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# Antibacterial efficacy of a novel plasma reactor without an applied gas flow against methicillin resistant *Staphylococcus aureus* on diverse surfaces

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ABSTRACT

The use of nonthermal plasma in the clinic 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 plasma reactor based on a high current version of sliding discharge and operated by nanosecond voltage pulses without an applied gas flow. This modification is advantageous for both portability and convenience. Bacterial inactivation was determined within a chamber by direct quantification of colony Jing units. Plasma exposure significantly inhibited the growth of *Escherichia coli* and *Staphylococcus epidermidis* following a 1-min application ($p < 0.001$). *S. epidermidis* was more susceptible to the plasma after a 5-min exposure compared to *E. coli*. Temperature and pH measurements taken immediately before and after plasma exposure determined neither heat nor pH changes play a role in bacterial inactivation. Because of the notable effect on *S. epidermidis*, the effect of plasma exposure on several isolates and strains of the related opportunistic pathogen *Staphylococcus aureus* was quantified. While *S. aureus* isolates and strains were efficiently inactivated on an agar surface, subsequent testing on other clinically relevant surfaces demonstrated that the inactivation level, although significant, was reduced. This reduction appeared to depend on both the surface texture and the surface moisture content. These findings suggest this novel plasma source lacking an applied gas flow has potential application for surface bacterial decontamination.



## 1. Introduction

Nonthermal plasmas are usually formed by high voltage electrical discharges in gases when an induced electric field accelerates free electrons, which dissociate, excite, or ionize gaseous molecules [1]. The applied gas for plasma generation can vary in composition from noble gases (i.e. helium or argon) [2–4] nitrogenated [5] or oxygenated mixtures [6], or ambient air [7]. These systems are attractive for clinical and biological applications [8–10] including bacterial decontamination [11–18], dental care [19–22], wound healing [4,23–25], dermatology [26], and food preservation [27–31], providing effective plasma treatment on the order of seconds to several minutes. In addition, some nonthermal plasmas are generated with temperatures of the neutral particles below 40 °C, making them safe for use on heat-sensitive materials, such as living tissue [7,32].

The predominant bacterial inactivation mechanism of nonthermal plasma is the induction of oxidative stress [9]. Reactive species generated from the plasma device must be disseminated away from the plasma source to damage bacterial cells. However, the portability of these devices remains a challenge. The need for an external gas source limits the application in environments, i.e. clinics or emergency medical transport, where space and or resources are scarce. An efficacious plasma device that does not rely on an applied gas flow would be advantageous in these circumstances.

The device presented generates plasma in ambient air in the exposure environment without the use of applied gas flow. Nanosecond high voltage pulses are applied to electrodes having edge-to-edge geometry adhered to a dielectric layer. As a result, the electrical discharges form in the inter-electrode gap sliding at a solid-gas interface. Sliding discharges generally have higher current flow and, consequently, higher energy density and faster rate of production of reactive species [33,34]. Extending one electrode to cover the inter-electrode gap on the opposite side of the dielectric layer increases the current flow by an order of magnitude compared to the simple sliding discharge [35]. To the best of the authors' knowledge, the high current version of sliding discharge without applied gas flow was employed in this study for the first time for the bacterial decontamination. It was studied because it not only results in increased rate of production of reactive species but it also induces strong electric wind in and around the discharge gap [36]. The discharge is comprised of multiple thin plasma channels (streamers) that are initiated from close to

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the edge of the anode and propagated in the discharge gap towards the cathode. Huge current flows through the streamers. For example, >15 A peak current was recorded in the present study. The current is a flow of charges, i.e., electrons moving to the anode and an almost equal number of positive charges moving to the cathode in the plasma channels. Since the positive charges are significantly bulky and heavy compared to electrons, they drag the ambient gas molecules, causing a net gas flow in and around the plasma channels along the direction of their movement that is referred as "electric wind". Since each cathode strip is sandwiched between two parallel anode strips, the two opposing electric winds will be deflected away from the electrode plane towards the treatment surface. The electric wind facilitates the transport of the reactive species by convection in addition to diffusion in gas phase that is expected to compensate for no applied gas flow condition in this study. The device configuration fits over the geometry of a petri dish, creating a closed plasma exposure chamber. In the current work, several surfaces relevant to clinical and environmental applications were seeded with *Escherichia coli*, *Staphylococcus epidermidis*, and methicillin resistant and sensitive *Staphylococcus aureus* strains and clinical isolates (MRSA and MSSA respectively) and separately exposed to plasma. Bacterial inactivation after plasma exposure was quantified by direct colony counts.

## 2. Materials and methods

### 2.1. Plasma system

The experimental setup illustrated in Fig. 1a, was similar to previous reports [16,37]. A compact pulsed power modulator (MPC3000S-OP1, Suematsu Electronics Co., Ltd., Japan) delivered positive high voltage pulses to the plasma device. A Tektronix P6015A voltage probe and a Pearson Electronics Current Monitor model 110A connected to the oscilloscope with equal length and Tektronix TDS 2024C oscilloscope were used to measure the temporal development of voltage and current. The energy per pulse (Ep) was calculated by integrating the product of voltage and current pulses over a sufficient period of time, i.e. $Ep = \int VIdt$, where V and I are the pulsed voltage and current, respectively. Power was calculated from the product of the energy per pulse and the pulse repetition rate. Representative voltage and current waveforms are shown in Fig. 2. It can be observed from Fig. 2 that the peak voltage was ~10 kV, voltage rise time (10% to 90%) was ~100 ns and pule duration (full width at half maximum) was ~200 ns. The pulse repetition rate was fixed at 500 Hz. The energy per pulse was ~2.7 mJ, which means the power dissipated in the plasma was ~1.4 W in this study.

