

Biomedical Application of Non-Thermal Atmospheric Pressure Plasma and Its Usefulness

Guest Editor: Tetsuo Adachi

Low temperature plasma equipment applied on surgical hemostasis and wound healings

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Low temperature plasma (LTP) coagulation equipment, which avoids causing burn injuries to patients, has been introducing into minimally invasive surgery. The mechanism by which this equipment stops bleeding is to directly occupy the injury with the formed blood clots, and different from the mechanism of the common electrical hemostatic devices that cauterize the tissues around the bleeding to stem the blood flow. A noteworthy point is that LTP treatment with our equipment is not confined only to the blood coagulation system, but it has significant effects on the other blood components to form clots with or without hemolysis, and that there is a plasma current threshold that determines whether the treatment makes stable clots. In this review, we introduce the clinical benefits of LTP current and describe the clot formation it facilitates.

Key Words: low temperature plasma, clot formation, coagulation equipment, surgical hemostasis, serum protein

Current Topics on Low Temperature Plasma (LTP) Equipment at Atmospheric Pressure

Attempts have been made to introduce technologies associated with LTP at atmospheric pressure into medical practice for various situations, and LTP's feasibility for use has been demonstrated in hemostasis, wound care, and anti-cancer therapy. Translational studies of the basic technology for LTP at atmospheric pressure have remarkably proceeded at the Max Planck Institute for Extraterrestrial Physics (Gerching, Germany),⁽¹⁻⁸⁾ Leibniz Institute for Plasma Science and Technology Greifswald e.V. (INP Greifswald e.V.; Greifswald, Germany),⁽⁹⁻¹³⁾ the National Institute of Advanced Industrial Science and Technology (AIST) of Japan,⁽¹⁴⁻¹⁷⁾ Nagoya University,^(18,19) and Drexel University.⁽²⁰⁻²²⁾ Now, its commercial viability has been studied at companies such as terraplasma GmbH, neoplas tools GmbH, and CINOGY Technologies GmbH. Indeed, these companies have released product announcements about LTP equipment for clinical use. Relevant products are SteriPlas—formerly known as MicroPlaSter⁽²³⁾ manufactured by ADTEC Plasma Technology Co., Ltd. (Hiroshima, Japan and London, UK),⁽²⁴⁾ kINPen[®]Med,^(10,25) and PlasmaDerm[®].⁽²⁶⁻²⁸⁾ An example describing the clinical benefit of SteriPlas is that it can be used to control bacterial infection in chronic wounds and to eradicate infected microorganisms, providing new clues for

solving issues related to the increase in bacteria that are resistant to multiple antibiotics.^(1,2)

For hemostasis, different types of LTP equipment are being developed by AIST, Nagoya University and Drexel University that will be used for clinical trials. Indeed, the discovered advantages of LTP use may have an impact on changing the basic approaches to and concepts for hemostasis and minimally invasive surgery. This review describes the clinical benefits of LTP for hemostasis and LTP's underlying mechanisms.

Differential Targets of Hemostasis by LTP and Other Energy Devices

Increasing the safety of surgery is closely linked with the availability of energy devices for hemostasis, such as high-frequency coagulation equipment (HFCE), laser coagulation equipment, ultrasonic coagulation equipment,⁽²⁹⁾ and high temperature plasma coagulation equipment.⁽³⁰⁾ Their basic mechanism of inducing hemostasis is tissue ablation due to generated heat, and bleeding point shrinkage is induced by cauterized tissue. Many efforts have been made to reduce the occurrence of associated the heat injuries, such as nerve paralysis and cartilage damage, which occur near the cauterized tissue with hemostatic electro-surgical equipment. However, based on the general concept of energy devices, it can be hard to establish new hemostatic instruments that do not cause heat injury.

