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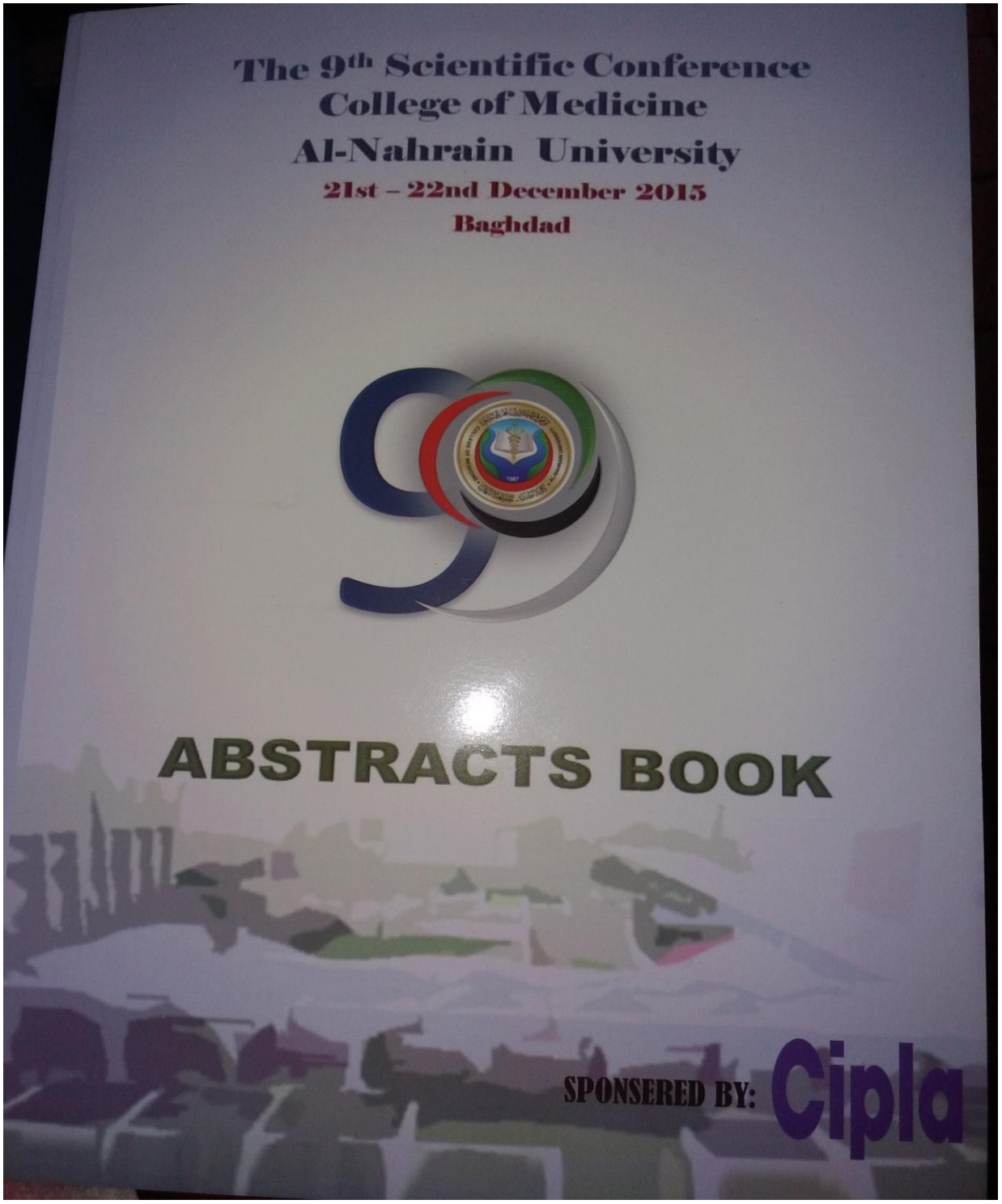
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NHCO-71-2015

Title: Enhancement of Healing wounds by Atmospheric Non- Thermal Plasma on Iraqi Patients

Author(s) & Affiliation:
Mohammed Ubaid Hussein,
Rana Talib Muhsan

Background and Objectives:
Cold plasma composed of low temperature particles and relatively high temperature electrons and they have a low degree of ionization.The dielectric barrier discharge (DBD) is most frequently used as a non-thermal plasma source that can be operated with different gasses at elevated pressures (up to atmospheric pressure).

