Iranian Journal of Medical Physics
Vol. 10, No. 1-2, Winter & Spring 2013, 31-36
Received: April 11, 2013; Accepted: Jan 10, 2013

Original Article

In Vitro and in Vivo studies of the Effects of Cold Argon Plasma on Decreasing the Coagulation Time

Elham Janani[1], Mahsa Ale-Ebrahim[2*], Pejman Mortazavi[3]

Abstract

Introduction
Cold plasma is a self-sterilized, painless, and non-contact method in surgeries. These properties allow it to be applied to the living tissues and heat-sensitive parts. The aim of this study was to design a new cold plasma producer device and evaluate the effects of cold argon plasma on decreasing the coagulation time of blood drop *in vitro* and that of the injured liver blood *in vivo*.
Materials and Methods
In an experimental study, two blood drops of a normal healthy human were placed on a glass slide. The experimental sample was irradiated by plasma until the complete coagulation occurred, while the control sample remained intact. The complete coagulation time was then measured for both samples. In another part of our study, 20 rats were divided into two experimental and control groups and anesthetized for experimentation. Livers of the rats in the control group were incised and the bleeding time was measured until complete coagulation. Livers of the experimental rats were irradiated by plasma after being incised, and the complete coagulation time was measured.
Results
Cold plasma treatment increased the speed of blood coagulation in both blood drop *in vitro* and the injured liver blood.*in vivo*. Histopathological examinations revealed that plasma treatment caused no significant tissue damages as compared with the control group.
Conclusion
The use of argon plasma coagulation device at the time of surgery, in addition to accelerating blood coagulation, caused no injury and burning on tissues. Plasma increases the platelets activation, fibroblasts proliferation and fibrin production. the mechanism of action is likely mediated by exogenous nitric oxide.

Keywords: Cold Argon Plasma, Coagulation Time, Liver, Rat

1- *Sama Technical and Vocational Training College, Islamic Azad University, Andisheh Branch, Andisheh, Iran.*
2- *Young Researchers and Elite Club, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*Corresponding author: Tel: +98 21 88582036; Email: mahsa.alebrahim@yahoo.com

3- *Department of Pathology, Faculty of Specialized Veterinary, Science and Research Branch, Islamic Azad University, Tehran, Iran*

1. Introduction

Cold plasma technology may well serve as a viable alternative to electric knives in surgical operations, since it accelerates blood coagulation, stops bleeding, and sterilizes the injured part at the same. Currently, blood coagulation is an important issue in medicine, in particular regarding wound treatment [1-5]. This project was undertaken to evaluate the effects of cold argon plasma on decreasing the coagulation time *in vitro* and *in vivo*. Plasma has so far been the subject of many biological and medical studies. It is an ionized gas made up of charged particles including ions, electrons, free radicals, and neutral atoms. It can also be produced out of helium or argon at atmospheric pressure. The air in the atmosphere and other gas mixtures may also be used for making plasma [4,6-8]. Since 30 years ago, high-temperature plasma technology has been employed locally in many surgeries for purposes such as coagulation, treatment of vascular constriction (microvascular abnormalities, especially in the digestive system), and removal of tumors [9-12]. However, due to inflicting serious histological damages, it was gradually replaced by semi-thermal and non-thermal (cold) plasma method. Cold plasma refers to the fact that argon is characterized by slow ionization and the produced ions take the room temperature very rapidly (within a fraction of a second). Cold plasma is a self-sterilized, painless, and non-contact method. These properties would allow it to be applied to the living tissues and heat-sensitive parts without significant tissue damages [13]. The cold plasma method is now widely used in medical science for accelerating the coagulation of blood in particular during surgical operations [9,10]. It would be highly desirable if the plasma produced by our student-made device can both increase the speed of blood coagulation and cause no damages or necrosis in tissues. Because of the sterilizing property of plasma, our device may prove useful for accelerating blood coagulation time and recovery of injuries in medical practices.

