Irradiation Experiments on a Mouse Using a Mild-Plasma Generator for Medical Applications

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Plasma technologies using an argon plasma coagulator have been used in endoscopic therapy to induce blood coagulation and ablate residual tumors. However, present devices have a risk of perforating the stomach wall during endoscopic submucosal dissection. Therefore, to reduce this risk, irradiation is performed for a limited time, which leads to incomplete cessation of bleeding and recurrence of residual tumors. Therefore, a device with greater controllability and safety is strongly desired for clinical applications. In this study, we have evaluated the irradiation efficiency of an atmospheric-pressure plasma jet based on a dielectric barrier discharge to control bleeding. Bleeding from a mouse femoral artery was induced, and then plasma was irradiated onto the bleeding area. Prompt coagulation in the disrupted blood vessel was observed, and there was no histological evidence of either burns or tissue necrosis caused by the plasma jet. These results suggest that postoperative scarring and adhesion may be prevented using the proposed plasma generator because of the reduced tissue damage.

Keywords: blood coagulation, dielectric barrier discharge, atmospheric-pressure plasma jet, histopathological observation, C57BL6 mouse, femoral artery, mouse brain

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1. Introduction

Plasma technologies have been applied in many areas, such as nuclear fusion; production of semiconductors, diamonds, and photovoltaic cells; and coating and sterilization of material surfaces. The main system used to generate plasma is generally operated under vacuum and requires a large space, which has limited the use of plasma in other areas. Recent advances in plasma technologies have enabled the practical use of atmospheric-pressure plasmas [1, 2]. The advantages of this type of plasma are its simple operating system and reduced heat load compared to arc plasma, a low-temperature plasma is more useful in biotechnology applications [3–6]. An example of the practical use of atmospheric-pressure plasmas in medical care is endoscopic submucosal resection, which is performed to stop bleeding and ablate residual tumors. This type of plasma known as an argon plasma coagulator (APC) [7], is used to induce currents through the human body. Although APC is effective in inducing blood coagulation over a wide region, it is difficult to control during medical operations. Since the current can accidentally increase because of the formation of arc-like plasma, the APC has a risk of causing severe damage, such as perforation of the stomach or esophageal wall. Therefore, a new type of blood coagulator with greater controllability and safety is strongly desired by physicians and endoscopists [8]. We have designed a mild-plasma generator [9] with reduced current flow and high-frequency electric field leakage due to a dielectric barrier discharge [10–12] to reduce the possibility of such accidents.

Here we have described an experiment demonstrating blood coagulation using the proposed atmospheric-pressure plasma generator [9], and studied the histological effects of irradiation on tissues. Moreover, we have discussed the results of plasma irradiation on the mouse brain, and the potential use of the proposed device.

2. Experimental Setup

We adopted a plasma source based on a dielectric barrier discharge for irradiation experiments. This type of plasma source produces glow-like plasma, and in principle, does not transit to an arc discharge. The peak-to-peak voltage $V_{\text{p-p}}$ applied to the electrode is within the range of 8 to 20 kV, and the frequency is between 10 and 100 kHz, although it is typically set at 20 kHz. A disadvantage of this type of discharge is the relatively high voltage. Therefore, the electrode should be completely covered by a grounded electrode, and leakage of the high-frequency electric field must be suppressed. It is also important to decrease the current flowing through the human body. The current is given by $I = C\, \mathrm{d}V/\mathrm{d}t$; thus, a plasma system with an extremely small power supply is necessary. A frequency of less than ~1 GHz is favorable for clinical use to avoid in-

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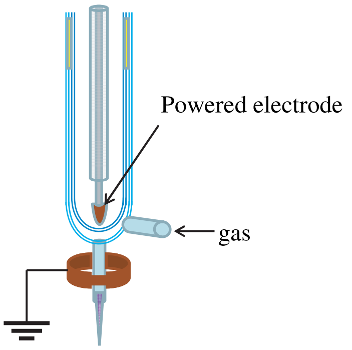

Fig. 1 Schematic drawing of an example of the plasma device that we have used. Powered electrode is covered completely by a dielectric layer.

terference with other electronic devices. In this setup, helium gas of 99.995 % purity is used as the working gas. We also used argon plasma for comparison with helium plasma (data not shown). The gas flow rate is ~2.0 l/min. Figure 1 shows an example of the plasma device used here. The powered electrode is completely covered by a dielectric, layer made of a substance such as quartz to protect it from the plasma. To better illustrate the device, the grounded electrode is not covered completely in the figure. In this case, the plasma is transported through a pipe such as a catheter. This type of plasma device has good directivity (controllability).

3. Experimental Results

A helium plasma jet, which in principle does not produce the growth of an arc discharge [9], was used to irradiate the bleeding site in a C57BL6 mouse (Fig. 2). The bleeding was induced by cutting the femoral artery under anesthesia with isoflurane. Following irradiation, coagulation promptly occurred in the disrupted blood vessel, which stopped the bleeding in less than 10 s. Here, the distance between the edge of the dielectric nozzle and the blood is ~ 10-30 mm.