Methodology:
The DBD plasma effects on clotting time and bleeding time were shown. Eighty patients complaining from different clinical signs and symptoms were admitted to Ramadi Teaching Hospital for seeking medical advise were included in the present study.

Results:
Plasma treatment reduced the bleeding time approximately 50%. The decreasing in the mean values of bleeding time to 2.4 minutes, while before treatment was 4.2 minutes

Conclusion:
Plasma treatment decreases the clotting time of the blood and the decreasing was increased when the applied voltage increases. It means finally the plasma treatment accelerates the blood clotting.

Enhancement of Wounds Healing by Atmospheric Non- Thermal Plasma on Iraqi Patients

Mohammed Ubaid Hussein[1],Rana Talib Muhsan[2]

[1]Department of physiology and medical physics, College of Medicine, Anbar University, Anbar, Iraq.

Email: mmphysics361@gmail.com

[2]Department of biochemistry, College of Medicine, Anbar University, Anbar, Iraq.

Key words: Atmospheric non- thermal plasma, clotting time, bleeding time

Abstract:

Non-thermal atmospheric pressure plasma has emerged as a new promising tool in medicine. The effectiveness of an Atmospheric Non Thermal Plasma (ANTP) for clinical and biological applications is studied. This research effort includes a designing of plasma generating system and examine it on some medical and biological parameters. No patient had previous operative or chemotherapeutic treatment, so the patients with hematological disorders (hemophilia) and those with open heart surgery that receiving warfarin and other antithrombotic agents were excluded from the study. A total of eighty (80) Iraqi patients seeking for a medical consultation in Al-Ramadi Teaching Hospital were included in this study. The plasma treatment is applied both *in vitro* and *in vivo* experiments on those patients. In the *in vivo* experiments, the bleeding time (BT) tests were carried out on these patients; the results showed the BT of the patients was reduced as compared with control. *In vitro* experiments regarding clotting time (CT). The results have demonstrated that this plasma device clots blood rapidly via a (control). The dependencies of the degree of clotting on the exposure time, distance have been recorded. A series of experiments reveal that this effectiveness is due to the ability of direct discharge to bring ions to blood samples due to generation of so many mediators like reactive nitrogen species and free radicals.

Introduction:

Plasma in physics is the fourth state of matter and most in the universe, as fire in the sun, stars **[1]**, while .This term was introduced by Irving Langmuir in 1928, because it resembles the ionic liquids in medicine and biology, it consists of positively and negatively charged ions, electrons and neutral species (atoms, molecules), it divides to types; hot and cold plasma.

Hot plasma or non-equilibrium plasma consists of very high temperature particles and they are close to the maximum degree of ionization, while cold plasma composed of low temperature particles and relatively high temperature electrons and they have a low degree of ionization **[2]**.

Recently there has been increased interest and development in cold plasma processes working at atmospheric pressure by the growing requirements of new plasma technology that can allow continuous plasma processing ,like plasma needle **[3,4]** ,the hair line plasma**[5]** ,micro capillary plasma jet **[6]**.

Cold plasma is used in many areas such as, surface modification of polymers **[7]**, sterilization **[8]**, and inactivation of bacteria **[9]**.

The dielectric barrier discharge (DBD) is most frequently used as a non-thermal plasma source that can be operated with different gasses at elevated pressures (up to atmospheric pressure) **[10, 11]**.

The plasma is created between two conductive electrodes connected to an ac or pulsed power source. At least one of the DBD electrodes is covered by a dielectric layer, which prevents the arc formation after breakdown. DBD discharge usually consists of a large number of short-living micro channels (filaments) that are randomly distributed over the entire area of the dielectric barrier. Despite a high breakdown voltage in gas at atmospheric pressure (several kV); the average electric current is low. Therefore, DBD plasma can be applied directly to living tissue and open injuries without causing them damage **[12, 13]**.

