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Contributions to Plasma Physics

ORIGINAL ARTICLE

Effect of nonthermal atmospheric pressure plasma on plasma coagulation in healthy persons and patients under treatment with Warfarin

E.S. Musavi[1] | **S.M. Khorashadizadeh**[1] | **R. Fallah**[1] | **A. Rahmanian Sharifabad**[2]

[1]Department of Physics, University of Birjand, Birjand, Iran
[2]Atherosclerosis and Coronary Artery Research Center, Birjand University of Medical Sciences, Birjand, Iran

*** Correspondence**
R. Fallah, Department of Physics, University of Birjand, Birjand, Iran.
Email: rfallah@birjand.ac.ir

Recently, nonthermal atmospheric pressure plasma has been used in medical devices for sterilization, blood coagulation, induction of apoptosis in cancer cells, etc. The purpose of this study is to evaluate the impact of cold atmospheric plasma on coagulation time in patients under treatment with warfarin as an anticoagulant agent (group A) and to compare this impact in healthy persons (group B). To measure the coagulation time, Clotting Time (CT) is used. After obtaining informed consent from each subject, two venous blood samples are taken to check CT. One sample is processed with plasma (case sample) and the other sample is not processed with plasma. CT in both samples is measured by a physician and recorded in a form in addition to demographic characteristics and drug history. The data are analysed using Statistical Package for the Social Sciences (SPSS) software. The Mann–Whitney test is used for comparison between groups and the Wilcoxon signed-ranks test is used to compare the difference between CT before and after plasma processing. The results show the significant effect of plasma on the reduction of plasma coagulation time, and this reduction is higher in the warfarin-treated group.

KEYWORDS
clotting time, cold atmospheric plasma, plasma coagulation, plasma medicine, warfarin

1 | INTRODUCTION

In recent decades, atmospheric pressure plasma has attracted a great deal of attention and has been used for various applications, including polymer surface modification,[1–4] air purification,[5–7] and sterilization.[8–10] Scholars have also studied the use and application of atmospheric pressure plasma in various biochemical contexts.[11] The application of this plasma, which is the result of electron avalanche at atmospheric pressure, is progressing day by day due to its lack of need for a vacuum system.[1] In nonthermal atmospheric pressure plasma, although the temperature remains at the ambient temperature, their electron temperature is very high. These types of plasma have a low degree of ionization, and the density of various types of charged particles is low in them and their electrons and ions never reach the local thermodynamic equilibrium, retaining the plasma temperature at room temperature.[1]

The progress in plasma medicine research has been rapid and fruitful in recent years from both scientific and clinical points of view.[12,13] The ability of cold atmospheric plasmas (CAPs) to kill bacteria and to accelerate the proliferation of specific tissue cells opened up the possibility to use this plasma in various medical research and clinical applications. The CAP produces chemically reactive oxygen and reactive nitrogen species and these reactive species play an important role in the interaction between CAP and cells (prokaryotic and eukaryotic cells) triggering various signalling pathways in cells. Additionally, the chemically active species produced by CAP have a relatively short life span. Hence, after the plasma flow sustainment stops, there is no more such active, i.e. containing short-lived species, gas flow; in addition, plasma is not accompanied by significant harmful radiation but exhibit transient intense electric field.[14] Recently, due to the ability of CAP to inactivate and degrade components of prokaryotic cells such as proteins, it has been well recognized that plasma can inactivate adhesion proteins of cancer cells,[15] thus a new route is offered to plasma-assisted cancer therapy. Although these are early evidence in finding a successful therapeutic solution, the study of interactions between CAP and cell components represents a more detailed enquiry at a cellular level and may lead to new application implications not easily imaginable with current understanding of plasma-cell interactions.

