

# Cell Permeabilization and Molecular Delivery Following Helium Plasma jet Treatments

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# Cell Permeabilization and Molecular Delivery Following Helium Plasma jet Treatments

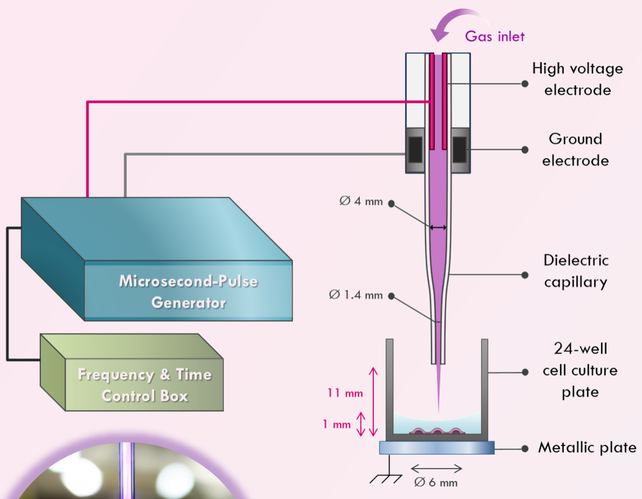
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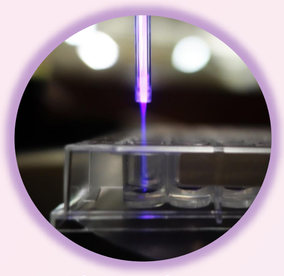
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## Introduction

Molecular delivery in cells combining low cytotoxicity and high efficiency using physical techniques remains to be optimized. In this work, we report on the use of a cold atmospheric pressure plasma jet device called Plasma Gun as an innovative strategy for cell membrane permeabilization. In our previous study[1], we focused on evaluating key parameters needed to achieve an efficient molecular delivery. We showed that a low number of pulses (between 1,000 and 100,000) and a 30-minute incubation at 37° C of the treated cells were enhancing molecular uptake in a ring shape pattern with no significant toxicity. In this report, we investigated cell permeabilization kinetics and surprisingly, best permeabilization levels were reached when molecular injections in the cell medium and plasma treatments were not simultaneous. Indeed, best efficiency was measured when the injection was performed a few minutes after plasma. This transient permeabilization was also measured for longer delays of molecular injection which confirms the non-toxic nature of our plasma treatment conditions.

## Materials & Methods



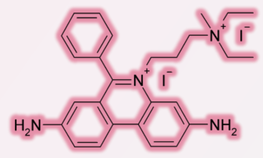
Plasma plume

**Plasma parameters**
**Voltage :** 14 kV
**Gas :** He
**Flow rate :** 0.5 SLM
**Frequency :** 100 Hz
**Exposure time :** 100 s
**Capillary-cells distance :** 1 cm

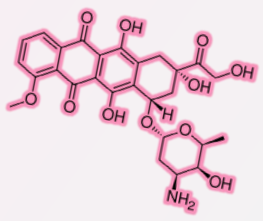
**HeLa : Human cervical cancer cells**
**4T1 : Murine breast cancer cells**

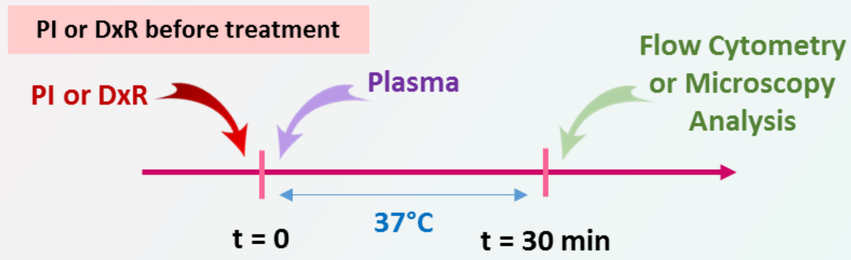
**Molecular markers for permeabilization :**

