Influence of Atomic and Singlet Molecular Oxygen Generated by RF Plasma on Reduction of Protein

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RF plasma was applied for the reduction of human blood and bovine blood in which essential proteins to lives were included. Atomic oxygen and singlet molecular oxygen are significantly important for the treatment of protein by RF oxygen plasma. The reduction rate of the protein was analysed by FTIR spectra, and the light emitted from oxygen plasma was detected by spectrometer. The ratio of emission intensity from atomic oxygen and singlet molecular oxygen strongly depended upon the pressure in the chamber. Irradiation time of the oxygen plasma was obviously related to the reduction rate of the protein. It was found that the reduction rate of protein correlated with the light intensity from atomic oxygen at 777 nm and singlet molecular oxygen at 762 nm.

Keywords: oxygen RF plasma, plasma sterilization, protein, atomic oxygen, singlet molecular oxygen

1. Introduction

Medical tools with insufficient sterilization, which have infectious factors such as bacteria, virus and protein, are one of the main causes of second infection. Autoclaving method (high-pressure steam sterilization) and ethylene oxide gas (EOG, C_2H_4O) sterilization are practically used for eliminating infectious factors. However, these sterilization methods have difficulties; medical tools made out of poor resistance to high temperature and humidity, such as plastic materials, are impossible to be sterilized by autoclave treatment, and toxicity of EOG treatment is pointed out as a health issue of a operator of sterilization unit.

Plasma sterilization is actively studied in a decade as substitutional/complemental sterilization method of auto-claving and EOG treatments[1-5]. Plasma sterilization is generally operated in a dry process and a low temperature around 70 degrees. Many materials of medical tools can be conformed to the plasma sterilization. Moreover, the health issue of an operator and a hazard to the environment contamination caused by toxic gases are resolved by plasma sterilization because the non-highrisk gases such as oxygen, nitrogen and vaporized water will be applied to plasma sterilization.

The pollution of medical tools is concerned not only by a bacteria or virus but also by a protein. It is widely accepted that the root of the outbreak of Bovine Spongiform Ecephalopathy (BSE) in England in 1986 is mutated protein called “prion protein” that perform like bacteria or virus. Creutzfeldt-Jakob Disease (CJD) and its variant (vCJD) on human being are supposed almost same disease of BSE. It is supposed that CJD and vCJD are affected by an intake of abnormal prion protein, and it is concerned that the protein remaining on medical instruments are one of the important fears for secondary contagions.

Atomic oxygen generated by oxygen plasma has been mainly applied on sterilization in study phase against virus or bacteria on medical tools[1,2] or human being[3-5]. We have researched the reduction of protein by RF oxygen plasma. In this paper, the influence of atomic and singlet molecular oxygen produced by RF plasma to reduce the protein is reported.

2. Method and material

2.1 Method

Bovine blood and human blood, which include important proteins such as albumin, globulin and fibrinogen, was chosen in this test as a sample. Two types

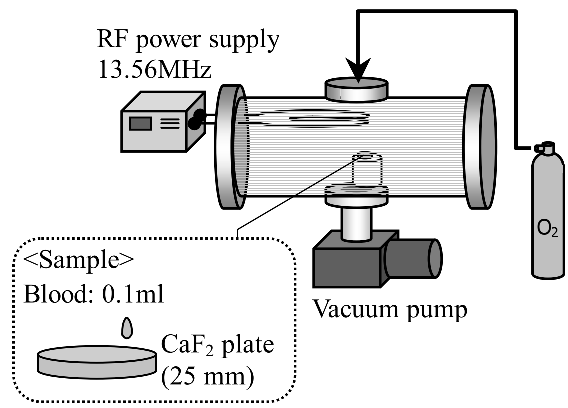

Fig.1. Schematic of the experimental apparatus.

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of the sample diluted with pure water were dropped on CaF_2 plate with 25 mm in diameter and then were dried naturally.

