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Molecular Diffusion Rates of Supported Lipid Bilayer in Phosphate Buffered Saline Irradiated with Oxygen Radicals

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Non-equilibrium atmospheric-pressure plasmas (NEAPPs) are widely employed in recent biological researches. In the applications of NEAPPs, it is important to understand the reaction mechanism between plasma-generated oxygen and nitrogen reactive species (RONS) and cell membrane. Recently, our work revealed that the oxygen radicals formed nanopores on the artificial cell membrane, phospholipid bilayer, due to the lipid oxidation [1]. The size of nanopores in a range from 10 to 50 nm in diameter was observed by atomic force microscope (AFM). It is expected that the RONS can penetrate into the cell through the nanopores, resulting in the oxidization of intracellular organelles and the enhancement of pore formation on the cell membrane.

In this study, we measured the diffusion coefficient of the supported lipid bilayer (SLB), which is an artificial planar lipid bilayer system formed at a solid-liquid interface, by the immersion time of the radical irradiation solution with fluorescence recovery after photo-bleaching (FRAP) using a confocal laser microscope.

In the experiment, we used a phospholipid molecules 1,2-Dioleoyl-sn-glycerol-3-phosphocholine (DOPC) of 0.26 mM and 1,2-Dioleoyl-sn-glycerol-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rb-DOPE) of 1.6 μM. The phospholipid solution drop of 200 μl was dripped on the glass bottom dish and incubated for 2 h in a constant temperature at 45 °C. In order to remove excess vesicles on the developed SLB, the sample was washed by Phosphate buffered saline (PBS). We treated PBS using the oxygen radical source. Oxygen radicals were generated by a radical source (Fuji Machine MFG. Co., LTD. FPA-10). The radical source is based on an atmospheric-pressure high-density O_2/Ar plasma, which produces a high electron density of about 10^{16} cm^{-3}. Charged species and optical radiation from the O_2/Ar plasma were blocked using electrodes and the structural shape of the exit aperture so that only neutral species were supplied to the sample. For generating the highest density of atomic oxygen (^3P_j), a mixed gas of O_2 (30 sccm) in buffered Ar (4.97 slm). The O(^3P_j) density of about 10^{14} cm^{-3} was measured in our previous study [2]. Distance between the nozzle exit and the surface of the sample was fixed at 10 mm. Using a confocal laser scanning microscope associated with FRAP method, [3] where we measured fluorescent intensity of phospholipid for 5 min. Then, we measured the diffusion coefficient of SLB in unirradiated PBS of 3ml. After removing 3ml of PBS from the glass bottom dish, we dripped the radical-irradiated PBS into the SLB and reacted them for 5min. Following this, we stopped the reaction between them by removing the radical- irradiated PBS and dripping unirradiated PBS of 3ml. Finally, we measured the diffusion coefficient of the SLB.

Figure 1 shows the time-dependence of the SLB fluorescent intensity as a function of elapsed time from 0 to 300 s. From the results, the diffusion coefficients were calculated to be 1.93 $\mu m^2/s$ for pristine SLB, 0.89 $\mu m^2/s$ for 5min-immersed SLB. Decrease of the diffusion coefficient clearly showed the immersed SLB seems to be damaged by RONS, which forms nanopores on the SLB. Also we observed long term effect for 2 h.

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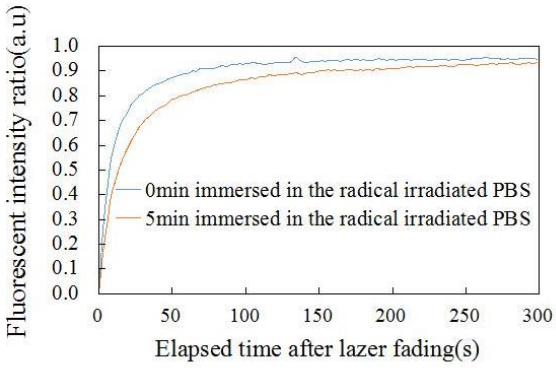

Fig.1 Fluorescent intensity of SLB as a function of elapsed time after laser fading for SLB immersed in PBS irradiated with or without oxygen radicals

References

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