Application of a Novel Cold Atmospheric Plasma for Water Decontamination

Hosan Kim*, Salman Izadjoo*, Huan Truong**, and Mina Izadjoo*

*Trideum Biosciences, Frederick, MD, USA, mizadjoo@trideumbiosciences.com
**General Vibronics LLC, Tempe, AZ, USA, huan@generalvibronics.com

ABSTRACT

In recent years, non-thermal atmospheric plasma has been considered as a potential technology for decontamination. The plasma can interact with the ambient atmosphere and generate a variety of reactive species such as ozone, hydrogen peroxide, radical, atomic, and ionic species. In this study, we used a novel cost effective cold atmospheric plasma (CAP) system developed by our collaborator General Vibronics, LLC. We conducted laboratory testing on the effect of this CAP technology as a mosquito control measure on both larvae and eggs living in aqueous environments. In addition, its antimicrobial effects in an aqueous environment was tested against *E. coli* as an indicator organism. The results demonstrated this novel CAP technology has mosquito larvicidal activity, and is very efficient in inactivating and/or killing mosquito eggs. There is great promise in the application of this novel approach for decontamination of water and improving water quality.

Keywords: water decontamination, mosquito larvae, *E. coli*

1 INTRODUCTION

Cold atmospheric plasma application is found various fields such as biomedical, industrial, cosmetic, agricultural due to the plasma's ability to interact with the ambient atmosphere and generate a variety of reactive species such as ozone and hydrogen peroxide, radical, atomic, and ionic species.

The device driver (Figure 1A) acts as a power supply and is connected to a plasma array pad (Figure 1B). The array is powered by the device driver and utilizes atmospheric oxygen to generate reactive atmospheric species. In a dark room, the atmospheric plasma is visible by emitting a glowing blue light (Figure 3C). One of the most important reactive oxygen species produced by the plasma device is O_3 (ozone). Ozone does exhibit toxic activity against microorganisms including bacteria. This is the reactive oxygen species we have chosen to measure in correlation to cold atmospheric plasma in this study [1-2].

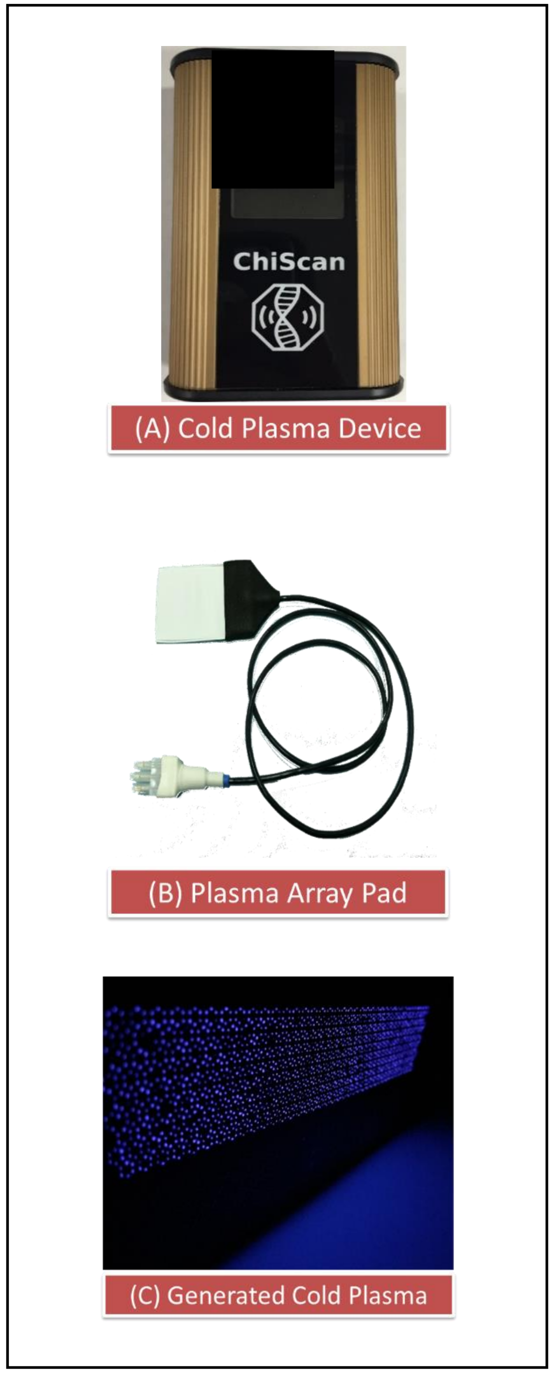

Figure 1: Cold Atmospheric Air Plasma (CAP) Technology

2 OBJECTIVE

- To evaluate the effect of this CAP technology as a mosquito control measure on both larvae and eggs living in aqueous environments.