The CaF_2 plate was located in almost centre of the plasma chamber, which has 300 mm in length and 8 inch in diameter (Fig.1). The chamber was once evacuated until 1.0 Pa by rotary pump, and then oxygen gas was supplied, and the pressure varied from 10 Pa to 100 Pa. RF power (13.56 MHz) varied from 10 W to 150 W was transferred to the wavy shaped ICP type antenna to generate activated species effectively. The CaF_2 plate with thin blood was exposed to oxygen plasma ranging from 1 hour to 5 hours.

Effect on RF oxygen plasma for removing protein was analysed by absorbance spectra of FTIR (Shimadzu Co., FTIR-8900), and light emission from plasma was measured by emission spectrometer (Hamamatsu Photonics K.K., PMA-C8808). The reduction rate was estimated as percentage between the initial absorbance intensity of amide I and the value after irradiation of oxygen plasma.

2.2 Material

CaF_2 is transparent and colourless material and it has broad transmittance in infrared region, and also has the characteristic that hardly dissolves in water. Therefore, wet sample becomes easily analysable with using CaF_2.

Typical absorbance spectra of the blood detected by FTIR were indicated in Fig.2. Several strong peaks related to the length between each constituents of protein (Fig. 3) were detected. Protein is composed of amide group, carboxyl group and side chain, and its bonds are clearly identified in the spectra. The spectra around 3300 cm^{-1} and 1400 cm^{-1} are indicated as N-H stretching vibration and side chain respectively. Amide I (C=O stretching vibration) and Amide II (N-H bending vibration and C-N stretching vibration) are identified respectively at around 1650 cm^{-1} and 1545 cm^{-1}. In this research, the reduction rate of protein by irradiation of oxygen plasma was estimated on the peak of amide I around 1650 cm^{-1}, which principally intends secondary structure of protein.

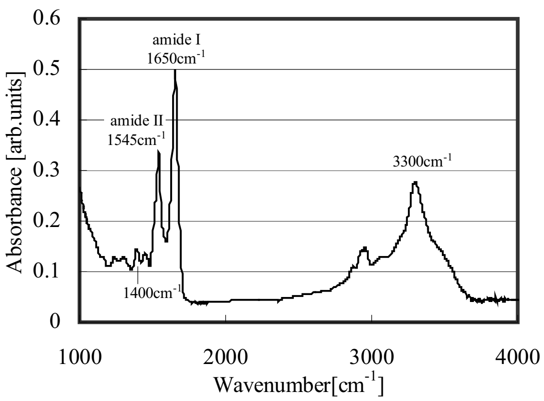

Fig. 2 Typical FTIR spectra of protein.

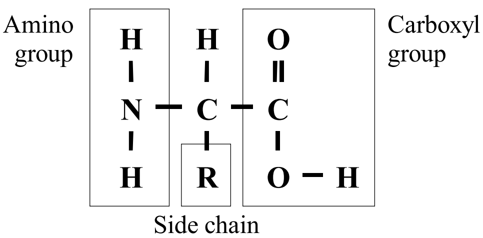

Fig. 3 Structure of amino acid that is basic constituent of protein.

3. Results and discussions

The reduction rate of amide I gradually increased with the length of the irradiation time of oxygen plasma and then had a tendency to become progressively saturated (Fig. 4). It was observed that the reduction rate of amide I was obviously related to the irradiation time of oxygen plasma. Approximately 50 percent of amide I was removed after 5 hours by irradiation of plasma generated by 30 W in 10 Pa of the pressure in the chamber.

It is considered that oxygen plasma reacted with only the surface of the sample on CaF_2. It is assumed that oxygen plasma gradually removed the protein from the whole surface of the sample; therefore, typical peaks of protein decreased without a remarkable denaturalisation and a reaction product, which were not detected in FTIR

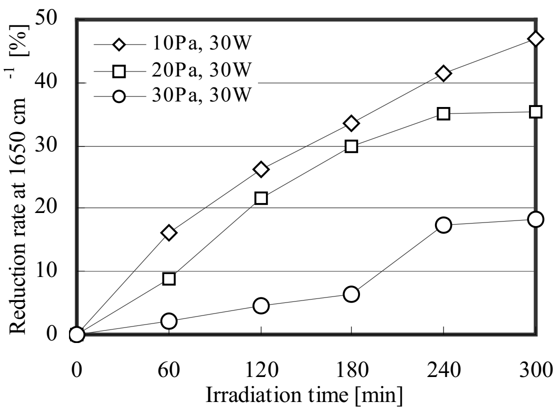

Fig. 4. Relationship between the reduction rate and the irradiation time.

