Influence of non-thermal argon plasma needle on blood coagulation

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Abstract

Non-thermal argon plasma needle at atmospheric pressure was constructed. The experimental setup was based on a simple and low cost electric component that generates a sufficiently high electric field at the electrodes to ionize the argon gas which flow at atmospheric pressure. A high AC power supply was used with 1.1 kV and 19.57 kHz. Non-thermal Argon plasma used on blood samples to show the ability of non-thermal plasma to promote blood coagulation. Three tests have been done to show the ability of plasma to coagulate both normal and anti-coagulant blood. Each blood sample has been treated for varying time from 20sec. to 180sec. at different distances. The results of the current study showed that the cold plasma produced from argon significantly increase the in vitro speed of blood coagulation, the plasma increases activation and aggregation of platelets, causes proliferation of fibroblasts and fibrin production accelerates blood coagulation.

Key words

Plasma treatment, blood coagulation, Plasma needle, Plasma medicine.

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تأثير بلازما الأركون غير الحرارية على تخثر الدم

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الخلاصة

تم تكوين أبره أركون بلازما غير الحرارية عند الضغط الجوي. واستندت الإعدادات التجريبية على مكونات كهربائية ذات تكلفة بسيطة ومنخفضة تعمل على توليد حقل كهربائي عالي عند الأقطاب لتأين غاز الاركون المستخدم الذي يتدفق عند الضغط الجوي. تم استخدام مجهز قدرة عالي ذو تيار متردد AC عند القيمة 1.1كيلوفولت وبالتردد 19.57 كيلوهيرتز. كما تم استخدام بلازما الاركون غير الحراريه في اظهار قدرتها على تخثر الدم. حيث اجريت ثلاث تجارب لاظهار قدرة البلازما على تخثير الدم الاعتيادي والدم المضاف له مانعات تخثر كل عينة دم تم معالجتها بزمن يتراوح مابين 20 ثانية الى 180 ثانية وعند مسافات مختلفة. النتائج اظهرت ان البلازما الباردة لها القدرة على تخثير الدم خارج الجسم خلال فترة قصيرة وكذلك تعمل على تفعيل تجمع الصفائح الدموية وتسريع انتشار الخلايا الليفية وبالتالي انتاج الفايبرين الذي يسرع تخثر الدم.

Introduction

Over the past few years, non-thermal atmospheric pressure plasma has emerged as a novel promising tool in medicine. As compared to the effects of the more conventional thermal plasma, non-thermal plasma is selective in its treatment since it does not burn tissue [1]. This enables many new medical applications, including sterilization of living tissue without damage [2], blood coagulation [2], induction of apoptosis in malignant tissues, and modulation of cell attachment [3,4].

Coagulation is the formation of a blood clot and occurs when blood changes from a liquid to a solid. Coagulation is an important primary step of homeostasis and occurs immediately after damage to the endothelium of the blood vessel to

prevent blood loss from the injured vessel and to seal the area from infection [5]. Formed elements and blood plasma contribute to blood coagulation during hemorrhage. In our work we examine the effectiveness of non-thermal atmospheric plasma jet on coagulation applications.

Experimental work

1. Plasma needle

Plasma needle consists of a hollow stainless steel pipe of 100mm long with inner diameter 1mm and outer diameter 2.7 mm inserted inside a Teflon pipe as shown in Fig. 1. The stainless steel connected to the high voltage AC power supply. As put between Teflon pipe and stainless steel pipe filled with Teflon tape. Under certain conditions an argon plasma needle can be extracted from the downstream tube end since there is no discharge inside the plastic tube. The plasma needle obtained by this method is cold enough to be put in direct contact with human skin without electric shock and can be used for medical treatment and decontamination. All configurations the high voltage AC power supply generates high voltage of sinusoidal shape of 1.1kV (peak to peak) and frequency of 19.57 kHz.

Fig. 1: Photograph of plasma needle system.

2. Plasma needle system

Plasma needle system includes four main parts:

1. High Ac. voltage power supply.
2. Plasma needle.
3. Argon gas.
4. Flow meter.

Fig. 2 shows the schematic diagram of plasma needle system, which consist of high voltage source, plasma torch, Argon gas and gas flow meter.

