Nanopore formation process in artificial cell membrane induced by plasma-generated reactive oxygen species

Ryugo Tero a, b,*, Ryuma Yamashita c, Hiroshi Hashizume d, Yoshiyuki Suda c, Hirofumi Takikawa c, Masaru Hori b, Masafumi Ito e, **

a Department of Environmental and Life Sciences, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan
b Electronics-Inspired Interdisciplinary Research Institute, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan
c Department of Electrical and Electronic Information Engineering, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan
d Plasma Medical Science Global Innovation Center, Nagoya University, Nagoya, Aichi 464-8601, Japan
e Department of Environmental and Life Sciences, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan

* Corresponding author. Department of Environmental and Life Sciences, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan.
** Corresponding author.
E-mail addresses: tero@tut.jp (R. Tero), ito@meijo-u.ac.jp (M. Ito).

Abstract
We investigated morphological change of an artificial lipid bilayer membrane induced by oxygen radicals which were generated by non-equilibrium atmospheric pressure plasma. Neutral oxygen species, O(3Pj) and O2(1Dg), were irradiated of a supported lipid bilayer existing under a buffer solution at various conditions of dose time and distances, at which the dose amounts of the oxygen species were calculated quantitatively. Observation using an atomic force microscope and a fluorescence microscope revealed that dose of the neutral oxygen species generated nanopores with the diameter of 10–50 nm in a phospholipid bilayer, and finally destructed the bilayer structure. We found that protrusions appeared on the lipid bilayer surface prior to the formation of nanopores, and we attributed the protrusions to the precursor of the nanopores. We propose a mechanism of the pore formation induced by lipid oxidation on the basis of previous experimental and theoretical studies.

1. Introduction

Reactive oxygen species (ROSs) are reactive factors and/or mediators in biological and physiological reactions shown to be either beneficial or harmful [1–3]. Atmospheric pressure plasma is a novel and valuable tool to generate ROSs intentionally, as well as reactive nitrogen species (RNSs) and electric field, and is applied in the fields of medicine and biology in these decades [4–6]. Recent studies demonstrated the multifarious applications of atmospheric pressure plasma in these fields: sterilization [7–13], selective killing of tumor cells [14,15], cell regulation [16,17], and gene transfection [18,19]. Various kinds of ROS and RNS are generated and included in these reactions induced by atmospheric pressure plasma [12], and play crucial roles in these phenomena. Some studies showed that radical species are requisite for the transportation of materials through a cell membrane [19,20]. Recently we developed a neutral oxygen radical source on the basis of non-equilibrium atmospheric pressure plasma [21], which effectively inactivates Penicillium digitatum spores [22–26] and proliferates/ inactivates Saccharomyces cerevisiae [27]. This radical source excludes the effects of electric field and lights, and supply only ROSs. The ROSs provided by this radical source are characterized and their dose amounts were quantitatively evaluated [21,25–27]. The active species critical for these reactions are attributed to the atomic oxygen at the ground state O(3Pj), and its amount is on the order of 1019 cm−3.

In spite of the various applications of the atmospheric pressure plasma, however, what ROSs causes on cell membranes and how ROSs pass through cell membranes are still unclear. Biological cell membrane is the outermost cell organ and acts as a barrier and gates controlling the transportation of materials and information. Everything needs to pass through a cell membrane when it enters into a cell and causes an effect inside the cell. The plasma-generated ROSs also access to the cell membranes first. The critical effect of ROSs on cells may be a direct effect on cell membranes, or indirect one through physiological cascades inside cells and/or genetic damages. Some previous studies showed that cell...