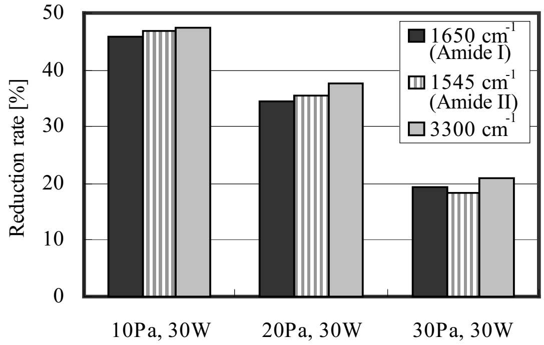

Fig.5. The reduction rate between 1650cm^{-1}, 1545cm^{-1} and 3300 cm^{-1} after 5 hours irradiation.

spectra. Oxygen plasma sterilization may not have properties of permeability to sterilized objects.

The reaction between oxygen plasma and the sample was not selective because the reduction rate indicated almost same tendency between typical peaks ($1650cm^{-1}$, $1545cm^{-1}$ and $3300\ cm^{-1}$) of protein (Fig. 5). Oxygen sterilization possibly has difficulties to remove some materials, from the reason of the fact that the reduction rate tended to become saturated, such as a fatty materials existing in the blood.

Typical light emission spectra from oxygen plasma analysed by emission spectrometer is shown in Fig. 6. There are several characteristic peaks between 200 nm and 900 nm such as 777 nm and 845 nm emitted from atomic oxygen and 762 nm emitted from singlet molecular oxygen. Atomic oxygen and singlet molecular oxygen are the major light emitter of oxygen plasma. The small broad peak detected around 310 nm is supposed the peak of hydroxy (OH) radical generated from residual moisture in the chamber. It is supposed that the atomic oxygen and singlet molecular oxygen chiefly affect the reduction of amide I, because of the fact that the emission intensity of OH radical is extremely smaller than the peaks of atomic oxygen and singlet molecular oxygen.

The light emission spectra from oxygen plasma vary according to plasma conditions. Dependence of atomic oxygen and singlet molecular oxygen against the pressure in the chamber and RF energy is indicated in Fig. 7 and Fig. 8. Emission intensity of atomic oxygen tends to be high under the low pressure around 10 Pa, and light emission spectra of other pressures indicated similar tendency. It is supposed that the amount of atomic oxygen in 10 Pa is higher in comparison with other pressures in the chamber. Moreover, intensity of singlet molecular oxygen tends to be high under the high pressure around 100 Pa and 70 Pa. The emission intensity of both atomic oxygen and singlet molecular oxygen also depends on input RF power to the wavy antenna.

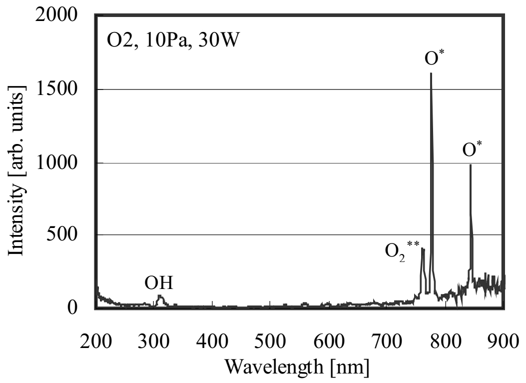

Fig. 6. Typical PMA spectra of oxygen plasma.

The micro organisms such as bacteria, virus and yeast are sterilized by active species produced in plasma[6~8]. It is supposed that active species also affect the reduction rate of amide I at $1650\ cm^{-1}$. Figure 9 shows the relationship between the reduction rate of amide I at $1650\ cm^{-1}$ and the light emission intensity of the active oxygen species, which are 777 nm from atomic oxygen and 762 nm from singlet molecular oxygen. It is clearly found that the reduction rate has been highly correlated with intensity of atomic oxygen species (r=0.80, p>0.0001).

