Potentiality of a Fractionated Cold Atmospheric Pressure Plasma Treatment for Bacterial Decontamination
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Abstract: This work deals with the comparaison of two methods of plasma treatments on *E. Coli*: continuous and fractionated treatment. It was realized with a micropulsed DBD jet reactor operating in a mixture of He and O_2. Bacterial inactivation areas were measured versus the effective time of treatment. It is demonstrated that a fractionated treatement is twice more efficient than a continuous plasma exposition. Optical diagnosis and KI-starch experiments were realised to infer about the inactivation mechanisms of the two protocols.

Keywords: CAPP, Bacteria, Decontamination, Fractionated treatment

1. Introduction

In the past few years, the field of Plasma medicine has been widely investigated [1-8]. The bactericidal effects of the cold atmospheric pressure plasma (CAPP) are nowadays established [2-4,8]. They may offer an alternative to conventional and sometimes inoperative antibiotic approach. There are several physical and chemical phenomena induced by the CAPP. Among of all of them, some play the roles of biogical-active agents, such as ultra-violet, charged species, electrical field, oxygen and nitrogen reactive species (RONS) [5-6,8]. For bacterial decontamination and inactivation at atmospheric pressure, dielectric barrier discharge reactors (DBD) have been deeply studied for their important RONS creation rate [7]. These non-thermal plasma reactors create CAPP jets. Few seconds of continuous treatment at close distance (in the order of few centimetres) are sufficient to inactivate bacteria even resistant strains [8].

However, the treated area is narrow due to the diameter of the jet (c.a. few millimeters). Moreover, some reactors generate jet with a temperature exceeding 40 °C. Thus, the treatment times on human tissues of these jets can not last a long time on the same location.

In the aim of overcoming these drawbacks, an other method of treatment was experienced in this work. A fractionated treatment was studied in the idea of a sweeping treatment, where a same location is treated multiple times.

In this work, we first compared influence of the time of treatment on bacterial inactivation area on Petri dishes, for continuous and fractionated treatment. Then, optical diagnosis were realized to understand mechanisms that could play a role in the differences between continuous and fractionated treatments. Finally, agar with KI-starch Petri dishes were treated both ways, to observe oxidant species storage and diffusion in an agar-medium through time [9].

2. Experimental set up

Plasma source

The different cold atmospheric pressure plasma treatments were realized by Plasma Gun (PG) reactor developed by the GREMI [10]. It is a micropulsed DBD jet reactor operating, in this work, in a mixture of He and O_2. The reactor is made of the thermoplastic PEEK as dielectric, with a 100 mm long capillary and an inner diameter of 4 mm . The PG generator delivers 2 µs discharges, with a voltage up to +10 kV and a pulse repetition rate up to 30 kHz.

Bacteria

The bacteria used are from a *E. coli* (CIP54 117) non pathogenic strain provided by the *Institut Pasteur*.

As pre-sowing protocol, 100 µL of bacteria are inoculated in 5 mL nutritive broth to grow for 12 hours at 37 °C in a shaking incubator to reach 10^8 bacteria/mL. Solid and liquid nutritive media for bacteria are sterilized in autoclave then dispensed in 9 cm diameter Petri dishes. 100 µL of the bacteria solution are inoculated in 5 mL liquid medium.

Bacteria sowing was operated by the flooding method. The surplus was removed after 10 min. The Petri dishes were left under a laminar flow cabinet to dry for 20 minutes.

KI-Starch agar plates

Agar with 0.3 % of KI and 0.4 % of starch Petri dishes were used to monitor reactive species storage and diffusion in the agar medium. Agar and KI-starch solution are dispensed in 9 cm diameter Petri dishes.

Optical diagnosis

A camera, model *Canon 6D*, and a light pad were used to take photos of the Petri dishes with an homogeneous lightning.

An ICCD high speed camera, model *PIMAX3 Princeton Instruments*, and a highspeed camera, model *Fastcam mini AX Photron*, associated with a Schlieren set up were used to characterize the generation and propagation of the plasma jet.

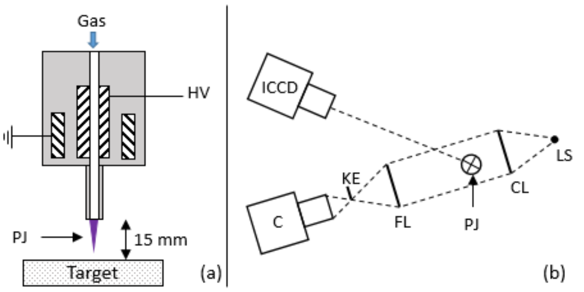

Fig. 1. Experimental set up. (a) Plasma Gun. (b) ICCD camera and Schlieren set up: light source (LS), collimating lens (CL), plasma jet (PJ), focusing lens (FL), knive edge (KE), highspeed camera (C)

3. Methods and Analysis

Treatments methods

Two different treatment methods were studied and compared: continuous (Tc) and fractionated treatment (Tf). For both treatments, the PG generator delivered discharges of 7 kV at 20 kHz in a mixture of He with 0.5% O_2, for a gas flow of 1 slm. The reactor-target gap was 15 mm long.

