RPE | Radiation Physics & Engineering
An International Peer-Reviewed Journal
Published by K.N. Toosi University of Technology
http://rpe.kntu.ac.ir
Research Article

Radiation Physics and Engineering 2020; 1(2):7–11
https://doi.org/10.22034/RPE.2020.63475

Determination of coagulation time of in-vivo cut bleeding treated by non-thermal atmospheric pressure plasma

Zahra Shahbazi Rad[a,*], Fereydoun Abbasi Davani[a], Gholamreza Etaati[b], Samaneh Seifi[c]

[a] *Radiation Application Department, Shahid Beheshti University, P.O. Box 1983969411, Tehran, Iran*
[b] *Physics and Energy Engineering Department, Amirkabir University, Hafiz Avenue, Tehran, Iran*
[c] *Nuclear Physics Department, Physics Faculty, Shahrood University of Technology, Shahrood, Iran*

HIGHLIGHTS

- The coagulation time of in vivo bleeding treated by cold atmospheric pressure plasma was determined.
- The optical emission spectrum of plasma showed the existance of ROS and RNS species.
- The coagulation time of treated cut was compared with control cut.

ABSTRACT

The aim of this research was determination of the required time for coagulation of in vivo cut bleeding treated by non-thermal atmospheric pressure plasma. To meet this, an atmospheric pressure plasma jet device was designed and constructed. Helium was used as working gas. The electrical parameters and optical emission spectrum of helium plasma were measured. The averaged treatment time to coagulate the incision bleeding on the mouse liver was obtained 8.6 μs, and the average time of naturally incision bleeding coagulation was 10 min.

KEYWORDS

Helium plasma jet
Non-thermal Atmospheric pressure plasma
In vivo blood coagulation

HISTORY

Received: 1 April 2018
Revised: 10 May 2018
Accepted: 24 May 2018
Published: June 2020

1 Introduction

In recent years, prominent researches have been performed on therapeutic application using non-thermal atmospheric pressure plasma (Howatson, 1965; Fridman et al., 2005; Laroussi et al., 2000). Recently, it has been found that the non-thermal atmospheric pressure plasma can be applied to living tissues (Fridman et al., 2006), inducing blood coagulation and killing bacteria on the wounds without significant side effect or heating (Stoffels, 2002). Non-thermal plasmas can initiate, promote, catalyze and control complex behavior and responses in biological systems (Aleynik et al., 2012).

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) exist in non-thermal atmospheric pressure plasma (Benstaali et al., 2002, 1998; Laroussi and Leipold, 2004). Relevant parameters of medical plasmas are the electron and ion temperature and density, UV irradiation, optical and infrared emission, the density of free radicals, the temperature of the neutral gas, the gas composition and the gas flow (Heinlin et al., 2010). Decisive factor is the flow of active, charged particles (electrons, positive and negative ions) and uncharged atoms and molecules (such as O_3, OH, H_2O_2, NO, OH radicals etc.) (Kolb et al., 2008; Uhm et al., 2007). It has been shown that reactive oxygen and nitrogen species (RONS) can cause oxidative damage to biomolecules which can contribute to the development of a variety of diseases. However, recent evidence has suggested that intracellular RONS are an important component of intracellular signaling cascades. It reveals that deleterious effects do not appear if only one primary species (superoxide radical, nitric oxide) even at high concentrations is present in a biological system. The negative effects are the formation of highly reactive secondary species (hydroxyl radical, peroxynitrite), emerging exclusively upon reaction with another primary species or a transition metal. The secondary species are toxic, which is not well controlled and causes irreversible damages to all classes of biomolecules. In contrast, primary RONS are well controlled (superoxide dismutase, catalase), and their reactions with biomolecules are reversible, which make them ideal for physiological/pathophysiological intracel-

*Corresponding author: z_shahbazi@sbu.ac.ir

lular signaling (Weidinger and Kozlov, 2015). The "controllable plasma" has the capability to provide RONS with possible positive effect.

A number of studies have been accomplished on the effect of non-thermal atmospheric pressure plasma on blood coagulation. The effect of floating electrode dielectric barrier discharge plasma in air on the blood coagulation and living tissue sterilization was investigated by Fridman *et al.* (Fridman et al., 2006). They found that treatment time of 15 s for the in vitro blood drop leads to complete coagulation in 1 min. Also, they showed that the treatment of human spleen (in vivo application) for 30 s results in blood coagulation with the cut temperature remained at room temperature. Janani *et al.* (Janani et al., 2013) investigated the effect of the cold argon plasma device on decrement of the coagulation time for in vitro and in vivo experiments. Their results show that the blood drops on the slide coagulated completely after plasma exposure treatment, whereas the coagulation took 10 min without plasma treatment. Also, they had found that the required time for complete blood coagulation in incised liver of rats is 12 s and 4.5 min with and without treatment, respectively. Miyamoto *et al.* (Miyamoto et al., 2016) had investigated the red blood cell coagulation induced by low-temperature plasma treatment. They authenticated that a different clot formation mechanism involving hemolysis of erythrocytes to coagulate from the already known clot formation process linked with plasma treatment.

