Surface reaction analysis of plasma-treated bio-molecules using sum frequency generation spectroscopy

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Abstract: It is important to analyse the molecular structure of the cell surface treated by atmospheric pressure plasmas(APPs) in order to clarify the interaction between cells and reactive oxygen/nitrogen species(RONS). In this study, sum frequency generation (SFG) spectroscopy measurement of a glucose film was carried out after an APPs or oxygen radical treatment. The SFG spectra obtained from a surface of the glucose film was compared with the Raman spectra obtained from a bulk of the glucose film.

Keywords: sum frequency generation spectroscopy, surface reaction

1. Introduction

Biological applications using atmospheric pressure plasmas (APPs) have been intensively studied in medical or agricultural fields.[1]-[3] It is important to analyse the molecular structure of the cell surface treated by APPs in order to clarify the interaction between cells and reactive oxygen/nitrogen species(RONS).

Sum Frequency Generation (SFG) spectroscopy was applied to observe the molecular structure of cell surface. SFG is a spectroscopic technique based on the second order nonlinear effect. When a sample is irradiated by infrared light (ω_1) and visible light (ω_2) simultaneously, SFG light ($\omega_1+\omega_2$) is generated at interface between a cell and ambient phase (gas or liquid) as shown in Fig.1. Fig. 2 shows energy diagram of SFG light. The SFG signal is observed when the frequencies of molecular vibration and the infrared light are harmonized. Thus, the chemical reactions between molecules of cell surface and RONS can be analysed by using SFG spectroscopy.

In this study, SFG spectroscopy measurement of a glucose film was carried out after the APP treatment.

2. Experimental

Fig.3 shows the optical system of SFG spectroscopy. Wavelength-scanned infrared laser light (ω_1: 2500-10000 nm, energy: 0.1 mJ, pulse width: 20 ps) and visible laser light (ω_2: 532 nm, energy: 5 mJ, pulse width: 25 ps) were simultaneously irradiated to the specimen. SFG signal was detected by a photomultiplier through a monochromator to eliminate the laser lights.

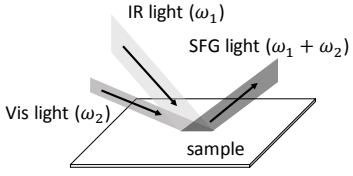

Fig. 1 Generation of sum frequency light at interface between a sample and ambient phase (gas or liquid).

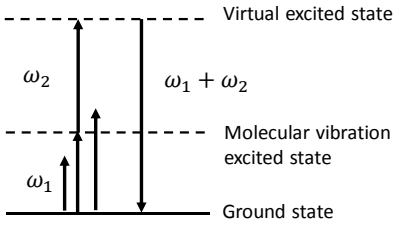

Fig. 2 Energy diagram of SFG light ($\omega_1+\omega_2$).

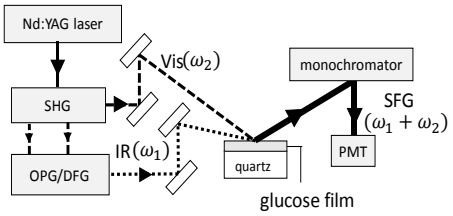

Fig. 3 Optical system of SFG spectroscopy.

3. Results

Fig. 4 shows SFG spectra of glucose film from 2800 to 3000 cm^{-1} with various plasma irradiation time. The SFG signals of C-H stretching vibration of the 6th C atom in carbon chain at 2868 cm^{-1}, asymmetric C-H stretching vibration of the 6th C atom at 2887 cm^{-1}, C-H stretching vibration of the 1 and 4th C atom at 2939 cm^{-1}, asymmetric C-H stretching vibration at the 1 and 4th C atom at 2952 cm^{-1} was observed.[4] SFG intensities of both symmetric and asymmetric C-H stretching vibration of the 6th C atom at 2868 cm^{-1} and 2887 cm^{-1} decreased with the plasma irradiation time. These results indicate that a primary alcohol of glucose film was changed to a

carboxylic acid via an oxidation reaction by ROS produced from the plasma.

Fig. 5 shows SFG spectra of glucose film from 1650 to 1850 cm^{-1} The SFG signals of C=O stretching vibration attributed to carboxylic acid was observed at 1732 cm^{-1}. The SFG intensity of C=O stretching vibration increased with increasing plasma irradiation time. In general, alcohol is oxidized to aldehyde, and then C-H bond is changed into C=O bond. The oxidation reaction initiated by the APP treatment at the air / glucose interface was observed by the SFG spectroscopy.

