Treatment of Protein Using Oxygen Plasma Produced by RF Discharge

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Removal of proteins from the surface of medical equipments are attempted using oxygen plasma produced by RF discharge. FTIR spectra indicate that the bonds of C-H and N-H in the casein protein are reduced after irradiation of oxygen plasma. Also, the second order structure of a protein such as α-helix and β-sheet are modified by the oxygen plasma. Complete removal of protein as remnants on the medical equipments requires several hours avoiding the damage to medical equipments.

Keywords: Sterilization, Inactivation of protein, Second order structure of proteins, Oxygen radicals

1. Introduction

In the field of medicine, the sterilization has been one of the important procedures for the disinfections of medical equipments. Recently brand-new sterilization method, plasma sterilization, has been studied and put to practical use in hospitals as well as the conventional methods such as autoclave and EOG sterilizer [1-3]. These sterilization processes are successful to diminish or reduce bacilli and viruses on medical equipment due to destruction of cell wall and decomposition of DNA.

Recently, a kind of proteins with the infectious ability in the living body has been a serious problem in the field of medicine and food industry. Since this infectious protein called prion [4] has a robust β-sheet in the second order structure [5], it possesses significantly high receptivity to the EOG, autoclave and formalin as well as heat and radiation [6]. In order to inactivate the robust protein, all bonds of atoms including the β-sheet structure in the protein particle must be decomposed completely. Even though the sufficient heating and radiation is able to destruct the protein completely, medical equipments tend to be damaged by them. In this study, the oxygen radicals produced by the RF discharge are adopted to remove proteins on medical equipments, especially to decompose proteins including the robust β-sheet structure. The decomposition effect of protein is estimated changing the parameters of RF power, gas pressure and treatment period. Also, the optimum parameters to remove protein from medical equipments are found out avoiding the damage to the equipment.

2. Experimental procedure

Figure 1 shows the schematic diagram of experimental apparatus. The plasma chamber is made of stainless steal with the dimension of 450 mm in length and 200 mm in diameter. After evacuation below 1 Pa, pure oxygen gas was supplied to the chamber by the bombe. ICP type antenna used in this experiment has a one-turn loop shape for effective and spatially uniform generation of oxygen radicals. When RF power (13.56 MHz) is applied to the antenna, the glow discharge plasma with high uniformity is produced below the antenna.

It is important for sterilization of medical equipments with tiny gaps to diffuse radicals uniformly in the chamber. Since the lifetime of oxygen radical in the low-pressure circumstance (around several Pa) is approximately 10 ms, oxygen radicals are difficult to reach tiny gaps of medical equipments by the diffusion due to a density gradient. Therefore the gas pressure in the chamber was varied from 3 Pa to several 10^2 Pa, repeatedly. If the pressure in the chamber is varied from 3 Pa to 300 Pa, the oxygen radicals are accelerated to the velocity of approximately 30 m/s by the injected gas flow, and a flying range of the radicals is estimated to be 30 cm [7]. Therefore, the oxygen radicals are penetrated into every gap of medical equipments within the lifetime of 1 ms.

Generation of atomic oxygen radical was confirmed by light emission spectra of the plasma. The prion protein

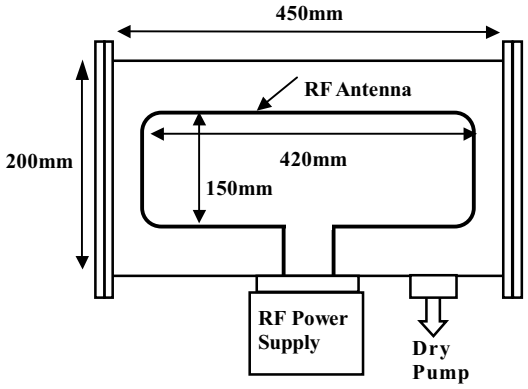

Fig.1 Schematic diagram of experimental apparatus.

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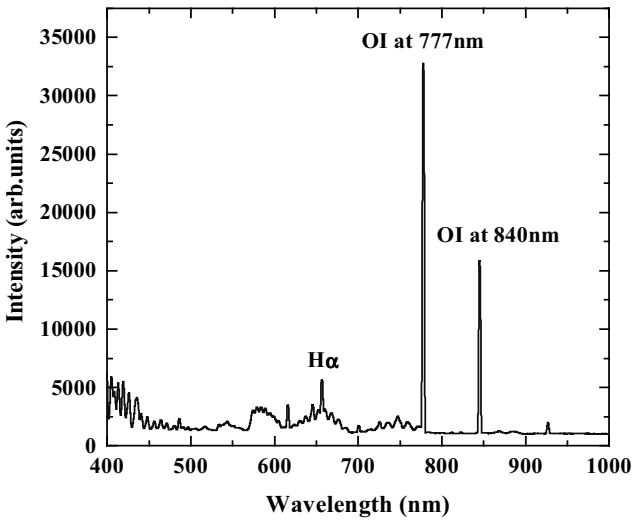

Fig.2 Typical light emission spectrum of oxygen RF plasma.

