Plasma Jets In Interaction With Liquids In The Practical Case Of In Vitro Treatments

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Abstract— The majority of atmospheric pressure plasma jet (APPJ) applications involves the interaction between the plasma and a target. The understanding of the mechanisms underlining this interaction is a key step for the future development of APPJ technology. Nevertheless, many widely adopted target configuration, such as those encountered in in vitro biomedical studies have barely been investigated from a physical point of view. The present study shows how APPJ can induce vortex formation and lead to strongly non-uniform distribution of plasma-generated long-lived reactive species in a vessel with the characteristic length scale of the wells commonly adopted for *in vitro* biomedical studies.

Keywords—atmospheric pressure plasma, liquid target, schlieren, convection, fluid dynamic, plasma medicine

I. INTRODUCTION

Atmospheric plasma jets were supposed to generate a "free jet" able to propagate out of the plasma reactor, to be then delivered on any kind of target (as for e.g. biological tissues). This characteristic overcomes geometrical and size-dependent limitations of DBD and even FE-DBD plasma sources where target is inside the plasma reactor or is part of it. Nevertheless, many studies recently focused on the effects of target materials and electrical connections on the properties of atmospheric pressure plasma jets [1,2]. The presence of the target induces a perturbation of the discharge itself, that cannot be avoided because the discharge is sensitive to the surrounding [3]. Several works support the thesis according to which the target could change the electric field distribution between the electrode and the target thus affecting the dynamic and density of the ionization wave, the generation of reactive oxygen and nitrogen species and the corresponding treatment effects. In plasma science the targets could be living tissues, metal materials or dielectrics. Although many works have emphasized the interaction between plasma and targets of different materials many points remain still unclear on the mechanisms governing the process. In particular in plasma medical studies, there are some *in vitro* configurations (e.g. APPJ impinging on a Petri dish containing agarose) that while being widely adopted have been very scarcely investigated from a physical point of view. As already cited, plasma is affected by the target and it′s surrounding in such a way that even changing the target support can play a major role [1,3,5].

As a result, it appears quite critical and relevant to undergo a physical investigation of the adopted setups even when the main focus of the research maybe purely biological.. In the context of plasma medicine the understanding of plasma-liquid interaction is of major importance in biomedicine where water is a main component of most of the encountered targets (e.g. culture medium, tissues, …) [4].

As an example, Sasaki et al. [6] reported on a how the plasma-induced enhancement of the cell membrane permeability decreased markedly with increased solution thickness probably due to the decay of the plasma-produced OH that is characterized by a diffusion length on the order of several hundred micrometers. The addition of few milliliters of solution while irrelevant from

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a purely biological point of view could completely change the plasma treatment outcome. Previous studies already demonstrated that for example natural convection can take place inside Petri dishes in incubators and lead to non-uniform cell culture [7]. Other studies demonstrated that an APPJ impinging on a liquid surface can induce modification of the fluid dynamic regime in the liquid phase affecting therefore the reactive species transport [8–10].

Awareness is rising that only a combined characterization of both the treatment conditions and the treatment effects could provide powerful insights on the leading mechanisms of plasma action.

With this in mind, the present study aim is to investigate the fluid dynamic behavior taking place in a common plasma medicine *in vitro* setup with a plasma jet impinging on a 24 well microplate for biological studies.

II. METHODOLOGY

The atmospheric plasma jet used in this work is a Plasma Gun (PG) already described in detail in [11]. Briefly, the PG is a coaxial DBD reactor with a quartz capillary flushed with helium and powered by a micropulsed high voltage generator. The 12 cm long capillary was tapered at the outlet ($Ø_{in}$ = 1.5 mm, $Ø_{ext}$ = 3mm). The source was flushed with a helium flow and powered by 4 µs duration voltage pulses of 12 kV peak that could be set to be either with positive or negative polarity. The repetition rate of the pulses was fixed at 1kHz. The plasma source was positioned vertically over the sample (Figure 1). The discharge gap (distance between the nozzle and the surface of the liquid) was varied between 5 and 15mm.

