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Dissertation Summary

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MONTE CARLO SIMULATIONS AND EXPERIMENTAL CHARACTERIZATIONS OF AIR MICROPLASMA INDUCING PORATION OF CELL MEMBRANES FOR GENE TRANSFECTION

Gene transfection is a technique of deliberately introducing nucleic acids (DNA) into cells through membrane to give them specific characteristics. The cold atmospheric plasma irradiation is potentially a new alternative, safe and damage-free technique. It can lead to a transient permeabilization of the cell membrane allowing processes of gene transfection in which DNA and cells are both exposed to fluxes of active plasma species (electrons, ions and neutral radicals). The mechanisms of more particularly membrane poration are far to be clear and controlled. Therefore, the aim of this thesis is to numerically study the mechanisms of plasma-induced membrane permeabilization using a specific micro-air plasma that is also characterized in the framework of this thesis.

The first chapter is devoted to bibliographic overview on the different plasma setups used for gene transfection in literature which clearly confirm that the cold plasma irradiation is effective to gene transfection. Moreover it is shown, from comparison performed at Ehime University that the air micro-plasma is the most promising, safe and high efficiency plasma configuration.

The aim of this thesis consists more precisely to develop and exploit a specific Monte Carlo poration model. This model is aimed to simulate the pore formation of few nanometer of width through cell multilayer membranes when irradiated by micro-air plasma. This developed model requires a prior input data on density of charged particles (electrons and N_2^+ ion), and the temperature of gas T_g and electrons T_e arriving to the cell membrane. Thus, the second chapter is devoted to the experimental characterization by optical emission spectroscopy OES of the micro-air plasma. This is a corona discharge generated in ambient air from the tip of a pulsed high voltage micro-tube placed 2 mm in front of petri dish containing deionized water and set over a grounded copper plate. Rotational temperature T_{rot} estimated from comparison of synthetic and experimental spectra of OH(A-X), N_2^+(FNS) at 391.4 nm, and N_2(SPS) at 337nm are respectively equal

to 2350K, 2000K, and 700K. This clearly underlines a thermal non-equilibrium of the corresponding excited species generated inside the thin streamer filaments. But, due to the high dilution of these species in the background gas, these high T_{rot} do not affect mean T_g that remains close to 300K. Then N_2^+(FNS) and N_2^+(FNS) head bands spectra at 391.4 nm and 388.4 nm allowed to estimate vibrational temperature which is around 3000K near the tip electrode up to about 6500K near the plate. Moreover, T_e equal to about 6.75eV has been estimated from an interesting approach based on the experimental ratio of the closest nitrogen emission spectra of N_2^+(FNS) at 391.4nm and N_2(SPS) at 394.3 nm. Based on measured Hα spectrum, electron density η_e has been estimated from Stark broadening versus the inter-electrode position. η_e of about $1x101^5cm^{-3}$ near the tip is coherent with the usual magnitude of electron density in the streamer head developed near the tip of corona discharges. Last, the density of nitrogen ion has been also estimated near the tip to about $10^{15}cm^{-3}$ from the relative intensities of the same close wavelength spectra N_2^+(FNS) and N_2(SPS).

In the third chapter, we performed a literature review on the numerical modelling of cold plasma interactions with cells and tissues. We underlined that there are no literature simulations devoted to membrane permeabilization and pore formation when impacted by plasma actives species. Therefore, we developed for the first time in literature a specific Monte Carlo poration model. It is aimed to statistically simulate, at a global scale, the pore formation during air micro-plasma interactions with cell membrane. In the framework of this model, we assumed each plasma species (electrons, ions and neutral radicals) as a super-particle grouping a large number of particles. The species fluxes were estimated from a plasma reaction kinetic model and OES study of chapter 2. The membrane layers were assumed as a simple membrane model superposing four layers of phospholipids and proteins. Each layer was constituted by a succession of super-sites subjected to specific super-processes (recombination, reflection, activation of site, opening, etc) during the membrane impacts by the plasma super-particles.

For an accurate exploitation of our model, the estimation of probability of occurrence of the whole considered super-processes is absolutely necessary. Thus, in the last chapter, a large parametric study is conducted. This is aimed to evaluate the effects of the initial simulation parameters as well as the magnitude of the occurrence probabilities of each reaction process on the cell membrane permeabilization and pore formation. This parametric study enabled to emphasize several important results. First, energetic electrons play a main role on super-site activations and openings due their strong anisotropy in the forward direction. In addition, due to their lower energy close to background gas, reflection processes due to ions and radicals, have shown their role to widen and deepen the pore dimension. Overall, it is more particularly shown that the initial particle number Np is the most efficient parameter of the membrane poration. We observed a direct correlation between N_p and the exposure time of the cell membrane to the air micro-plasma. This means that Monte Carlo poration model is an interesting tool of the prediction of the optimal exposure time versus the input data of the low temperature plasma parameters, the cell membrane structure and the needed pore sizes. Under the specific chosen simulation conditions coming from the parametric study, it is shown a dynamics of formation of membrane pores having dimensions pore (diameters~10nm) compatible for the gene transfection. Last, our Monte Carlo simulation results are qualitatively validated from a first comparison with the measured transfected rate of DNA plasmid and the surviving cell rate in the case of mouse fibroblast cells. The present Monte Carlo poration method is therefore a very promising tool for a better understanding of the plasma gene transfection mechanisms.