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Influence of Plasma Irradiation on Silkworm

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ABSTRACT: Silkworms have recently been proposed as an animal model for safety testing in basic research. We propose using silkworms for *in vivo* trials of direct plasma treatment. In this study, the influence of plasma irradiation on silkworms was investigated using a non-thermal atmospheric pressure plasma. Silkworm survival rate decreased with increasing low-frequency voltage and plasma irradiation period. Further investigation of the plasma-generated agents (oxygen related radicals, UV light, and charged particles), revealed that the contribution of charged particles significantly increases silkworm mortality.

KEY WORDS: silkworm survival rate, DBD plasma torch, non-thermal atmospheric pressure plasma, ozone, discharge current

I. INTRODUCTION

Non-equilibrium plasmas are characterized by the electrons in the plasma having a higher mean energy than the ions or atoms. This higher energy can produce sufficient active chemical radicals for the high-speed surface processing of materials without causing thermal damage. Recently, non-equilibrium atmospheric pressure plasma has been studied in direct contact with living tissues in order to deactivate pathogens,[1] stop bleeding with no damage to healthy tissue,[2] disinfect wounds and accelerate wound healing,[3] and to selectively kill some types of cancer cells.[4] However, much research is still required to clarify and minimize the health risks of plasma treatment. In particular, *in vivo* trials using animal models are essential for predicting the risks of plasma treatment. The use of mammals for experimental models has many associated problems, such as high cost and ethical issues. However, silkworms have been proposed recently as an animal model for safety testing in basic research, because the mechanisms of chemical absorption, distribution, metabolism, and excretion are similar in silkworms and mammals.[5] Until now, silkworms have been used for assessing the therapeutic effects of chemicals and in tox–icity research.[6–11] Thus, silkworms are considered appropriate for evaluating the effects of plasma treatment on animal bodies. In this study, we made a preliminary investigation of the influence of plasma irradiation on live tissue using silkworms.

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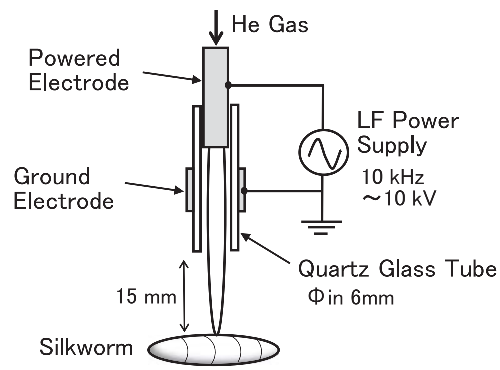

FIG. 1: Schematic diagram of the experimental setup

II. EXPERIMENTAL APPARATUS AND METHOD

Figure 1 shows the torch type of dielectric barrier discharge (DBD) plasma equipment used in this study. The quartz glass tube of 6-mm inner diameter was covered with copper tape as a ground electrode, and a copper tube inside the quartz glass tube functioned as the powered electrode. A DBD plasma jet was generated by applying low frequency (LF) high voltage (10 kHz) to the two electrodes under atmospheric pressure. The working gas was helium. The LF voltage was varied from 6 to 9 kV, while the gas flow rate was fixed at 10 SLM in all cases. The length of the plasma plume increased from about 20 mm to 50 mm as the LF voltage was increased from 6 kV to 9 kV. The life cycle of a silkworm consists of four stages: embryo, larva, pupa, and adult moth. The larval stage lasts for about 27 days, and the silkworm goes through five growth stages called instars during this time. Fifth instar silkworm larvae used in this study were purchased from Kogensha Co. Ltd (Nagano, Japan). The plasma irradiation was performed with a distance of 15 mm between the silkworm and the torch nozzle; the silkworm was in direct contact with the plasma. Therefore, the silkworms were exposed to all the plasma-generated agents (reactive species, UV photons, and charged particles). Ten silkworms were exposed individually to the plasma for each of the plasma treatment conditions. Ten untreated silkworms were used as a control. After plasma irradiation, both the treated and untreated silkworms were raised in a plastic case and fed artificial food twice a day. The amount of food administrated each time was 150 g per silkworm. The number of living silkworms was counted every day to obtain the survival rate after plasma irradiation.

