（第６号様式）

学位論文審査の結果の要旨

氏　名	AMEL ZERROUKI
審査委員	主査　神野　雅文 副査　門脇　一則 副査　下村　哲 副査　Mohammed YOUSFI 副査　Ahmed RHALLABI

論　文　名 MONTE CARLO SIMULATIONS AND EXPERIMENTAL CHARACTERIZATIONS OF ~~ASIR~~ AIR MICROPLASMA INDUCTING PORATION OF CELL MEMBRANES FOR GENETRANSFECTION

審査結果の要旨

Amel Zerrouki has defended her Ph. D thesis on 29 August 2016 at the main meeting room of the Faculty of Engineering of Ehime University during the requested time in the presence of the full jury.
The presentation has been considered very clear and very pedagogic. It is based on a very good selection of slides well representative of the multiple interesting aspects of her research works.
The jury has underlined the very high quality and quantity of the research works undertaken in the frame work of the joint supervision between Toulouse and Ehime Universities supported by Erasmus Mundus Program. The scientific rigor of the candidate, her strong ability to conduct the research works and to analyze the obtained results enabled her to develop a very original and promising tool of Monte Carlo simulation of the membrane poration for plasma gene transfection application.
Amel Zerrouki has brilliantly responded to the numerous interesting questions of the jury members thus showing her great skills, her scientific maturity and her perfect mastery of the subject of research.
Consequently the jury awarded to Amel Zerrouki the title of Doctor of Ehime University. In the same time, the jury congratulated Amel Zerrouki for the excellence of her research works.

The contents of Amel Zerrouki's thesis is as follows:
Gene transfection is a technique of deliberately introducing DNA into cells through the membrane. The cold atmospheric plasma CAP 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. More precisely, is to develop and exploit a specific Monte Carlo poration model. This model is aimed to simulate the pore formation of few nm of width through cell membranes when irradiated by micro-air plasma. This developed model requires a prior input data on the density of charged particles and the temperature of gas and electrons. Thus, an experimental

characterization by OES of the micro-air corona discharge is performed. Rotation temperature was determined (between 700 K to 2350 K) even though under our non-equilibrium conditions T_g remains about 300K. OES also has given the space variation from the high voltage tip to the grounded plate of vibration temperatures (between 3000 K up to about 6500 K) and T_e (about 6.75 eV down to 3.4 eV near the plate). A magnitude around $10^{15}cm^{-3}$ for the electron and ion densities have been also determined. Moreover, knowing that there are no literature simulations devoted to membrane permeabilization and pore formation when impacted by plasma actives species, we developed for the first time in literature a specific Monte Carlo poration model. In this framework, 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. 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 a site, opening, etc.). For an accurate exploitation of our model, the estimation of the probability of occurrence of the whole considered super-processes is absolutely necessary. Thus, a large parametric study is conducted. The aim is to evaluate the effects of the initial simulation parameters as well as the magnitude of the occurrence probabilities of each reaction process on pore formation. Several important results are emphasized. First, energetic electrons play a main role on 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 dimensions. Overall, it is more particularly shown that the initial particle number N_p 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 microplasma. 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 ~10 nm) compatible for the gene transfection. 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 method is, therefore, a very promising tool for a better understanding of the plasma gene transfection mechanisms.