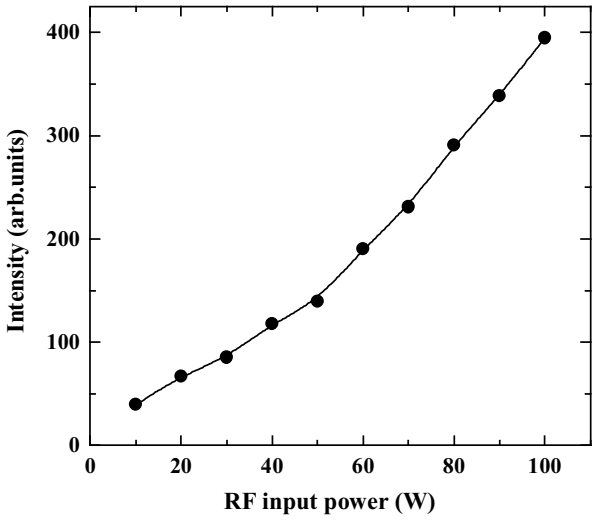

Fig.3 Dependency of emission intensity of atomic oxygen at 777nm on the RF input power.

that is rich in the β-sheet in the second order structure is highly infectious agent. In this experiment, the casein protein and the albumin those have the β-sheet structure are adopted, instead of the prion protein. The decomposition of β-sheet structure of the proteins indicates the ability of decomposition of the prion protein. The solution of the casein protein is put uniformly on the CaF_2 substrate with the diameter of 20 mm using a spin coater with a fixed rotation speed and different deposition time in order to prepare different densities of the casein protein. The casein protein on the substrate forms fine crystals with the size of several ten μm that is identified using a microscope. The casein protein on the substrates has a sufficient thickness to cover the CaF_2 substrate uniformly, even in the case of 0.21 mg/cm^2. Therefore, the spatial uniformities of the casein protein on the substrate with different densities are considered to be similar according to the objective of this study. The thickness of the casein protein on the substrate is different, when the casein density is varied. The crystallized protein powder on the CaF_2 substrate is fixed in a plasma diffusion region at 15 cm below the RF antenna.

The treatment effect is determined by the height of major peaks in FTIR spectra around 1600 cm^{-1} and 3300 cm^{-1} that indicate the amide I bonds of C-O and N-H, respectively. Also, the decomposition of β-sheet and α-helix of the protein second order structure is confirmed by the second derivative of FTIR spectral peaks at 1635 cm^{-1}and 1655 cm^{-1}, respectively [8].

3. Results and discussion

3.1 Characteristics of oxygen plasma

Figure 2 illustrates the light emission spectra of RF oxygen plasma. Intense peak at the wavelength of 777 nm implies the generation of oxygen radicals (singlet atomic oxygen, OI). Oxygen radicals those are accelerated in a plasma by the charge exchange or collisions of energetic ions etch organic compounds physically and chemically, such as the resist striping in the semiconductor fabrication process. The bonds in proteins such as C-H, C-N and N-H are decomposed and oxidized into CO_2, NO_2 and H_2O. This process proves the decomposition of proteins by oxygen radicals in the plasma. In addition, it is known that an intense UV light with the wavelength shorter than 250 nm that is emitted from excited NO and CO molecules can decompose the bonds in protein, because the energy of the photon is comparable to that of oxygen radicals produced in the plasma. In this study, the UV detection label indicates that intensity of the UV light below 250 nm is significantly small around the substrate. Therefore, the UV light is not effective for the protein decomposition. The spectral peak of 777 nm is detected up to 15 cm below the ICP antenna. Therefore, the atomic oxygen that is produced around the ICP antenna diffuses to whole of the chamber.

Figure 3 indicates the generation of oxygen radical changing the RF input power. The light emission intensity that represents the density of atomic oxygen increases with the RF input power. However, the temperature in the chamber and the object to be treated also increases with the input power. In order to keep the temperature below 70 °C avoiding damage to objects to be treated, the input power is restricted to 60 W.

