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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.

Key words: sterilization, protein, second order structure, 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 with 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. EXPERIMANTAL 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. 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 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 injected gas flow, and the flying range of the radicals is estimated to be 30 cm [7]. Therefore, the oxygen radicals are penetrated into every gap of the equipments within the lifetime of 1 ms.

Generation of atomic oxygen radical was

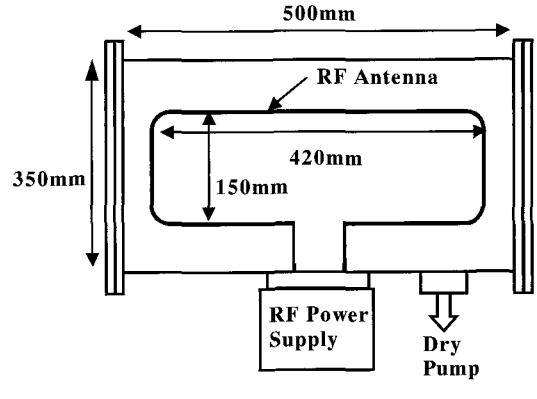

Fig.1 Schematic diagram of experimental apparatus.

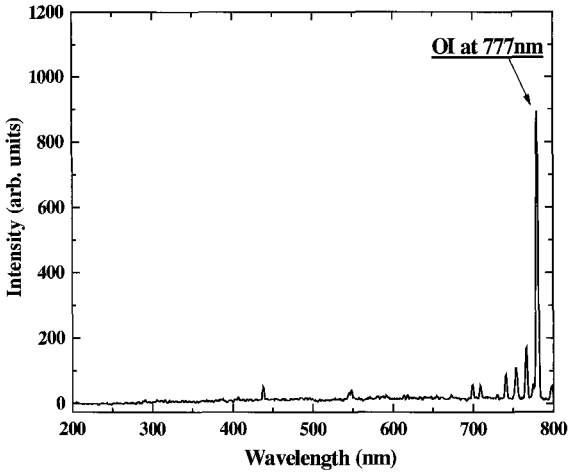

Fig.2 Typical UV-vis emission spectrum of oxygen RF plasma.

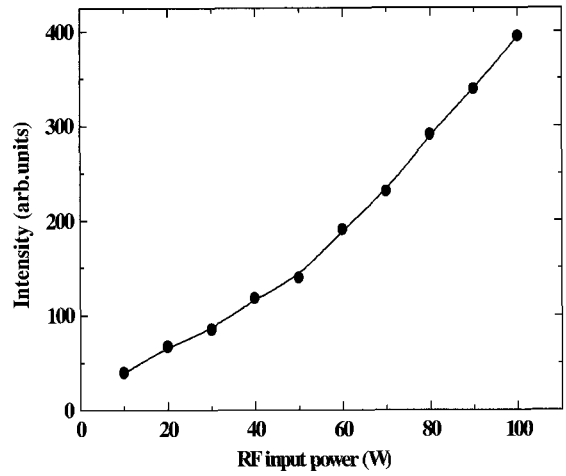

Fig.3 Dependency of emission intensity of atomic oxygen at 777nm on the RF input power.

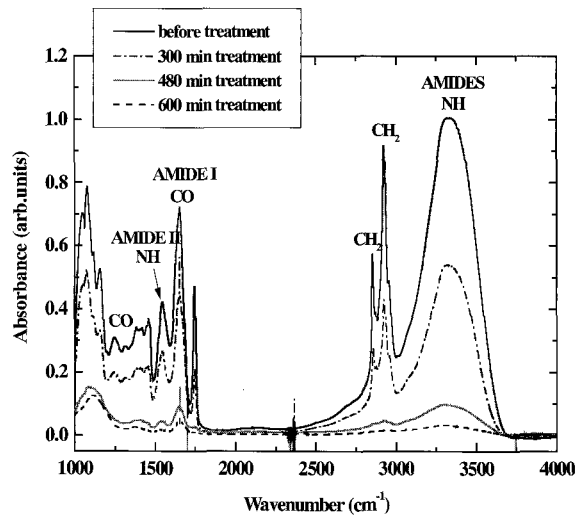

Fig.4 Typical FTIR spectra of casein protein on CaF_2 substrate for different treatment time.

