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Surface-dependent inactivation of model microorganisms with shielded sliding plasma discharges and applied air flow

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ABSTRACT

Cold atmospheric plasma inactivates bacteria through reactive species produced from the applied gas. The use of cold plasma clinically has gained recent interest, as the need for alternative or supplementary strategies are necessary for preventing multi-drug resistant infections. The purpose of this study was to evaluate the antibacterial efficacy of a novel shielded sliding discharge based cold plasma reactor operated by nanosecond voltage pulses in atmospheric air on both biotic and inanimate surfaces. Bacterial inactivation was determined by direct quantification of colony forming units. The plasma activated air (afterglow) was bactericidal against *Escherichia coli* and *Staphylococcus epidermidis* seeded on culture media, laminate, and linoleum vinyl. In general, *E. coli* was more susceptible to plasma exposure. A bacterial reduction was observed with the application of air alone on a laminate surface. Whole-cell real-time PCR revealed a decrease in the presence of *E. coli* genomic DNA on exposed samples. These findings suggest that plasma-induced bacterial inactivation is surface-dependent.

1. Introduction

Conventional atmospheric-pressure cold plasmas are produced when strong electric fields accelerate free electrons, which dissociate, excite, or ionize gaseous molecules [1]. The applied gas can vary in composition from noble gases (i.e. helium [2,3] or argon [4–6]), nitrogenated [7] or oxygenated mixtures [8], or ambient air [9]. These systems are attractive for clinical and biological applications [10,11] including bacterial decontamination [8,12–14], dental care [15–17], wound healing [6], dermatology [18], biosensor formation [7,19,20] and food preservation [21,22], allowing for effective plasma treatment on the order of seconds to several minutes. Furthermore, cold plasmas are generated with gas temperatures below 40 °C, making them safe for use on living tissue and heat-sensitive materials [23,24].

The advantages of using these devices are hindered by several factors: scalability, portability, and sufficient ion deposition. The treatment of larger areas outside the region of direct plasma exposure remains an obstacle, as the fabrication of an efficacious device in a compact system is more convenient for use in a healthcare setting. The use of ambient air for plasma generation is thus attractive, as it eliminates the need for a large external gas tank. The predominant bacterial inactivation mechanism of cold plasma is induced oxidative stress [10]. Reactive oxygen and nitrogen species generated from the reactor need to be disseminated away from the plasma source to damage cells outside the directly exposed region.

Although use of cold plasmas for bacterial inactivation has been reported, there is a need for more translational studies to determine clinical applicability. Over the last decade, the global prevalence of hospital-acquired infections has increased [25]. While establishing effective prevention strategies, such as hand-washing and smart antibiotic-use has reduced infection occurrence, multi-drug resistant bacteria remain a relevant threat [26–28]. The role of surfaces in pathogen transmission is facilitated by their persistence on dry inanimate objects. Ranging from a period of minutes to months, pathogens are able to survive despite arid conditions, suggesting variance in susceptibility to desiccation [29–31]. Cold plasma serves as a potential alternative or additive therapy for infection prevention and antibacterial or antiseptic treatment on live and inanimate surfaces.

In the current study, we assessed the antibacterial efficacy of a novel cold plasma reactor producing cathode-directed surface streamers [32–34] on both biotic and inanimate surfaces inoculated with the model microorganisms, *Escherichia coli* and *Staphylococcus epidermidis*. Materials exposed were similar to those found in a healthcare setting to facilitate the translation of plasma treatment to appropriate surface types. Bacterial inactivation after exposure to plasma or applied air was quantified by direct colony counts and by real-time quantitative PCR (qPCR) coupled with the use of propidium monoazide (PMA) dye to differentiate live and dead bacterial cells.

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2. Materials and methods

2.1. Plasma system

The reactor consists of two glass dielectric plates, separated by spacers, with 50 μm thick aluminum foil electrodes placed perpendicular to the applied air flow, forming an inter-electrode gap of 2.0 cm between the exposed edges of the anode and cathode (Fig. 1). Located at the exit side of the reactor, the ground electrode was extended to form a conductive shield at the dielectric layer opposite to the discharge area. This created an enhanced electric field in the discharge gap and a higher energy density compared to other nonthermal plasma reactors [34]. The high voltage electrode was pulsed biased positively. The plasma reactor was operated by applying a pulsed voltage of 24 ± 0.5 kV peak voltage, 50 ns rise time, and 100 ns duration (full width at half maximum) at a repetition rate of 500 Hz (Fig. 2). Ambient air was used as the working gas. At operating flow rates of five and ten standard liters per minute (SLM), sliding discharges were formed along the glass in a discharge chamber measuring 2.6 cm × 2.0 cm × 0.038 cm. Cathode-directed streamers were generated within the discharge gap. The total energy consumption of the device was 6.8 ± 0.6 W. Plasma-activated gas exits at the base of the slit opening. Inactivation studies at durations of 1 and 3 min, were performed at a 10 mm distance from the slit to the exposed surface.

2.2. Ozone and nitric dioxide (NO_2) measurements

Ozone was analyzed based on UV absorption technique using a gFFOZ ozone analyzer (Semiconductor Ozone Solutions, Albany, OR). The NO and NO_2 in ppm units were monitored by a ENERAC Model 500 NOx analyzer (ENERAC, Holbrook, NY) with a resolution of 1 ppm and a reading accuracy of 4%. Ozone reacts with NO in a one to one molar ratio as long as there is sufficient NO present [32,35]. Therefore, ozone concentration was also estimated based on the amount of NO removed when the latter was mixed in the plasma after glow.

