukemia Cancer Cells. (Galveston, TX: 10th Annual Pathways Research Conference, 2012).


17. Eduardo Valdez* and Magesh Thiyagarajan. Characterization of Diffused Atmospheric Pressure Cold Plasma Reactive Gas Species for Surface Treatment. (Corpus Christi, TX: 8th Annual LSAMP Conference (Best Paper Award), 2012).


23. Heather Anderson†, Megan Norfolk*, Magesh Thiyagarajan, Xavier Gonzales‡. In Vitro Cold Plasma Treatment Inducing Apoptosis in Acute Myeloid Leukemia Cancer Cells. (Corpus Christi, TX: TAMUCC 2nd Annual S&E Graduate Student Research Forum, 2012)


35. M Norfolk and Magesh Thiagarajan. Pre-programmed Cell Death in Acute Monocytic Leukemia Cancer Cells Induced by Nonthermal Ionized Plasma. (Corpus Christi, TX: Sigma Xi 11th Annual Undergraduate Research Symposium (Best Paper Award), 2011).


Research News Highlights, Media Articles & Non-Refereed Publications


15. Local firm eyes mass production - Technology speeds the healing process, can sterilize surfaces, Caller Times, 2010.
Invited Research Presentations & Demonstrations


3. Magesh Thiyagarajan, "Plasma Research Projects Presentation," Texas Engineering Experiment Station (TEES) leadership Team visit, Plasma Engineering Research Lab, TAMUCC. (May, 2013)


5. Magesh Thiyagarajan, "Plasma Research Projects Presentation” U.S. Senator John Cornyn visit, Coastal Bend Innovation Center, TAMUCC. (May, 2013)


15. Magesh Thiyagarajan, "Plasma Research Projects Presentation,” Dr. Dennis O’Neal, Deputy Director of TEES visit, Plasma Engineering Research Lab, TAMUCC. (October 2011).


Dissertation, Thesis, Project and Research Supervision

1. Postdoctoral Research Associates – Research Projects
   a. Dr. Abdollah Sarani (Plasma Applied Physics) "Non-thermal Plasma Jets for Materials and Biomedical Applications" (2012 - Present)
   b. Dr. Xavier Gonzales (Biomedical Sciences) "Cold Plasma Induction and Biological Mechanisms of Apoptotic Behavior in THP-1 Leukemia Cancer Cells" (October 2011 - Present)
   c. Dr. Kenneth Williamson (Mechanical Engineering) "1064 nm IR High Power Laser Induced Plasmas and Optical Diagnostics" (August 2011 - 2012)
   d. Dr. Guadalupe Vidal (Microbiology) "Cold Plasma Induced Cell Deactivation Mechanisms in Various Infection Causing Bacteria" (October 2011 - 2012)

2. Postdoctoral Visiting Scholar – Research Projects
   a. Dr. Zhen (John) Ma, Canadian Space Agency (CSA) (Mechanical Engineering) "Conceptual Design of a Non-thermal Plasma Source" (November 2010 - February 2011)
   b. Dr. Cosmina Nicula (Electrical Engineering) " Non-thermal Plasma Jets for Materials and Biomedical Applications" (October 2011 - Present)
3. Master's Graduate Thesis Committee Chair
   
i. **Heather Anderson**, TAMUCC (Biology) "Non-thermal Plasma Induced Apoptosis in THP-1 Leukemia Cancer Cells" (August 2011 – August 2013)

   ii. **Jennifer Chancellor**, TAMUCC (Biology) "The effects of non-thermal plasma on various phases of wound healing" (August 2012 - Present)

4. Graduate Student Research Projects
   
i. **Chelsea Perez** (English) "Impacts of Plasma Decontamination in the Food Industry" (2011) “TAMUCC Outstanding Islander”

   ii. **Anudeep Reddy Kandi** (Computer Science) "Optical Characterization and Diagnostics of High Power 1064 nm Infrared Laser System" (2011)

5. Undergraduate Student Research Projects
   
1. **Valerie Ferdin** (Mechanical Engineering) "Atmospheric Pressure Cold Plasma Jet" (2012 - 2013) “TAMUCC Outstanding Islander”

2. **Amanda Whitmill** (Biomedical Sciences) "Plasma Induced Apoptosis on various Cell Lines" (September 2009 - August 2011) “TAMUCC Outstanding Islander”