Wounds classically are categorized as acute, that is, abrasions, scalds, burns, or post-operative incisions, or chronic, that is, long-term wounds such as diabetic ulcers, venous ulcers, arterial ulcers and pressure sores. Acute wounds can develop into a non-healing state and/or become infected, which limits their capability of successfully going through the phases of healing, and so can also become chronic in nature**.**

Wound healing is a complex biological and biochemical process with a multitude of variables governing the process. However complicated the healing process might be, some things are clear: for wound healing to occur, tissue damage during treatment needs to be minimized or eliminated and wound needs to be sterilized to prevent bacterial invasion **[14].**

Experiment setup:

Dielectric barrier discharge DBD system is based on a conventional dielectric barrier discharge and is basically a system driven by alternating current high voltage applied between two conductors where one or both are covered with a dielectric to limit the current and to prevent transition to an arc. A simplified schematic of the treatment setup is shown in Figure (1). Here, the signal of any frequency, amplitude, and waveform is current-amplified and then voltage is stepped up in the transformer which is then connected to the "powered electrode". The electrode is, basically, a

conductor covered by a dielectric. Plasma is then generated between the surface of the dielectric and the treatment target.

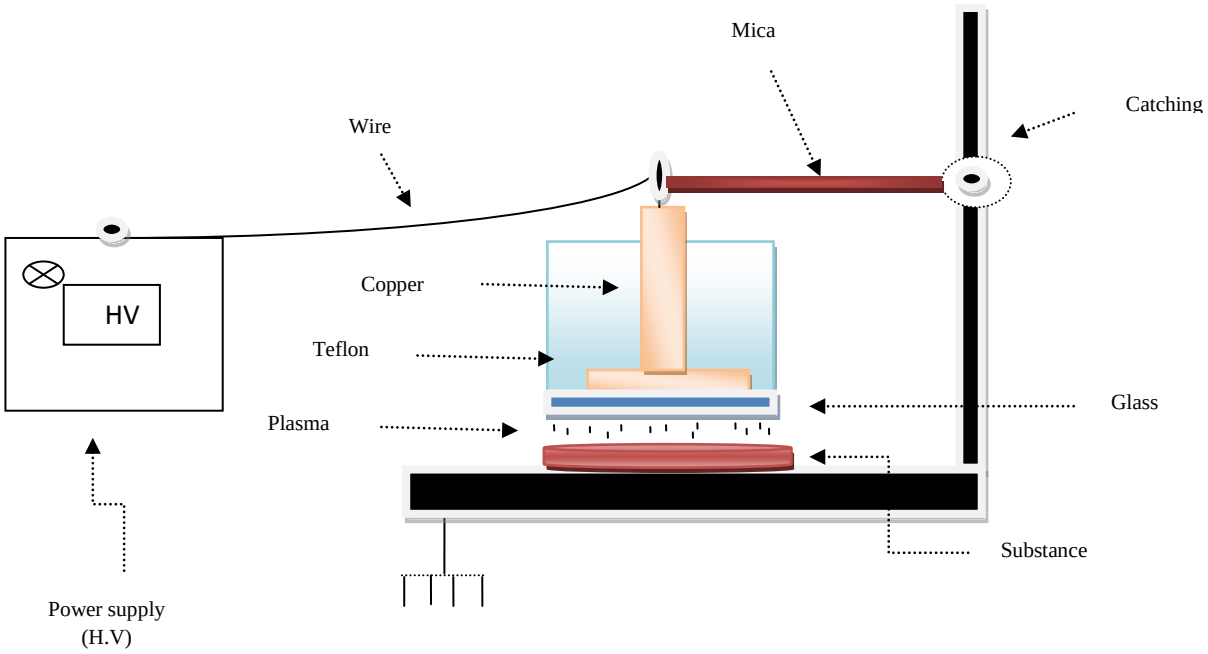


Figure (1) the device for DBD

Method:

Eighty patients complaining from different clinical signs and symptoms were admitted to Ramadi Teaching Hospital for seeking medical advise were included in the present study. No patient had previous operative or chemotherapeutic treatment, so the patients with hematological disorders (hemophilia) and those with open heart surgery that receiving warfarin and other antithrombotic agents were excluded from the study. Patients from both sexes and different age groups (20-45 year) were included in this study.