3.2 Decomposition of proteins

Figure 4 shows FTIR spectra of the casein protein on the CaF_2 substrate varying a treatment period. The initial concentration of the casein is approximately 1.0 % diluted by pure water. The density of the casein on the substrate is 0.63 mg/cm^2, which is same as or larger than the remnant of protein on medical equipments after the first stage

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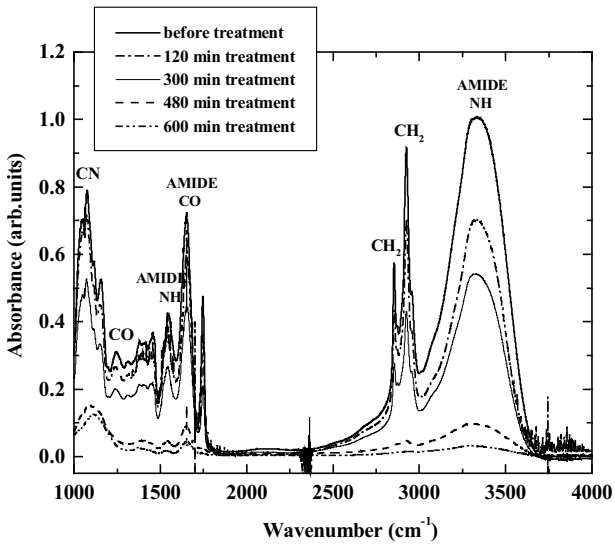

Fig.4 Typical FTIR spectra of casein protein on CaF_2 substrate for different treatment time.

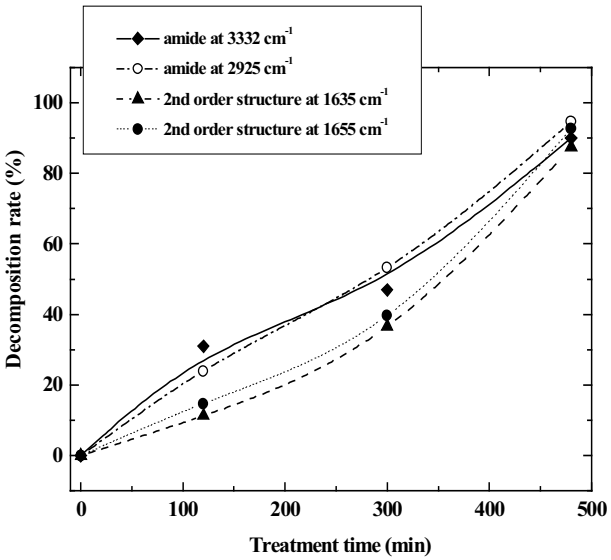

Fig.5 Temporal evolution of decomposition rate of each structure of casein protein.

wash and before the sterilization sequence in hospitals. The input RF power to produce radicals is kept at 60W. The pressure in the chamber is varied from 3 Pa to 300 Pa in every 5 minutes during the treatment, controlling the flow rate of oxygen gas. Oxygen radicals in a plasma reach the CaF_2 substrate directly and decompose proteins on the substrate, since oxygen radicals without electric charge are not affected by a sheath electric field in front of CaF_2 substrate.

In the spectra, amide peaks ranged from 1600 to 1700 cm^{-1}, peak of C-H side chain at 2800 cm^{-1} and peak of N-H amide bond at 3300 cm^{-1} are observed. The amide peaks from 1600 to 1700 cm^{-1} are attributed to the second order structure of protein such as α-helix and β-sheet. The β-sheet structure of protein that appears at 1635 cm^{-1} is significantly robust bindings and resistive to the heat and chemical agents. When a protein is rich in the β-sheet structure, the protein is difficult to destruct using conventional treatment methods of autoclave and formalin avoiding the damage to the medical equipment. The FTIR spectra indicate the significant peaks of the protein described above decrease with the treatment time. And the spectrum becomes almost flat after ten hours. When the initial concentration of the casein is 0.21 mg/cm^2 with the similar surface condition, the complete decomposition of the casein requires approximately 1 and half hours. The decomposition efficiency is larger than that of the concentration of 0.63 mg/cm^2. The obtained result that the thicker casein protein requires the additional treatment period considering the thinner casein protein case. This fact suggests that some byproduct of the protein decomposition would remain on the substrate. Therefore, the oxygen plasma treatment can be adapted to the treatment of proteins with lower density.

Figure 5 shows the time evolution of peak heights of the major bonds in the casein protein appeared on the FTIR spectra of Fig. 4. Both peaks of C-H and N-H bonds of the amide structure in protein at 2925 cm^{-1} and 3332 cm^{-1}, respectively, reduced with treatment time. The peaks of the α-helix and β-sheet of the second order structure of proteins at 1655 cm^{-1} and 1635 cm^{-1}, which are embedded in the amide band spectral peak, also decrease with time. This figure suggests that the amide structure is decomposed faster than the second order structure of proteins. The resistively of the second order structure against the oxygen radical is higher than those of amide bonds such as C-N, C-H and N-H, which is same situation as another sterilization method, which would be due to the hydrogen bonds in the structure. Amide structure diminished almost completely after a treatment for eight hours, while the second order structure remains approximately 12% of original amount.