Because LTP can stop blood flow by sealing the bleeding point without inducing heat injuries, it can be distinguished as a new energy device in surgery. Fig. 1 compares the different sites of hemostatic action for LTP coagulation equipment and HFCE. Importantly, the mechanism by which LTP stops bleeding is analogous to that of surgical hemostats, which is why it is feasible to use it to reduce heat injury, resulting in decreased invasiveness (Table 1). Based on results from a series of basic studies in plasma science, we have succeeded in developing LTP coagulation equipment that has a minimal risk for burn injury by reducing the plasma current that flows through the tissue.⁽³¹⁾

Because the basic safety and essential performance requirements of a LTP coagulator are different from those of other energy

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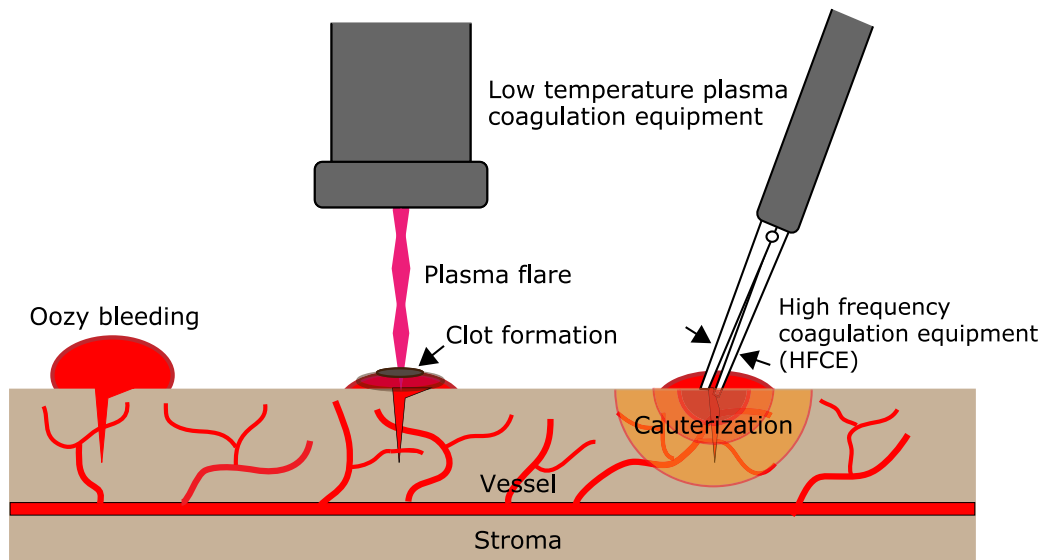


Fig. 1. Differences in mechanisms for hemostasis between typical electrical hemostatic devices and low temperature plasma coagulation equipment. The figure on the left shows blood oozing at the wound site. The figure on the right shows that the bleeding is stopped by cauterization made by HFCE. A burn injury is left at the wound site. The figure in the center shows that the bleeding is stopped by covering the wound site with a blood clot created by low temperature plasma coagulation equipment. A burn injury is not left at the wound site.

Table 1. Hemostats and electric surgical device for hemostasis

Category	Operating point	Action	Burn injury	Clot formation
Surgical hemostats	Bleeding point/Blood	Astriction/Coagulation	-	-/+
Low temperature plasma*	Blood	Platelet clot/Protein clot	-	+
High temperature plasma	Stroma	Cauterization	+	-
Laser	Stroma	Laser ablation	+	-
High frequency coagulation equipment	Stroma	Cauterization	+	-
Ultrasound	Stroma	Cauterization/Crush wound	+	-

*Standardize: IEC/TC62-SC 62D WG34 for IEC60601-2-76. Low-energy ionized gas coagulation equipment.

Table 2. From the view point of essential performance

Category of equipment and hemostats	Activation of platelets and coagulation factors	Formation of serum proteins aggregation	Formation of clot from RBCs
Category I (Drexel Univ., etc.)	+	-	-
Category II (Nagoya Univ.)	+	+	-
Category III (AIST)	+	+	+
High frequency coagulation equipment	-	-	-
Mech. hemostats	+/-	-	-
Active hemostats	+	-	-

devices, we are working with a group to make a consensus document for low-energy ionized gas coagulation equipment.