1. Coagulation time:

The patient's finger was cleaned with spirit and allow the spirit to dry, it was pricked by lancet; the first drop of blood was removed, it was squeezed, to obtain a larger drop of blood and the capillary tube with blood was filled, capillary tubes were sealed plasticine and immersed in water bath at 37^0C, after one minute start breaking small pieces of the capillary tube every 30 second until a fibrin thread seen between the two broken ends[**15**], also by same above method , capillary tubes exposure to plasma DBD.

2. Bleeding Time

The lobe of the patient's ear was Cleaned with alcohol and was let to dry, for ear-glass slide is placed behind the ear lobe and held firmly in place this provided a firm site for incision, discard the glass slide if ear lobe has been incised, the ear lobe was Pierced with the lancet .making the incision 3mm deep start the stopwatch, the blood was blooted with the filter paper at regular 30 second intervals; the filter paper was moved so that each drop touches a clean area, when the filter paper no longer shows singe of blood stop the stopwatch and record the time, normal values of 1 to 5 minutes.**[16]** , also by same above method , the lobe of the patient's ear exposure to plasma DBD.

Results and Discussions

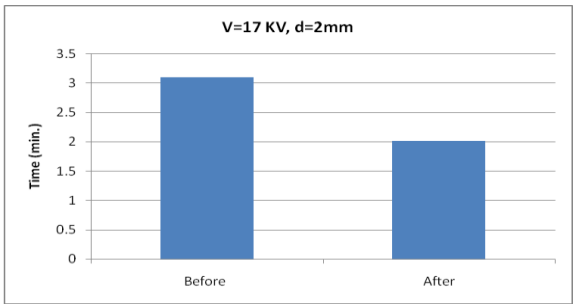
The DBD plasma effects on clotting time and bleeding time were shown. Eighty patients complaining from different clinical signs and symptoms were admitted to Ramadi Teaching Hospital for seeking medical advise were included in the present study.

1 .The Clotting Time (CT)

The blood clotting time is the time required for blood to clot in a glass tube. This time was measured for 40 patients before and after DBD plasma treatments, and a comparison between the two cases was shown in figure (2). The DBD plasma was generated under the conditions of 17kV, 22kV, 25kV applied voltages, and 2mm gap distance.

The figure (2) appears clear effect for the DBD plasma on the clotting time. The plasma decrease it approximately from 60% to 30% according to the applied voltage as in a, b and c of the figure. In other words the plasma treatment decreases the clotting time of the blood and the decreasing was increased when the applied voltage increases. It means finally the plasma treatment accelerates the blood clotting.

(a)



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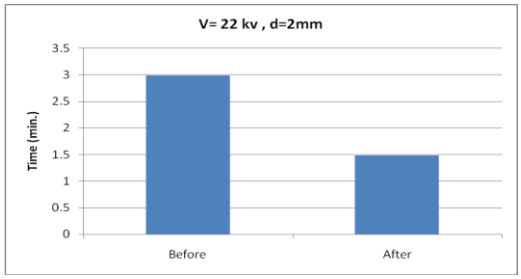


(c)

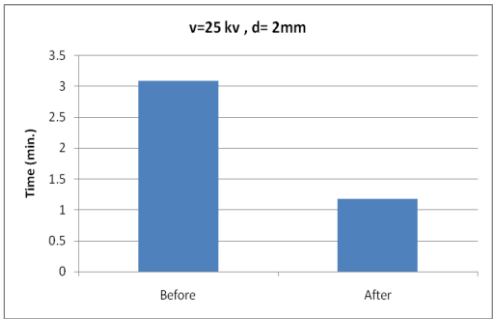
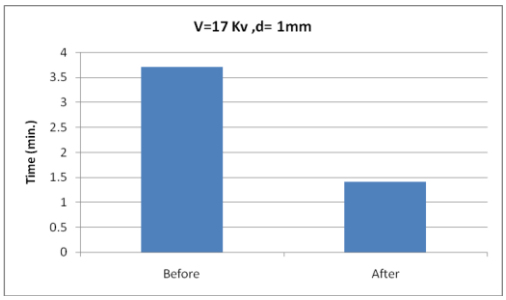


Figure (2): *Mean values of blood clotting time before & after DBD plasma treatments when the gap distance is 2mm and applied voltages are a) 17kV, b) 22kV, and c)25 kV.*

(a)