As a target we adopted a custom-made 3D-printed vessel with two parallel glass windows. The vessel was designed to present a cubic cavity of 16x16x16mm. These dimensions were chosen in order to reproduce as close as possible those of the well of a common 24-multiwell microplate (cylinder Ø16mm, H:16mm; Nunclon® Delta Surface, Thermo Fisher Scientific, DK) while having parallel walls so to allow Schlieren visualization. Even if with different geometry (cubic instead of cylindrical) the custom vessel has the same characteristic length scale of a standard well adopted for *in vitro* experiments and should therefor present comparable fluid dynamic regimes. Moreover, the small capillary nozzle ($Ø_{in}$ = 1.5mm) is pointed at the center of the vessel so that the influence of the wall is minimized.

The vessel bottom (1mm thick) was maintained 1cm above a grounded plate by a dielectric support.

The liquid adopted for the test was DPBS (Dulbecco's Phosphate Buffered Saline by Gibco). A total amount of 2ml of fresh liquid was inserted into the vessel before each test.

Visualization of helium and liquid flows was performed on a Z-type Schlieren [12] optical test bench, equipped with a 3 W green LED light source, a set of parabolic mirrors, a knife-edge mounted vertically on a precision translation and a high frame rate camera (IDT-Streal XS-3, shutter speed: 1/10000s, fps: 60) [13].

The overall plasma-produced oxidizing species distribution in the solution was detected by KI-starch reaction [14]. KI-starch reagent was mixed into the liquid target previous to plasma exposure.

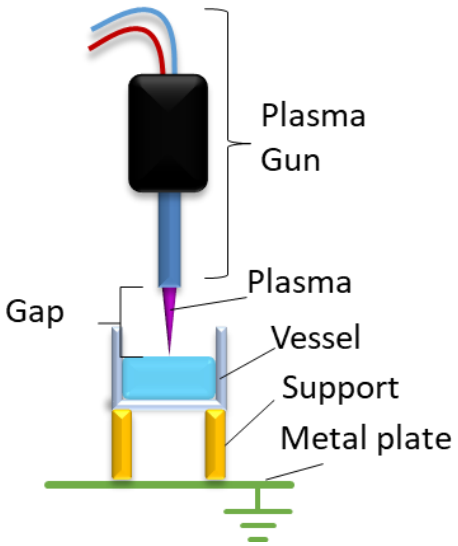

Figure 1: Schematic representation of the experimental setup

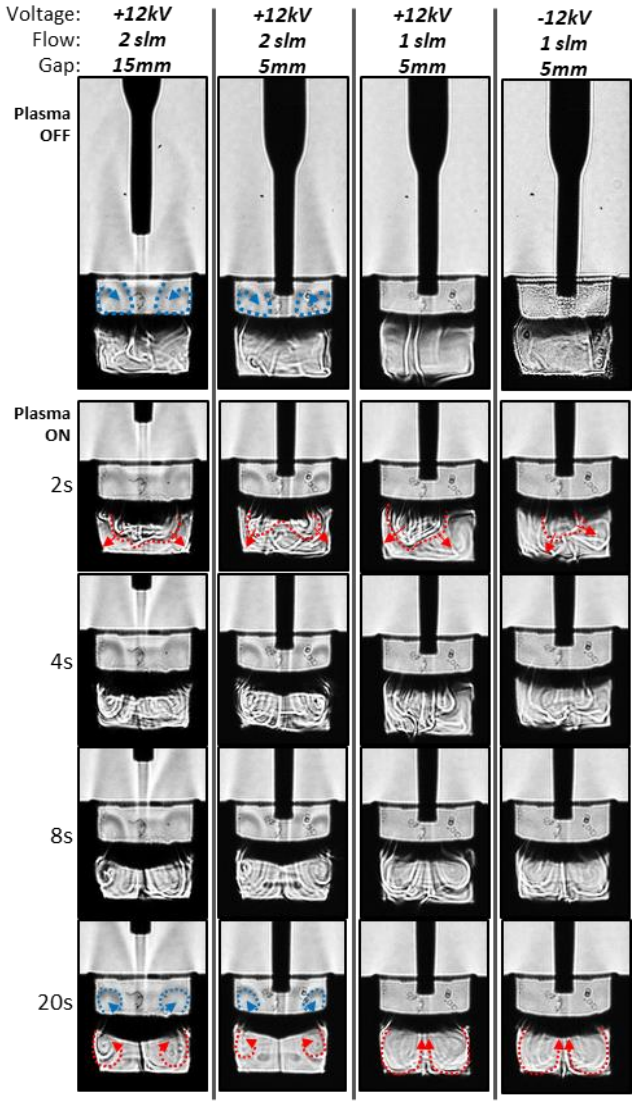

Figure 2: Schlieren acquisitions. Superimposed blue arrow are to help the visualization of vortexes in the gas phase while red lines/arrows are used to highlight turbulent fronts and vortexes in the liquid phase.

