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Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms

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ABSTRACT

Non-thermal atmospheric pressure plasma jets (APPJs) are capable of generating cold plasma plumes that are not confined by electrodes, which makes them very attractive for bio-medical applications. In the present work, the inactivation efficiency of cold APPJ was evaluated against three pathogenic microorganisms with different cell wall characteristics. The Gram-positive bacterium *Enterococcus faecalis* (ATCC 29212), the Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 15442) and the fungus *Candida albicans* (SC 5314) were plated on standard Petri dishes filled with specific culture media. The plasma jet with mean power of 1.8 W was directed perpendicularly on agar plates and the system was flushed with pure helium at two different flows, 2.0 and 4.0 SLM. During the treatments, time and distance between nozzle and agar were varied. The presence of excited reactive species was confirmed by optical emission spectroscopy. Scanning electron microscopy (SEM) was applied for investigation of cell morphology. The microbicidal efficiency was evaluated by measuring the area of inhibition zone (where there was no cell growth). For different flows of helium, no significant difference of inhibition zone size was noted for the same microbial species. However, high flows led to formation of non-homogenous inhibition zones, presenting microcolonies distributed through the inactivated region. The Gram-positive bacterium was more susceptible to the plasma antimicrobial effects than the other microorganisms.

1. Introduction

Non-thermal atmospheric pressure plasmas have attracted much attention due to their innumerous advantages over low-pressure plasmas [1–4]. Since they can be generated under ambient pressure and temperature conditions there is no limitation in the size of the treated objects [3,4]. Cold atmospheric pressure plasmas are also characterized by reactive chemistry at close-to-room temperature, which allows the treatment of heat sensitive materials [3].

Among other plasma sources, the atmospheric pressure plasma jet has emerged as a promising tool capable of generating cold plasma plumes that are not spatially confined by electrodes. Depending on the operation conditions, the resulting plasma jet can propagate up to several centimeters into surrounding ambient, which makes it appropriate for treating irregular surfaces or 3D objects [4]. Plasma jets present relatively low operational cost and when launched into air generate large amount of reactive and exited species under ambient temperature conditions. Because of their rich chemistry and the possibility for easy application to any target, APPJs have become very attractive for biomedical applications [5–8]. They have drawn significant attention in applications such as microbial inactivation [9–11], blood coagulation [12], decontamination of medical equipment [13], wound healing [14], for medical therapy and applications in dentistry [15,16].

Most APPJ systems consist of a high voltage electrode embedded in a dielectric tube or a capillary. The device is fed with a noble gas or a mixture of a noble and a reactive gas. The plasma is generated inside the tube and expands into the open air where the plasma plume interacts with air molecules forming reactive oxygen (ROS) and nitrogen species (RNS) [17]. These reactive species combined with UV photons and charged particles can cause microbial inactivation. Besides, humidity present in air can enhance the production of ROS by the plasma jet [18]. The exact mechanism of cell inactivation by cold plasma jet as well as the precise contribution of each plasma component to the process are not well-understood [19]. The damaging effects of reactive oxygen and nitrogen species (RONS) on cells can be attributed to their ability of reacting with some cellular biomacromolecules such as proteins, lipids and DNA [20]. Some ROS can cause oxidative damage to microorganisms [21]. However, it is important to consider the synergetic effect of multiple species presented in APPJs. It was shown

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that jet inactivation efficiency is greatly enhanced in the presence of UV radiation [19].

The precise control of plasma parameters is an important concern in plasma medicine. When physical parameters are changed, different amount of plasma species are driven to target, which can lead to more efficient surface decontamination. In the present work, three species representative of different microbial groups were treated in order to evaluate the inactivation efficiency of cold atmospheric pressure plasma jet. Gram-positive bacteria possess thick cell walls composed by several layers of peptidoglycan. Differently from Gram-negative bacteria, they are absent of bacterial outer membrane. Gram-negative bacteria exhibit clearly layered structure with three main sections: the outer membrane, the peptidoglycan cell wall and the inner membrane. The outer membrane plays important role in protecting the cell against toxic molecules and providing an extra stabilizing layer around the cell [22]. Fungi are eukaryotic cells and their cell walls are completely different from the prokaryotic ones (bacteria). Fungal cell walls are very thick and composed by rigid polysaccharide layers, such as chitin. It is responsible for providing structural strength to fungi cell walls making them more resistant to harmful extracellular agents [23]. Due to these noticeable structural differences of the selected microorganisms, it is expected that different responses to the plasma treatment would occur. Here, the Gram-positive bacterium *Enterococcus faecalis* (ATCC 29212), the Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 15442) and the fungus *Candida albicans* (SC 5314) were plated on standard Petri dish filled with culture media and then exposed to plasma. The effect of gas flow and the distance between plasma plume and agar surface was also investigated.