- To evaluate antimicrobial effect in an aqueous environment against *Escherichia coli* (*E. coli*) as an indicator organism.

3 METHODS

Plasma device

An electric vital energy generator (eVEG) developed by General Vibronics, LLC (Tempe, AZ). The voltage can be modulated to enhance the effectiveness of the array against pathogens that are sensitive to specific frequencies without using any kind of noble gas. The modulation frequencies such as 1550-, 740-, 482-, and 241-hertz (Hz) and irradiation time can be controlled to generate different concentrations of plasma. In this study we selected low (1550 Hz) and high (241 Hz) strength frequencies to compare their efficacy. An Ozone sensor (Eco Sensors, Inc., Santa Fe, New Mexico, USA) was used to monitor the level of ozone actively generated by the CAP device.

Bacterial Strain

Escherichia coli 11775 from ATCC was used in this study.

Mosquito Larvae and Eggs

Mosquito larvae and eggs (*Aedes aegypti*) were fed on a liver powder suspension made in our laboratory.

Ozone Measurement

Ozone level in water were measured using a commercial N, N-diethyl-*p*-phenylenediamine (DPD) visual kit (K-7423) and instrument kit (I-2019) with a single ozone concentration range of 0-5 ppm (CHEMetrics Inc, Midland, VA).

Survival of Mosquito Larvae in CAP Treated Solution

A rectangular plasma array (3" x 5") was placed vertically in a container to prepare CAP treated solution. Next, mosquito larvae were added to the treated water as shown in Figure 2.

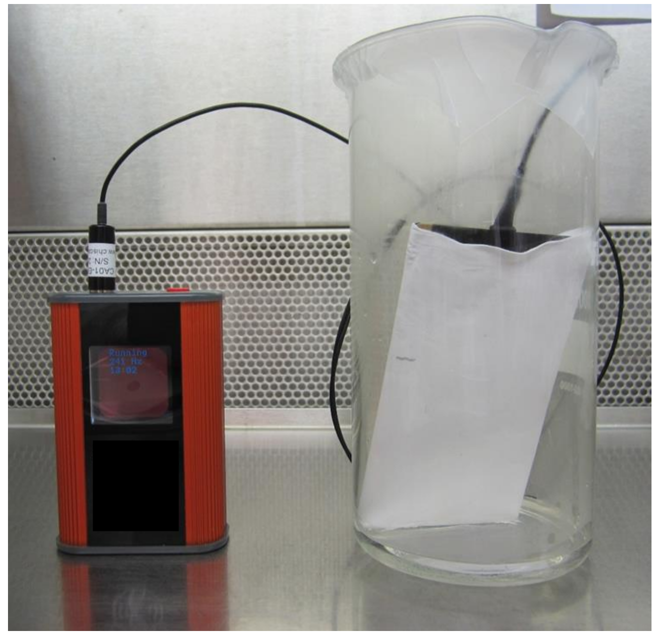

Figure 2: CAP Setup and Treatment

Survival of Mosquito Eggs in CAP Treated Solution

(1) We prepared mosquito egg patches containing approximately 200 eggs. For the post-treatment experiments, a rectangular plasma array (3" x 5") was placed vertically in a container to prepare CAP treated water. A single mosquito egg patch was added to the treated water.

(2) The effect of CAP treatment on mosquito eggs was monitored for 72 hours.

The Effect of CAP on *E. coli*

(1) Overnight broth cultures were diluted to make 10^5, 10^6, 10^7 to 10^8 colony forming units (CFUs)/ml.

(2) A rectangular 3" x 5" array was placed vertically in a container containing *E. coli* cultures and ozonized as shown in Figure 2. The serial dilutions were plated to determine the surviving bacteria.

4 RESULTS

Cold Atmospheric Air Plasma (CAP) Efficacy on Mosquito Larvae

To investigate whether this novel CAP has any larvicidal efficacy against *Aedes aegypti* mosquito larvae, we applied CAP with minimum (1550 Hz) and maximum (241 Hz) strength for 20 minutes, respectively.

The CAP treatment at 1550 Hz for 20 minutes was less effective against mosquito larvae showing 53% mortality. However, the treatment condition at 241 Hz for 20 minutes showed 100% mosquitocidal activity with low pH levels close to pH 3.0 within the 1-hour incubation period (Table 1).