The optical emission from the torch plasma was collected along the axial direction of the quartz tube using an optical fiber and was directed into a multi-channel spectrometer (Ocean Optics HR4000CG-UV-NIR). The ozone concentration at a distance of 20 mm from the torch nozzle, and the surface temperature of the silkworm, were measured using an ozone sensor (Eco Sensors, A-21ZX) and an infrared thermometer (Mather Tool, MT-10), respectively. The discharge voltage and current were measured with a high-voltage probe (Tektronix, P6015A) and current probe (Pearson, 4100), respectively, and monitored using a digital oscilloscope (Iwatsu, DS-5107).

III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Characteristics of Torch Plasma

In order to evaluate species in the discharge region, we investigated the optical emission spectra with the LF voltage maintained at 7 kV. The typical optical emission spectrum is shown in Fig. 2, where the emission peaks of atomic oxygen (O) and the hydroxyl radical (OH) are observed. It is considered that these radicals were generated from the dissociation of oxygen molecules and water molecules in the ambient air. They are highly reactive and able to oxidize most chemicals. Moreover, significant UV emission spectra were not observed at wavelengths below 300 nm. UV radiation in the 200 to 300 nm wavelength range with doses of several mW/sec/cm^2 is known to cause lethal damage to cells.[12] Thus, we expect the plasma-generated UV to not significantly affect the silkworms.

Another important reactive species generated in non-equilibrium atmospheric pressure plasma is ozone (O_3), because it is very long-lived at atmospheric pressure and so can be transported over long distances after being generated in the plasma. Figure 3 shows the O_3 concentration at a distance of 20 mm from the torch nozzle as a function of LF voltage. It is found that the O_3 concentration increases with increasing LF voltage up to a maximum concentration of 0.36 ppm at a voltage of 9 kV. On the other hand, nitrogen-based reactive species, such as NO and NO_2, were not detected by the gas detecting tubes (Kitagawa, 11L).

We evaluated the oxidation efficiency of oxygen-based reactive species generated in the torch plasma using chemical indicators (Sakura Color Products, Plazmark for O_2 cleaning). The color of the chemical indicator changes gradually from purple to green by reaction with oxygen radicals, such that the oxidation efficiency can be evaluated. The plasma-irradiation-time dependence of the color of the chemical indicator with different LF voltages is shown in Fig. 4. The color of the chemical indicators was found to change

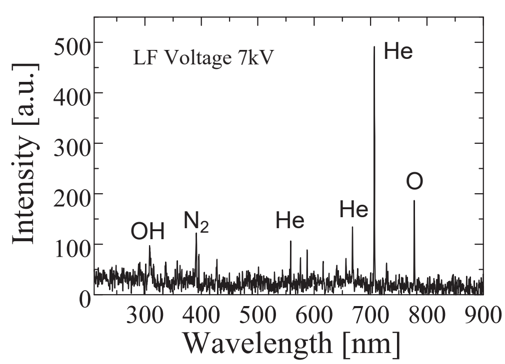

FIG. 2: Optical emission spectra. LF voltage: 7 kV

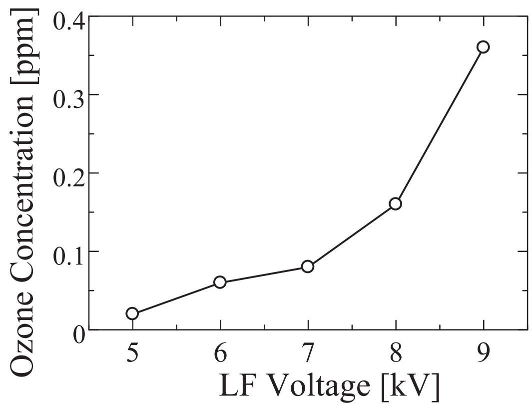

FIG. 3: Ozone concentration as a function of LF voltage

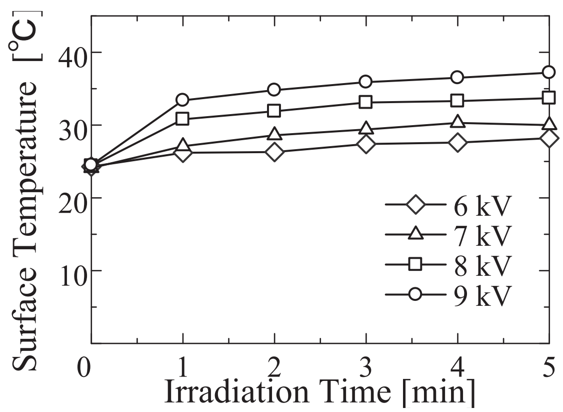

FIG. 4: Plasma irradiation time dependence of the color of the chemical indicator with different LF voltages