Oxygen as ambient gas is commonly used in the plasma based sterilization techniques, and it is often the case that the effect of the sterilization is evaluated by atomic oxygen[6~8]. However, the atomic oxygen cannot explain the reduction rate of protein sufficiently. The relationship between the intensity of atomic oxygen at 777 nm and the reduction rate has shown the poor sequential correlation (r=0.58, p>0.005), because some data, in the case of high pressure and high RF power, do not fit to the entire tendency of the reduction rate. Furthermore, the relationship between the emission intensity of singlet oxygen molecule (762 nm) and the reduction rate also indicates poor correlation (r=0.61, p>0.005).

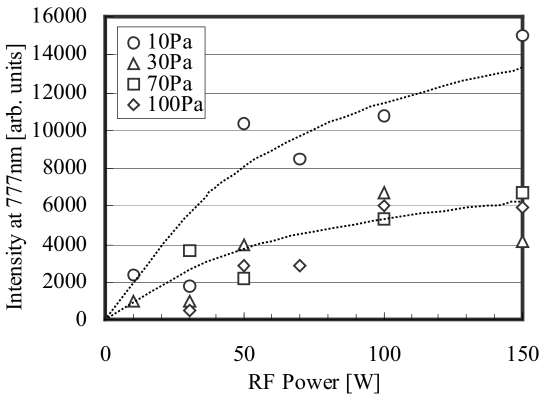

Fig. 7. Relationship between 777 nm of atomic oxygen against the pressure and RF energy.

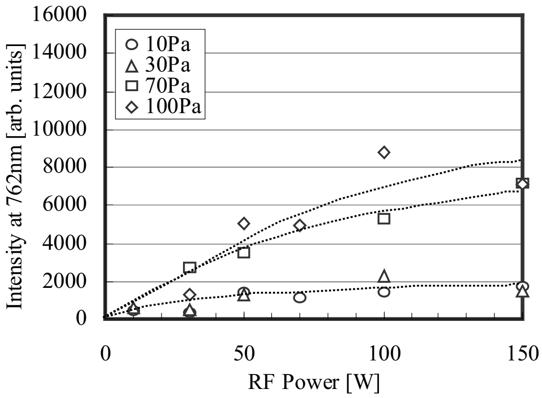

Fig. 8. Relationship between 762 nm of singlet molecular oxygen against gas pressure and RF energy.

Therefore, it is supposed that both atomic oxygen and singlet molecular oxygen contributed to the reduction of protein. The atomic oxygen has tendency to react with the protein in the case of low pressure around 10 Pa, and the singlet molecular oxygen reacts with the protein in the case of high pressure in the chamber.

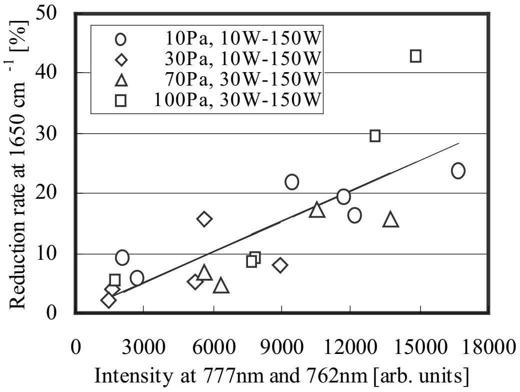

Fig.9. Reduction rate of 1650 cm^{-1} (amide I) correlated strongly with intensity at 777 nm from atomic oxygen and 762 nm from singlet molecular oxygen.

4. Conclusion

Active oxygen species such as atomic oxygen and singlet molecular oxygen are significantly important for the treatment of protein by RF oxygen plasma. Intensity of the light emission from atomic oxygen at 777 nm is strongly correlated with the pressure in the chamber and the RF power. It was found that the reduction rate of protein around 1650 cm^{-1} (amide I) significantly relates to the intensity of light emission from atomic oxygen at 777 nm and singlet molecular oxygen at 762 nm.

5. References

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