3. **Eduardo Valdes** (Mechanical Engineering) "Atmospheric Pressure Cold Plasma Jet" (September 2010 - Present)

4. **Daniel Cantu** (Biology) "The effects of non-thermal plasma on various phases of wound healing” (2012 - Present)

5. **Andres Ramos** (Mechanical Engineering) “Large volume resistive barrier discharge” (June 2013 – Present)

6. **Samantha Valdez** (Biomedical Sciences) "THP-1 cancer cells treatment using Cold Plasmas” (2012 - 2013)

7. **Cody Torno** (Mechanical Engineering) "Atmospheric Pressure Cold Plasma Jet" (2013)

8. **Jennifer Ausland** (Biology) "Assessment of the efficiency of Bacillus cereus sterilization by cold plasma as an alternative decontamination method and its influence on bacterial growth" (August 2011 - 2013)

9. **Bokang Yang** (Mechanical Engineering) "Atmospheric Pressure Cold Plasma Induced Surface Energy Measurements” (August 2011 - 2012)

10. **Hoang (Kim) Thi Pham** (Biology) "Sterilization of Staphylococcus aureus and Pseudomonas aeruginosa using a Novel Cold Plasma Source and its effect on the bacterial re-growth” (January 2011 - 2012)
11. Megan Norfolk (Biomedical Sciences) "THP-1 cancer cells treatment using Cold Plasmas" (April 2011 - 2012)


13. Alison Doyungan (Biology) "Experimental Deactivation of Escherichia coli using a Novel Cold Plasma Source and its effect on the bacterial re-growth" (January 2011 - August 2011)


15. James Shames (Mechanical Engineering) "Design and Construction of Large Volume Plasma Discharge Chamber" (February 2011 - July 2011)

16. Katherine Johnson (Biomedical Sciences) "TUR cancer cells treatment using Cold Plasmas" (November 2010 - July 2011)

17. Thurman Walling (Mechanical Engineering) "Characterization of Atmospheric Pressure Cold Plasma" (September 2009 - May 2011)

18. Keisha Hardeman (Biomedical Sciences) "Plasma Decontamination Study on various Bacteria's" (September 2009 - July 2010)

19. Ciera Johnston (Biomedical Sciences) "Effects of Plasma on various Bacteria" (May 2010 - October 2010)

20. Christos Elias (Mechanical Engineering Technology) "Air Plasma & Nitrogen Oxide (NOx) Characterization" (September 2009 - February 2010)


6. Undergraduate and Honors Research Thesis
   i. Hoang (Kim) Thi Pham (Biology) "The effect of non-thermal plasma on Pseudomonas bacterial sterilization and re-growth" (August 2011 - December 2011)
   
   ii. Valerie Ferdin (Engineering) “Surface energy modification of polymeric substrates for biomedical applications by atmospheric pressure non-thermal plasma jet” Completed (2011 – 2013)
   
