Albumin aggregation by atmospheric pressure plasma discharge using needle electrode

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Abstract: One mechanism of blood coagulation by low-temperature atmospheric pressure plasma is protein aggregation. In this study, we aim to understand the relation between the electrical parameters for plasma discharge and aggregation. A plasma discharge was produced between a needle electrode and albumin solution. It was observed that the albumin aggregate was produced below the plasma discharge and a size of the aggregate increased with plasma treatment time.

Keywords: plasma medicine, protein aggregation, plasma exposure on liquid

1. Introduction

Low-temperature (non-thermal) atmospheric pressure plasmas have been investigated for biomedical applications [1-3] and a new scientific field ‘plasma medicine’ has been built up. It is possible to treat (living) substances at room temperature and at atmospheric pressure. From the plasmas, there are charged particles, reactive nitrogen and oxygen species, (UV) photons, and the plasma treatments without thermal damage are possible. Many investigated biomedical effects by the plasmas have been caused by reactive species.

Since the plasma exposure is suitable for a superficial treatment, sterilization and wound care [4,5] were the main applications in the early stages of plasma medicine. Afterwards, a number of biomedical applications using plasmas increased due to beneficial property of plasma. Currently, the spectrum of applications is broad, and many other biomedical applications have been studied including bleeding control [6,7], cancer therapy [8], agriculture [9].

Bleeding control is one of the promising applications using low-temperature atmospheric pressure plasmas. There are three major mechanisms in plasma-induced blood coagulation such as aggregation of protein, aggregation of fibrinogen and platelets, and hemolysis [7,10]. In this study, we focus on the aggregation of protein using albumin that is the most abundant protein in blood. In earlier reports, the mechanism of protein aggregation using helium plasma jet was discussed. So far, it is suggested that the charge balance and charge fluctuation on the surface of albumin solution could be a key parameter for the albumin aggregation [11,12] because the albumin molecules have a negative charge in solution.

In this contribution, we aim to understand the mechanism of albumin aggregation using plasma from the viewpoint of electrical parameters. Plasma discharges were produced between a needle electrode and solution surface. Time evolutions of albumin aggregation on the albumin solution by the plasma exposure were measured. By changing electrical parameters for the plasma production, the mechanism of albumin aggregation is discussed.

2. Experimental setup

Figure 1 shows our experimental setup for the albumin aggregation. A quartz dish (30 mm in diameter, 15 mm in height) filled with the albumin solution was placed on an electrically grounded plate. The density of albumin solution was 50 mg/ml using bovine serum albumin (Sigma-Aldrich Co. LLC, A3294). A needle electrode made of Tungsten was placed 1 mm above the surface of albumin solution. Between the needle electrode and the electrical ground, a high voltage of 3-5 kV_{pp} at 1 kHz in frequency was applied to produce a plasma discharge [13]. The high voltage was generated from a high voltage amplifier (NF Corporation, HVA4321) and a function generator. The applied voltage was monitored by an oscilloscope. Please note that no external gas flow was applied, and all the experiments were carried out at atmospheric pressure.

By the plasma exposure, a white substance (albumin aggregate) was produced on the surface of albumin solution. In order to observe a time evolution of albumin aggregate, a digital camera was used for recording.

Before and after the plasma treatment on the albumin solution, pH and electrical conductivity of the albumin solution were measured using a pH meter (HORIBA, Ltd., LAQUAtwin-pH-11B) and an electrical conductivity meter (HORIBA, Ltd., LAQUAtwin-EC-33B), respectively.

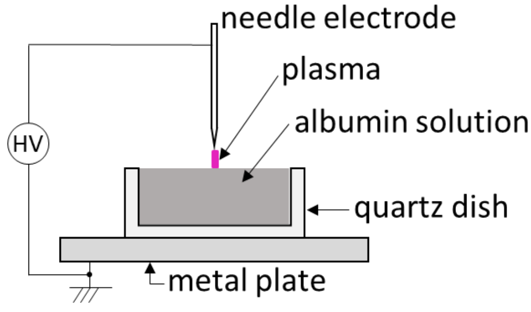

Fig. 1. Experimental setup for albumin aggregation.

3. Experimental result

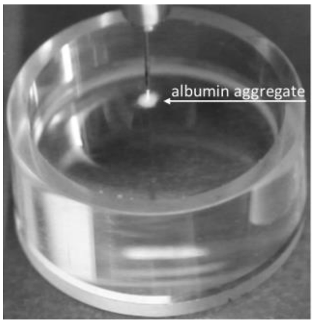

Fig. 2. Albumin aggregate on the albumin solution.

By the plasma treatment, albumin was aggregated on the albumin solution as shown in Fig. 2. In this experiment, the plasma discharge was produced using a high voltage of 5 kV_{pp} at 1 kHz in frequency. This photo was taken at 40 s after the plasma treatment started. The aggregate was formed just below the plasma discharge (needle electrode) and the shape of aggregate was almost symmetrical.

After recording the evolution of albumin aggregate by the digital camera, a size of albumin aggregate was estimated for each frame from the video computationally. Figure 3 shows a size evolution of albumin aggregate during the plasma treatment.

It is observed that the size of the albumin aggregate increased almost monotonically from 0 to 50 s. At 50 s, the size reached about 5 mm^2 and was almost constant for 300 s with several fluctuations.

By the plasma treatment, reactive species are generated in the treated solution. Before the plasma treatment, pH and electrical conductivity of the albumin solution were 7 and 0.56 mS/cm, respectively. It was confirmed that both pH and electrical conductivity after the plasma treatment were almost same as before. It means that the electrical parameter of the albumin solution was almost constant during the plasma treatment.

4. Summary

We demonstrated that the albumin was agglomerated on the albumin solution by the plasma discharge produced between the needle electrode and solution surface. The shape of the albumin agglomerate was almost symmetrical, and the size of the agglomerate reached about 5 mm^2 at 50 s after the plasma treatment started. With longer plasma exposure, no more growth of the agglomerate size was observed.

In the conference, we discuss a dependence of electrical parameters such as input power to plasma and a waveform of the applied voltage on the albumin aggregation. Moreover, plasma characteristics will be measured and a relation between the albumin aggregation and the characteristics is considered.

Acknowledgements

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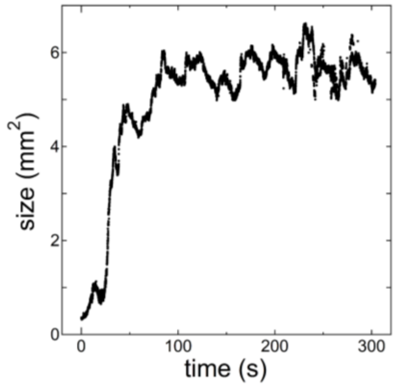

Fig. 3. Size evolution of albumin aggregate when the plasma discharge was produced using high voltage of 5 kV_{pp} at 1 kHz.

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