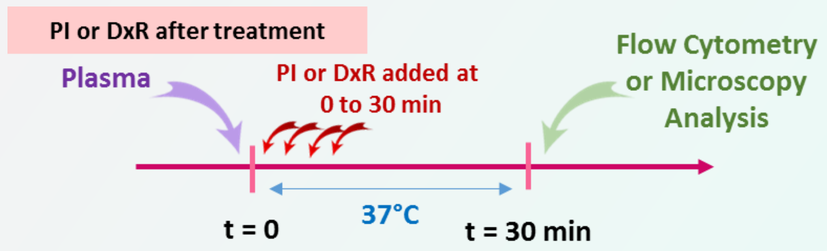
**Propidium iodide (PI)** : 668 Da



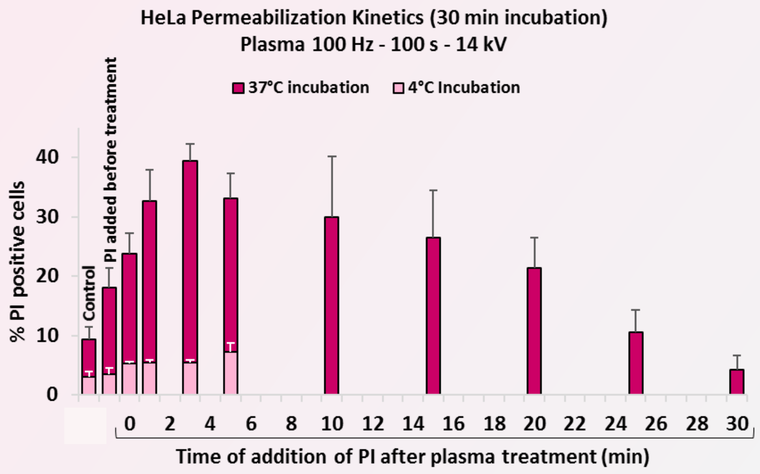
**Doxorubicin (DxR)** : 544 Da





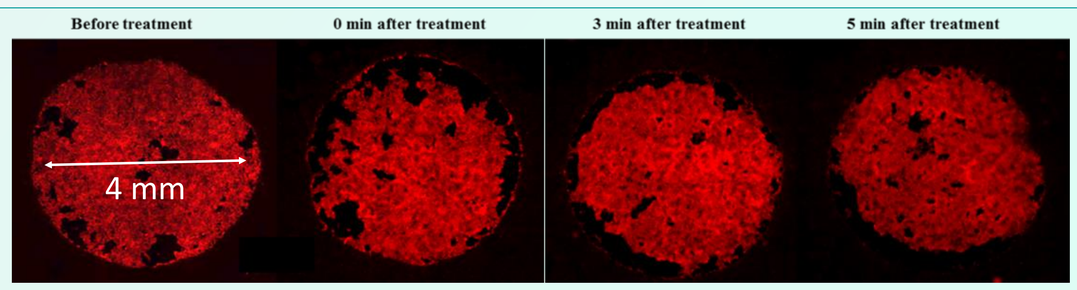



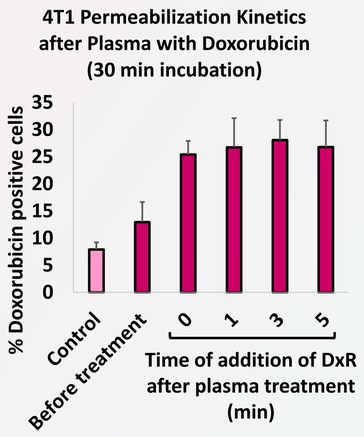

## Results




In Hela cells, permeabilization efficiency increased when PI was added after plasma treatment. It peaked when PI was added 3 minutes after plasma; 39.3% of PI positive cells compared to 18% when PI is added before treatment, and slowly decreased until it reached the control condition (4.25%). Our first hypothesis was that PI might have been degraded by plasma which could explain why molecular uptake was higher when PI was added after plasma treatment. However, we measured the fluorescence of PI solutions treated by plasma and no molecular damage was assessed (data not shown). The decrease in the uptake after 3 minutes illustrates the non-toxicity of the treatment as pores seem to close and restore cell activity as it was before plasma treatments. A continuous increase in molecular uptake would have shown to be a sign of cytotoxicity in cells. Viability measurements in our previous study also confirms these results[1]. These results also shows that when cells were incubated at 4°C, permeabilization efficiency severely decreased (highest value of 7.24% of PI positive cells compared to 33.1% when incubated at 37°C). It is probably correlated with either a decrease in RONS production, a decrease in the speed of the chemical reactions involved in this RONS production and/or the cell mechanisms involved.

Similar to HeLa[1], a circular impact zone is observed with 4T1 cells in all conditions. Our hypothesis is that plasma electric field might *prepare* the cells during treatments and that only the cells subjected to a certain amount of plasma electric field are prone to molecular uptake. And that cells are still activated few minutes after plasma treatments. Another hypothesis is that because cells have been subjected to an electric field, when PI is added after treatment, it is not presented to the cells the same way as when PI is present during the electric field sensitization of the cells. Molecular accessibility is then modified and is improved when the molecule is added after treatment.




Similar experiments with direct plasma treatments were conducted using Doxorubicin as an anticancer drug in 4T1 cells. Results were similar as those with PI as molecular uptake was more efficient when doxorubicin was added after plasma treatment. For the four conditions of treatment with the addition of doxorubicin after plasma, the percentage of PI positive cells were above 25% compared with the addition of the molecule prior treatment (12.95%). Efficient delivery of doxorubicin was achieved in 4T1 cells.

**Application in Drug Delivery**




## Conclusion

- Efficient molecular uptake was assessed in HeLa and 4T1 cell lines
- Interesting results in permeabilization kinetics were observed which opens a new way of plasma treatments
- Efficient delivery of an anticancer agent (doxorubicin) in 4T1 cells was assessed
- Exact plasma mode of action, mechanisms involved in the ring shape permeabilization pattern and specific role of plasma electric field still to be investigated

**Perspectives for Cosmetic Applications**

- Evaluating conditions where cell permeabilization is possible in skin cells (keratinocytes, melanocytes…)
- Evaluating skin reaction towards plasma treatments in the stated conditions



**References :**

[1] V. Vijayarangan, A. Delalande, S. Dozias, J.-M. Pouvesle, C. Pichon, and E. Robert, IEEE Transactions on Radiation and Plasma Medical Sciences, 2, 1-7 (2017).