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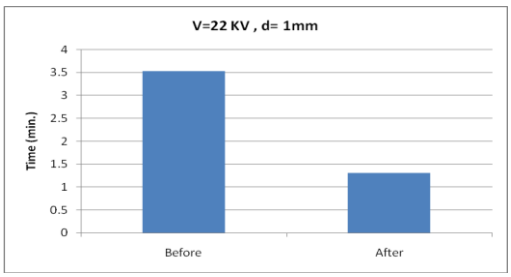

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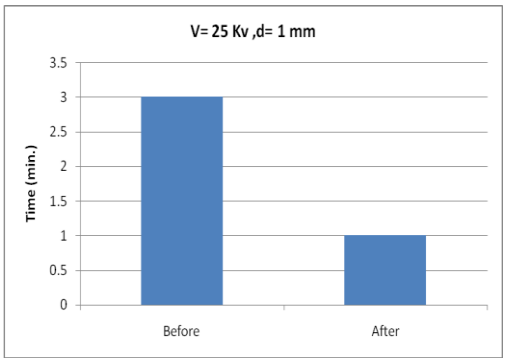


Figure (3): *Mean values of blood clotting time before & after DBD plasma treatments when the gap distance is 1mm and applied voltages are a) 17kV, b) 22kV, and c)25 kV.*

Regarding the three pervious figures (3), it is obvious that the clotting time of the studied patients was affected by the applied voltage variation, where the increasing in the applied voltage affects the properties of (DBD) plasma such as the electric field, electron density, and the concentration of active species which depend on the applied voltage.

The physical and medical affects of (DBD) significantly depend on the duration and the applied voltages. That was affecting the homogeneity of the plasma and composition activity biology of types generated plasma.

Then, to show the DBD plasma treatment effect on the clotting time, when the gap distance becomes less than the previous. The above measurements were repeated at gap distance 1mm. The results of this case were shown in figure (3).

Figure (3) shows the mean values of clotting time of the studied patients before and after DBD treatment. It is obvious the mean values of clotting time of patient before DBD treatment and after DBD treatment. It is clear that clotting time decreased with the DBD plasma treatment.

The reducing of the distance between the electrodes an important role in the generation of plasma, where DBD plasma characteristics in air depending on applied voltage as well as inter-electrode distance **[17].** That was lead to reduce the blood clotting time and thus accelerates blood clotting.

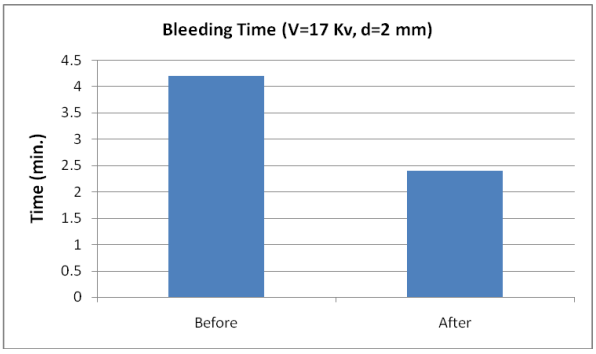
When comparing figure (2) with figure (3) for the same voltage, and variable distance, it is clear the mean values of clotting time before and after treatment by DBD was different, this refers to the role of gap distance in the acceleration of blood clotting.

The discharge ignites when the powered electrode approaches the surface to be treated at a distance (discharge gap) less than about 3 mm, depending on the form, duration and polarity of the driving voltage, so the increase in distance leads to the generation of plasma slim, thus decreasing the concentration of the ion, the acceleration does not occur in the blood clotting **[14].**

2. The Bleeding Time (BT)

Bleeding time is the time required for blood vessel constriction and platelet plug formation. It is the duration of bleeding after controlled standardized puncture of the earlobe. It was measured for 40 patients before and after DBD plasma treatments. The treatment is by exposing, to the DBD plasma, the ear lobe after acupuncture. That is under the conditions of gap distance of (1,2mm) and applied voltage of 17kV.The results were presented in figure (4).