At the present time, blood coagulation is an important issue of medicine, in particular regarding wound treatment. The recent development of medical nonthermal plasma systems allows for the effective blood coagulation without thermal effects.[16,17] The floating electrode dielectric barrier discharge (FE-DBD) plasma produced by Kalghatgi et al.[18] has experimentally demonstrated plasma coagulation. Under normal conditions, blood coagulates on the stainless steel for about 15 min, which can be reduced to less than 1 min via using plasma processing for 15 s. To determine the mechanism responsible for the plasma-assisted blood coagulation, additional experiments were performed, which allowed the exclusion of factors such as the temperature, electric field, plasma-induced change of blood pH, light, and the increase in the concentration of calcium ions due to their release from calcium-bound proteins.[18] The fibrinogen aggregation was supposed to be an important factor in blood coagulation. Thus, nonthermal plasma has been demonstrated to induce blood coagulation, but the influence of coagulation parameters and the underlying biochemical mechanisms remains poorly understood. One of the biggest challenges in medical interventions, for example in outpatient or complicated surgeries, is the prevention of bleeding, and it is especially more important in cases where the patient's blood coagulability is impaired for some reasons (metabolic, heritable, medication, toxins, etc.).[19] There are several factors that affect blood coagulation, including the coagulation factors, platelets, vascular walls, and anticoagulation factors in the blood. Different criteria are used to measure blood coagulability. Some of these criteria, such as prothrombin time (PT), thrombin time (TT), and international normalized ratio (INR), only assess the function of coagulation factors, and are not reliable due to the involvement of other factors in blood coagulation under some conditions (e.g. platelet function disorder, etc.) because, although a coagulation disorder exists, the results of these tests are within the normal range.[20] Some other measurement criteria, such as bleeding time (BT) and clotting time (CT), examine the final outcome of the function of the factors involved in coagulation. In the BT criterion, the time required for the bleeding on the body surface to stop is measured, and in CT, the time required for fibrin strand formation in the whole blood in vitro is measured.

The present study investigates the effect of plasma on the rate of blood coagulability in healthy individuals as well as those who have a longer coagulation time due to the use of the anticoagulant drug, warfarin. For this purpose, the CT criterion was used to measure blood coagulability and investigate the effect of plasma. The results of this study can be considered and used by medical and biomedical researchers.

2 | EXPERIMENTS AND METHODS

2.1 | Experimental setup

In this study, the nonthermal atmospheric plasma was produced by a plasma jet with a maximum input power of 500 W. For this purpose, an AC high voltage, with a pick-to-pick voltage of 7.5 kV and a frequency of 25 kHz, was applied when helium gas was injected with a flow rate of 3 L/min. The plasma effluent of the torch was directed downward toward the sample plate placed on the sample holder. In addition, the diameter of the nozzle at exit was 4 mm and *nozzle sample* was kept at a distance of 9 cm. The sample *spread over* a glass slide, which was clamped to a holder. A schematic of the experimental setup is depicted in Figure 1.

2.2 | Methods

CT indicates the final result of the effective factors in blood coagulation. On the other hand, any disturbances in the process of coagulation are influential on this criterion. Therefore, this criterion was utilized to measure the effect of nonthermal atmospheric plasma on blood coagulation status. Considering that this study aimed to investigate the effect of plasma on coagulation time, the confounding factors were applied in the sampling in the form of the inclusion criteria. The inclusion criteria involved:

- not taking anticoagulants (except warfarin)
- not suffering from congenital coagulation diseases (Glanzmann's thrombasthenia syndrome, Bernard Soulier syndrome, haemophilia, etc.)
- not suffering from severe liver diseases (fatty liver [grade 3 or more], viral or drug-induced hepatitis)
- not taking drugs that affect hepatic metabolism

