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COMPARISON OF PLASMA AND ELECTRIC PULSE TREATMENTS ON CELL MEMBRANE PERMEABILIZATION

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Abstract: In this preliminary work, we showed that a kHz plasma jet can induce cell membrane permeabilization. Those results have been compared to electroporation and indicate that permeabilization by plasma is less efficient, which is probably due to the fact that in electroporation a current gets through the cells. We also showed that chemistry and/or electric field induced by plasma plays a role in cell permeabilization.

Keywords: Plasma medicine, cell membrane permeabilization, electric field, plasma jet, electroporation.

1. Introduction

Cold atmospheric pressure plasmas have demonstrated their potentiality in biomedical applications thanks to their low gas temperature and their capacity to produce radicals, ions, electrons, UV radiation and electric field.

One of the first evidence for cold atmospheric pressure plasma inducing cell permeabilization was reported a few years ago by Ogawa *et al.* They were able to introduce nucleic acids into cells [1]. This technique could be useful for medicine and biology applications such as gene therapy or cancer treatment [2-3]. Leduc *et al* demonstrated that an atmospheric pressure glow discharge torch was able to induce cell permeabilization with molecules with a maximum radius of 6.5 nm [3]. However, the understanding of the interactions between plasma, living cells and tissues is still far from being completely understood and the mechanism responsible for cell permeabilization is still unclear.

It has been demonstrated that the electric field plays a very important role in cell permeabilization, especially in electroporation for drug delivery where electric pulses are used [4]. Indeed, electric pulses are able to permeabilize cell membranes and thus to increase the uptake of non-permeant molecules such as chemotherapies [5]. Actually, the range of the electric field produced in cell electroporation protocols is in the same order of magnitude than the electric field produced by certain plasma jets. But it must be pointed out that the pulse duration is different. It rises at around 100 μ s for the electric pulses while it is between 100 ns and some μ s for kHz plasma jets. Robert *et al* measured the electric field at the output of a Plasma Gun, a kHz plasma jet, and showed that the electric field from this plasma can in some specific conditions propagate deeply up to several millimeters in tissues [5].

The goal of this work is to get more insight in the role of the electric field and the reactive species produced by a kHz plasma jet on cell permeabilization. Electric pulses were used to study the effect of an electric field equivalent to that produced by the plasma jet, while the jet was used to study the effect of the combination of reactive species and electric field. A comparison between those two techniques will be presented, but it must be stressed that electric field is not applied in an equivalent manner in the two cases.

2. Experimental setup and method

The so called Plasma Gun, plasma jet used in this study was a coaxial dielectric barrier discharge reactor with a quartz capillary tube. A scheme of this reactor is shown on Fig. 1. In the tube, a ring electrode was connected to the high voltage, while a second ring electrode around the tube was connected to the ground. The device was powered by microsecond-duration voltage pulses of 14 kV with a repetition frequency between 10 Hz and a few kHz. Pure helium was flowing through the device at 0.5 slm flow rate. The plasma was generated in a 4 mm inner diameter quartz capillary having a 1.4 mm inner diameter tapered outlet.

The biological model used in this work was Chinese hamster fibroblasts DC-3F cells. The cells were cultured at 37°C with 5% CO₂ in a complete culture medium (Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) and 2% penicillin/streptomycin). Before treatment, the cells were rinsed with PBS, trypsinized with Trypsin-EDTA, centrifuged 5 min at 1000 rpm and counted. The cells were then diluted in DMEM and placed in a 96-well plate. Yo-Pro (629 Da) was used at a concentration of 1 μ M as fluorescent molecular marker to detect the cell permeabilization (1Da = 1.66 x 10⁻²⁷ kg). A couple of minutes after the plasma treatment the cells were analyzed

using a flow cytometer. Fluorescence due to impurities was subtracted from the total fluorescence signal, and only fluorescence from live and dead cells will be presented.

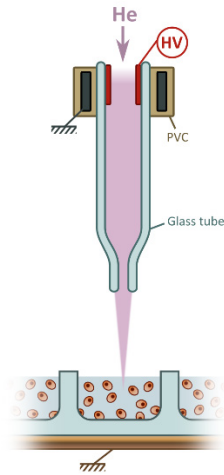


Fig. 1. Scheme of the plasma setup.

A metallic grounded plate lied under the 96 multi-well plate, with the cells in suspension, while the plasma jet was put vertically above the plate with the plasma plume toward the bottom. The plasma was in direct contact with the liquid and the treatment time was 80 s.

3. Results and discussion

Fig. 2 (a) shows a histogram of the fluorescence measured with no treatment (*ie*, control, black line) and with 80 s plasma treatment (red line). The main peak at 10^4 AU represents fluorescent cells, while the second peak at 3×10^6 AU represents highly fluorescent cells. Fluorescence of cells after plasma treatment is comparable to the control condition, apart a slight shift on the second peak. This indicates that under this condition, plasma has no major effect on cell permeabilization. Fig. 2 (b) is the same experiment condition as (a) unlike that a magnetic stirrer is used to make the solution homogeneous. In that case, plasma induces a change in the fluorescence. The shape of the first peak is modified and the peak amplitude is shifted to the right side. This reveals a slight enhancement of cells fluorescence. Adding a magnetic stirrer improves the mixing of the solution.