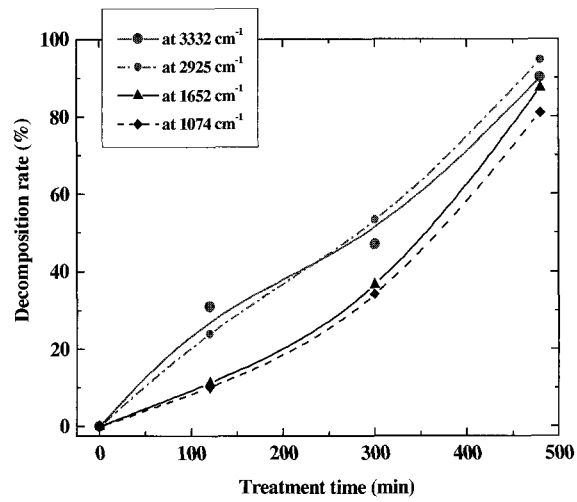

Fig.5 Temporal evolution of decomposition rate of each structure of casein protein.

confirmed by light emission spectra of the plasma. The prion protein that is rich in the β-sheet in the second order structure is highly infectious agent. In this experiment, the casein protein extracted from milk that has the β-sheet structure is adopted, instead of prion protein. The decomposition of β-sheet structure of the casein protein indicates the ability of decomposition of the prion protein. 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.

3. RESULTS AND DISCUSSION

3.1 Characteristics of oxygen plasma

Figure 2 illustrates the UV-vis light emission spectra of RF oxygen plasma. Intense peak at the wavelength of 777 nm implies the generation of oxygen radicals (singlet atomic oxygen). 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 changed into CO_2, NO_2 and H_2O. This process proves the decomposition of proteins by oxygen radicals in the plasma. In addition, an intense UV light with the wavelength shorter than 250nm that is emitted from excited NO and CO molecules can decompose the bonds in protein, because the energy of the photon is comparable to

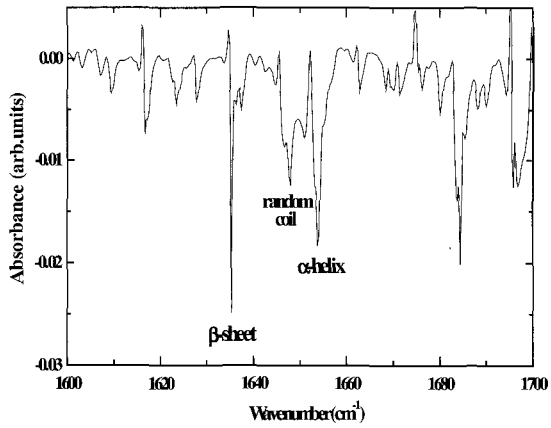

Fig.6 Second derivative of FTIR spectra before and after the treatment for eight hours.

that of oxygen radicals produced in the plasma. In this study, however, the UV light is not effective for the protein decomposition, because intensity of the UV light below 250 nm is significantly small. The spectral peak of 777 nm is detected up to 10 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 degree avoiding damage to objects to be treated, the input power is restricted to 60 W.

3.2 Decomposition of protein

Figure 4 shows FTIR spectra of casein protein on the CaF_2 plate varying treatment period. The casein is extracted from cow's milk, and the initial concentration of casein is approximately 1.0 % diluted by pure water. The density of the casein on the plate is 0.63 mg/cm^2, which is approximately same as the remnant of protein on medical equipments after the first stage wash and before the sterilization sequence in hospitals. Oxygen radicals accelerated in a plasma hit the CaF_2 substrate and decompose proteins on the CaF_2 substrate, since oxygen radicals without electric charge are not affected by a sheath electric field in front of CaF_2 substrate.

In the spectra, peak of C-N amide bond at 1074 cm^{-1}, 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, the complete decomposition of casein requires approximately 1 and half hours. The decomposition efficiency is larger than that of the concentration of 0.63 mg/cm^2. Therefore, oxygen radical is suitable for the treatment of protein with lower concentration.

Figure 5 shows the time evolution of peak heights of the major peaks appeared on the FTIR spectra of Fig. 4. Both peaks of C-H and N-H bonds of amide structure in protein at 2925 cm^{-1} and 3332 cm^{-1}, respectively, reduced with treatment time. Spectral peaks around 1655 cm^{-1} and 1635 cm^{-1} are attributed to the second order structure of proteins those indicate the structures of α-helix and β-sheet, respectively, and also decrease with time. 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. Structures of amide almost diminished after treatment for eight hours, while the second order structure remains approximately 15% of original amount.

The β-sheet of second order structure of protein has significantly robust due to the strong bond of the sheet structure. 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 fibrinogen, which brings the reduction of the second structure of protein into relief. In this figure, α-helix and 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 several hours. Therefore, the oxygen radicals such as atomic oxygen and hydroxyl radical enable to decompose the β-sheet structure of proteins. Above results implies that the oxygen radical would remove prion proteins from medical equipments.

4. SUMMARLY

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

complete removal of protein with the concentration of 0.63 mg/cm^2 requires approximately ten hours, respectively, avoiding the damage to the equipment.

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