As shown in Fig. 4, SFG intensities of both symmetric and asymmetric C-H stretching vibration of the 1 and 4th C atom at 2939 cm^{-1} and 2952 cm^{-1} increased with the plasma irradiation time. On the other hand, SFG intensities at 2939 cm^{-1} and 2952 cm^{-1} were not change by the atmospheric-pressure atomic oxygen radical treatment. These results indicate that ROS would have no contribution to the change in C-H stretching vibration of the 1 and 4th C atom.

Fig. 6 Raman spectra of glucose film from 2800 to 3000 cm^{-1} with various plasma irradiation time. The Raman signals of C-H stretching vibration of the 6th C atom in carbon chain at 2893 cm^{-1}, and symmetric H-C-H stretching vibration at 2917 and 2936 cm^{-1} were observed. The Raman signal intensities decreased with increasing plasma irradiation time as well as SFG intensities at 2868 cm^{-1} and 2887 cm^{-1}. The Raman signals were mainly obtained from the bulk of the glucose film. These results indicate that the oxidation reactions to produce carboxylic were occurred at both the surface and bulk of the glucose film. In addition, the change in C-H stretching vibration of the 1 and 4th C atom by APPs treatment was occurred only at the glucose film and this phenomenon was observed by using SFG spectroscopy.

3. Summary

The molecular structure at the surface of the SFG a glucose film after the APPs and oxygen radical treatments were observed by SFG spectroscopy. C-H stretching vibrations of the 6th C atom were oxidized produce carboxylic by the treatments at both the surface and bulk of the glucose film. The C-H stretching vibrations of the 1 and 4th C atom were increased by the APPs treatment, and this reaction was occurred at the surface of the glucose film, which was observed by using SFG spectroscopy.

4. References

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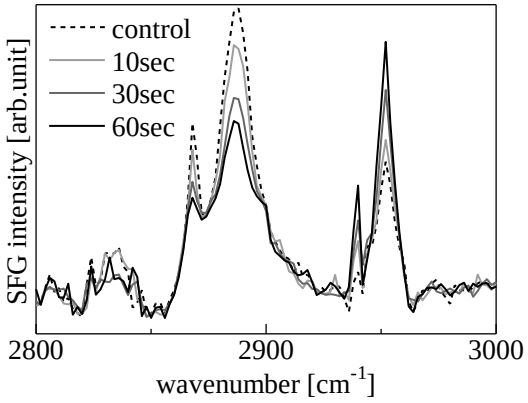

Fig. 4 SFG spectra of glucose film from 2800 to 3000 cm^{-1} with various plasma irradiation time.

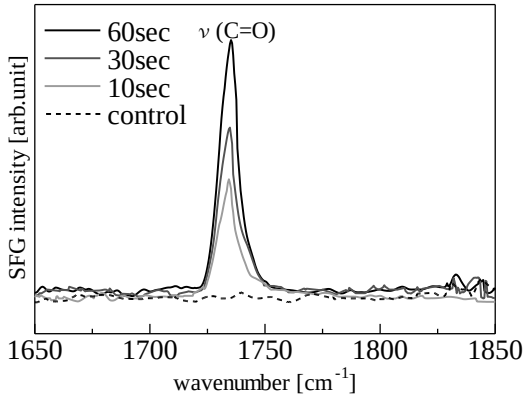

Fig. 5 SFG spectra of glucose film from 1650 to 1850 cm^{-1} with various plasma irradiation time.

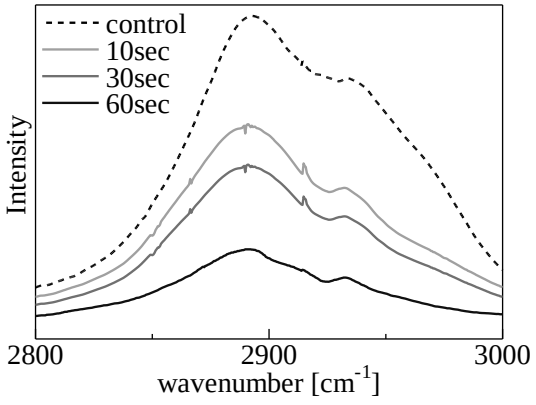

Fig. 6 Raman spectra of glucose film from 2800 to 3000 cm^{-1} with various plasma irradiation time.