STUDY OF PLASMA COMPONENT EFFECT ON CELL MEMBRANE PERMEABILIZATION FOR DRUG DELIVERY APPLICATIONS

Vinodini Vijayarangan, Anthony Delalande, Claire Douat, Sébastien Dozias, Jean-Michel Pouvesle, Chantal Pichon, Eric Robert

STUDY OF PLASMA COMPONENT EFFECT ON CELL MEMBRANE PERMEABILIZATION FOR DRUG DELIVERY APPLICATIONS

Vinodini Vijayarangan[1, 2], Anthony Delalande[1], Claire Douat[2], Sébastien Dozias[2], Jean-Michel Pouvesle[2], Chantal Pichon[1] and Eric Robert[2]

[1] *CBM UPR4301 CNRS, Orléans France*
[2] *GREMI UMR7344 CNRS/Université d'Orléans, Orléans France*

Abstract: The parameters needed for helium plasma jet to induce a cell membrane permeabilization on cancerous cells for drug delivery purposes have been investigated. Using our optimal settings, different fluorescent molecular markers were used in order to determine pore size, opening kinetics and the importance of endocytosis in the process. It is shown that plasma induced chemistry but also electric field play a role in drug uptake and that endocytosis is one of the mode of membrane permeabilzation.

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

1. Introduction

Cold atmospheric pressure plasmas have demonstrated their ability 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]. However, the understanding of the interactions between plasma, living cells and tissues is still far from being completely understood.

It has been demonstrated that electric field can play a very important role in cell permeabilization, especially in electroporation for drug delivery where electric pulses are used [4]. Robert *et al.* measured the electric field produced at the output of the plasma. The range of this electric field measured was in the same order of magnitude than electric fields used in cell electroporation protocols. They also showed that the electric field from a plasma jet can propagate deeply, up to several millimeters in tissues [5].

In this work, we investigated the parameters needed for helium plasma jet to induce a cell membrane permeabilization on cancerous cells for drug delivery purposes. Using our optimal settings, different fluorescent molecular markers were used in order to determine pore size, opening kinetics and the importance of endocytosis in the process.

Furthermore, in order to get more insight in the role of the electric field produced by the plasma jet in cell membrane permeabilization, a comparison between the plasma and the electric field alone will be presented.

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 with 0.5 slm flow rate. The plasma is generated in a 4 mm inner diameter quartz capillary having a 1.4 mm inner diameter tapered outlet.

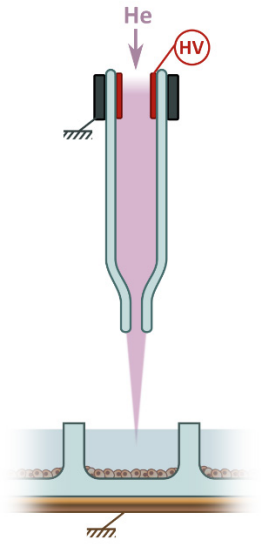

Fig. 1. Scheme of the plasma setup.

The biological model used in this work was human cervical cancer cells (HeLa). The cells were adherent and seeded on multi-well plates. They were incubated at 37°C with 5% concentration of CO_2 in a complete culture

medium until the number of cells reached the 100% confluency.

Propidium iodide (668 Da) and FITC-Dextran were used as fluorescent molecular markers to detect the permeabilized cells, respectively with a concentration of 0.05 mg/mL and 1 mg/mL (1Da = 1.66 x 10^{-27} kg). Two different sizes of FITC-Dextran were used: 70 kDa and 150 kDa.

A metallic grounded plate lied under the multi-well plate, while the plasma jet was put vertically above the plate with the plasma plume toward the bottom. The treatment time was between 10 and 100 s.

3. Results and discussions

Fig. 2 presents pictures of fluorescence microscopy of HeLa cells 30 minutes after treatment by helium plasma in the presence of propidium iodide (PI) and FITC-Dextran. The left and right columns show bright field and fluorescence images of permeabilized cells, respectively. In all conditions, a circular empty space was observed with permeabilized cells forming the borders. This result is surprising, since there is no direct interaction between the plasma and the cells standing a few millimeters below the liquid solution. The plasma interacts with the liquid which covers the cells. The empty circle comes likely from cell detachment. The diameter of the circle rises most of the time up to 1 mm like shown on the pictures (a) and (c) of the Fig. 2, but can sometimes fluctuate and reach some mm like in the picture (e) of the Fig. 2.