with the plasma irradiation, meaning that oxygen radicals with the ability to undergo surface oxidation exist at the plasma irradiation point.

The surface temperatures of the silkworm at the plasma irradiation point as a function of plasma irradiation time for different LF voltage are shown in Fig. 5. Under our plasma irradiation conditions, the surface temperatures of the silkworm were lower than about 38°C. The viable temperature of silkworms is 7°C to 40°C, so plasma irradiation is unlikely to cause thermal damage to silkworms.

B. Plasma Irradiation of Silkworms

Figure 6 shows the time variation of the survival rate of silkworms after plasma irradiation in several LF voltages with a fixed plasma irradiation period of 2 min. The control silkworms that were not irradiated by plasma exhibited 100% survival. No color changes were observed on the surface of the silkworm immediately after plasma irradiation in all conditions. However, the color of the silkworm surface changes to black about three hours after the plasma irradiation. It is demonstrated that the survival rate of irradiated silkworms begins to decrease more rapidly in cases of higher LF voltage, as shown in Fig. 6. Figure 7(a) shows the survival rate of the silkworm 10 days after plasma irradiation for 2 min as a function of the LF voltage. It is found that the survival rate decreased proportionally with increasing LF voltage. In addition, the survival rate of the silkworms 10 days after plasma irradiation as a function of the plasma irradiation period for an LF voltage of 7 kV is shown in Fig. 7(b). It is found that the survival rate decreased proportionally with increasing plasma irradiation period.

To evaluate only the effect of ozone on the silkworm, we performed a further O_3 treatment experiment. Ten silkworms were placed in a sealed plastic container (160 × 100 × 30 mm), and ozone gas was introduced. Ozone was generated by a self-designed ozonizer that utilized surface discharge. The working gas was O_2, and O_3 concentration in the container was controlled by varying the LF voltage. Ozone concentration was measured using a gas detecting tube (Kiragawa, 17S). Figure 8 shows the survival rate of silkworms as a function of O_3 dose (O_3 concentration multiplied by irradiation period). It is found that the threshold O_3 dose for death of the silkworm is about 5,000 ppm-min. This value is much larger than that of the previously mentioned plasma irradiation using the plasma torch. Thus, it seems that the contribution of O_3 to silkworm

	Treatment time [min]			
Voltage [kV]	0	1	3	5
6				
8				

FIG. 5: Surface temperature of silkworms as a function of plasma irradiation time for different LF voltages