In this study, a non-thermal atmospheric pressure plasma jet including working gas of helium was constructed. The homemade sinusoidal power supply with intermediate frequency has been used to drive discharges. The purpose of this article is to determine the appropriate treatment time for in vivo blood coagulation of mice skin. The constructed helium plasma jet has been applied to the in vivo cuts on the livers of mice to obtain the required treatment time for blood coagulation. Also, the voltage and the current waveforms as well as the optical emission spectrum of the helium plasma jet have been measured.

2 Materials and Methods

2.1 Plasma set-up and diagnostics

An atmospheric pressure plasma jet with working gas of helium has been used in this research. A photograph of the homemade helium plasma jet is shown in Fig. 1-a. Moreover, Fig. 1-b shows a schematic cross-sectional view of the plasma jet.

The helium plasma jet has been constructed using quartz tube with the height of 80 mm and the inner and outer diameters of 4 mm and 6 mm, respectively. This device includes two electrodes: one as an outer aluminum electrode placed at 10 mm from the tube nozzle and another as 1 mm diameter grounded needle placed at the tube center. The constructed plasma jet works with the homemade sinusoidal power supply. The power supply produces sine waves with the output values up to 30 kVp-p and the frequency range between 10-12 kHz. The high voltage is applied to the outer electrode and the centered needle is grounded. The plasma jet has been allowed to work with two different gases (argon and helium), either individually. In this research, the 99.999% pure helium gas with the flow rate between 0.5-2 slm was used as the working gas. The discharge plasma was produced with the high voltage of 9 kV and the frequency of 11 kHz in all tests.

The Tektronix P6015A (wide bandwidth probe) was used for measuring the applied voltages. A digital phosphor oscilloscope (Tektronix DPO7104, 1 GHz bandwidth) was used for recording and observing the voltage and current waveforms.

The Optical Emission Spectrometer (OES) ranging between 200-1000 nm with the compact CCD spectrometer (CCS200 with the FWHM spectral accuracy of <2 nm) was used for measuring the device spectrum. The spectra were subtracted from the dark baseline.

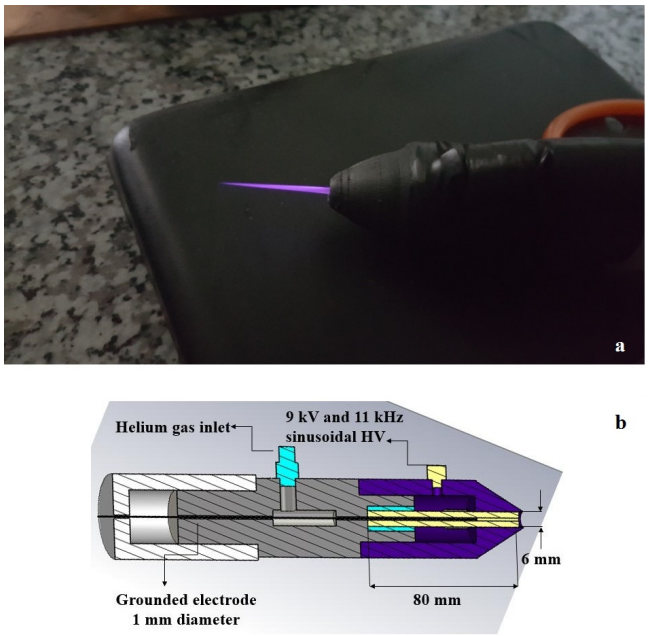

Figure 1: a) A photograph of the homemade helium plasma jet, b) A schematic cross-sectional view of plasma jet.

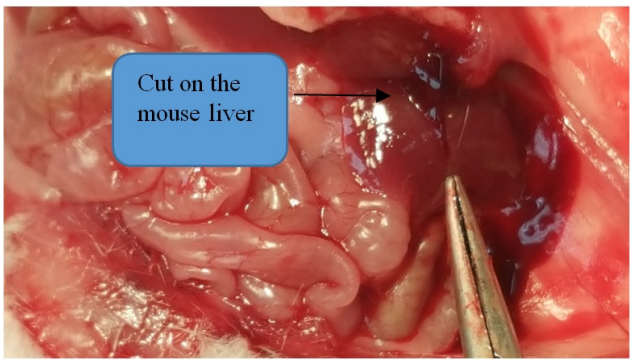

Figure 2: The cut induced with blazer on the mouse liver.