With PI and 70 kDa FITC-Dextran the fluorescence exhibits a ring shape. The majority of permeabilized cells were located at the edge of the plasma spot. Almost 30% of permeabilized HeLa cells was obtained after an incubation time of 30 minutes at 37°C for a plasma treatment generated at 14 kV with a repetition frequency of 100 Hz and a time treatment of 100 s. This value is probably underestimated since the measure takes into account only the adherent cells. It has to be pointed out that a cytotoxicity of 20% was measured 24 h after treatment. Efficient drug delivery was obtained using molecules up to 70 kDa (Fig. 2 – (b) and (d)), while with 150 kDa Dextran a low fluorescence was observed (Fig. 2 – (f)).

In order to get more insight on the permeabilization of cell membrane by plasma, the influence of the electric field generated by the plasma jet was also investigated. For this purpose, a dielectric barrier was used between the plasma and the biological target. Electric field alone has a weak but non-negligible contribution to the cell permeabilization. Therefore the main contribution with plasma treatment is probably due to the chemistry in the gas and liquid phases.

Moreover, experiments using plasma activated media were also done to understand the contribution of radical formation on cell membrane e and will be presented (data not shown). We also found the percentage of permeabilized cells depends also on the time when the florescence molecular maker is injected into the solution.

Finally, to know whether endocytosis could be involved in cell membrane permeabilization as observed for other physical methods, chlorpromazine was used as a clathrin – mediated endocytosis inhibitor. When cells were treated with this compound, the percentage of permeabilized cells decreased, suggesting that endocytosis could be partly responsible for cell membrane permeabilization.

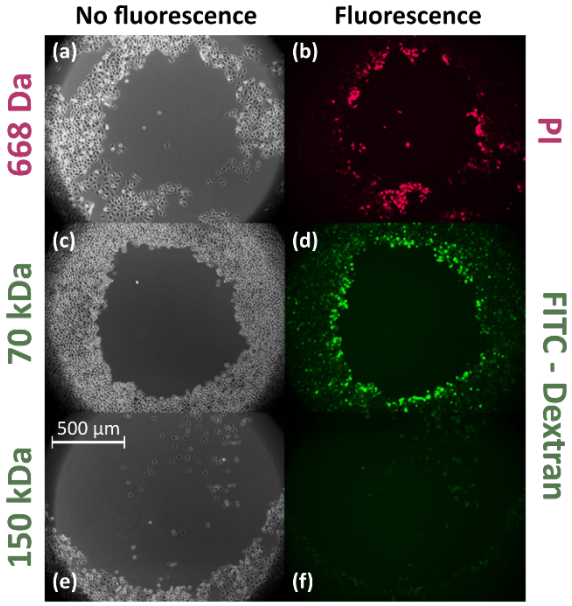

Fig. 2. Fluorescence microscopy images of HeLa cells 30 minutes after treatment by helium plasma in the presence of propidium iodide, 70 kDa FITC-Dextran or 150 kDa FITC-Dextran.

4. Conclusion

In this work, we have studied the cell membrane permeabilization following plasma treatment using a plasma gun under various conditions. It has been shown that permeabilization is very sensitive to plasma parameters such as pulse repetition rate and discharge pulse number. Plasma action not only results from the generation of reactive species, but also from the transient electric field produced at the tip of the plasma plume. Dedicated experiments realized with plasma electric field alone seem to indicate that part of drug penetration resulted from that plasma component. It also seems that endocytosis mechanisms are partly responsible for cell permeabilization while transient pore formation in the cell membrane may also be involved in drug uptake. Our results partly correlate with recently reported role of both electrical and chemical factors in plasma induced gene transfection [6].

5. References

[1] Y. Ogawa *et al*, vol. 92, no. 7, pp. 865–870, 2005.

[2] W. Zhu *et al*, Sci. Rep., vol. 6, no. October 2015, p. 21974, 2016.

[3] M. Leduc *et al*, New Journal of Physics, **11** (2009) 115021 (12pp)

[4] J. Gehl *et al*, vol. 4, pp. 3–13, 2006.

[5] E. Robert *et al*, Phys. Plasmas, vol. 22, no. 12, p. 122007, Dec. 2015.

[6] M. Jinno *et al*, Arch. Biochem. Biophys., vol. 605, pp. 59–66, 2016.