2. Materials and methods

2.1. Plasma generation

The atmospheric pressure plasma jet used in this study is a DBD type APPJ with a single electrode and it was detailed in [24]. It consists of a 2.3 mm diameter copper rod, which is embedded inside a closed-end quartz tube. The quartz tube with wall thickness of 1.7 mm serves as a dielectric barrier. It is centered in a syringe-like dielectric enclosure made of Delrin, which terminated with 2.0 cm long, 1.5 mm inner diameter nozzle. The system is flushed with helium (99.5% purity) and gas flow rate was adjusted within the range 0.1–10.0 SLM by a rotameter.

The electrode is connected to a Minupuls4 AC power supply (GBS Elektronik GmbH, Germany). A grounded sample holder was placed under the plasma plume allowing fine vertical displacement of the sample and electrical characterization of the discharge. The discharge current and the transferred charge were measured across a low inductance resistor of 100 Ω (for current) or a capacitor of 10 nF (for transferred charge) connected in series to the grounded stage. The signals were monitored on a digital oscilloscope (Tektronix TDS 2024B, 200 MHz). The mean discharge power of the plasma jet (1.8 W) was calculated by the Q-V Lissajous figure method. More detailed information about electrical characterization of the plasma jet can be found in [24].

2.2. Microorganisms

The following microorganisms were used in this study: *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 15442 and *Candida albicans* SC 5314. All organisms were kept in BHI broth supplemented with 20% glycerol at −80 °C. Fresh cultures were obtained by plating *C. albicans* on Sabouraud dextrose agar, *E. faecalis* on M-enterococcus and *P. aeruginosa* on BHI agar. After incubation for 24 h at 37 °C and normoxia, cells were suspended in saline solution (NaCl 0.9%) and the turbidity was adjusted to the McFarland 0.5 standard. To evaluate the antimicrobial effect of plasma jet, 0.1 mL of cells suspensions were plated on the surface of solid culture medium distributed in standard Petri dishes (90 mm diameter) using a sterile swab. Dishes were kept in aseptic environment (air flow chamber) to dry for 15 min and then exposed to plasma jet. In order to evaluate the effect of plasma treatment on cells, the area of microbial inhibition zones formed on agar were calculated.

2.3. Treatment conditions

Plasma jet operated at frequency of 31.0 kHz and voltage amplitude of 13.0 kV for all treatments. Each experiment was performed in triplicate in order to ensure reproducibility and the plasma plume was directed perpendicularly to the surfaces of agar plates. The three microorganisms were exposed to plasma jet for different time intervals (60, 90, 120, 150 and 180 s). After treatment, all plates were incubated for 24 h at 37 °C. For control experiments, samples were subjected to helium flow at the same flow rate without plasma ignition.

The effect of gas flow on inactivation of different microorganisms was studied by varying the applied helium flow between 2.0 and 4.0 SLM. This flow range was determined according to the conditions to obtain optimal jet length (around 2.0 cm). In this first experiment, the distance between the agar surface and nozzle exit was kept fixed to 2.5 cm. Since increase of treatment distance can prevent some active species of reaching the surface, a second experiment was done. The distance between nozzle and agar was varied while the gas flow was kept constant in 2.0 SLM. The treatments were performed with three different distances (2.0, 2.5 and 3.0 cm).

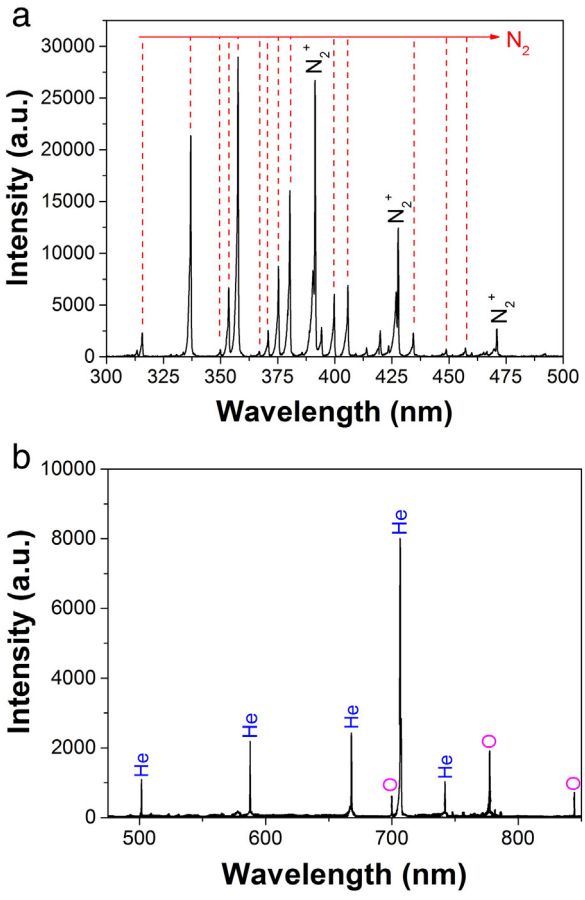

Fig. 1. Emission spectrum acquired at the tip of the plasma plume (a) for wavelengths below 500 nm and (b) for wavelengths between 475 nm and 850 nm.

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