The β-sheet of second order structure of protein has significantly robust against heat and chemical agents due to the strong bonds between sheet structures. Therefore, protein that is rich in β-sheet such as fibrin and prion is difficult to destruct by heat and chemical agents such as formaldehyde. The β-sheet structure is attempted to decompose using the oxygen plasma. Parameters of oxygen plasma are same as that described above. Figure 6 illustrates the second derivative of a FTIR spectrum of the casein, which brings the reduction of the second structure of protein into relief. In this figure, the α-helix and the β-sheet can be identified clearly as the peaks of 1655 cm^{-1} and 1635 cm^{-1}, respectively. Negative peaks indicate the decomposition of structures by oxygen plasma. When the protein is immersed in the oxygen plasma that is produced by the low-pressure RF discharge, both the α-helix and β-sheet in the FTIR second derivative spectrum are reduced after the 3 hours.

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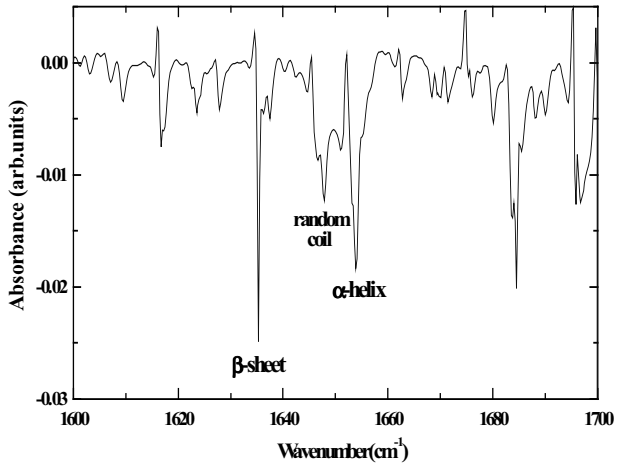

Fig.6 Difference of the FTIR second derivative spectra before and after the treatment for eight hours.

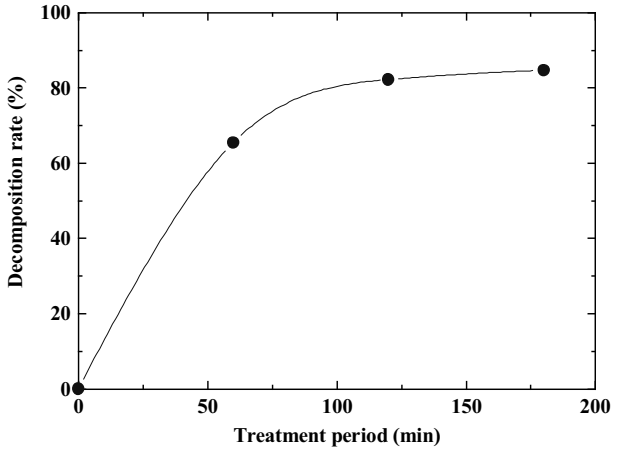

Fig.7 Time variation of the decomposition rate of the albumin.

The albumin from chicken eggs is rich in the β-sheet structure as well as the casein protein, and is utilized as a substitute of the prion protein in experiments. The albumin on the CaF_2 substrate with the initial concentration of 0.3 mg/cm^2 is immersed in the oxygen plasma. Figure 7 shows the time variation of the decomposition rate of the second order structure of albumin estimated by the FTIR spectral peak at 1635 cm^{-1}. The decomposition rate increases with the treatment time till 120 minutes and then almost saturates as the concentration of albumin on the substrate becomes lower. The β-sheet structure is destructed with 85% after the treatment for 180 minutes, and is decomposed over 95% after eight hours. The significant difference of the tendencies between results of Fig.5 and Fig.7 in first 100 min would originate from the sample type, due to amount of the hydrogen bonds between β-strands. However, unfortunately, the detailed mechanism of the difference of the tendencies is still under investigation.

Above results suggest that the oxygen radical sterilizer enables to remove the β-sheet structure of proteins from medical equipments. Complete decomposition of the β-sheet structure would require the treatment for more than ten hours.

4. Summary

The oxygen radicals produced by the low-pressure oxygen RF discharge enables to decompose the second order structure including β-sheet, as well as the amide bonds and side chains of proteins. The decomposition rate of the casein protein with the concentration of 0.63 mg/cm^2 and the albumin with 0.3 mg/cm^2 are 88% and 95%, respectively, avoiding the damage to the equipment. Therefore, the oxygen radical sterilizer would act as a remover of the prion protein from medical equipments.

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