(a)



(b)

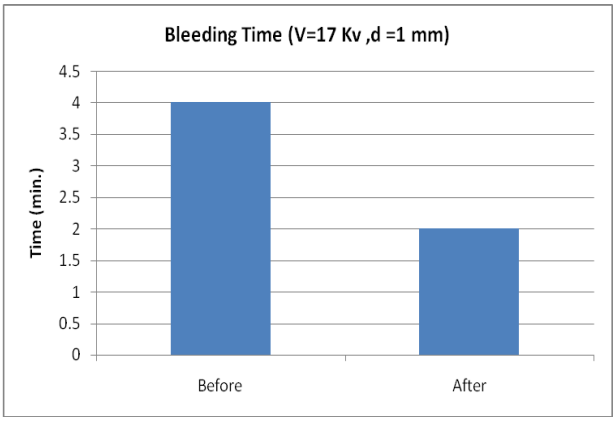


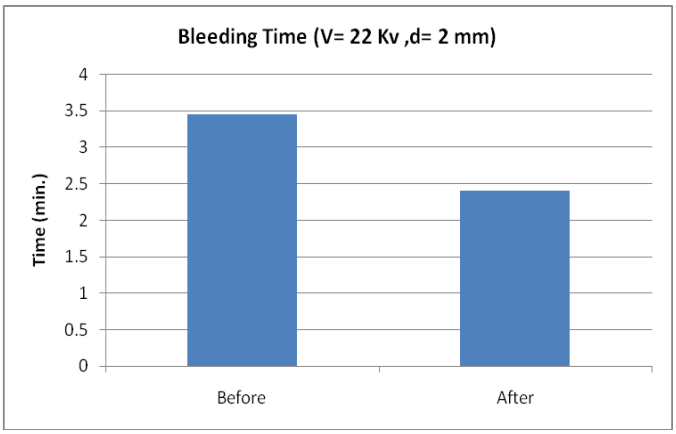
Figure (4): *Mean values of bleeding time before & after DBD plasma treatments when applied voltages are 17kV and the gap distance is a) 2mm b) 1 mm .*

Figure (4) shows clearly that, the plasma treatment reduced the bleeding time approximately 50%. The decreasing in the mean values of bleeding time to 2.4 minutes, while before treatment was 4.2minutes in case (a).In case (b) , the decreasing in the mean values of bleeding time to 2 minutes , while before treatment was 4 minutes , the bleeding time more decreases because of gap distance decreasing.

For other applied voltage (22kV) and other gap distances (2,1mm) the bleeding time was presented in figure (5). The figure shows same previous behavior but larger effect; the bleeding time more decreases because of the applied voltage increasing and

gap distance decreasing. The mean values of bleeding time after plasma treatment is 2.4 minutes, while before treatment was 3.4 minutes in case (a). In case (b), the mean values of bleeding time after plasma treatment is 1.7 minutes, while before treatment was 3.7min.

(a)



(b)

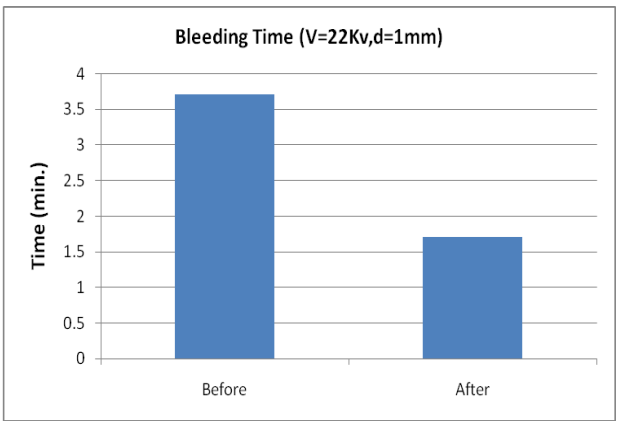


Figure (5): *Mean values of bleeding time before & after DBD plasma treatments when applied voltages are 22*Kv *and the gap distance is a) 2mm b) 1 mm.*

Increasing applied voltage affects the discharge, also decreasing gap distance and therefore, the plasma ions induce a number of clotting processes, thus accelerates blood clotting and stop bleeding **[18].**

The effective non-thermal plasma-medical system permits to achieve effective blood coagulation and stop bleeding without any thermal effects, ability of plasma to hasten wound healing through wound sterilization **[19].**

That DBD plasma is able to promote platelet activation and formation of fibrin filaments, platelets release a multitude of growth factors including platelet-derived growth factor (**PDGF**),a potent chemotactic agent, and Transforming Growth Factor-β (**TGF-β**), which stimulates the deposition of extracellular matrix. Both of these

growth factors have been shown to play a significant role in the repair and regeneration of connective tissues **[20]**. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor(**VEGF**) [**21**].