Histopathological examinations were performed to examine the effect of plasma irradiation on tissue. In brief, tissues containing coagulated blood were fixed in 10 % formalin solution (v/v), and embedded in paraffin. Tissue sections of 5 μm thickness were obtained and stained with hematoxylin eosin. Figure 3 shows microscopic images of the coagulated blood after plasma irradiation. Coagulated blood covered the non-coagulated blood surfaces (Figs. 3 (a) and (b)). Coagulative degeneration of red blood cells was observed under high magnification (indicated by arrows in Fig. 3 (b)). Unlike a laser or radiofrequency knife, the plasma irradiation caused no damage by burning, which suggests that the method of helium plasma coagulation can help to control bleeding effectively. However, the detailed mechanisms of blood coagulation due to helium plasma irradiation remain to be elucidated.

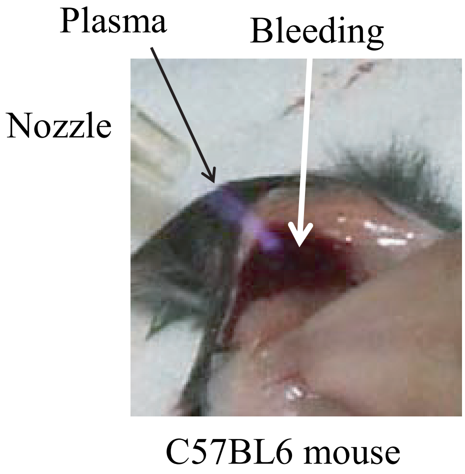

Fig. 2 Photograph of experiment involving plasma irradiation of bleeding from the femoral artery. Violet area is the helium plasma jet.

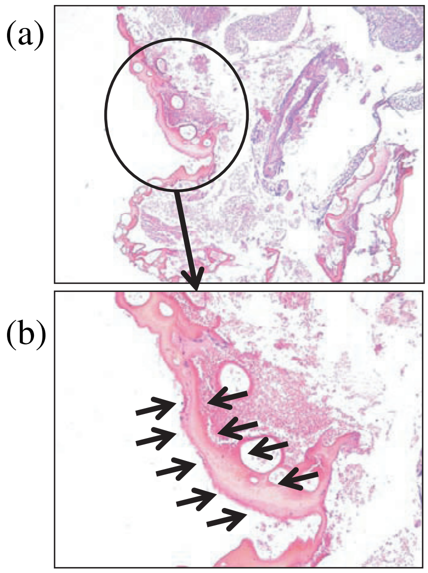

Fig. 3 Photomicrographs (histopathological observations) after plasma irradiation of the bleeding area and tissues. Image magnifications: (a) ×40 and (b) ×100. Hematoxylin-eosin staining was performed; arrows indicate coagulated blood.

Figure 4 (a) shows a photograph of a mouse brain before plasma irradiation, and Fig. 4 (b) shows plasma jet irradiation of the right lobe of the mouse brain. This type of plasma clearly has strong directivity, and thus good controllability. The distance between the edge of the dielectric nozzle and the brain was ~30 mm, and the irradiation speed was ~1 mm/s. After plasma irradiation, we observed that the surface color of the right lobe had become dark white to milky, which indicates severe and deep coagulation.

To study the effect of plasma irradiation on the brain, tissue sections were prepared and the changes were histologically evaluated. Representative results from a series of

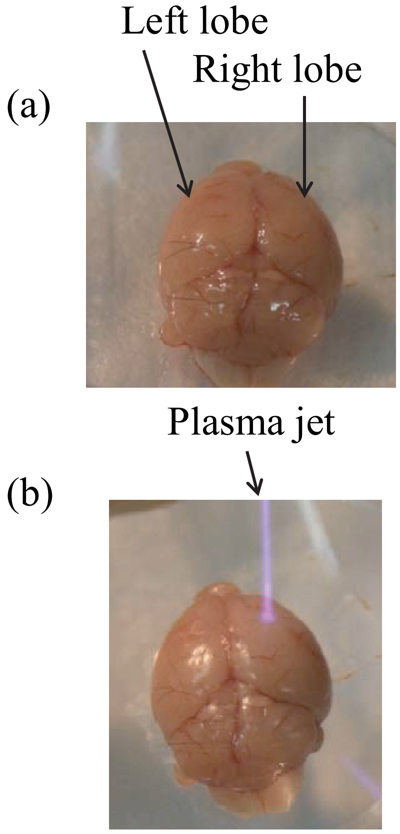

Fig. 4 Photographs of a mouse brain (a) before and (b) during plasma irradiation of the right lobe. Violet line is the helium plasma jet.