Table 1. Evaluation of CAP efficacy on mosquito larvae

No.	Condition (Hz/ min)	Mortality (%)	pH change
1	None	0%	8.17
2	1550/ 20	53%	3.98
3	241/ 20	100%	2.91

Evaluation Of CAP-Efficacy on Mosquito Eggs In CAP-Treated Water

We placed mosquito eggs into the CAP treated water to determine any inactivation or killing effects. Each of four experimental combinations were tested at (1550 and 241 Hz frequency at 10 and 20 minutes For 72 hour, we monitored the rate (%) of egg hatching and also if there were dead larvae. The pH change was measured after the incubation. The longer treatments resulted in more acidic pH changes.

Eggs in the untreated water showed hatching rates of 75.1% for 48 hours and almost 100% within 72 hours without pH change (Table 2). Relatively low hatching rates were observed in all CAP treatment conditions. Interestingly, all the hatched larvae did not survive, indicating CAP treatment of the eggs may be a good strategy to control mosquitos.

Table 2. Evaluation of CAP efficacy on mosquito eggs

No.	Condition (Hz/ min)	% of Dead larva (% of Hatched rate)		pH change
		48 h	72 h	
1	None	0% (75.1%)	0% (99.0%)	8.17
2	1550/ 10	100% (17.1%)	100% (17.7%)	2.50
3	1550/ 20	100% (16.2%)	100% (29.7%)	2.17
4	241/ 10	100% (6.7%)	100% (33.3%)	2.48
5	241/ 20	100% (5%)	100% (5%)	2.10

Antibacterial Effect of CAP on *E. Coli*

To investigate antibacterial efficacy of CAP on environmental *E. coli* in an aqueous solution, we treated 10^5 to 10^8 CFU/ml of this organism with CAP at 241 Hz for 20 minutes. After treatment, we checked for any changes in ozone and pH levels. The bacterial cultures were serially diluted, plated and incubated for 18 hours at 37˚C to determine viable bacterial count.

CAP treatment resulted in 100% bactericidal activity within 20 minutes at 241 Hz in the experimental condition. The CAP device was effective even at the highest concentration (10^8 CFU/ml) of *E. coli* (Table 3). The ozone levels in all treatments were more than 5 ppm. All of the treatments resulted in less than pH 3.0. The low pH value may be a good indicator for the ozone level in water.

Table 3. Evaluation of CAP efficacy on *E. coli*

Factors	Bacterial number (CFU/ml) applied							
	1.00E+05		1.00E+06		1.00E+07		1.00E+08	
	C[1)]	T[2)]	C	T	C	T	C	T
Bacterial reduction (log_{10} CFU/ml)	0	5	0	6	0	7	0	8
pH changes	5.8	2.52	5.8	2.44	5.8	2.8	5.8	2.48
Ozone level	0	>5	0	>5	0	1.24	0	>5

Four concentrations of *E. coli* cultures (10^5 ~ 10^8 CFU/ml) were treated with CAP at 241 Hz for 20 minutes. Ozone levels and pH changes were measured after treatment. Serial dilutions were plated to determine surviving bacterial numbers.
[1)] Control, [2)] CAP treatment

5 CONCLUSIONS

In this study, we showed mosquitocidal activity of CAP on larvae and mosquito eggs. In addition, we demonstrated bacterial killing effects of cold plasma in water by this novel device. The CAP-treated aqueous solution may contain various types of primary and secondary species penetrating or dissolving into the liquid based on the discharge type, gas, and the chemical composition in the surrounding environment [3]. The CAP technology generates plasma by utilizing oxygen, therefore, we primarily assessed the concentrations of ozone as relatively long-lived reactive species and monitored the change of pH as the final reactive effect upon the plasma accumulation in the liquid sample. In summary, the General Vibronics Cold Plasma technology has shown;

- Significant mosquitocidal activities in larvae and egg stages.
- Can decontaminate water due to its bacterial killing effect.
- Can be used for water decontamination and improving water quality in a field setting.

REFERENCES

[1] Cahill OJ, Claro T, O'Connor N, Cafolla AA, Stevens NT, Daniels S, Humphreys H. 2014. Cold air plasma to decontaminate inanimate surfaces of the hospital environment. Appl Environ Microbiol. 80(6):2004-10.

[2] Gorbanev Y, O'Connell D, Chechik V. 2016. Non-Thermal Plasma in Contact with Water: The Origin of Species. Chemistry. 22(10):3496-3505.

[3] Shen J, Tian Y, Li Y, Ma R, Zhang Q, Zhang J, Fang J. 2016. Bactericidal Effects against S. aureus a Physicochemical Properties of Plasma Activated Water stored at different temperatures. Sci Rep. 6:28505

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