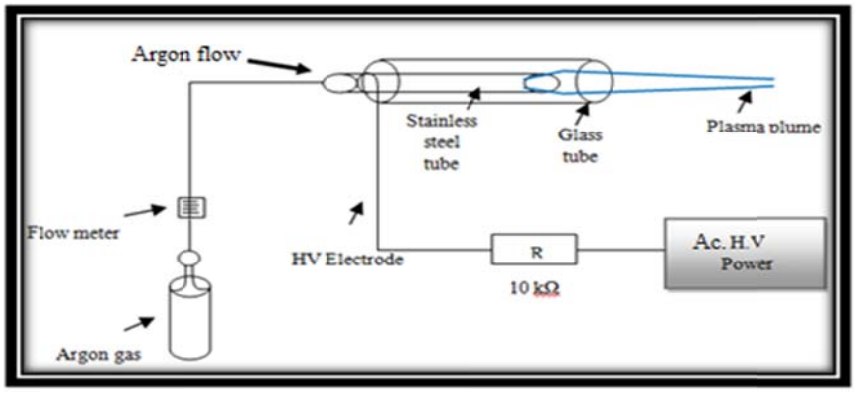

Fig. 2: Diagram of plasma needle system.

3. Medical applications (blood coagulation)

The Non- thermal plasma needle that designed and constricted locally was used to treat two types of blood sample (normal blood samples and anti-coagulant blood samples) at different exposure time and distances.

3.1. Sample preparation

Blood samples were taken from healthy female donor. These samples were used to test the effect of laboratory made plasma needle system on blood coagulation, the effects of plasma needle were tested on both normal blood samples and anti-coagulated blood samples, anti-coagulated blood samples were tested in EDTA (ethylene demine tetra acetic acid) anti-coagulant tubes.

3.2. Sample treatment by plasma needle

Blood samples were treated with plasma needle at argon gas flow rate of L/min. Three tests has been made to show the ability of plasma to coagulate both normal and anti- coagulant blood, each blood samples treated for varying time between 20sec. to 180sec., and at different distances. All blood samples have been tested at room temperature. The effect of argon plasma on blood has been measured by studying the plasma effect on red blood cell and platelets activation also studying its effect on the concentration of calcium ion in blood.

Results and discussions

1. The effect of argon plasma on non-coagulant blood

This first test has been made to show the ability of plasma to coagulate fresh blood (non-coagulant blood) not by heat but by plasma effluent (oxygen radicals). This test were performed by exposing five drops to five different exposure time (20, 25, 30, 40 and 50 sec.), at distance 2.5cm from the plasma tip. From this test one can conclude that the degree of clotting is increased by increasing the exposure time, as soon as the blood starts clotting a shell is formed on the surface of the blood drops as shown in Fig. 3. The degree of blood clotting differs as the dissimilar blood sample surface structure led to conclude. As the degree of dryness and hardness of a shell increases, the bubble on that shell surface tends to collapse and consequently forms dimples. Drop (b) has smoother surface than drop (c) and drop (c) has smoother surface than both (d, e) while drop (f) the bubble on the surface is collapsed.

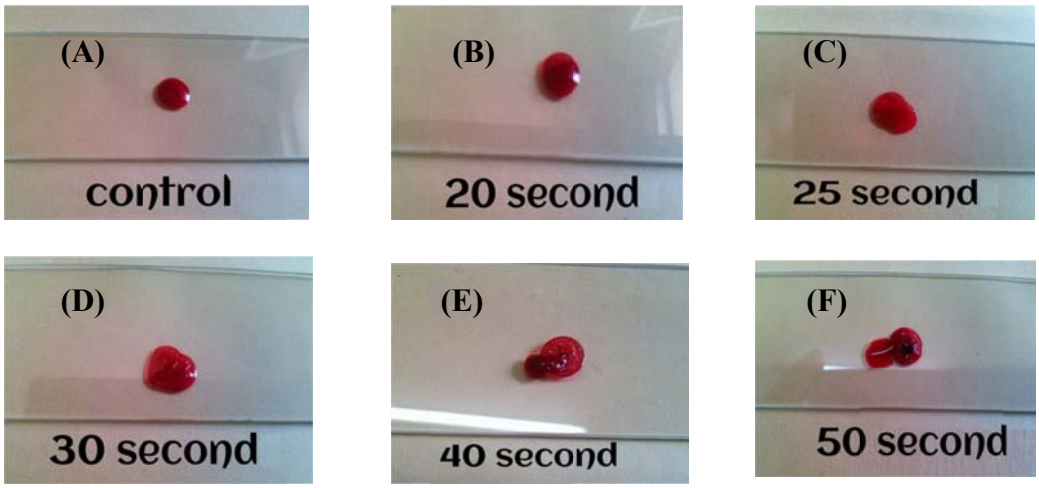

Fig. 3: Control and treated blood samples for different exposure time.