III. RESULTS

In Figure 2 are presented a selection of frames extracted from the Schlieren acquisitions. The horizontal shadow visible in the middle of the well is casted by the meniscus formed at the the surface of the liquid in proximity of the glass wall. As visible in the first row of Figure 2, for none of the considered cases the helium flow alone (plasma off) was able to induce the formation of a stable regime in the liquid sample even after several tens of minutes. Random fluctuation in the liquid density appear probably caused by the helium flow cooling effect but no stable regime sets. For the first two cases on the left, at higher helium flow, we observe that stable vortexes are generated above the liquid surface.

Moreover, a column of helium moving upward is clearly visible to enclose source capillary probably reducing the mixing with ambient air. Moving to the frames after the plasma ignition (plasma on) we see in the first frames (Figure 2 row at 2s after plasma is turned on) a turbulent front start propagating from the impinging point and moves radially away from it. This turbulence is visible in all cases but more pronounced in the cases at 2 slm. The turbulence induced by the plasma evolves in the following seconds (Figure 2 rows 4s and 8s) to form stable vortexes. According to the complete video acquisition these vortexes appear to rotate faster and have smaller dimensions for the cases at higher flow rate (2slm) where they nearly mirror the vortexes formed in the gas phase (see Figure 2 row 20s). We can speculate that the gas flow features above the liquid surface are at least partially at the origin of the observed liquid patterns.

The cases at lower flow rate (1slm) induce wider vortexes that follow the vessel borders and converge at the centre of the vessel in an upward direction toward the surface. No significant influence of the voltage pulse polarity is observed (Figure 2 third and last column from the left) on the fluid dynamic induced inside the liquid.

In Figure 3 we observe the results achieved from the addition of KI-starch reagent in the liquid. For all considered case in the first 20s after the plasma ignition we observe an accumulation of reactive species at the surface of the liquid. These species are transported into the bulk of the liquid only starting from 30s after the start of the plasma. The propagation of the oxidation of the reagents follows the vortex structures previously identified (compare Figure 2 and 3). For the case at higher flow rate the vortex are faster and lead to a nearly uniform diffusion of the plasma induced species within 50s. The two cases at lower flowrate are characterized by larger and slower vortexes which induce reactive species flow localized

