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Fast Blood Coagulation of Capillary Vessels by Cold Plasma: A Rat Ear Bleeding Model

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ABSTRACT: Cold atmospheric pressure plasmas previously have been shown to be effective in vitro in generating active species, radicals, and charges, which may aid in various processes of interest to medicine, including blood coagulation. Floating electrode dielectric barrier discharge microsecond-pulsed, high-voltage plasma was used in an animal model of hairless Sprague-Dawley rats to treat an incision on the ear. The results confirm cold plasma's ability to coagulate blood in a live animal model. A small incision in the rat ear, cutting the thin epidermis and slicing through the capillaries, creates a small and controllable wound from which bleeding is observed. Without plasma treatment, the animal bleeds for approximately 2 minutes, whereas a 10-second treatment is sufficient to coagulate blood. Cold plasma floating electrode dielectric barrier discharge was shown to effectively coagulate bleeding small vessels, which may prove beneficial in surgical intervention treatments of, for example, vocal cord surgery, eye surgery, or brain surgery, for which other means of coagulation may be prohibitive. Future animal trials will include larger animals and different bleeding sites, with the potential to move on to human trials.

KEY WORDS: cold plasma, nonthermal plasma, dielectric barrier discharge, floating electrode, blood coagulation, animal model, rat, plasma medicine

I. INTRODUCTION

Blood presents a challenging environment to every branch of science that studies it, from the physiology[1] and biochemistry[2] of blood coagulation to the fluid dynamics of the process.[3–5] During the wound treatment process, for example, issues related to blood coagulation need to be addressed because blood provides the basis for closing the wound and for the pre-forming matrix, which is later used by cells and proteins to repair and close the wound, culminating in the re-formed tissue.[6–8] This article is concerned with the process of blood coagulation in a wound model where rat ear capillaries were cut and treated by cold plasma, promoting fast coagulation and closure of the wound. It is of interesting note that *electric plasma* was so named after blood plasma by Irving Langmuir because of the complexity and ionic nature of both mediums.[9] Although thermal plasma is well known to coagulate blood through thermal desiccation,[10] some authors previously have reported the ability of *cold plasma* treatment to coagulate stationary blood in vitro and in explanted organs and tissues.[11–13] Some elucidation of the mechanisms of

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blood coagulation has been made, showing plasma's ability to interfere with the coagulation cascade, serving as a source of charged species that may catalyze the process,[14] crosslinking blood plasma polymers, and activating platelets.[13] Cold plasma, in general, has been shown to be an effective new tool with the potential for use in the medical setting. Plasmas have been shown to effectively sterilize various inanimate surfaces such as medical instruments[15–20] as well as living human and animal tissues and cells.[11,12,14,21–23] Nitric oxide generated in plasma has been shown to effectively promote wound closure and to speed up wound healing processes.[12,24–26] For these reasons, we believe it is important to show the effective coagulation of capillary vessels in an in vivo model.

II. MATERIALS AND METHODS

Floating electrode dielectric barrier discharge (FE-DBD), previously described by Fridman et al,[11] was used in this work, and a standard rat ear bleeding model was modified for the use of cold plasma as a treatment modality.

A. Floating Electrode Dielectric Barrier Discharge

In this study we used an FE-DBD generated between the insulated high-voltage electrode and the sample (floating electrode) undergoing treatment. This setup was previously described by Fridman et al[11] in detail, so only a brief description is given here. One-millimeter-thick polished, clear, fused quartz (Technical Glass Products, Painesville, OH) was used as an insulating dielectric barrier. The setup and high-voltage electrode schematic are shown in Fig. 1, and a handheld, pen-like device with a quartz tip was used for treatment. The discharge was generated by applying high-voltage pulses with the following characteristics: 20 kV (p-p), 1.6-μs pulse duration, 1-kHz frequency. The average power density for the active area of the high-voltage electrode was kept at the level of approximately 0.74 W for a 6-mm electrode diameter measured electronically by integrating current and voltage signals, as has been previously described.[11] Of course, in the case of a hand-held electrode, as shown in Fig. 1, the actual surface power density is difficult to estimate because it changes depending on the angle of application and distance from the tissue; for this reason, here we report the plasma treatment dose in seconds rather than Joules per centimeters squared; the actual treatment area varies and is under the subjective control of the operator.