2. Materials and Methods

2.1. Cold plasma generating device

The device used in this work for the production of cold argon plasma (Figure 1) was designed and made by the students majoring plasma physics at the Science and Research Branch, Islamic Azad University, Tehran. This device that is formally registered under patent number 74562 can be used under atmospheric pressure at room temperature. It is made up of a glass tube 5 cm in length and 8 mm in outer diameter in which a movable nickel-chromium (Ni-Cr) electrode of 11 cm in length and 1 mm diameter is inserted. Since the electrode inside the tube is movable, it can be adjusted to the extent that the high performance of plasma is obtained. Seven cm of the total length goes inside the tube (Figure 1).

The electrode is covered by a 4 mm diameter insulating material made of Pyrex. Another electrode made of aluminum in the form of a thin layer with 2 cm in length is wrapped around the glass tube to produce plasma with higher quality and length. The device is kept fastened by an aluminum holder. This metal holder has a gas inlet to which a flow meter is connected.

Figure 1. Cold-plasma generating device. Seven cm of the length of the movable electrode is inside the glass tube

Flow of argon gas is 1.5-2.5 liters per minute in the device and the produced plasma finds way out of the glass tube as the output. Experiments show that argon under 2 liters per minute pressure results in plasma with higher quality and length. Our device operates under 50 HZ, 1.1 kV and 0.1 mA.

2.2. Rats

For *in vivo* experiment, adult male Wistar rats weighting 180-200 g were purchased from the Pasteur Institute of Iran. The rats were kept in the animal room under controlled temperature 23±3 °C and a photoperiod of 12 hours light – 12 hours dark. During the experimental period, the rats were fed with enough food and water. The tests for both sample and control groups were administered under similar environmental conditions and ethics in animal works were observed to handle the animals.

2.3. The effect of cold plasma on in vitro blood drop coagulation

Two drops of blood of a healthy man were dropped on a glass slide; one drop was taken as the control sample and the other as the experimental sample. Both drops were equal in size. The experimental sample was immediately irradiated by plasma until complete coagulation, took place but the control sample remained intact. The complete coagulation time for both samples, were examined and measured by naked-eye and by an optical microscope.

2.4. The effect of cold plasma on in vivo coagulation of injured liver blood

Twenty rats were divided into two groups, sample and control. The rats were anesthetized by subcutaneous injection of ketamine and xylazine and were shaved before undergoing surgery. An incision one cm in length way made in the rats liver. For the control group, bleeding time was measured until complete coagulation and clot formation. For experimental group, the livers were irradiated by plasma immediately after incision and the bleeding time was measured until complete coagulation. Thereafter, liver tissues were removed and fixed in 10% formalin buffer solution for tissue study. It should be noted that plasma irradiation was used in monotonous and uniform manner to avoid pain and localized lesions.

2.5. Experiments on tissues

Liver tissue samples were placed in 10% formalin buffer solution. Using conventional methods, the fixed samples were processed and then molded in paraffin and cut into 3-5 micrometer sections by microtome. The sections were then stained with hematoxylin and eosin by standard methods. Histopathological examination of the samples was performed by an optical microscope.

2.6. Statistical analysis

All the data are presented as means± standard error of the mean (SEM). Statistical analysis was carried out by one-way ANOVA followed by a Tukey's post-hoc test. The criterion for statistical significance was $P<0.05$.

3. Results

3.1. The effect of cold plasma on in vitro blood drop coagulation

In vitro examination by the naked-eye and optical microscope revealed that in control group, after about 8 minutes, the blood drops on the slide were thoroughly coagulated, whereas in the experimental group, it took only 10 seconds to be completely coagulated after plasma exposure treatment (Figure 2).

