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REGULAR PAPER

Reduction in lateral lipid mobility of lipid bilayer membrane by atmospheric pressure plasma irradiation

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Plasma medicine is an emerging research field in which various applications of electrical discharge, especially in the form of nonequilibrium plasma at atmospheric pressure, are examined, for example, the application of plasma to biological targets for various purposes such as selective killing of tumor cells and blood stanching. We have focused on the behavior of an artificial cell membrane system at the solid–liquid interface. To evaluate the lateral lipid mobility, we measured the diffusion coefficient of the supported lipid bilayer (SLB) composed of dioleoylphosphatidylcholine with fluorescence recovery after photobleaching by confocal laser scanning microscopy. It was found that the diffusion coefficient was decreased by plasma irradiation and that the diffusion coefficient decreasing rate proceeded with increasing plasma power. We investigated the effects of stimulation with an equilibrium chemical, H_2O_2, on the SLB and confirmed that the diffusion coefficient did not change at least up to a H_2O_2 concentration of 5 mM. These results indicate that transient active species generated by plasma play critical roles in the reduction in SLB fluidity. The effects of the two generated major oxidized lipid species, hydroxyl- or hydroperoxy-phosphatidylcholine (PC) and acyl-chain-truncated PCs terminated with aldehyde or carboxyl group, on lateral lipid mobility are discussed.

1. Introduction

A research field on the application of electrical discharge, especially in the form of nonequilibrium plasma at atmospheric pressure, to biological targets has been developed, which is known as "plasma medicine".[1,2] Recent research results show that plasma irradiation is effective for the inactivation of bacteria, fungi, and viruses (sterilization),[3–9] wound healing, blood stanching (hemostasis),[10] cellular regulation,[11,12] tooth bleaching, and selective killing of tumor cells.[13–15] Development of a gene transfection technique using plasma has been studied.[16,17] In most of these experiments, plasma firstly comes in contact with water that surrounds cells and the active species generated by plasma irradiation dissolve in the water. In another method, the water irradiated with plasma, called plasma-activated medium, whose property can be maintained for several hours without plasma irradiation, is used for the treatment of biological targets.

Tian and Kushner[18] have recently reported on the generation of reactive oxygen and nitrogen species (ROS and RNS: RONS) including short-lived species of •OOH and •OH and long-lived species of H_2O_2 and NO_3^-. They analyzed a dielectric barrier discharge (DBD) plasma, a type of atmospheric pressure plasma, in a $N_2/O_2/H_2O$ gas mixture and described the dissociation of N_2, O_2, and H_2O molecules due to electron collision, the generation of radicals, the reaction between radicals and molecules, and their dissolution in liquid. Owing to many practical studies of plasma irradiation of biological cells and the use of plasma-activated medium,[19–21] it is now commonly understood that RONS are key species for treatment applications in plasma medicine. However, the reaction of plasma-generated active species with the cell and cell membrane is not well understood. To ensure the safety of plasma medicine in clinical applications, a basic study of the plasma effect on biological targets at different tissue levels, which range from biological molecules to living tissues, is required.[22,23]

We have studied the effect of atmospheric pressure DBD plasma on the supported lipid bilayer (SLB) and found an increase in the number of defects on SLB.[24] Although a simulation study has similarly shown that nanopores were formed on a lipid bilayer by applying an intense electric field (~0.01 V/nm) for a very short time (5–6 ns),[25] our experimental results show that the defects formed on the SLB remained for longer than 1 h, which is beneficial for long-term examination techniques, e.g., atomic force microscopy (AFM) and fluorescence microscopy. This is an advantage in SLB to study the interaction between plasma and the cell surface. The results also show the formation of micrometer-sized pores after the plasma irradiation longer than 30 s, indicating the existence of a preliminary process before the poration. Recently, several groups have studied the effects of atmospheric plasma on phospholipid bilayer membranes using lipid vesicles. These studies showed that the atmospheric plasma irradiation of vesicle suspensions induces the leakage of the contents in vesicles, which reasonably corresponds to the plasma-induced poration observed in our previous study.[26–30]

In this study, we investigated the plasma-induced change in a physical property of the SLB before the poration. The fluidity of SLBs, which is the lateral mobility of lipids, is a principal property of lipid bilayer membranes and sensitive to the chemical components and physical states of SLB. We measured the diffusion coefficient of SLB before and after the DBD plasma irradiation by fluorescence recovery after photobleaching (FRAP) to investigate the plasma effect on the fluidity of SLB. We compared the change in the diffusion coefficient of the SLB induced by plasma irradiation with that induced by chemical stimulation using H_2O_2, and examined the mechanism of the reduction in lateral lipid mobility.

2. Experimental procedure

2.1 DBD apparatus

The DBD-plasma irradiator (MVS Engineering MV016-0000) of the parallel plate type developed for this study is