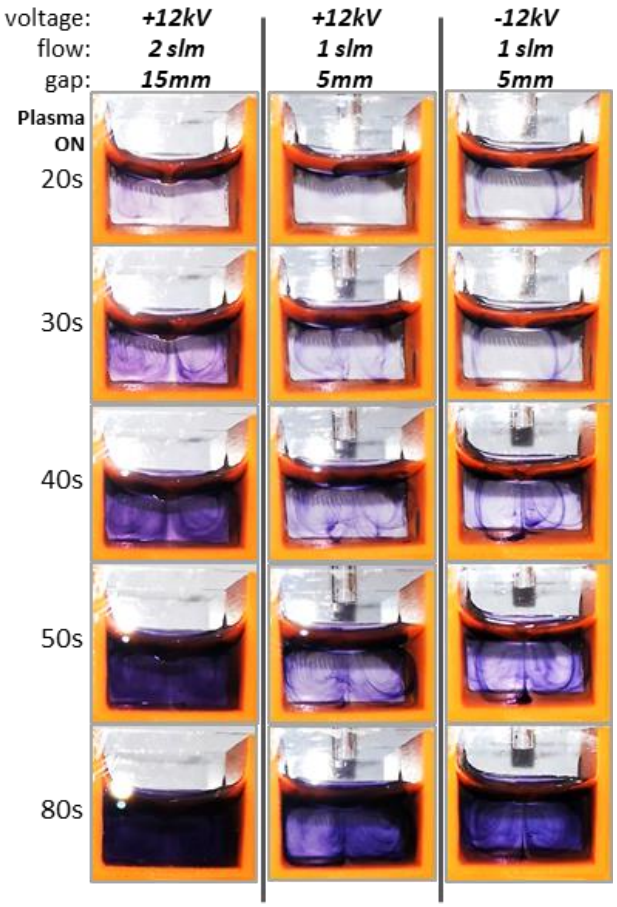

Figure 3: Temporal evolution of target solution containing KI-starch reagents during plasma exposure for different operating conditions

close to the vessel wall and a central column in correspondence of the impinging point. The volume of liquid at the centre of this vortexes forms a torus that remains relatively clear of plasma induced reactive species up to 80s after the start of the plasma.

IV. DISCUSSION

Starting from cases where the gas flow only does not induce vortex formation in the liquid, we observed the formation of these vortexes when the plasma was turned on. We tend to exclude a possible heating of the liquid due to the plasma as a cause of these vortexes as it would probably not lead to the formation of structured patterns, such as the vortexes, but rather to stratification due to the accumulation of hotter and less dense liquid at the top of the well.

Other possible causes are the gas flow instabilities induced by the plasma discharge, electrohydrodynamic forces and buoyancy forces due to the reactive species formation and liquid heating. The combinations of these plasma related effects cause the liquid to circulate only in the presence of the plasma.

The vortex dimension and position is strongly influenced by the gas flow rate and the gap, resulting in faster and smaller vortexes for higher helium flowrates and shorter gaps.

As reported by Kaneko et al [5] short-lived reactive oxygen species generated at the solution surface are prevented to reach deeper layer in the liquid solution. This observation is confirmed also by our findings even when stable vortexes take place in the solution as their velocity results too low to transport short-lived species to the bottom of the well if not with time scales of several seconds.

The results here presented are complementary with those reported by [6] where very shallow thickness (≤1mm) of the liquid and short treatment time (≤5s) were investigated. In their case there is no effect due of the liquid recirculation and the reactive species distribution in the liquid is mostly dominated by the diffusion and the distribution in the gas phase. This is also in agreement with our results where in the first seconds of the treatment we essentially observe an accumulation of reactive species in the close proximity of the surface.

Partially in contrast with what reported in other works [9,10] we did not observe an homogeneous plasma induced color change of the solution. On the contrary, the observed reaction of KI-starch is strongly anisotropic and mainly governed by the vortex induced inside the liquid phase. Assuming an initial uniform distribution of the KI-starch reagents, this could suggest a transport mechanism for long lived reactive species generated at the surface of the liquid by the plasma. It appears evident that, especially on short timescales, some zones of the well can be exposed to reactive species concentrations significantly higher than the average one of the whole solution.

V. CONCLUSION

Presented results show how the interaction between plasma and target can not only affect the two but also the treatment conditions. It was observed that for a liquid contained in a vessel, with the dimension of a typical multiwell plate commonly adopted in biomedical studies, the impinging of an APPJ can induce the formation of stable vortexes in the liquid phase. These vortexes strongly affect the distribution of long-lived reactive species inside the liquid, leading to non-uniform distributions but also to a relatively fast transport from the surface to the bottom of the vessel.

It is shown that discharge gap and especially gas flowrate have a strong influence on the formation of vortexes and the distribution of liquid species. Surprisingly, the influence of applied voltage polarity has been found to be minimal for the investigated cases.

The findings here reported suggest that special attention should be payed to the setup adopted during plasma treatment of small liquid volumes as in *in vitro* biomedical experiment.

The formation of vortexes in the liquid phase can significantly alter the transport and distribution of long-lived reactive species and in turn the effect of the treatment. Especially comparison between plasma treatment and various controls that may include exposure to gas flow only or to liquid solutions with various concentrations of reactive species mimicking those produced by the plasma, should take this mixing dynamic into account.

Future studies will focus on the investigation of a wider range of operating conditions, including different volume of liquid and the presence of grounded surfaces under the bottom of the vessel as well as the possibility to ground the treated liquid.

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