During the plasma treatment procedure, the operator was asked to hold the electrode steadily for a preset time interval. Training time for the operator was no more than a few minutes. All procedures were performed in compliance with the Animal Welfare and Protection Act after the approval of Drexel University's Institutional Animal Care and Use Committee.

B. Animal Model

Thirty hairless Sprague-Dawley rats, weighing approximately 250 g, arrived and were

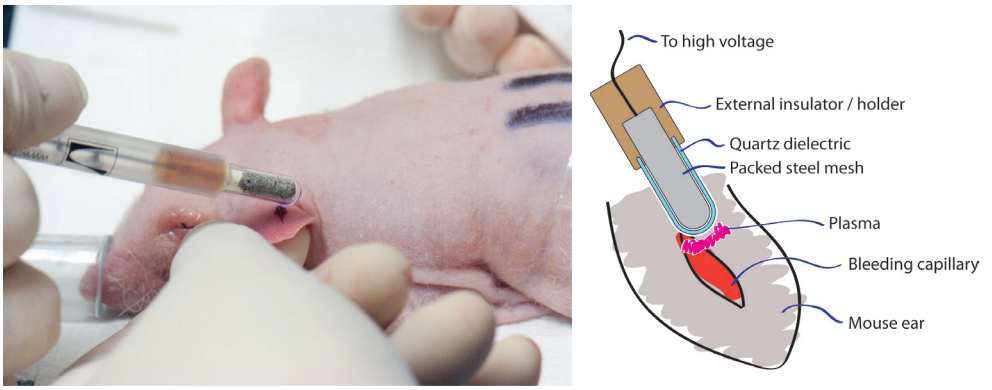

FIGURE 1. Photograph (left) and schematic (right) of the cold plasma treatment for capillary blood coagulation.

acclimated for 3–5 days in the facility. Animals were transferred from the vivarium to the approved surgical suite. Animals were kept anesthetized using an inhalational gas anesthetic, Isoflurane (Vedco, St. Joseph, MO), at 2–3% induction plus oxygen (2 L/min), then 1.5–2% maintenance anesthetic was administered via a face mask for the appropriate length of time, and the rats were placed in the supine position. Animals received analgesia (1 mg/kg subcutaneously, Meloxicam, Boehinger-Ingleheim, Germany) at the onset of anesthesia but before surgery. A full-thickness wound of the auricular vein was inflicted on the bilateral pinnas with a sterile blade. One pinna served as the control, and coagulation allowed to occur naturally. Bleeding time commenced upon incision until hemostasis was achieved, with no rebleeding within 30 seconds. FE-DBD plasma was applied to the opposing pinna upon the onset of incision. Two techniques were applied to control blood coagulation using plasma. The first involved discharge treatment to the wound for 30-, 20-, 10-, and 5-second increments. Blood coagulation was monitored without removal of the clot that formed as the result of FE-DBD treatment. The second technique involved discharge treatment of the wound for 5-second increments during continuous removal of blood from the affected site until hemostasis was achieved. Control experiments performed were identical except plasma treatment was not applied. All data is reported with 95% confidence interval ($P < 0.05$), and the number of samples in each experiment is identified.

