Decomposition of Proteins Using a Microwave Air Plasma Sterilizer

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The decomposition of proteins on a surface was investigated using a low-temperature microwave plasma torch sterilization method. Albumin and keratin, used as model proteins, were irradiated with reactive species produced by an air microwave discharge plasma and their decomposition rates were approximately 0.1 mg/cm² per 30 min. All chemical bonds in the proteins, including those involved in forming secondary structures, are decomposed by the reactive species. These findings suggest that the microwave plasma torch method could be useful for removing prion proteins from the surfaces of medical devices.

Key words: Microwave plasma, Protein removal, Albumin, Keratin, Prion protein

1. INTRODUCTION

Reusable medical and surgical tools that have been appropriately disinfected and sterilized are required in medical facilities. All proteins adhering to these tools must be removed prior to their reuse. Medical tool sterilization devices used in hospitals include high-pressure steam, ethylene oxide gas (EOG) [1–3], and hydrogen peroxide gas sterilizers [4–7].

Some protein often remains adhered to washed medical tools, and the irregularly shaped prion protein (PrP^{Sc}) has recently become problematic in this regard in the medical care and food industries [8–14]. PrP^{Sc} is believed to be a structurally deformed version of the properly shaped prion (PrP^C) protein, and PrP^{Sc} accumulates in the brain and causes diseases. The accumulation of PrP^{Sc} in the human brain can destroy brain function and may result in death. PrP^{Sc} is infectious and can be transmitted between species. Although there have been attempts to develop pharmaceuticals for prion diseases, their effects remain limited [2]. Therefore, the removal and breakdown of proteins adhered to medical tools is necessary to prevent the spread of prion diseases in hospitals. At present, proteins on medical devices are inactivated using high-pressure steam sterilizers by treating for 18 min at 134 °C. However, this method cannot be used for non-heat-resistant materials.

Plasma sterilization methods have attracted attention as safe, low-cost alternatives to traditional sterilization methods such as EOG or high-pressure steam sterilization. A previous study reported that NO radicals generated by microwave discharges in air can sterilize surfaces at low temperatures. In this study, we used NO radicals in an air microwave plasma torch to observe the

Fig. 1. Schematic of the microwave plasma generation apparatus.

decomposition of the secondary structures of proteins adhered to medical tools [15].

2. EXPERIMENTAL PROCEDURE

A low-pressure plasma source that generates plasma by microwave discharge was used and a schematic is shown in Fig. 1. The experimental apparatus consists of a vacuum chamber, a vacuum pump, a glass tube, an antenna for plasma generation, and a microwave power supply. The microwave source was operated at a frequency of 2.45 GHz. A glass tube with an inner radius of 5 mm was placed coaxially inside the cylindrical-type antenna. This antenna has a 6 cm long slit, corresponding to the half-wavelength of the microwaves [1]. The antenna is placed inside the microwave waveguide to absorb the microwaves and plasma is generated inside the glass tube. The plasma is

Fig. 2. Photograph of plasma emitted from the open end of the torch.

then transported to the 17.5 L processing region (vacuum chamber) by the airflow in the glass tube. Low-temperature sterilization of non-heat-resistant materials such as plastic and resin must be conducted at temperatures below 60 °C. This is achieved by limiting the microwave power to 250 W and setting the pressure inside the chamber to 80 Pa. Medical tools are sterilized within a few hours using the above parameters. The microwaves penetrate a relatively small region of the glass tube (a discharge volume of 1.2 cm³), ensuring a high density of high energy electrons. Reactive species such as NO radicals are produced by these electrons, and were measured using light emission spectroscopy [1].

Two model proteins were used in this experiment rather than potentially dangerous prion protein: cow blood-serum albumin, which contains several long α -helices, and wool keratin, which contains some α -helical structure and a large amount of β -sheet structure. Keratin is an abundant protein in feathers, has a robust structure due to its many disulfide bonds, and is tolerant of chemical agents. The α -helical structure of PrPsc is lower and the amount of β -sheet structure is higher compared to PrPc. Thus, the ability of low temperature plasma to degrade keratin would indicate its ability to degrade prion protein. A protein surface concentration of 0.2 mg/cm² was used to provide strong infrared spectra and represents the maximum amount of protein that can adhere to a medical tool; this concentration is far lower than the lethal concentration of PrPsc (1 mg/cm²) [2]. CaF₂ substrate is transparent to a wide range of wavelengths and is thus useful for Fourier transform infrared (FTIR) analyses. CaF₂ substrate was positioned 3 cm below the open end of the torch. The presence or absence of CaF₂ substrate had no effect on the spectra. Decomposition of the proteins was evaluated from changes in the height of specific peaks in the FTIR spectra.

3. RESULTS AND DISCUSSION

Fig. 3. Typical light emission spectra of microwave plasma at various gas pressures.