2.3. Plasma application on agar plates

Shielded sliding atmospheric plasma discharges were applied to model organisms *E. coli* ATCC 25922 and *S. epidermidis* ATCC 12228. Overnight nutrient-rich broth cultures were serially diluted to a final concentration of 10^4 cells/mL. One-hundred microliters of each bacterial suspension was uniformly spread on Brain Heart Infusion (BHI) agar plates. Seeded plates were air-dried to insure proper adherence of cells to the solid medium. Bacterial species were exposed separately. The reactor was positioned laterally across the seeded petri dish for an exposure duration of 3 min (Fig. 3). Unexposed and air sham controls, performed at a flow rate of 5 SLM, were also included. Post-exposure, agar plates were incubated overnight at 37 °C. The initial bacterial inoculum was determined from serial dilutions, and the percent of control was determined for both air and plasma exposed samples. To quantify the bacterial $\log_{10}$ reduction beyond the area of direct plasma exposure, ImageJ software (National Institutes of Health, Bethesda, MD) was used to construct a region of interest in the area of inhibition and compared to unexposed controls.

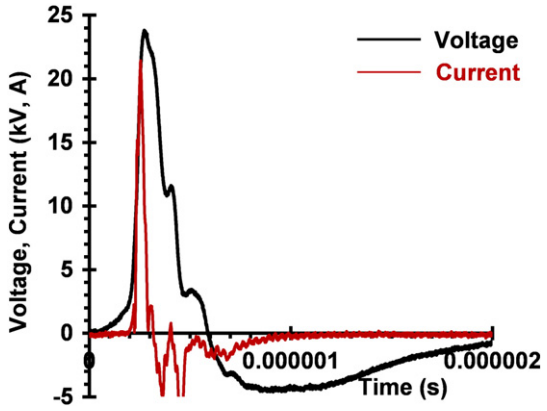

Fig. 2. Voltage and current waveforms for shielded sliding discharge employed in this study.

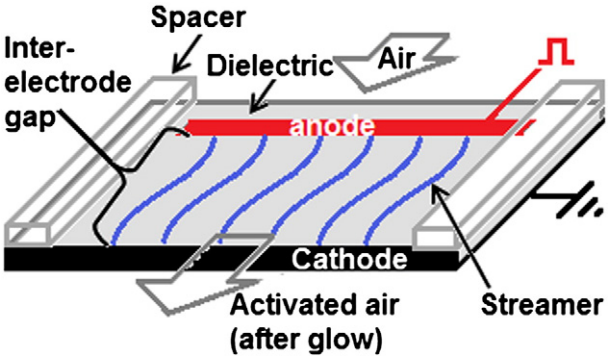

Fig. 1. Schematics of the plasma device showing dielectric layer with spacers. Anode and plasma located on top surface and cathode on face extending to the bottom as a shield. The top dielectric layer with cathode/shield on the outer side is not shown.

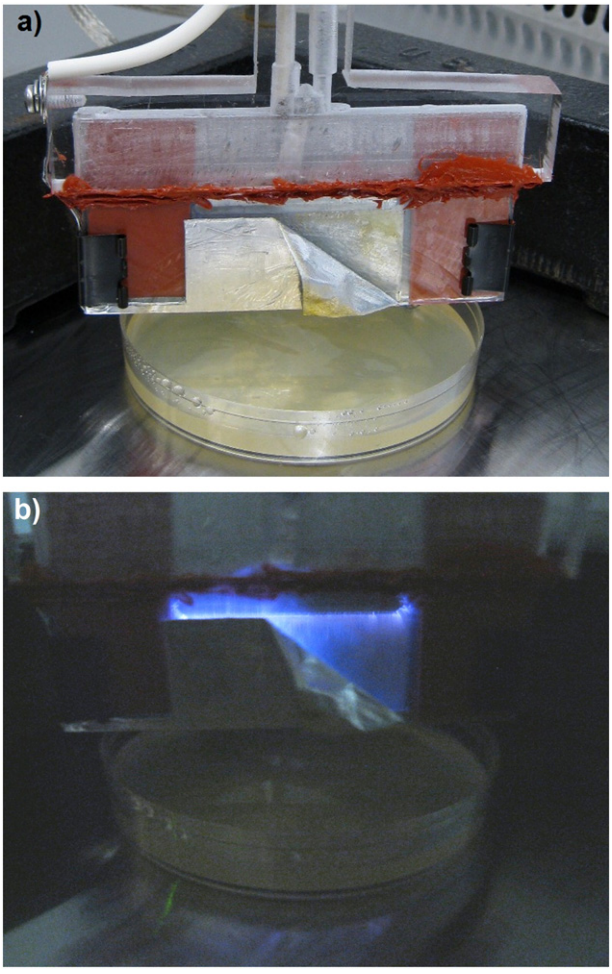

Fig. 3. Lateral orientation of plasma device across a seeded petri dish prior to exposure (a). The outer electrode is folded down to appreciate geometry within parallel glass sheets and to visualize the plasma recorded with lights OFF (b).