## Teaching Activities

### Courses Taught

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<tr>
<th>Sl.No.</th>
<th>Semester</th>
<th>Course Title</th>
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<tbody>
<tr>
<td>1</td>
<td>Fall 2013</td>
<td>ENGR 4390: Introduction to Plasma Engineering (Mechanical Engineering)</td>
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<td>2</td>
<td>Fall 2013</td>
<td>BIOL 5940: Master’s Graduate Thesis Research</td>
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<td>3</td>
<td>Summer 2013</td>
<td>BIOL 5102: Master’s Graduate Research Seminar</td>
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<td>4</td>
<td>Summer 2013</td>
<td>BIOL 5394: Master’s Graduate Thesis Submission</td>
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<td>5</td>
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<td>MEEN 4396: DIS: Advanced Topics in Plasma Engineering</td>
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<td>6</td>
<td>Spring 2013</td>
<td>ENGR 2322: Lec/Lab: Materials Science (Mechanical Engineering)</td>
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<td>7</td>
<td>Spring 2013</td>
<td>ENTC 3410: Lec/Lab: Materials Science (Engineering Technology)</td>
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<td>8</td>
<td>Spring 2013</td>
<td>BIOL 4396: DIS: Advanced Topics: Plasma Science in Biology</td>
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<td>9</td>
<td>Spring 2013</td>
<td>BIOL 5392: Master’s Graduate Thesis Proposal</td>
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<td>BIOL 5393: Master’s Graduate Thesis Research</td>
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<td>13</td>
<td>Fall 2012</td>
<td>BIOL 5396: DIS: Cold Plasma Induced Wound Healing</td>
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<td>14</td>
<td>Summer 2012</td>
<td>BIOL 5940: Master’s Graduate Thesis Research</td>
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<td>Spring 2012</td>
<td>ENGR 2322: Lec/Lab: Materials Science (Mechanical Engineering)</td>
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<td>16</td>
<td>Spring 2012</td>
<td>ENTC 3410: Lec/Lab: Materials Science (Engineering Technology)</td>
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<td>17</td>
<td>Spring 2012</td>
<td>BIOL 5392: Master’s Graduate Thesis Proposal</td>
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<td>18</td>
<td>Spring 2012</td>
<td>BIOL 4396: DIS: Ionized Non-Thermal Plasma effects on THP-1 cells</td>
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<td>19</td>
<td>Fall 2011</td>
<td>HONR 4195: Senior Capstone: Honors Research-Project of Excellence</td>
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<td>20</td>
<td>Fall 2011</td>
<td>ENGR 2322: Lec/Lab: Materials Science (Mechanical Engineering)</td>
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<td>21</td>
<td>Fall 2011</td>
<td>ENTC 3410: Lec/Lab: Materials Science (Engineering Technology)</td>
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<td>22</td>
<td>Summer 2011</td>
<td>ENTC 4496: DIS: Design and Fabrication of Conveyor System</td>
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<td>ENGR 2322: Lec/Lab: Materials Science (Mechanical Engineering)</td>
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<td>27</td>
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<td>ENGR 2322: Lec/Lab: Materials Science (Mechanical Engineering)</td>
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<td>28</td>
<td>Fall 2009</td>
<td>ENTC 3410: Lec/Lab: Materials Science (Engineering Technology)</td>
</tr>
</tbody>
</table>

DIS: Directed Independent Study
Invited Talks / Presentations Presented (Teaching & Education Based)


3. Magesh Thiyagarajan. *Materials Engineering Course Design and Improvement for Effective Research Based Learning Environment*. 1st Faculty Symposium: Course Design for the Millennial Student, Texas A&M University – Corpus Christi, 2011. *(Showcased by the Center for Faculty Excellence)*

Trainings / Workshops Participated (Teaching & Educational)

1. Trainee, Integrating Sustainability into Courses: Principles and Tools to Expand Your Eduative Capacity, National Science Foundation, NSF grant DUE, Anaheim, CA, October 2010.


Non-Credit Instruction Presented

1. Plasmas in the Engineering & Science Field: Instruction presented to the ENGR 1211 - Foundation of Engineering Class - Fall 2011.

2. Plasmas in the Engineering & Science Field: Instruction presented to the ENGR 1211 - Foundation of Engineering Class - Spring 2011.

3. Plasmas in the Engineering & Science Field: Instruction presented to the ENGR 1211 - Foundation of Engineering Class - Fall 2010.

4. Plasmas in the Engineering & Science Field: Instruction presented to the UCCP 1101/1102 - TAMUCC Freshman Seminar Series.

Mechanical Engineering/Technology Capstone Project Supervision

1. Ruben Longoria (ME), Alex Iben (ME), Matt White (MET) "Capstone Project - Plasma Actuators for Efficient Wind Energy Conversion" (August 2011 - Present)

2. Joseph Gloria (MET), Ryan Ramon (MET), Alberto Salazar (MET) "Capstone Project - Automated Plasma Decontamination Unit for Food Industry" *Best Capstone Project Award*, Awarded by IEEE Corpus Christi Section. (September 2010 - May 2011)
Directed Independent/Research Study Supervision


2. Megan Norfolk (Biology) “Ionized Non-Thermal Plasma effects on THP-1 cells” Completed (Spring 2012)

3. Jennifer Chancellor, TAMUCC (Biology) “The effects of non-thermal plasma on various phases of wound healing” Completed (Fall 2012)

4. Jennifer Ausland, (Biology) “The effects of non-thermal plasma on Deactivation of Vibrio vulnificus, Salmonella Typhimurium, and Bacillus cereus Vegetative Cells and Spores for Food Industry Applications” Completed (Spring 2013)

5. Valerie Ferdin (Engineering) “Surface energy modification of polymeric substrates for biomedical applications by atmospheric pressure non-thermal plasma jet” Completed (Spring 2013)