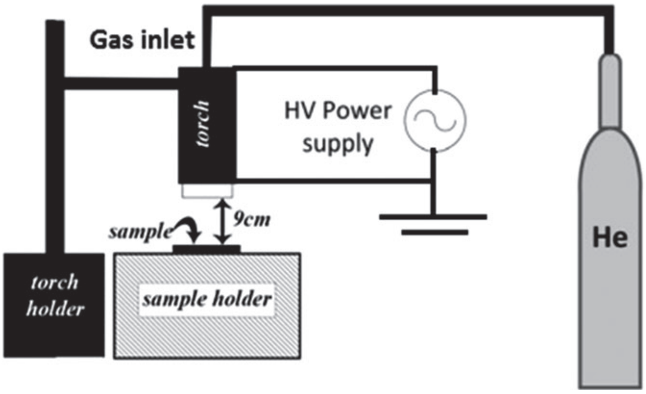

FIGURE 1 Schematic layout of the laboratory setup

TABLE 1 Demographic data of the participants

	Criterion	
	Sex	
Group	Male	Female
Group A	10	7
Group B	12	12

In addition, the inclusion criterion for the warfarin-treated group was regular consumption of warfarin for at least 1 year; hence, those who took warfarin and did not have one of the above-mentioned criteria were excluded from the study. To conduct the research, first, the code of approval was received from the Ethics Committee of Birjand University of Medical Sciences (ir.bums.REC.1396.53). Afterwards, a representative of the research team attended one of the laboratories in the city of Birjand; then, informed consent was obtained from people referring to the lab for normal tests, the standard questionnaire for the inclusion criteria and demographic information was completed, and 3 cc of blood was taken from the volunteers. To prevent spontaneous blood clotting, considering the long time interval (more than an hour) between blood sampling and analysis, the samples were kept in a citrate standard vial and after centrifugation, the platelet-rich samples were stored at 4 °C. The individuals' information was then recorded in the electronic form and a unique code was assigned to each person to prevent bias in the study. After transferring the samples to the plasma lab and maintaining the cold chain, two volumes of 0.1 cc were separated from each sample. Then, CT was measured for a sample after plasma processing (intervention sample) and without plasma processing (control sample) for another sample. To investigate the effect of plasma on the coagulation time, the sample was subjected to a nonthermal plasma torch from a 9 cm distance for 15 s. For eliminating the effect of the exiting air flow from the plasma torch on the control sample, similar to the intervention sample, this sample was also exposed to the air flow exiting from the torch (without plasma) from a 9 cm distance for 15 s. Moreover, to eliminate the effect of selection bias on CT measurement, the measurement was done by a collaborator physician who was not aware of the type of sample and the group of the individuals (blinding) and the information was recorded in an electronic questionnaire based on the unique sample codes. Eventually, the data were statistically analysed using Statistical Package for the Social Sciences (SPSS) software.

3 | RESULTS AND CONCLUSION

In the present study, from among 55 subjects in the study population, 41 subjects had the inclusion criteria for the study, of which 17 were assigned to group A (regular warfarin consumption for at least 1 year) and 24 were assigned to group B (no anticoagulant consumption) (Table 1).

The Wilcoxon signed-ranks test was used to compare CT before and after plasma processing, and as shown in Table 2, there was a significant decrease in CT in both groups after the intervention. In addition, the Mann–Whitney test showed that the CT level before the intervention was significantly different between the two groups. Considering the use of warfarin in group A, this difference was predictable; therefore, analysis of covariance (ANOCOVA) was used to compare the effect of plasma on CT variation in the processed sample to obtain more accurate results and consider the initial differences in the two groups before the processing. Table 2 clearly shows that in group A, the mean CT before plasma processing was 84 ± 26.3 s, which reduced to 22 ± 3.3 s after plasma intervention, indicating the significant impact of plasma on the mean CT in group A. Similar results are observed for group B, so that the mean CT in this group was 49.5 ± 10.6 s before the intervention, which reduced to 19 ± 1.2 s

TABLE 2 Clotting time (CT) results based on the tests

Variable	Group (A) mean, (SD), median	Group (B) mean, (SD), median	*p* value
CT before intervention	84, (26.3), 88.5	49.5, (10.6), 48	<.001[a]
CT after intervention	22, (3.3), 22.2	19, (1.2), 19.2	<.001[b]

[a]Mann–Whitney test results.
[b]Matched with CT before intervention and ANOCOVA.