The second test has been made to show that heat has no effect on blood coagulation and the coagulation is done by plasma effluents (oxygen radicals). This test has been performed by exposing the blood drops to plasma for 40 second at distance 2.5cm the first with aluminum foil (to transfer all the heat generated by plasma to the blood drop) and the other uncovered[7]. Fig. 4 shows the covered and uncovered blood sample before and after plasma needle treatment.

Fig.4a: Covered blood sample before and after treatment.

Fig. 4b: The uncovered blood sample before and after treatment.

The both samples are treated at time of 40sec. The uncovered blood sample (see Fig.4b) showed immediately formation of clot layer after treatment, while the sample covered with aluminum foil (as shown in Fig. 4a) did not exhibit any clot formation. Therefore we conclude that average heating is not responsible for plasma induced blood coagulation [6].

2. The effect of Argon plasma on anti-coagulant blood

This test has been made to show the ability of plasma to coagulate anti-coagulant blood, there are several types of anti- coagulants but in our research we used ethylene demine tetra acetic acid (EDTA) tubes, (EDTA) is designed to bind calcium, an important factor in coagulation process [2]. An EDTA tubes were chosen to achieve this part each tube contains 1ml of blood. Blood samples were treated for different exposure time and distances to show the ability of plasma to coagulate anti-coagulant blood. Fig. 5 illustrates the effect of argon plasma on red blood cells (RBC) and platelets (PLT) at distance 2.5cm.

At shorter exposure distance and longer exposure time red blood cells increase while platelets decrease, the significant reduction in platelets percentage was evidence of the plasma effluent on platelets. Because cold plasma produce long living and short lived neutral particles and charged particles ions and electrons. By production of nitric oxide plasma increases the aggregation and activation of platelets and production of fibrin production which accelerates blood coagulation. Fig.6 shows the effect of argon plasma on red blood cells and platelets at distance 3.5cm.

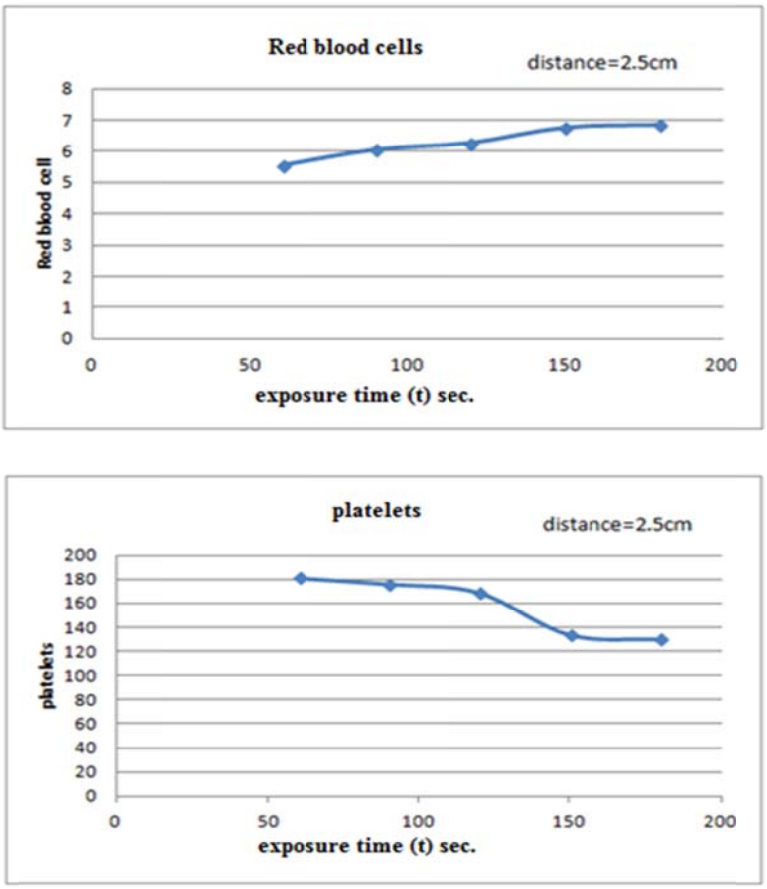

Fig. 5: Effect of argon plasma on red blood cells and platelets at distance 2.5cm.