6. Eduardo Valdes (Engineering) “Surface energy modification of polymeric substrates for biomedical applications by atmospheric pressure non-thermal plasma jet” Completed (Spring 2013)

Advisor - Coastal Bend Technology Business Plan Preparation

1. Ruben Longoria (ME), Alex Iben (ME), Matt White (ME) “Plasma Actuators for Efficient Wind Energy Conversion” (August 2011 - 2012)

2. Joseph Gloria (MET), Ryan Ramon (MET), Alberto Salazar (MET) “Plasma Conveyor System” (September 2010 - December 2010)

Academic Advising/Mentoring: 2009-2013

<table>
<thead>
<tr>
<th></th>
<th>Total Number of Undergraduate Student Advisees : 366</th>
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<tbody>
<tr>
<td><strong>Description of Advising Activities:</strong> Scheduling advising meetings, Reviewing student academic progress &amp; performance, Advising for course pre-reqs and enrollments, Releasing student academic holds</td>
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<tr>
<td>Fall 2013</td>
<td>44 Students</td>
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<tr>
<td>Spring 2013</td>
<td>49 Students</td>
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<tr>
<td>Fall 2012</td>
<td>50 Students</td>
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<tr>
<td>Spring 2012</td>
<td>55 Students</td>
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<td>Fall 2011</td>
<td>30 Students</td>
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<tr>
<td>Spring 2011</td>
<td>47 Students</td>
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<td>Fall 2010</td>
<td>56 Students</td>
</tr>
<tr>
<td>Spring 2010</td>
<td>35 Students</td>
</tr>
</tbody>
</table>
Service Activities

Program/Department/School Level Service

1. Faculty Search Committee Member - Assistant Professor of Mechanical Engineering, (2013)
2. Faculty Search Committee Member - Assistant Professor of Mechanical Engineering, (2011)
3. Faculty Search Committee Member - Associate Professor of Mechanical Engineering, (2011-2012)
4. Faculty Search Committee Member - Associate Professor of Mechanical Engineering, (2009-2010)
5. Faculty Search Committee Member - Assistant Professor of Mechanical Engineering, (2009-2010)
6. Staff Search Committee Member – Engineering Lab Coordinator II, (2011)
7. Staff Search Committee Member - Engineering Education Program Coordinator II, (2010)
8. Staff Search Committee Member – Engineering Lab Coordinator II, (2010)
11. Faculty Representative, TAMU System Engineering Network Meeting & Texas Engineering Experiment Station (TEES) Leadership Meeting, TAMUCC. (May, 2013)
12. Faculty Member – Mechanical Engineering ABET Accreditation Preparation, (2009 - 2011)
15. Committee Member, Engineering Labs and Machine Shop Reorganization. (2010)
16. Faculty Advisor – ASME Student section – Supported departmental, college and university events and represented the engineering student community in campus. (2010 – 2011)
17. Faculty Mentor - ASME - Engineering Seminar Series. (2010 - 2011)
18. FE Exam Faculty Instructor – Materials Science Topics – (2012)
19. Faculty Mentor - Computing Sciences and Engineering Representative at SACNAS, (2010-2011)
24. Master of Ceremonies (MC), 3rd Annual Coastal Bend Engineering Competition, (March 2012)
25. Judge - TAMUCC Engineering Summer Camp, (July 2010)
26. Attendee, Meeting - Industrial Advisory Board IAB, (2010 - Present)
27. Master of Ceremonies (MC) - 1st South Texas Engineering Alliance Design Fair, (2011)
28. Committee Member - TAMU System Level Engineering Program Meeting, Dallas, TX, (2009)
College Level Service

1. **Committee Member** - College of Science and Engineering Scholarship Committee, (2009 - 2012)
2. **Faculty Search Committee Member** – Assistant Professor of Atmospheric Science, (2010 - 2011)
4. **Committee Member** – TAMUCC Vivarium (Animal Testing Facility) committee, (2011)

