Formation of Membrane-like Structures in Clotted Blood by Mild Plasma Treatment during Hemostasis

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Keywords: mild plasma treatment, hemostasis, fibril formation

1. Introduction
During surgery, bleeding from capillaries, small vessels, and veins must be controlled, usually by cautery, with a high-frequency electrical coagulator, ultrasonic wave equipment, or laser. The basic mechanism responsible for such forms of hemostasis involves destruction of small blood vessels by heat. However, the tissue burns produced by these methods can cause prolonged postoperative disorders and therefore may limit the ability to receive a second surgery, if needed [1, 2].

Recently, low-temperature plasmas delivered in the atmospheric pressure region have been studied without the need for a vacuum system and biomaterials [3-5]. To apply plasma technologies for blood coagulation during a surgical procedure, we have developed a new device to produce a stable glow-like plasma using helium gas. Our equipment includes fully coated dielectric electrodes and uses a dielectric barrier discharge and a frequency of ~60 kHz with a quick start of the plasma and a stable discharge [6, 7]. The plasma produced (“Sakakita plasma”) promotes blood coagulation but keeps the surface temperature at less than 40°C without arc-like plasma formation.

In this study, we histologically evaluated plasma-induced coagulation to understand the possible clinical implications of the use of this mild plasma treatment for hemostasis.

2. Materials and Methods
Mice. Female C57BL/6 (B6) mice were obtained at 8 to 12 weeks of age from Charles River Japan Inc. (Yokohama, Japan) and kept under standard housing conditions.

Plasma technology to control bleeding. The details of the operating conditions of the equipment to generate plasma for bleeding control are described in our previous studies [6, 8]. Briefly, the peak-to-peak voltage, \( V_{p-p} \), applied to the electrode is within the range of 6 to 10 kV, and the frequency range of the sinusoidal wave is between 10 and 70 kHz.

Histopathology. Histological analysis was performed according to standard protocols [9]. In brief, samples obtained from animal experiments were fixed in phosphate-buffered 10% formalin (pH 7.5) for 24 h at room temperature, and 3 µm-thick sections were prepared from paraffin-embedded specimens and stained with hematoxylin–eosin. The sections were examined histologically, and the image data for the histology were acquired using a DM2500 microscope (Leica Microsystems, Wetzlar, Germany) with a DFC290 camera system.

Study approval. All animal experiments were performed using an experimental protocol approved by the Ethics Review Committee for the Animal Care and Use Committee of National Institute of Advanced Industrial Science and Technology (AIST).

3. Results
Plasma treatment caused faster coagulation of the blood bleeding femoral artery of B6 mice than did naturally occurring hemostasis (Fig. 1).
There was no apparent difference between the gross appearances of blood clots produced by plasma treatment and naturally occurring clots. Plasma generated with helium gas had greater coagulability of blood at sites of vascular injury compared with that produced with argon gas, even in the same electrical conditions. The injuries associated with heat and electrical treatments were not observed after treatment with plasma generated by either types of gas.

Histologically, the blood clots produced in the natural coagulation process usually contained erythrocytes, whereas those produced by helium plasma treatment comprised eosinophilic fibrous membrane-like (EFML) structures containing a small number of mononuclear cells (Fig. 2). The appearance of EFML structures was a characteristic finding for the blood clots produced by helium plasma treatment. An amorphous appearance similar to amyloid fibrils was evident in clots produced by argon plasma treatment (Fig. 2D).

4. Discussion

In this study, we describe our histological observations of the blood clots generated by mild plasma treatment. Blood clots generally retained the shapes of erythrocytes when produced by the natural coagulation process, whereas the plasma-induced blood clots comprised EFML structures that did not retain the shapes of erythrocytes. This result indicated that EFML structures were produced by both platelets and erythrocytes, suggesting that the plasma treatment is feasible to use bleeding bloods as dressing materials coating on injured parts.

Blood coagulation and platelet-mediated primary hemostasis act as physiological reactions to stop bleeding. Abnormalities in the balance between the procoagulant and anticoagulant systems can cause bleeding or thrombotic disease [10]. The hemostasis during surgical procedures is based on the mechanical obstruction of blood vessels (Fig. 3). From the viewpoint that plasma treatment promotes fibril formation, which stops bleeding, the basic concept underlying the Sakakita plasma may involve activation of the coagulation system through a physiological reaction rather than by mechanical obstruction of vessels. The plasma treatment promptly coagulated the blood from the hemoral artery in B6 mice but produced neither heat nor electrical damage. Taken together, these results suggest that minimally invasive plasma treatment effectively activates the blood coagulation system through physiological reactions.
5. Conclusion

Advances in equipment for bleeding control have helped decrease operative mortality by preventing unexpected blood loss. Japanese surgeons currently use various techniques and equipment for bleeding control during surgery. Sakakita plasma is a potential minimally invasive procedure for bleeding control.

Acknowledgement

This work was supported by Grants-in-Aid for Scientific Research on Priority Area (21590454, 24590498, and 24108006 to Y. I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

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