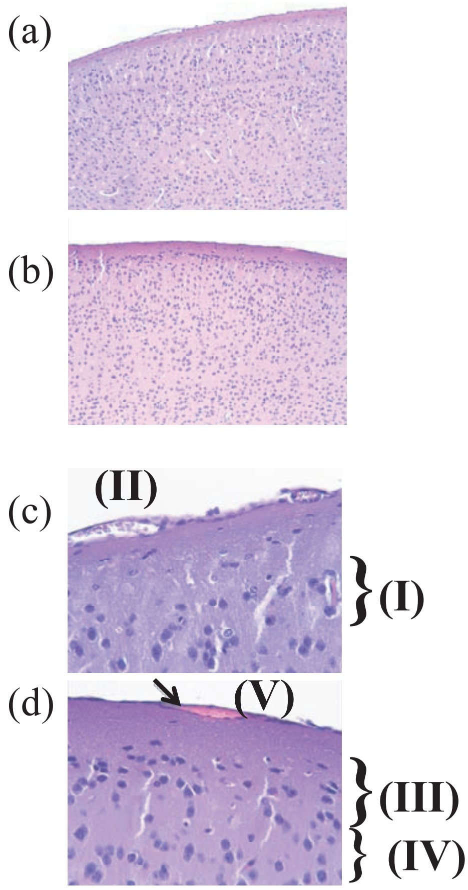

Fig. 5 Photomicrographs of tissue sections (histopathological observations) before and after plasma irradiation of the mouse brain surface. Hematoxylin-eosin-stained 4-μm-thick sections (a) before plasma irradiation (left lobe), magnification rate ×100; (b) after irradiation (right lobe), ×100; (c) of a part of the surface before plasma irradiation (left lobe), ×400; and (d) of a part of the surface after irradiation (right lobe), ×400 are shown. Regions (I) and (III) indicate the external granular layer. Region (IV) indicates the external pyramidal layer. Dark blue particles are the nuclei of neuronal cells.

irradiation experiments on the mouse brain are shown in Fig. 5. Plasma irradiation generated a pyknotic change and flattened the nuclei of neuronal cells in the external granular layer (region III) compared with cells in the same layer without plasma irradiation (region I). The neuronal cells in the external pyramidal layer (region IV) did not exhibit changes after plasma irradiation; this effect was exclusively observed in the external granular layer, which indicates that plasma-induced degeneration is localized within ~50 μm depth from the brain surface.

A number of erythrocytes were observed in the cavities of blood vessels in the left lobe, which was not irradiated with plasma (region II). After plasma irradiation, amorphous materials filled the blood vessels in the arachnoid membrane, which indicates intra-blood vessel coagulation (region V, indicated by an arrow). These results suggest that the effects of the plasma jet are localized exclusively in the superficial part of the brain, and the area of damage was shallower than that of tissue injury caused by lasers and electric scalpels.

4. Conclusion, Summary, and Future Prospects

We have developed an atmospheric-pressure plasma generator based on a dielectric barrier discharge, which in principle does not transit to an arc discharge [9]. We studied the coagulation of blood using this generator.

APC has the risk of causing severe damages to tissue. Thus, the irradiation time of the plasma is restricted, causing incomplete cessation of bleeding and recurrence of residual tumors. Moreover, APC is not controllable during operations. Therefore, a new type of blood coagulator is strongly desired for clinical applications.

Our proposed helium plasma jet was used to irradiate a bleeding site, which was induced by cutting the femoral artery under anesthesia, in a C57BL6 mouse. Coagulation promptly occurred in the disrupted artery, which stopped the bleeding in less than 10 s. In addition, histopathological analysis indicated that a unique change occurred in the irradiated tissue. Moreover, there was no evidence of tissue damage because of ablation as observed when a laser or a radio-frequency knife is used. Furthermore, the results of plasma irradiation of the mouse brain demonstrated that plasma irradiation is more appropriate for brain surgery than a laser or electric scalpel. In fact, the observed effects of plasma irradiation were pyknosis and flattened nuclei of the neuronal cells in the external granular layer, which appeared to be restricted to within 50 μm depth from the brain surface. Thus, it can be concluded that the mild-plasma method has high potential for controlling bleeding in medical procedures such as those used in conservative therapy.

We are currently measuring plasma characteristics, such as the current and energy to confirm the safety of plasma irradiation onto an object. A numbers of particle species (e.g., ions and radicals) are also being measured by mass analysis [13] and spectroscopy. Optimum conditions in terms of factors such as $V_{\text{p-p}}$, frequency, gas species (e.g., helium, argon, nitrogen, and oxygen), and irradiation time are also being systematically surveyed. These results will enable us to modify the plasma device and decrease the current and applied voltage. To investigate the possibility of other applications of the proposed device, plasma irradiation experiments on skin and several internal organs have also been conducted.

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