The fractionated treatments consists in repetitions with ten second plasma off intervals in between the active 5 s continuous plasma delivery periods. During the plasma off intervals, the Petri dishes were still positioned under the gas flow. It had been experienced that the gas flow without plasma exposure during 1 min does not inactivate bacteria.

The times of continuous treatments studied were [5, 10, 15, 20, 25, 30, 60] seconds for bacteria and [5, 10, 30] seconds for KI-starch agar plates.

The effective times correspond to the cumulated times of fractionated plasma treatment (e.g. 5s+(10s pause)+5s fractioned treatment correspond to 10s effective time). These effective times studied were [10, 15, 20, 25, 30] seconds for bacteria and [10, 20] seconds for KI-starch agar plates. This corresponded respectively to [2, 3, 4, 5, 6] and [2, 4] repetitions of 5 s treatments.

Areas measurement

The Petri dishes photos were processed by a Matlab program to obtain bacterial inactivation areas and KI-starch reaction area.

Presented values are means and standard deviations calculated from three experiments.

ICCD and Schlieren configuration

The aim of the ICCD and Schlieren diagnosis was to observe if the behaviour of the plasma jet was steady state during the treatment time and to investigate the likely influence of the targets versus time. It was previously measured that accumulated charged on some targets may repulse the plasma jet from the target in a periodic way [11]. The target used in this work was at a floating potential. This is probably a configuration where the charge accumulation may have a more drastic impact on the plasma jet than with the Petri dishes wer the agar meadi is little bit more conductive but still at a floating potential..

ICCD camera acquisition was synchronized with the PG generator. The acquisition mode was a repetitive gating mode with a gate width of 10 µs to cover the entire high voltage pulse (2 µs) and the following instant. The intensifier was set at 100.

The Schlieren camera had a frame rate of 1000 fps and a shutter speed of 1/40000 s.

The target was positioned at 15 mm from the reactor nozzle.

4. Results and discussions

Bacterial inactivation

After sowing, eight hours at 37 °C are necessary for the bacterial colonies to grow. Colonies create an opaque layer in the Petri dish. Thus, inactivated areas by plasma can be observed as quite transparent zones. **Fig. 2** illustrates the bacterial inactivation area for different time of the two treatment methods.

Inactivation areas increase when the effective time of treatment increases. Fractionated treatment is more efficient compared to the continuous treatment for this configuration of the PG.

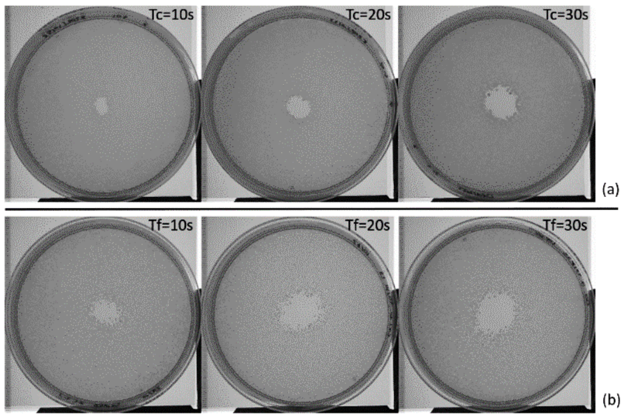

Fig. 2. Comparaison of the bacterial inactivation area between continuous (a) and fractionated treatment (b), for different treatment time : 10s (left), 20s (middle) and 30s (right). Corresponding respectively to 2, 4 and 6 repetitions of 5s.

Fig. 3 shows the linear growth of the bacterial inactivation area versus the treatment time. It highlights the fractionated treatment is twice more efficient compared to the continuous treatment.

The following sections report investigations on the mechanims that could play a role in the differences between the two methods of treatment.

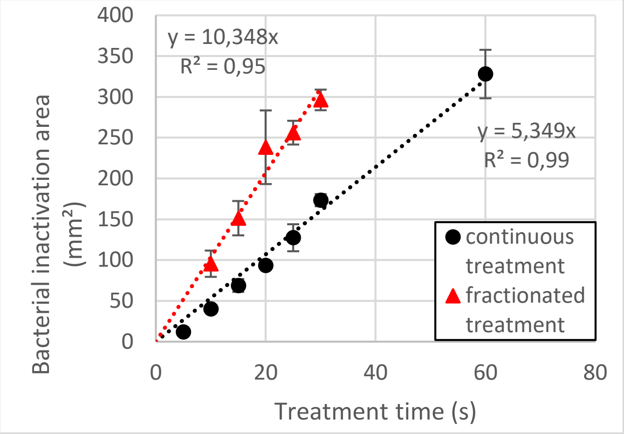

Fig. 3. Variation of the bacterial inactivation area surface as function of the treatment time, for continuous and fractionated treatment.