The plasma creates a multitude of components such as radicals, ions, electrons, UV radiation and electric field and interacts with the solution and the cells. A major challenge is the understanding of the role of each of those components. The results from Fig. 2 shows that solution mixing is an important parameter. Indeed, if the solution is not homogeneous, no cell permeabilization is observed. As the solution mixing does not induce a modification on the electric field and UV radiation, chemistry could be a

necessary component for cell permeabilization in that case. Another explanation would be that as DMEM media is a conductive liquid, the electric field induced by the plasma would have an effect on cells only on the edge of the liquid, which means on the liquid surface and at the bottom and on the wall of the well. A mixing of the solution would allow to the cells to be all exposed by the electric field.

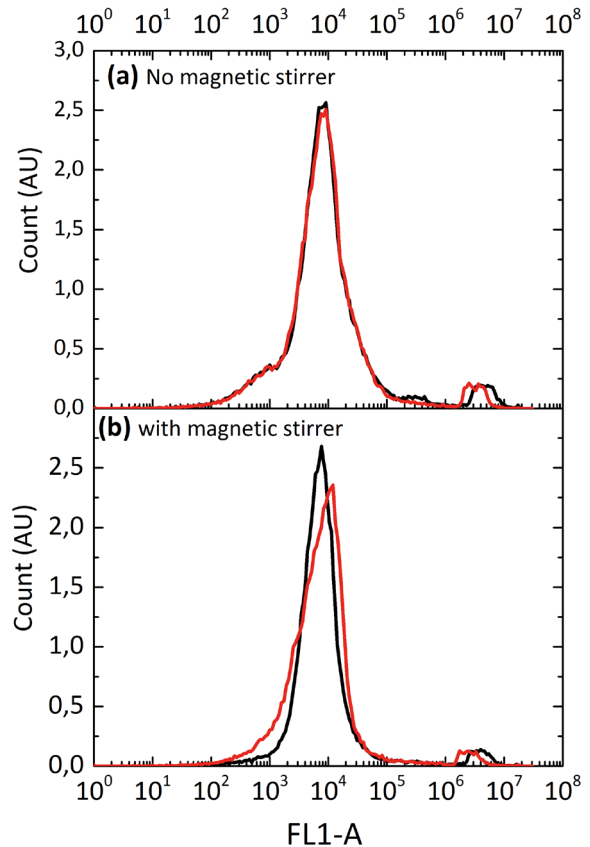


Fig. 2: Histogram of the fluorescence measured (FL1-A) without (a) and with (b) magnetic stirrer in the well. The black line is the control while the red line is after 80's plasma treatment.

Fig. 3 compares the fluorescence cell induced by a plasma treatment (red line) with an electric pulses treatment (blue line). A train of eight electric pulses of 100 V amplitude with a 100 μ s duration at 1 Hz was generated between two plate electrodes separated by a 1 mm gap, inducing an electric field of 1 kV/cm. In order to make data comparable, those histograms represent the same number of events per condition. We observe that with plasma treatment the fluorescence increases toward the right side compared to the control condition, while with electric pulses treatment, the fluorescence enhancement is even more pronounced. Initially 90% of cells were live cells and this percentage remains unchanged after electric pulses treatment, while it goes down to 83% after plasma treatment.

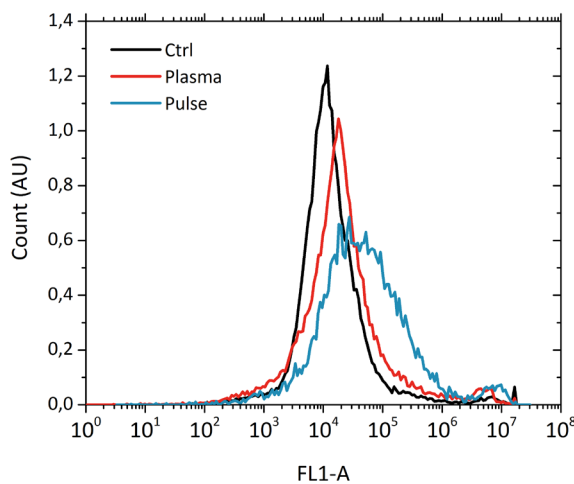


Fig. 3: Histogram of the cell YoPro fluorescence (FL1-A) measured with no treatment (*ie*, control, black line), with plasma treatment (red line) and with electric pulses treatment (blue line). Electric pulse condition: 8 pulses of 100V with a 100 μ s duration and a repetition frequency of 1 Hz.

Darny *et al* measured that the electric field induced by the plasma jet rises a couple of kV at 1 cm from the tube nozzle [7]. Darny's experiment condition were comparable to those displayed here, apart that a solution laying on a grounded metallic plate is in direct contact with the plasma, while in Darny's study there was no solution and the plasma reached directly the metallic plate. Thus we can assume than the electric field induced by the plasma is comparable to the electric field produced by the electric pulses.

In both cases, electric pulses and plasma jet enhance cell membrane permeabilization, but the plasma induces more cell death, while the electric fields magnitude are comparable. As we have shown, reactive species and/or electric field may play an important role in the case of permeabilization by plasma. But plasma creates others components like UV radiation or charged species which can also play a role. The main difference between electric pulses and plasma is the current which gets through the solution and then through the cells with electric pulses. It could explain why plasma is less effective than electric pulses.

Further improvements of cell membrane permeabilization is to be expected from the optimization of experimental parameters, including the repetition frequency, the gap between the tube nozzle and the gas composition.

4. Conclusion

In this preliminary work, we showed that a kHz plasma jet can induce cell membrane permeabilization. Those results have been compared to electroporation and indicate that permeabilization by plasma is less efficient,

which is probably due to the fact that in electroporation a current gets through the cells. We also showed that chemistry and/or electric field induced by plasma plays a role in cell permeabilization.

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