The cold plasma and using (DBD) has the ability to accelerate blood clotting effect on the concentrations of blood proteins normal, so the ions existing in cold plasma stimulates a number of thrombosis, and the greater the concentration of ions, this leads to an acceleration in the coagulation, hence conclude the plasma ion concentrations affects the blood and the likelihood of being able to stimulate blood clotting.

Analysis of changes in concentration of various blood proteins and clotting factors indicates that DBD plasma aids in promoting the advancement of blood coagulation, or in other words. Plasma is able to catalyze the complex biochemical processes taking place during blood coagulation **[18].**

Direct non-thermal plasma can trigger natural, rather than thermally induced, coagulation processes; it was observed that the release of calcium ions and change of blood pH level, which could be responsible for coagulation, is insignificant **[19].**

Plasma treatment is shown to "consume" coagulation factors (proteins and enzymes) and a visible film is formed on the surface of the treated samples. Increase in the sample volume and keeping the surface area fixed decrease the effect, indicating that plasma treatment initiates clot formation at the surface, not in the volume [**18, 19].**

Conclusions:

Plasma device clots blood rapidly. The dependencies of the degree of clotting on the exposure time, applied voltage and distance have been recorded. A series of experiments reveal that this effectiveness is due to the ability of direct discharge to bring charges to blood samples due to generation of so many mediators like reactive nitrogen species and free radicals. This DBD air plasma could be a promising technique in applications for biological and medical materials contaminated with

microorganisms. Results presented in this paper indicate that selective conversion of fibrinogen into fibrin is one of the potential mechanisms by which non-thermal

Plasma initiates blood coagulation. Further investigations are needed to determine the specific mechanisms of activation of fibrinogen by non-thermal plasma treatment.

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تحسين التئام الجروح باستخدام بلازما غير حرارية بضغط جوي على المرضى العراقيين

محمد عبيد حسين [١]، رنا طالب محسن [٢]

[١]قسم الفسلجة والفيزياء الطبية –كلية الطب –جامعة الانبار–الانبار–العراق

[٢]قسم الكيمياء الحياتية–كلية الطب –جامعة الانبار–الانبار–العراق

الخلاصة:

البلازما غير الحرارية بضغط جوي برزت كأداة ترويجية في الطب .التأثير لبلازما غير حرارية بضغط جوي للتطبيقات البايلوجية والسريرية كانت مدروسة.الجهد لهذا البحث تضمن تصميم لنظام توليد البلازما وتجريبها على بعض العوامل البايلوجية والطبية .تم استبعاد المرضى الذين لهم عمليات سابقة أو معالجات كيمياوية ،كذلك المرضى باضطرابات أمراض الدم (الهيموفيليا) وأولئك بجراحة القلب المفتوح الذين يستلمون الوارفارين وعوامل اخرى مضادة للتخثر كانت مستثناة من الدراسة. العدد الكلي كان ٨٠ مريضا عراقيا قصدوا الاستشارية الطبية في مستشفى الرمادي التعليمي المتضمنة في هذه الدراسة .معالجة البلازما تطابقت في كل من التجارب داخل وخارج الكائن الحي على هؤلاء المرضى.في تجارب داخل الكائن الحي،اختبارات زمن النزيف كانت محمولة على هؤلاء المرضى،النتائج بينت زمن النزيف للمرضى كان منخفضاً مقارنة مع القيمة الاعتيادية .في اختبارات خارج الكائن الحي بخصوص زمن التخثر .النتائج أثبتت بان هذا الجهاز البلازما يخثر الدم وبسرعة بالمقارنة مع القيمة الاعتيادية(السيطرة). اعتماد درجة التخثر على زمن التعرض ،المسافة كانت مسجلة .سلسلة التجارب توضح بان هذه التأثير ناتج من قدرة التفريغ المباشر لجلب الايونات إلى نماذج الدم بسبب توليد عدد من الوسطاء مثل انواع النتروجين المتفاعلة والجذور الحرة.