ICCD and Schlieren imaging

ICCD and Schlieren imaging revealed no significant alteration in the behaviour of the plasma jet during 30 seconds, as shown in the **Fig. 4**. For these configurations, accumulated charges does not repulse the plasma jet from the target. Thus, it is always in contact with the target during the treatment.

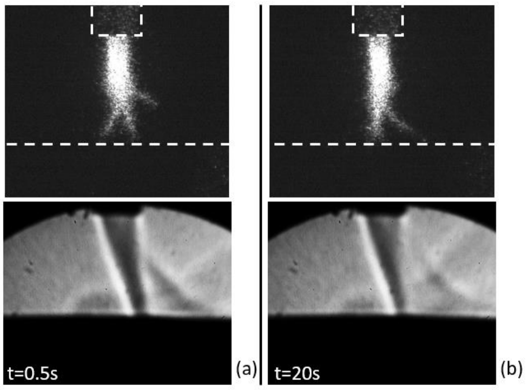

Fig. 4. Comparaison of ICCD (top) and Schlieren imaging (bottom) of the plasma jet at 0.5s and 20s

From a treatment time of only 10 s, The **Fig. 2** and **3** pointed out differences for the two methods. However, ICCD and schlieren imaging prove, for continuous and fractionated treatment with a similar effective time treatment, that the plasma jet contacts the target during the same amount of time. It is checked that the plasma jet operates in a steay state regime even in presence of the target

KI-Starch

The KI and the starch in the agar react with oxydant species such as the ROS created in the plasma jet. The reaction area becomes blue.

The diffusion and the storage of these species in the agar medium through time, may be observe by comparing photos of the same agar KI-starch Petri dish for different times after a plasma jet treatment.

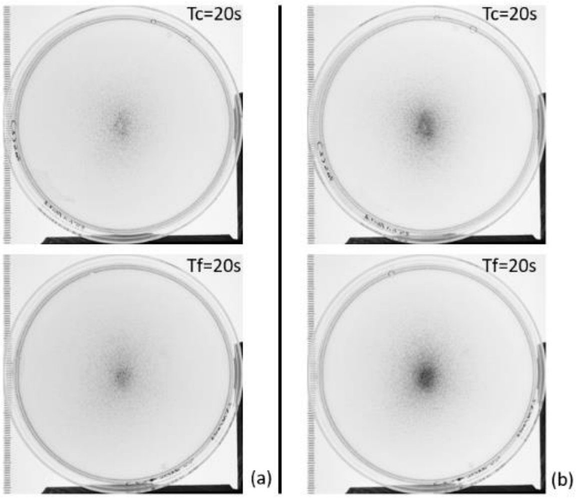

Fig. 5. Comparaison between photos taken 1min (a) and 1 day (b) after a continuous treatment of 20s on a KI-starch agar plate (up) and a fractionated treatment of 20s on a KI-starch agar plate (down)

Fig. 5 shows there is not significant discrepancy for the two methods for the same effective time of treatment 1 min and 1 day after the plasma treatments.

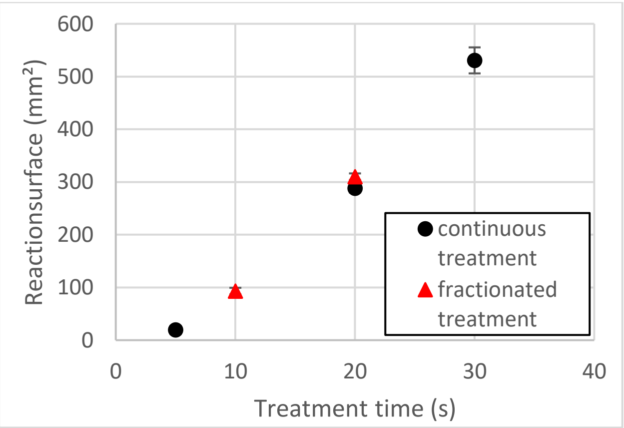

Fig. 6. Variation of the reaction area on a KI-starch agar plate 1day after treatment as function of the time treatment, for continuous and fractionated treatment.

Fig. 6 shows a linear expansion of the reaction areas with of the effective time treatment for both treatments. By contrast with the bacterial inactivation, Agar with KI-starch reacts identically whatever the treatment protocol (continuous or fractionated). Thus, oxydant species storage and diffusion in the agar medium can't explain the bacterial inactivation differences between the two methods of treatments for these configurations. Work is in progress to investigate the reactive species delivery on the surface of the agar plates and to check if biological response of bacteria are responsible for the fractionated antibacterial treatment improved efficiency.

5. Conclusion and perspectives

In this work, two methods of treatments by a micropulsed Dielectric Barrier Discharge plasma were compared for different times of treatment.

For the experiments using plasma gun configurations, the fractionated treatment is twice more efficient as the continuous treatment.

However, ICCD and schlieren imaging revealed the plasma jet is in contact with the target for the same time of treatment, for each experiments. Moreover, experiments with KI-starch proves the oxidant storage and diffusion in the agar medium do not differ in variation of these treatment methods.

6. Acknowledgments

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