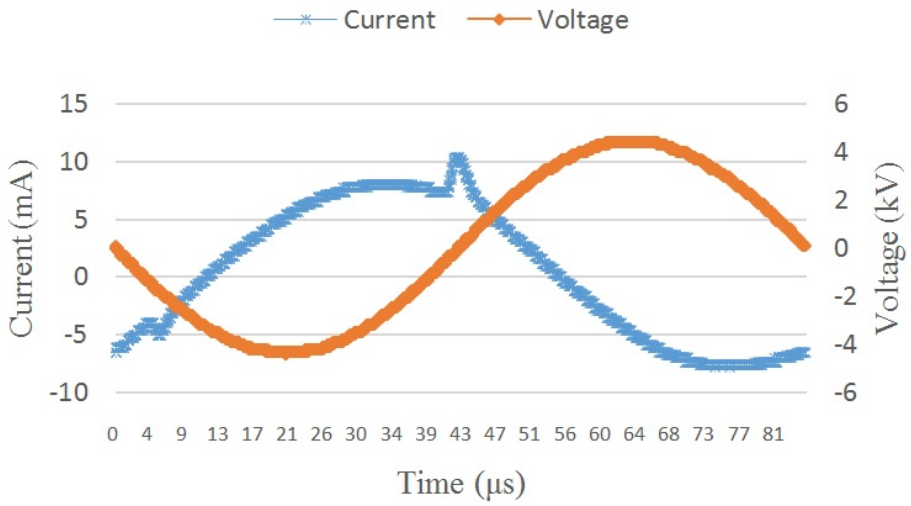

Figure 3: The waveforms of voltage and current for the helium plasma jet with the frequency of 11 kHz and the output voltage of 9 kV p-p.

2.2 Animal samples

Ten weighted 25-30 gr, 6-week old male Balb/c mice prepared from the Laboratory Animal Sciences Center of Baqiyatallah University, Iran, were used for in vivo blood coagulation tests. Another 25 mice with the same features were used for wound healing experiments. All the samples were kept under laboratory conditions, room temperature, atmospheric pressure and relative humidity of 1810%, 12h night and 12 h lighting. They had free access to standard laboratory food and water. The experimental conditions were in compliance with the requirements of the Ethics Committee of the animal sciences center of Baqiyatallah University.

2.3 Treatment procedure

Ten mice were anesthetized by intraperitoneal injection of Ketamine/Xylazine. The mice were autopsied. Two cuts on the liver of each mouse were induced with the blazer as shown in Fig. 2. One of the cuts was allowed to coagulate naturally, another one was treated with helium plasma jet until it coagulated completely. The distance from the plasma device was 2 cm. The flow rate of helium gas was about 0.5 slm.

2.4 Statistical analysis

The comparison between the blood coagulation time for cuts treated with helium plasma jet and the naturally coagulation time was performed with the one-way analysis of variance (ANOVA) using a least significance difference (LSD) test. Also, with in group comparisons of helium plasma jet treated wounds against the control wounds and the comparisons among 5 groups at each indicated day were done using ANOVA with LSD test. Data were reported as mean ± standard error. Furthermore, $P<0.05$ was considered statistically significant. All data were analysed used IBM SPSS statistics (Qiu et al., 2007).

2.5 Histological Analysis

On the 10^{th} day, 5 mice from each group (10, 20, 30, 40, and 50 s groups) were chosen for histological analysis. Tissue samples were extracted from slaughtered specimens in the form of slices normal to each incision trajectory, both from the treated and control sectors. The slices were fixed in 10% buffered formalin for 24 h and then was processed by histological techniques. The 5 m thickness was sectioned and stained by hematoxilin-eosin. The samples were analyzed using the optical microscope with the magnification of 40X.

3 Results and Discussion

3.1 Electric Parameters of plasma jet

Figure 3 shows typical current and voltage waveforms of the helium plasma jet.

The averaged instantaneous power resulting from multiplying voltage and current is 11.2 W.

3.2 Plasma OES spectroscopy result

Figure 4 shows the helium plasma jet emitted spectrum in the wavelength range of 200-1000 nm. The ROS and RNS are recognized in these spectra. OH and O with the important roles in biological applications such as blood coagulation and wound healing can be observed in this spectrum. The process is carried out in the ambient air and therefore the bands of the N_2 (in the wavelengths (in nm) of 316, 337, 353, 357, 375, 380, 394, 399, 406, 434, and 457) are observed in this spectrum. OH, O and N_2^+ can be observed based on 310, 377 and 427 nm in wavelength. Also the He bands are observed in the spectrum as mentioned.