III. RESULTS AND DISCUSSION

The expected bleeding time for the type of wounds inflicted in this model is in the 1- to 2-minute range.[27–29] In our experiments, as expected, we observe typical bleeding times of approximately 2 minutes in the control wounds (Fig. 2). As previously reported, there is no visible or microscopic adverse effect to tissue for long treatment times (more than a few minutes),[11,12,30] whereas the formation of blood clots was observed in vitro within approximately 15 seconds.[11] For this reason, we have chosen 30 and 15 seconds as initial

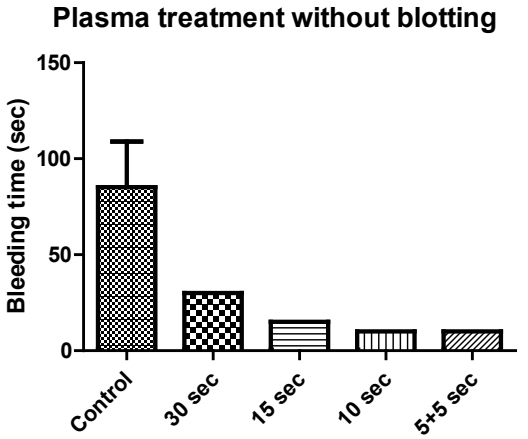

FIGURE 2. Results of observation of bleeding time without continuous removal of blood clot in control groups and plasma treatment groups.

treatment points. After the treatment, we observed clot formation in all of the animals studied (n = 4 for each experiment), with the same result achieved after 10 seconds of treatment both continuously and with a 30-second intermission between treatments (Fig. 2).

Two conclusions can be drawn from this experiment: Either plasma significantly speeds up coagulation in bulk (as has been previously suggested[11,13]), or there is a formation of solidified "scab," or thin film, which creates sufficient pressure to stop blood from flowing. This second possibility is less desirable in a medical setting because the solidified droplet of blood can be easily disturbed, potentially leading to reopening of the wound and further bleeding. Thus, we have conducted a second set of experiments (n = 16 for both control and plasma treatment groups). In this experiment we removed the forming droplet of blood by blotting it off with sterile gauze every 5 seconds. Removal of the droplet takes less than a second and the treatment then continues. Visually, this treatment more closely represents an actual clinical setting than the original model. During surgery, excess blood is continually removed using either vacuum suction or sterile gauze. As can be seen from Fig. 3, the blotting process does decrease bleeding time in the control group by a few seconds, although this small difference may be attributed to statistical variation (n = 4 for the first set and n = 16 for the second set). Plasma-treated wounds stopped bleeding, as before, in approximately 10 seconds (20 seconds was the longest bleeding duration, n = 1; 5 [n = 4] or 10 seconds [n = 7] was more typical).

IV. CONCLUSION

It was previously reported by Fridman et al[11,12] and by other groups[26,31,32] that blood coagulation is possible in vitro with the use of different types of nonequilibrium plasmas. Fridman et al[11] and Dobrynin et al[14] have previously reported on plasma's ability

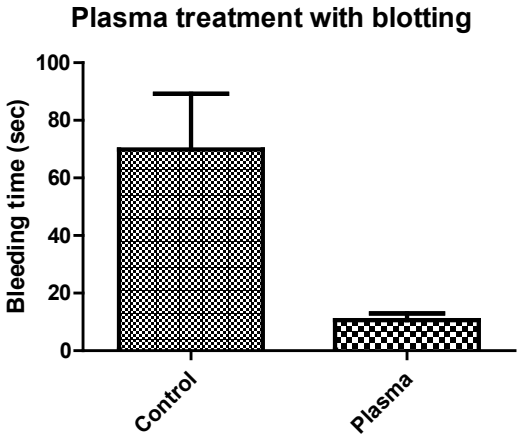

FIGURE 3. Results of observation of bleeding time with removal of blood clot every 5 seconds using sterile gauze.

to serve as a catalyst of various ion-related processes in the blood coagulation cascade, crosslinking various blood polymers and activating platelets, which prepares them for and speeds up natural coagulation processes.[13] This article presented the results of the verification of cold plasma interaction in vivo, where blood coagulation of a wound model was shown on a rat ear. These results further confirm a potential for cold plasmas to enter the medical field and serve as a new tool in the treatment of wounds, in a surgical theatre, or possibly in the treatment of diseases.

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