The electrodes were constructed of 50 μm thick aluminum foil (ALF200L from Intertape Polymer Groups, USA). The high voltage electrode had four strips, interconnected at the base. The counter-

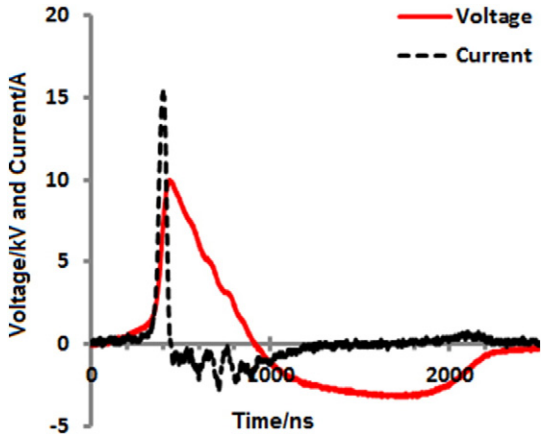


**Fig. 1.** Representative voltage waveform (solid line) and current waveform (dashed line).

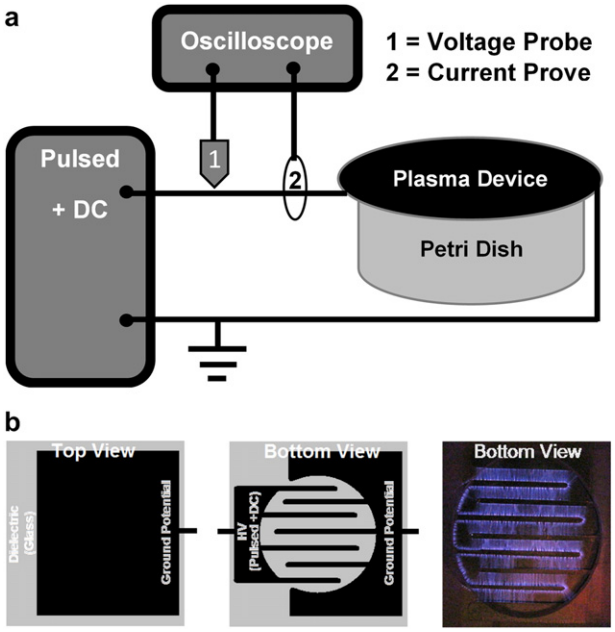


**Fig. 2.** Schematic of the experimental setup (top row) and schematic of top and bottom view of the plasma device along with time integrated image of the plasma (bottom row).

electrode at ground potential had five strips sandwiching the first electrode with an inter-electrode gap of 6.4 mm. An acrylic sheet was glued over the electrodes allowing for seating the device on a petri dish. The electrodes were glued to a dielectric sheet made of soda glass measuring 2.2 mm × 114 mm × 114 mm. The aluminum foil at ground potential was glued on the opposite side of the dielectric, covering the inter-electrode gaps. The extension of the electrode at ground potential on the opposite side of the dielectric enhanced the electric fields at the edges of the electrodes. It also modified the electric fields such that the plasma generated at the gas-solid interface stayed firmly fixed to the dielectric. These factors allowed more plasma channels to be formed and higher amount of current to flow through them [38]. A schematic of the electrode assembly with a time integrated image of the plasma are shown in Fig. 1b.

The plasma device was seated on the rim of a petri dish during exposure, thereby creating a closed system with the plasma on the inner side facing the surface to be treated in the petri dish. The plasma was formed in air close to solid-gas interface in the inter-electrode gap area without the application of an additional gas flow. Inactivation studies at durations of 1 and 5 min were performed at a fixed distance of 5 mm from the electrode to the surface.

### 2.2. Bacterial strains and isolates

*E. coli* (ATCC 25922), *S. epidermidis* (ATCC 12228), methicillin sensitive *S. aureus* (ATCC 29213), and methicillin resistant *S. aureus* (ATCC 43300) strains were purchased from the American Type Culture Collection (Manassas, VA). One methicillin sensitive and two methicillin resistant clinical *S. aureus* isolates cultured from a shoulder wound, a toe wound, and synovial fluid respectively from different patients were acquired from a local hospital. The susceptibility patterns of the two methicillin resistant isolates as determined by a Vitek 2 system (bioMerieux, Durham, NC) differed only with respect to clindamycin.

### 2.3. Plasma application to bacteria seeded on surfaces

Overnight nutrient-rich broth cultures were serially diluted and seeded onto agar plates, ex vivo porcine skin, or smooth or textured plastics. Agar plates. For *E. coli* and *S. epidermidis*, 5000 cells in 50 μL were uniformly spread on Brain Heart Infusion (BHI) agar plates. For *S. aureus*, the total