3.3 In Vivo blood coagulation

Figure 5 shows a mouse liver treated by helium plasma jet. As is shown in Fig. 6, the blood of the cut which was

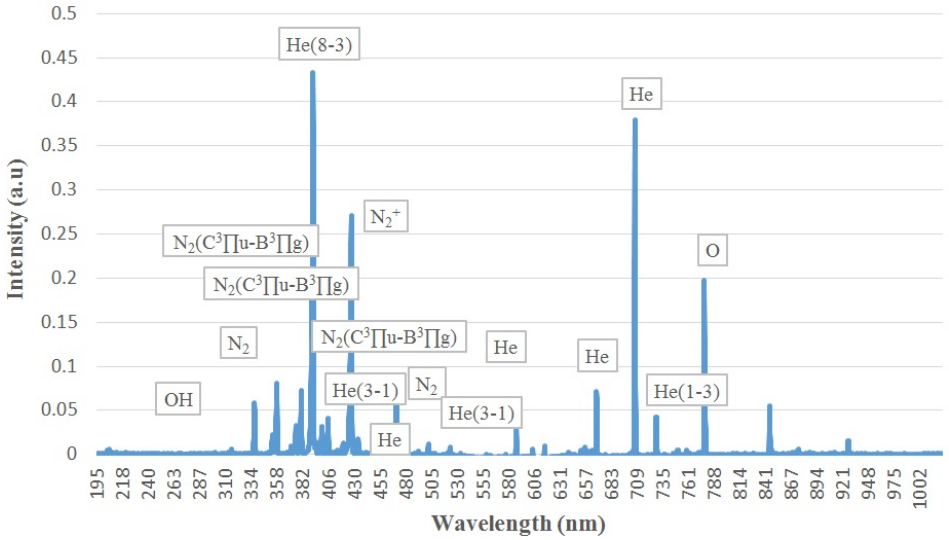

Figure 4: Optical spectrum corresponding to the helium plasma jet.

treated by non-thermal plasma was coagulated completely and no blood oozed from the cut after the treatment. The wound remained wet and the temperature of the cut remained at room temperature. Meanwhile, the control cut continued to ooze until 10 min after cutting.

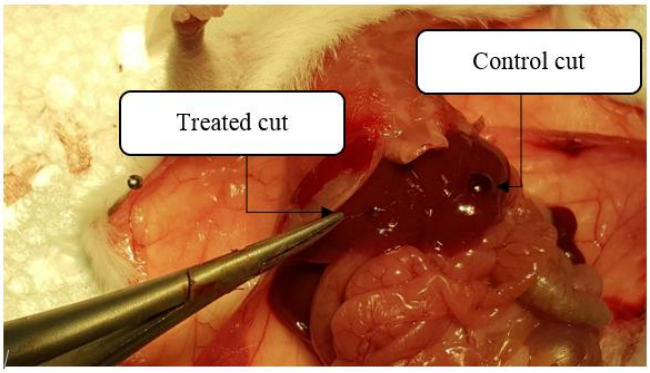

Figure 5: Treatment time of 10 s of mouse liver cut with helium plasma jet. Right cut: the control cut which continued to ooze until 10 min, left cut: blood coagulates completely after treatment.

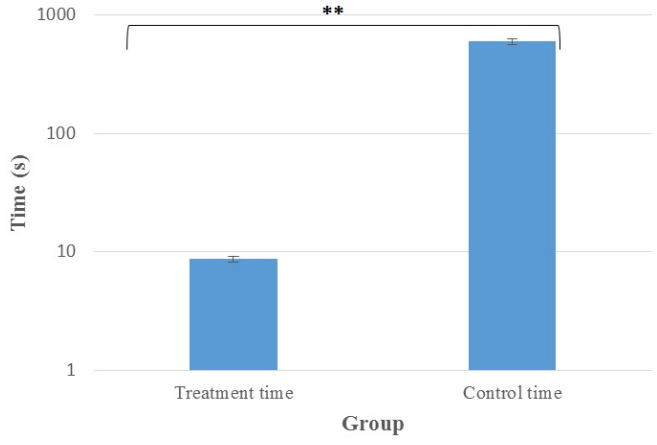

Figure 6: Coagulation time of in vivo cuts (**: P<0.001).

Figure 6 shows the 10 mice averaged in vivo coagulation time for both cuts (control and treated cuts). It can be seen that while the blood coagulation time of the treated cuts are about 8.6 s, this time is of about 60 min for control time. The difference between two times is considerably significant (P<0.001).

4 Conclusion

The aim of this work was to determine the appropriate treatment time for blood coagulation of the balb/c mice. For this reason, a plasma jet device working in helium gas was used. In these animal tests, the in vivo cuts on the ten mice livers showed an averaged coagulation time of 8.6 s. Naturally, the cuts bleeding stops after 10 min. As concluded, the treatment by helium plasma jet can speed up the in vivo blood coagulation.

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