after plasma processing. In this study, in addition to the reduction of the mean and median values after plasma intervention, it was observed that the dispersion of the data around the mean value was decreased, so (as shown in Table 2) the SD was strongly reduced in both groups after the intervention. Furthermore, in group A, the ratio of mean value after plasma processing to mean value before plasma processing was 22/84 that was much lower than the ratio of 19/49.5 in group B, which shows that the CAP has a more effect for the intended target group (group A) consisting of patients under treatment with warfarin. Based on the results of the ANOCOVA, as shown in the last column of Table 2, the mean CT reduction was higher in the warfarin-treated group than the anticoagulant group ($p < .001$), which confirms the assumption that plasma processing reduces the CT in both groups. The main mechanism of the effect of nonthermal atmospheric pressure plasma on reducing the coagulation time is not clear. However, according to Kalghatgi et al.[18] who had confirmed the formation of platelet plug in citrate blood via an electron microscope, it can be predicted that through the production of free radicals, plasma causes depletion of intra platelet granulomas and facilitates clot formation. The results of this study confirmed the significant effect of cold atmospheric pressure plasma on the reduction of blood coagulation time, and that this reduction is more statistically significant in warfarin-treated patients. Moreover, the findings of this study can be considered and used by health care and medical researchers and can lead to greater application of plasma in different medical and biomedical fields.

ORCID

R. Fallah http://orcid.org/0000-0001-6234-2768

REFERENCES

[1] X. Lu, M. Laroussi, *J. Appl. Phys.* **2006**, *100*(6), 063302.
[2] N. Y. Cui, N. M. D. Brown, *Appl. Surf. Sci.* **2002**, *189*, 31.
[3] R. Dorai, M. J. Kushner, *J. Phys. D: Appl. Phys.* **2003**, *36*(6), 666.
[4] N. K. Bibinov, A. A. Fateev, K. Wiesemann, *J. Phys. D: Appl. Phys.* **2001**, *34*, 1819.
[5] H. S. Ahn, N. Hayashi, S. Ihara, C. Yamabe, *Jpn. J. Appl. Phys.* **2003**, *42*, 6578.
[6] H. H. Kim, G. Prieto, K. Takashima, S. Katsura, A. Mizuno, *J. Electrostat.* **2002**, *55*(1), 25.
[7] R. Hackam, H. Akiyama, *IEEE Electr. Insul. Mag.* **2001**, *17*(5), 8.
[8] B. J. Park, D. H. Lee, J. C. Park, I. S. Lee, K. Y. Lee, S. O. Hyun, K. H. Chung, *Phys. Plasmas* **2003**, *10*, 4539.
[9] S. Lerouge, M. R. Wertheimer, Y. L'H, *Plasmas Polym.* **2001**, *6*(3), 175.
[10] N. S. Panikov, S. Paduraru, R. Crowe, P. J. Ricatto, C. Christodoulatos, K. Becker, *IEEE Trans. Plasma Sci.* **2002**, *30*, 1424.
[11] A. D. Morris, G. B. McCombs, T. Akan, W. Hynes, M. Laroussi, S. L. Tolle, *J. Dent. Hyg.* **2009**, *83*(2), 55.
[12] M. Keidar, E. Robert, *Phys. Plasmas* **2015**, *22*, 121901.
[13] M. G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. van Dijkand, J. L. Zimmermann, *New J. Phys.* **2009**, *11*, 115012.
[14] E. Robert, T. Darny, S. Dozias, S. Iseni, J. M. Pouvesle, *Phys. Plasmas* **2015**, *22*, 122007.
[15] G. C. Kim, personal communication, **2009**.
[16] Y. H. Kim, H. S. Rhim, H. S. Uhm, E. H. Choi, *2011 Abstr. IEEE Int. Conf. Plasma Science*, Chicago, IL, **June 26–30, 2011**, https://doi.org/10.1109/PLASMA.2011.5993283.
[17] C. Y. Chen, H. W. Fan, S. P. Kuo, J. H. Chang, T. Pedersen, T. J. Mills, C. C. Huang, *IEEE Trans. Plasma Sci.* **2009**, *37*, 993.
[18] S. U. Kalghatgi, G. Fridman, M. Cooper, G. Nagaraj, M. Peddinghaus, M. Balasubramanian, G. Friedman, *IEEE Trans. Plasma Sci.* **2007**, *35*(5), 1559.
[19] F. C. Brunicardi, D. K. Anderson, T. R. Billian, D. L. Dunn, J. G. Hunter, J. B. Matthews, R. E. Pollock, Schwartz's Principles of Surgery, McGraw-Hill Companies, New York **2010**.
[20] P. N. Malani, *JAMA* **2012**, *308*(17), 1813.

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