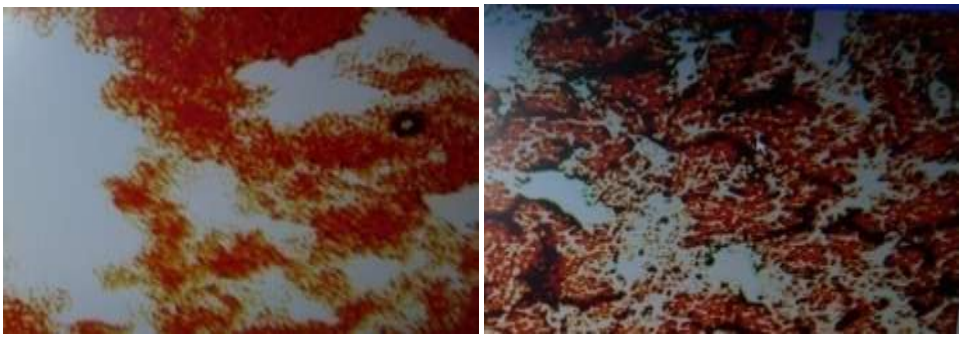

Figure 2. Optical image of blood drop coagulation. The experimental sample (left) has turned dark with observable clots 10 seconds after plasma treatment. The control sample (right) is still at partly-coagulated phase after 10 minutes.

3.2. The effect of cold plasma on in vivo coagulation of injured liver blood

Total coagulation time for blood samples from the incised liver in the control group was 4.5 minutes, while for the experimental group, plasma treatment reduced the coagulation time to 12 seconds which is significantly lower.

Histopatology examination of the liver in the control group showed a slight necrosis area with thickness of 10 microns (Figure 3). In the experimental group, the thickness of the necrotic area was approximately 12 microns and the cells in the lower layers were entirely normal with no sign of damage (Figure 4). No significant tissue damages were observed in the plasma-treated group (Figure 4) when compared with the control group (Figure 3).

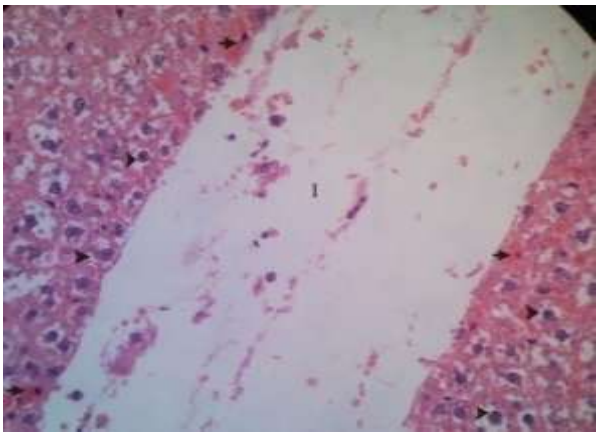

Figure 3. liver tissue sample of the control group: The incised area (I) with some taken-off liver cells surrounded by healthy hepatocytes (arrowhead). A slight necrosis (arrow) with thickness of 10 microns can be observed (H&E,640×).

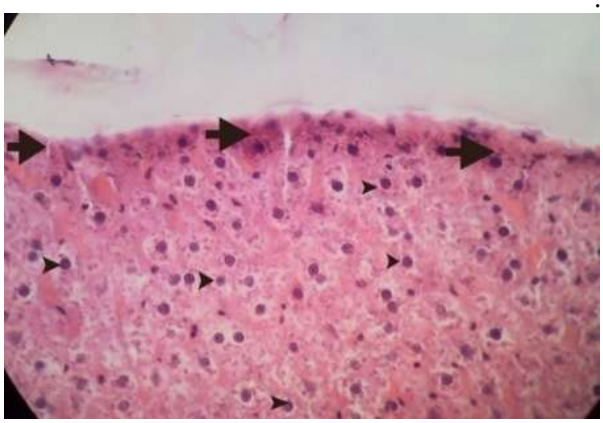

Figure 4. liver tissue sample of the experimental group (irradiated by plasma for 12 seconds) with greater magnification: the thickness of the necrotic area (arrow) is approximately 12 microns. Normal liver cells can be seen under the necrotic area (arrowhead) (H&E, 640×).

4. Discussion

The cold plasma generating device was designed and made by our team. It is a plasma-generator that uses argon to produce cold plasma at atmospheric pressure. Among the various gases used for making cold plasma, argon plasma has proven to be much more effective in sterilizing the micro-organisms [14].