Mechanism of Blood Coagulation by Supplied LTP

The mechanisms underlying blood coagulation by LTP treatment were revealed by a series of studies by AIST, Nagoya University,⁽¹⁸⁾ Drexel University,^(20,21) and others.^(32,33) We have summarized the effects on LTP equipment developed by each institute. An essential point is whether blood coagulation is confined to the blood coagulation system or extended to other blood components with or without hemolysis.⁽³⁴⁾ Drexel University reported that their equipment forms blood clots as a result of the coagulation of platelets and clotting proteins (Table 2 Category I), while

the Nagoya and AIST equipment formed clots from the other serum proteins such as albumin, fetuin, and immunoglobulin (Table 2 Category II). Moreover, the AIST equipment formed a membrane like clots from all of the blood components, including erythrocytes (Table 2 Category III).

LTP treatment with the Nagoya and AIST equipment extended to other blood components in the coagulation system, in contrast to LTP equipment by Drexel University and surgical hemostats that activate coagulation proteins.⁽²²⁾ Indeed, results of an experiment with albumin and immunoglobulin showed that the Nagoya and AIST equipment allowed protein aggregation.^(14,15) Blood is composed of erythrocytes, leukocytes, platelets, and plasma. The difference between serum and plasma is based on the presence or absence (decrease) of coagulation proteins (fibrinogen, pro-

thrombin, factor V, factor VIII), and they have in common that they contain proteins not involved in coagulation, such as albumin and immunoglobulin (Albumin comprises about 65% [w/w] of serum, and immunoglobulin comprises less than 20% [w/w] of serum). Furthermore, LTP treatment can form clots that more stable than those formed by the activation of the coagulation system because of the coagulation materials from red blood cells. The vulnerability of clots is based on leaking the liquid components of red blood cells (more than 45% of the blood's volume) upon hemolysis, while they are stable when coagulated to membrane like clots by LTP treatment.⁽³⁴⁾

Measuring Plasma Parameters to Control Clot Formation

We sought to establish a control method for blood clot formation in order to develop LTP coagulation equipment. Our most recent studies demonstrated that the efficiency of clot formation was linked to the current flowing through the solution, described as the "plasma current". Albumin aggregation appeared where the LTP flare touches, over the threshold of the plasma current.

Based on this information, we then sought to determine the plasma current that could form a stable clot from protein solutions without rupturing (Fig. 2). Plasma current is measured by a Rogowski coil (CTL-28-S90-05Z-1R1, U.R.D. Co., Ltd., Yokohama, Japan) every second, so values that are too small can be brought to a suitable value by adjusting the discharge voltage. Results from a series of experiments demonstrate that the plasma current value depends on the value of the discharge voltage and that the current value can be controlled arbitrarily by adjusting the discharge voltage. Additionally, immobilization is dependent on a length of 10 mm between the sample and discharge tube; a helium gas flow rate of 2 L/min; and a 50 mg/ml sample of albumin (Sigma-Aldrich, St. Louis, MO), as shown in Fig. 3. Interestingly, an aggregation formed during plasma treatment returned into a solution when the discharge voltage was stopped at 6.3 kV (Fig. 3-

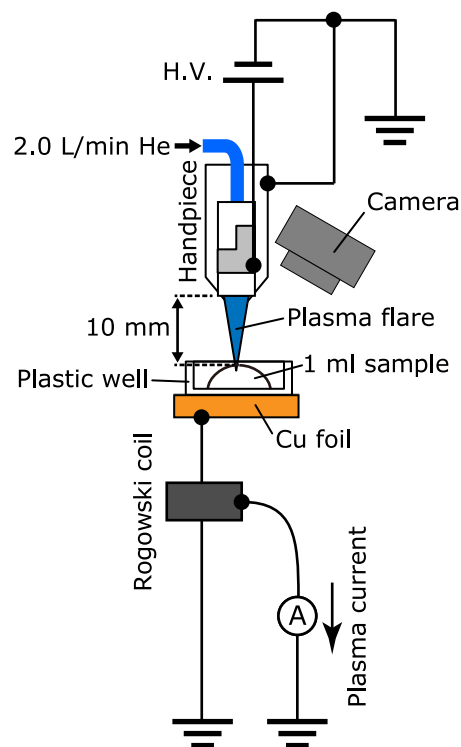


Fig. 2. The experimental set-up. A plastic well is on the stage covered with copper foil. A piece of the low temperature plasma coagulation equipment is right above the well to photograph the coagulation. The plasma current is measured with a Rogowski coil.