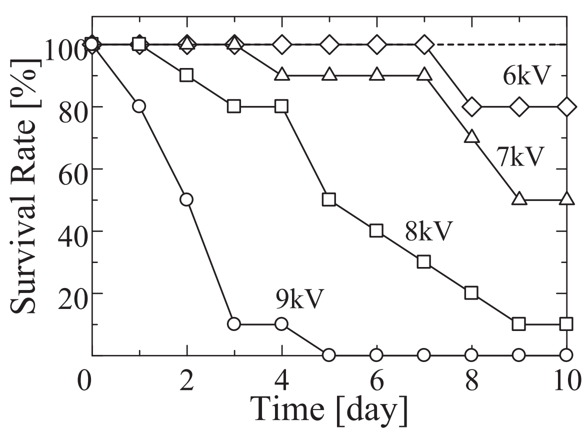

FIG. 6: Time variation of survival rate of silkworm with several LF voltages

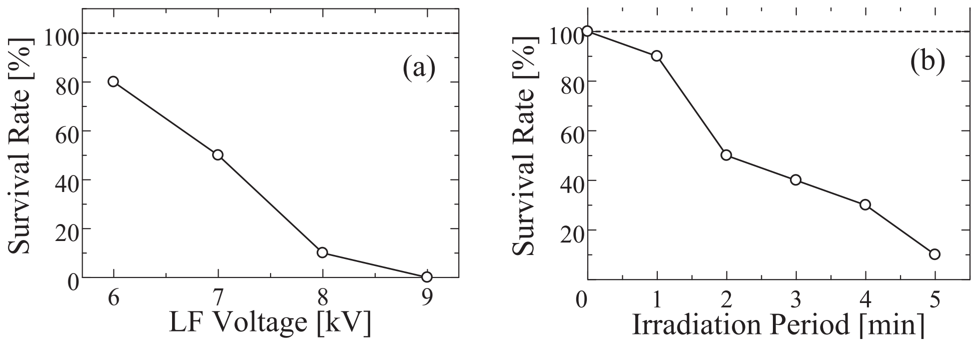

FIG. 7: Survival rate of silkworms 10 days after plasma irradiation for 2 min as a function of LF voltage (a), and as a function of plasma irradiation period for a voltage of 7 kV (b)

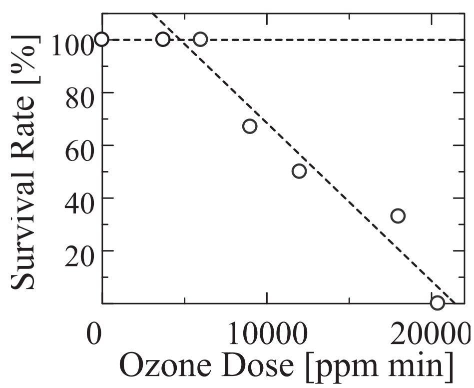

FIG. 8: Survival rate of silkworms as a function of the ozone dose (ozone concentration multiplied by irradiation period)

mortality in torch plasma irradiation is negligible.

Figure 9 shows the root-mean-square (RMS) of discharge currents and the optical emission intensity ratio between oxygen atoms (777 nm) and helium atoms (706 nm) as a function of LF voltage. These optical emission intensity ratios can be approximately regarded as the existence ratio between oxygen atoms and helium atoms in the plasma.[13] The optical emission ratio shown in Fig. 9 indicates the variation of the amount of oxygen atoms, since the helium gas flow rate was fixed in this experiment. It is found that the discharge current (which is the flux of charged particles) is proportional to the LF voltage, whereas the optical emission intensity ratio (namely, the amount of oxygen atoms) is almost independent of the LF voltage. As mentioned before, the survival rate of silkworms decreases with increasing LF voltage (Fig. 7[a]). Therefore, we conclude that the survival rate of the silkworm is inversely correlated with the presence of charged

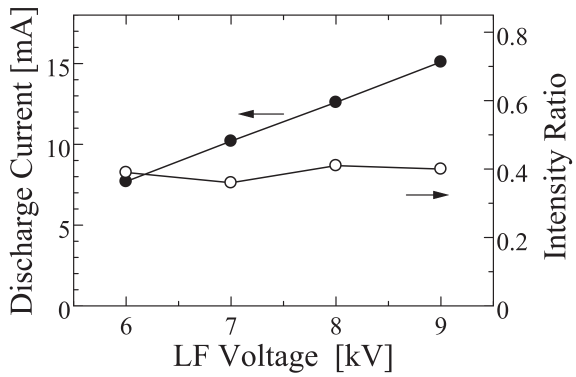

FIG. 9: RMS of discharge currents and optical emission intensity ratio between oxygen atoms (777 nm) and helium atoms (706 nm) as a function of LF voltage

particles. Consequently, we propose that the contribution of charged particles is a significant cause of silkworm mortality. Regarding the role of the charged particles, it has been reported that when direct non-thermal plasma treatment is employed, the charged particles play an important role in the inactivation of bacteria.[14–16]

IV. CONCLUSION

We investigated the influence of plasma irradiation on silkworms using a non-thermal plasma torch. The survival rate of the silkworm decreases proportionally with increasing LF voltage and plasma irradiation period. It was found that the contribution of charged particles significantly affects silkworm mortality. In this study, we have investigated the survival rate of silkworms when exposed to plasma. Further studies are still needed to better understand the mechanism of silkworm mortality by plasma irradiation.

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