Employing this device, in the present study, the effects of plasma on blood coagulation under both *in vivo* and *in vitro* conditions were investigated. Friedman et al. suggested that irradiation of cold plasma by Floating Electrode Dielectric Barrier Discharge (FE-DBD) device *in vivo* increases the coagulation speed of blood drops of normal healthy humans [15,16]. The results of the present study on blood droplets coagulation is in

agreement with the previous research findings. Plasma treatment in the experimental group significantly decreased the time required for coagulation of blood droplets from 8 minutes to just 10 seconds. Plasma is able to catalyze the complex biochemical processes involved in blood coagulation. The effect of cold plasma on blood coagulation depends on neither gas temperature nor blood temperature [10]. By activating the platelets and creating fibrin strands, plasma accelerates the process of blood coagulation and covers surface of the sample with a visible thin layer. The major advantage of plasma is that it only affects the surface of blood droplets in the coagulation process and since it does not penetrate deep in the blood, the risks of blood clotting and other post-surgery side-effects can be avoided. This technology is thus quite beneficial to thin layer tissues such as those of the liver, because it minimizes the risks of tissue burns and necrosis as compared with other conventional methods such as electric knife [9,17-19]. *In vivo* plasma treatment significantly reduced the time required for complete blood coagulation in the liver of experimental group as compared with the control group. Histological studies also revealed no significant tissue damages in the plasma-treated liver and just a 12-micron necrosis layer was observed, and the lower cells were entirely normal with no sign of damage. Plasma produced from argon has a high-sterilizing effect of the lesions and plays an important role in infection-reduction by deactivating the active bacteria. By producing nitric oxide (NO) radicals, argon plasma increases the platelets activation and aggregation, causes proliferation of fibroblasts and fibrin production, and stops the bleeding. This technology may thus be employed for rapid coagulation of blood during surgeries. Nitric oxide plays also a vital role in blood coagulation, regulating the immune system, inducting phagocytosis, proliferation of keratinocytes, collagen synthesis, and wound-healing [20-22]. Safety with cold plasma technology is the major factor that encourages its use in medicine and during surgeries. The findings of the present research indicate that plasma irradiation at the time of surgery not only accelerates the blood coagulation process, but also leaves no sign of tissue burn or injury [10,20,23].

5. Conclusion

The results of the current study showed that the cold plasma produced from argon significantly increases the *in vitro* speed of blood coagulation. It also accelerates *in vivo* coagulation of liver blood without causing significant tissue damages or necrosis. By production of nitric oxide, plasma increases activation and aggregation of platelets, causes proliferation of fibroblasts and fibrin production, accelerates blood coagulation process, and thus stops the bleeding by its sterilizing mechanism, plasma reduces the chance of infection as well. Therefore, plasma technology has the potential to be widely employed for blood coagulation and wound healing in various branches of medicine.

Acknowledgements

This research was supported by Plasma Physics Research Center, Science and Research Branch University, Tehran, Iran. The authors would like to express thanks to Dr. Ghoranneviss for his generous support and technical assistance.