University Level Service

1. **Maze Bearer** – Spring 2013 Commencement, Texas A&M University Corpus Christi.
2. **Committee Chair** – 2020 TAMUCC Strategic Planning – Research & Scholarly Activities Collaboration Subgroup, (2013)
3. **Lead Committee Member** - TAMUCC & Del Mar Community College Memorandum of Understanding (MOU) – Articulation Agreement for Mechanical Engineering, (2010 - 2011)
4. **Speaker** - FACTalk Islander Forum 2013, Texas A&M University Corpus Christi. (2013)
5. **Faculty Representative** – Corpus Christi Day at Capitol in Austin, Texas, (2013)
6. **Faculty Representative**, U.S. Senator John Cornyn visit to Coastal Bend Innovation Center, (2013)
7. **Faculty Representative**, Industry Day Presentation, Texas A&M System (2013)
8. **Faculty Representative**, Chancellors Breakfast, Board of Regents Meeting, TAMU System (2012)
9. **Event Director** - 2nd Annual Coastal Bend Engineering Competition, (2011)
10. **Event Director** – 1st Annual Coastal Bend Engineering Competition, (2010)
11. **Faculty Mentor** - Louis Stokes Alliance for Minority Participation Program, (2010 - Present)
12. **Faculty Mentor** - McNair Scholars Program, (2009 - Present)
13. **Representative Guest** - In Session with the President, Texas A&M Univ. Corpus Christi. (2013)
14. **Representative Guest** – Corpus Christi Business Hall of Fame, TAMUCC. (2013)
15. **Committee Member** - South Texas Engineering Alliance Consortium, (2009 - 2013)
16. **Faculty Advisor** - Students (engineering) visit to the members of the Appropriations and the Higher Education Committees at Capitol in Austin, Texas, (2011)
17. **Faculty Mentor** - Community College STEM Conference, TAMUCC, (2009)
Scholarly & Professional Service

1. **Advisory Committee Member** – Texas Emerging Technology Fund – Regional Center of Innovation and Commercialization - Office of The Governor Rick Perry, Texas, (2012 – Present)
2. **Advisory Committee Member** - NASA Texas Space Grant Consortium, (2011 - Present)
3. **Advisory Committee Member** - Del Mar College – Engineering Program, (2010 - Present)
5. **Grant Proposal Reviewer** - Coastal Bend Innovation Center, Corpus Christi, (2009 - Present)

Community/Public Service

1. **Community Collaborator** - Plasma Technologies Inc - Community Entrepreneurial Technology Development and Validation, (2009-2010)
2. **Faculty mentor**- Innovation Academy Mentors, (2010)
3. **Science Fair Judge** - School of Science and Technology Corpus Christi, (2011)
5. **Science Fair Judge** - School of Science and Technology Corpus Christi, (2010)
7. **Guest Speaker** - Plasma Lab Tour - Victoria County Outreach program, (2010)
8. **Member** Coastal Bend Business Plan Competition, (2010)
Awards and Honors

1. **Person of the Year Award** – Awarded by the Mayor of City of Corpus Christi. (2013)
2. **Corpus Christi 40-Under-40 Award** - Awarded by the Mayor of City of Corpus Christi. (2013)
3. **Faculty Excellence in Research Award** – Texas A&M University Corpus Christi. (2013)
4. **Distinguished Speaker of Academy Speaks** - Texas A&M University Corpus Christi. (2013)
6. **Distinguished TAMUCC Faculty Speaker FACTalks** – Islander Forum, TAMUCC. (2013)
7. **Honors Program Mentor** – Honors Program, Texas A&M University Corpus Christi. (2012)
8. **Outstanding Islander** - Texas A&M University Corpus Christi, TAMUCC. (2011)
9. **ASME Advisor Service Award** - Texas A&M University Corpus Christi, TAMUCC. (2009-2011)
12. **NSF Travel Award** for ASEE Integrating Sustainability in Engineering Courses, NSF. (2010)
17. **Dissertator Fellowship Award**, Dept. of Electrical Engineering, Univ. of Wisconsin Madison. (2007)
21. **Best Teaching Assistant Award**, Dept. of Electrical Engineering, Univ. of Wisconsin Madison. (2005)
22. **Best Paper Award**, SHPE - Society of Hispanic Professional Engineers. (2005)
25. **Citation Award for Professional Promise**, Chancellor, Univ. of Tennessee - Knoxville. (2004)
29. **Best Project Award**, University of Madras. (2001)
Recognitions

1. **DOTmed Daily News** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Two unexpected approaches to infection control” (August 2013).


3. **Healthcare Facilities Today Magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research “Infections may be getting two surprise foes” (August 2013).