3.1 Production of reactive species

The apparatus employed in this study uses microwave energy to produce a high density of active particles. The photograph in Fig. 2 shows the afterglow of the microwave torch plasma in the processing region of the vacuum chamber. The dimensions of the chamber are as follows: D 35 cm × W 32.5 cm × H 15 cm. Yellow plasma is ejected from the open end of the eight torches, ionizing air and spreading the plasma throughout the processing region of the chamber. As shown in Fig. 3, the spectra measured in the processing region comprise the superposition of excited nitrogen peaks and a characteristic broad peak between 350–700 nm. This broad peak is due to the NO radical, which emits yellow light [1]. The production of NO radicals increases with increasing pressure in the processing region. An increase in the number density of N and O atoms increases the probability of N and O atom recombination. The NO radicals produced by air plasmas can reduce organic compounds [1]. We anticipated that irradiating proteins with NO radicals would induce their reduction and decomposition.

3.2 Degradation of albumin and keratin

Active oxygen species induce the physical and chemical etching of organic compounds and chemical modification by oxidation. Figure 4 shows typical IR spectra of albumin after plasma irradiation, along with untreated albumin (control). The height of each peak decreases with increasing time of irradiation, and there is no conspicuous peak due to byproducts. Deconvolution of the FTIR spectra shows significant decreases in the peak height of N-H bonds around 3300 cm⁻¹, 1540 cm⁻¹ (amide II), and 1240 cm⁻¹ (amide III), whereas peaks due to CH and CH₂ at 2700–2850 cm⁻¹ change only slightly. PrPsc is characterized by many hydrogen bonds that control its secondary structure, and these bonds contribute significantly to the amide I band in IR spectra. The post-processing effects for amide I are indicated by C=O and N-H bonds, which determine the

secondary structure of a protein. These effects were reflected in the peak heights at approximately 1650 cm^{-1} in the FTIR spectra: as the plasma irradiation period

Fig. 4. Typical IR absorption spectra of albumin treated for various periods.

Fig. 5. Decomposition rates of proteins treated for various periods.

increased, the peak near 1100 cm^{-1} increased due to the formation of C–OH and C–O–C bonds and indicating oxidation of the proteins. The helical structure (α -helix) of keratin was confirmed by the heights of the 1635 and 1655 cm^{-1} peaks in the FTIR spectra.

We confirmed that an increase in processing time increases the amount of protein degradation. Figure 5 shows the decomposition rates of keratin and albumin determined from the amide I peak heights in the vicinity of 1650 cm^{-1} for various plasma treatment periods at a chamber pressure of 60 Pa and an input microwave output of 250 W. Plasma irradiation for 30 min decreased the amount of albumin by 31% and by 68% after 2 hrs. This indicates that the energy produced by air plasma is sufficient to decompose proteins. The maximum temperature during the 2 h plasma irradiation was $41\text{ }^{\circ}\text{C}$. Keratin irradiation for 30 min destroyed 34% of the protein and 64% was destroyed after 2 hrs of irradiation. This indicates that air plasma has sufficient

energy to break hydrogen bonds and peptide bonds.

Figure 6 shows the decomposition rates of keratin and albumin as the pressure in the chamber is varied from

Fig. 6. Decomposition rates of protein with different chamber pressures.

20–80 Pa. Pressure clearly affects the rate of decomposition. The initial concentration of both keratin and albumin on the CaF_2 substrate was 0.2 mg/cm^2 and the proteins were irradiated for 30 min. A chamber pressure of 20 Pa reduced the amount of keratin by 34% and pressures of 50 and 80 Pa resulted in a 59% and 64% reduction, respectively. These results confirm that the decomposition of keratin and albumin increases with an increase in pressure inside the chamber. High pressure results in the generation of a large number of reactive species, but the temperature is likely to rise. The maximum temperature attained during 30 min of plasma irradiation was $39\text{ }^{\circ}\text{C}$. Keratin degraded faster than albumin at all pressures, indicating that keratin decomposes readily when irradiated with air plasma.

The decomposition rates of different initial densities of keratin and albumin on the substrate were investigated. The albumin samples were irradiated for 30 min. An increase in initial density from 0.2 to 0.8 mg/cm^2 resulted in a decrease in decomposition from 42% to 24%. This result allows estimation of the rate of albumin decomposition by plasma treatment: approximately $0.2 - 0.4\text{ mg/cm}^2\text{ h}$, depending on the initial density of the protein. Different albumin concentrations provided the same FTIR spectra, suggesting that no toxic byproducts such as nitriles were produced during irradiation. The decomposition rate of keratin followed a tendency similar to that of albumin and its rate of decomposition was similarly estimated to be $0.2\text{ mg/cm}^2\text{ h}$. An autoclave can inactivate PrPsc in about 18 minutes at $135\text{ }^{\circ}\text{C}$. Our results demonstrate that although steam autoclaving is faster, the plasma torch method can destroy proteins at much lower temperatures.

4. CONCLUSION

Albumin and keratin were decomposed by irradiation by a microwave torch plasma that generates NO radicals. The temperature was maintained below 60°C, and the rate of decomposition was found to be approximately 0.2 - 0.4 mg/cm² h. These findings demonstrate that excited nitrogen molecules and atoms can decompose proteins. The obtained results suggest that this approach can be used to decompose prion proteins.

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