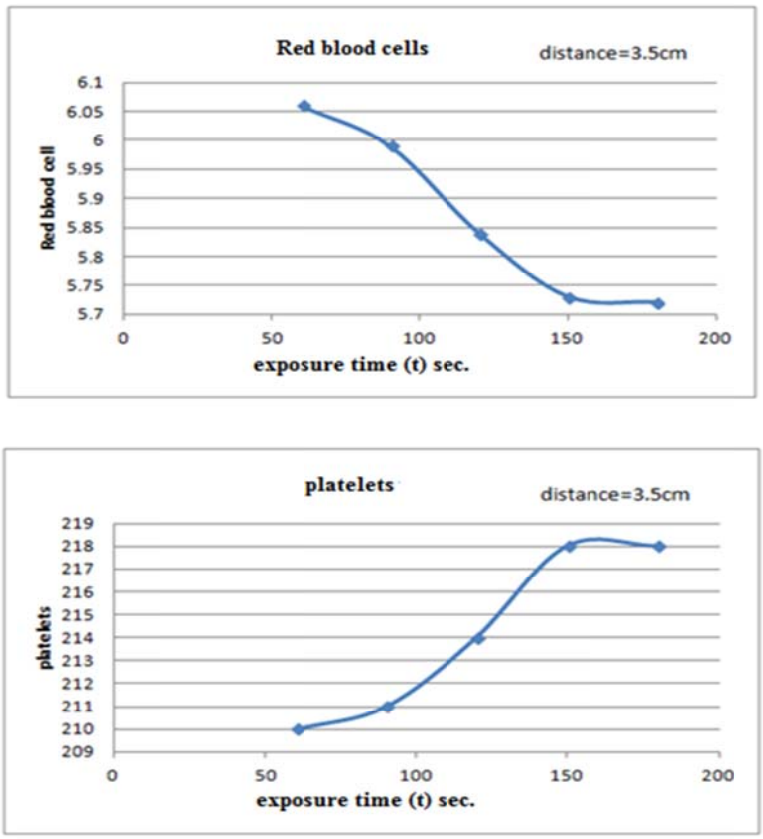

Fig. 6: The effect of Argon plasma on Red blood cells and platelets at distance 3.5cm.

At longer exposure distance RBC decrease and PLT increase because the plasma effluent is decreased by increasing the exposure distance [2,7].

Reactive oxygen species in the plasma effluent creates oxidants in the blood. These oxidants contribute to red blood cells, platelets, white blood cells interactions these interactions influence the concentration of cells suspended in blood.

3. The effect of Argon plasma needle on the concentration of Ca^{2+} in blood

Initial plasma coagulation hypothesis was focused on increase in concentration of calcium ion Ca^{2+}, which is an important factor in the coagulation process it is suggested that plasma stimulates generation of calcium ion [2]. Fig.7 demonstrates the effect of argon plasma on calcium ion at different exposure time.

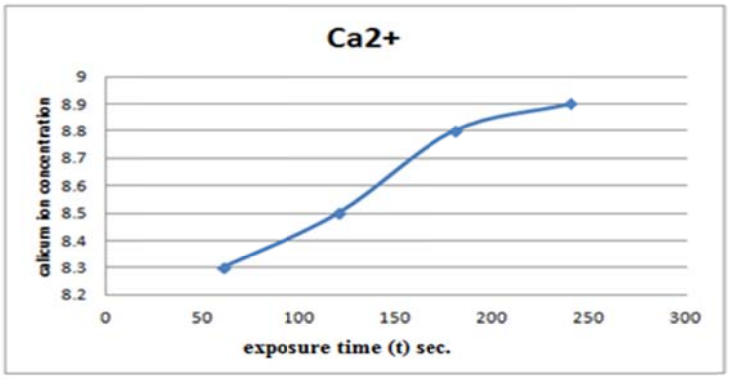

Fig. 7: The effect of plasma on concentration of Ca^{2+}.

Conclusions

The results of the current study showed that the cold plasma produced from argon significantly increases the *in vitro* speed of blood coagulation Cold plasma produces long living (O_3, NO, HO_2, H_2O_2) and short lived (OH, O electronically excited) neutral particles and charged particles (ions and electrons). 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.

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