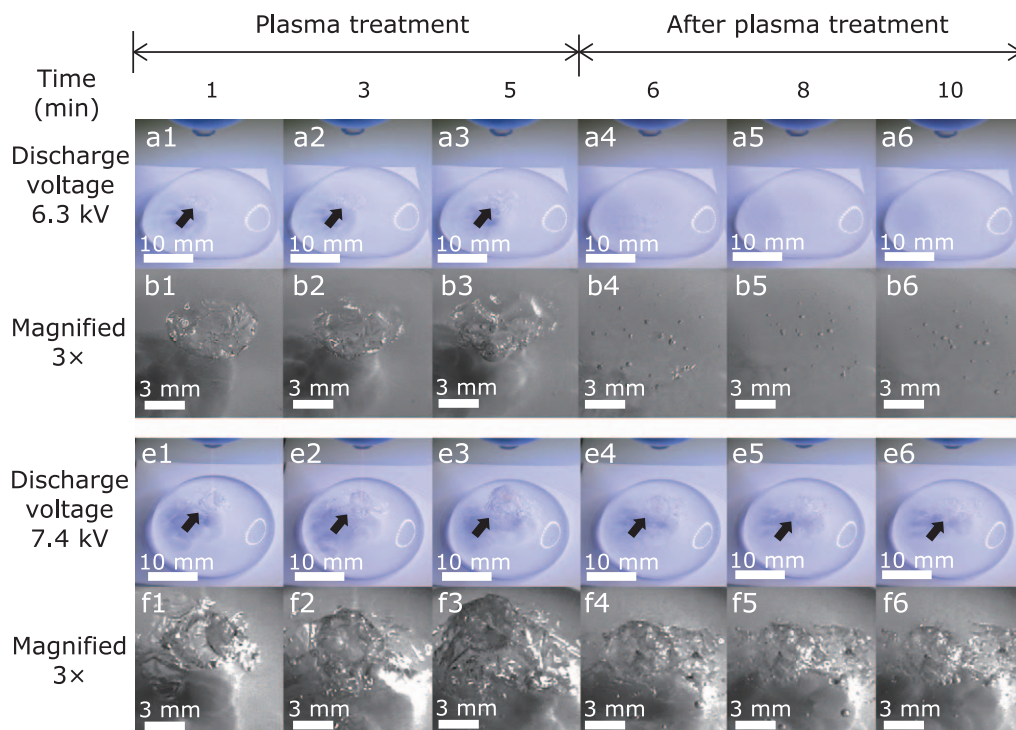


Fig. 3. Albumin clot formation depends on the value of the discharge voltage. The arrow in the figure indicates the albumin clots. When the discharge tube voltage is set at 6.3 kV, the albumin clot scatters in multiple directions after plasma treatment. In contrast, when the discharge tube voltage is set at 7.4 kV, the clot is left on the surface of the solution after treatment.

al to -a6 and -b1 to -b6), while the aggregation maintained its form when the discharge voltage was stopped at 7.4 kV (Fig. 3-e1 to -e6 and -f1 to -f6). These results suggest that, when using albumin at a concentration of 50 mg/ml, a threshold value for clot formation exists between 6.3 kV and 7.4 kV. It is important to note that various proteins and concentrations have their own discharge voltage thresholds to form stable clots. Thus, measuring and adjusting plasma parameters allows control of clot formation.

Future Prospects

We are developing a new plasma equipment system that will

establish the ideal clot formation by measuring the plasma current and providing feedback so that the user can adjust the discharge voltage. We expect that the new system will be used in the field of surgical hemostasis, regenerative medicine, and/or drug development in the future.

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