4. **Quality Assurance and Food Safety Magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research “University Touts ‘Superbug’ Killing Technology”, (July 2013).

5. **Surgical Products magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Killer “Superbugs” No Match For College Researcher” (June 2013).

6. **Operating Room Today – OR TODAY magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Texas A&M University-Corpus Christi researcher fights drug-resistant researcher with plasma technology” (June 2013).

7. **Medical Horizons magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Killer “Superbugs” are No Match for Texas A&M University-Corpus Christi Researcher” (June 2013).

8. **Infection Control Today magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Researcher Uses Cold Plasma Technology to Stop Superbugs” (June 2013).

9. **TAMUCC News** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Killer “Superbugs” are No Match for Texas A&M University-Corpus Christi Researcher” (June 2013).

10. **San Antonio Business Journal** recognized Dr. Magesh Thiyagarajan’s cold plasma research “Texas A&M-Corpus Christi researcher attacking ‘superbug’ bacteria” (June 2013).

11. **BioPortfolio magazine** recognized Dr. Magesh Thiyagarajan’s cold plasma research, “Researcher Uses Cold Plasma Technology to Stop Superbugs” (June 2013).

12. **Caller Times News** recognized Dr. Magesh Thiyagarajan for receiving the Corpus Christi ‘40-Under-40’ and the ‘Person of the Year’ Award. (2013).

13. **TAMUCC News** highlights Dr. Magesh Thiyagarajan’s award for the Corpus Christi ‘40-Under-40’ and the ‘Person of the Year’ Award. (2013).

14. **TEES Partner Facility - Plasma Engineering Research Lab listed in TEES. (2013).**


18. **Featured Researcher** - TAMUCC Office of Research and Graduate Studies Website, TAMUCC. (2011)


20. **Plasma Research Highlighted by TAMUCC President** at the 2011 State of the University Speech. (September 2011).


22. **Plasma Research Highlighted** - by **Mayor Joe Adame** at the 13th Annual State of the City Address Corpus Christi, Chamber of Commerce. (January, 2011).

23. **QEM Travel Award** for NSF-MRI Workshop, Quality Education for Minorities (QEM). (2010).


26. **The Islander Magazine TAMUCC** - Spring 2010 - Dr. **Magesh Thiyagarajan** and the Plasma Engineering Research Lab is featured in the cover story - Page 14. (March 2010).

27. **Caller Times** - News Paper Features a Cover Story on Research at Plasma Lab, Local firm eyes mass production - Caller Times News Media. (February 2010).

28. **Campus News** - TAMUCC - "**Dr. Magesh Thiyagarajan** Receives $700,000 Department of Defense Research Grant for Plasma-Biomedical Engineering Research", Texas A&M University Corpus Christi. (September 2010).

29. **Texas A&M University Corpus Christi - Facebook Page** - Features DOD Research Grant Award for Plasma - Biomedical Engineering Research, TAMUCC. (September, 2010).


31. **Panelist** - SBIR/STTR Workshop, Coastal Bend Business Innovation Center. (September, 2010).

32. American Society of Mechanical Engineers Membership Approved, TAMUCC. (December, 2010).

33. **TAMUCC Campus News** - Mechanical Engineering and South Texas Alliance of Academic Institutions - Coastal Bend Engineering Competition (March, 2010).

Research Student Mentees Awards & Successes

1. **2013 Gulf Coast Summer Institute Participant** – Xavier Gonzales, Louisiana State University, 2013.


9. **Best Research Paper Award** – Eduardo Valdes and Magesh Thiagarajan. 9th Annual LSAMP Symposium held at Texas A&M College Station, TX. 2013.

10. **Best Research Paper Award** - Valerie Ferdin and Magesh Thiagarajan. 9th Annual LSAMP Symposium held at Texas A&M College Station, TX. 2013.


21. **Conference Travel Award** - E Valdez and *Magesh Thiyagarajan*. (San Jose, California: SACNAS National Conference)., SACNAS. 2011.

22. **Conference Travel Award** - G Vidal, *Magesh Thiyagarajan*, and H Pam. (San Jose, California: SACNAS National Conference)., SACNAS. 2011.


25. **Conference Travel Award** - Megan Norfolk, *Magesh Thiyagarajan*, et. al. (San Jose, California: SACNAS National Conference., SACNAS. 2011.


- End of Curriculum vitae -