References

1. Vargo JJ. Clinical applications of the argon plasma coagulator. Gastrointest Endosc. 2004; 59(1): 81-8.
2. Kim YH, Rhim HS, Uhm HS, Choi EH. Atmospheric pressure air plasma jet assisted blood coagulation. Plasma Science (ICOPS), 2011 Abstracts IEEE International Conferenc. 26-30 June 2011.
3. Ginsberg GG, Barkun AN, Bosco JJ, Burdick JS, Isenberg GA, Nakao NL. et al. The argon plasma coagulator. Gastrointest Endosc. 2002; 55(7): 807-10.
4. Kieft I.E, Darios D, Roks A.J.M, Stoffels E. Plasma treatment of mammalian vascular cells: A quantitative description IEEE T Plasma Sci. 2005; 33(2): 771–5.
5. Laroussi M, Lu X. Room temperature atmospheric pressure plasma plume for biomedical applications. Appl. Phys. Lett. 2005; 87(11): 113902-3.
6. Kieft IE, Kurdi M, Stoffels M. Reattachment and Apoptosis After Plasma-Needle Treatment of Cultured Cells. IEEE T Plasma Sci 2006; 34(4): 1331-6.
7. Stoffels E, Gas plasmas in biology and medicine. J Phys D Appl Phys. 2006. 39(16).
8. Stoffels E, Kieft .IE, Sladek REJ, van den Bedem LJM, van der Laan EP, Steinbuch M. Plasma needle for in vivo medical treatment: recent developments and perspectives. Plasma Sources Sci T. 2006; 15(4): S169.
9. Fridman G, Peddinghaus M, Ayan H, Fridman A, Balasubramanian M, Gutsol B, et al. Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air. Plasma Chem Plasma P. 2006; 26(4): 425-42.
10. Fridman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. Plasma Chem Plasma P. 2008; 5(6): 503–33.
11. Gostev V, Dobrynin D. Medical microplasmatron. In 3rd International Workshop on Microplasmas (IWM-2006) Greifswald Germany; 2006.
12. Gostev V. Cold Plasma in Biological Investigations, in NATO Advanced Study Institute (ASI): Plasma Assisted Decontamination of Biological and Chemical Agents. Cesme, Turkey; 2007.
13. Heinlin J, Morfill G, Landthaler M, Stolz W, Isbary G, Zimmermann JL, et al. Plasma medicine: Possible applications in dermatology . J Dtsch Dermatol Ges. 2010; 8(12): 968-76.
14. Hong YJ, Seo YS, Lee HW, Choi J, Kang SK, Park GY, et al. Non-thermal atmospheric pressure plasma sources for biomedical applications. Plasma Physics and Controlled Fusion Create an alert RSS this journal R Foest 2005 Plasma Phys. Control. Fusion 2005; 47 (12B) :S38.
15. Pompl R, Shimizu T, Schmidt HU, Bunk W, Jamitzky F, Steffes B, et al. Efficiency and medical compatibility of low-temperature plasma sterilization. 6th International Conference on Reactive Plasmas; Matsushima, Japan; 2006.
16. Sosnin EA, Stoffels E, Erofeev MV, Kieft IE, Kunts SE. The effects of UV irradiation and gas plasma treatment on living mammalian cells and bacteria: a comparative approach IEEE T Plasma Sci. 2004; 32(4): 1544–50.
17. Yonson S, Coulombe S, Leveille V, Leask R. Cell treatment and surface functionalization using a miniature atmospheric pressure glow discharge plasma torch. J Phys D Appl Phys. 2006; 39(16): 3508–13.
18. Fridman G. Medical applications of floating electrode dielectric barrier discharge FE-DBD, in Proc. 1st Int Conf Plasma Med.; Corpus Christi, TX, 2007.
19. Fridman G, Peddinghaus L, Vinovrski T, Jah A, Fridman A, Balasubramanian M, et al. Use of Nonthermal Atmospheric Pressure Plasma Discharge for Coagulation and Sterilization of Surface Wounds. In 17th International Symposium on Plasma Chemistry (ISPC-17). 2005; Toronto, Canada; 2005.
20. Shekhter AB, Serezhenkov VA, Rudenko TG, Pekshev AV, Vanin AF. Beneficial effect of gaseous nitric oxide on the healing of skin wounds. Nitric Oxide-Biol Ch. 2005; 12(4): 210-9. 21.
21. Lu X. On the mechanism of plasma inducing cell apoptosis. Published in: Plasma Science (ICOPS); 2011 June 26-30; Abstracts IEEE International Conference. 2011.
22. Hoffmann M, Ulrich A, Schloericke E, Limmer S, Karsten Habermann J, Wolken H, et al. The application of cold-plasma coagulation on the visceral pleura results in a predictable depth of necrosis without fistula generation. Interact CardioVasc and Thoracic Surg. 2012; 14 (3): 239-43.
23. Shashurin A, Keidar M, Bronnikov S, Jurjus RA, Stepp MA. Living tissue under treatment of cold plasma atmospheric jet. Appl Phys Lett. 2008; 93(18): 181501 -3.