Journal of Engineering and Applied Sciences 13 (Special Issue 13): 10657-10663, 2018
ISSN: 1816-949X

Floating Electrode Dielectric Barrier Discharge (FE-DBD) System for Biolological Applications

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Abstract: The Floating Electrode-Dielectric Barrier Discharge (DBD-FE) was designed for production of cold atmospheric plasma to be used in biological applications. The designed Floating Electrode-Dielectric Barrier Discharge device was consist of two main parts: FE-DBD probe and high voltage power supply. Two types of probes were used according to the applications of the system: small probe of diameters 2-5 mm this is suitable for *in vitro* experiments and large probes of diameters 10-20 mm for *in vivo* applications. The HV power supply was designed, especially, to fid FE-DBD probe by alternative high voltage it can provide a variable Voltage (0-25 kV) and variable frequency (0-30 kHz). The device was designed in a way to be is able to change the output power of the produced cold plasma of five different level (1, 2, ..., 5) this is done by the main switch power fixed in the interface of the device. The output power was ranged 6-57 W and temperature of the produced plasma ranged 32-45°C, therefore, it is suitable for the biological applications. A cold plasma produced by the device was tested by exposure a natural tissue to the plasma it is found that it do not cause any toxicity to the natural tissue. Laboratory study was carried out on tissue culture in a circumvented circumference using breast tissue for tissue culture it was found that the cold plasma treatment would not cause any damage for, liver, kidney and spleen tissue. *In vitro* experiments, the cytotoxicity was calculated normal tissue (ref) under different doses of CAP, 5, 10 and 15 sec it was found the ref exhibition was very little or no cytotoxicity.

Key words: Dielectric barrier discharge, floating electrode, high voltage, cold plasma, laboratory, exhibition

INTRODUCTION

Plasma is the fourth state of matter where the substance is transformed from a solid state into a liquid and then into a plasma with an increase in the energy supplied to it (Stoffels *et al*., 2002). Plasma is an electrified gas included chemically reactive medium which consists of various species as positive or negative ions, electrons, gas atoms, free radical and molecules, either terrestrial or at any higher state (Fig. 1). It can be found at an extremely huge range of pressure and temperature, so, it can be generated at atmospheric pressure by the conjugation of energy with a gaseous media by using chemical, thermal, nuclear or radical means or by electromagnetic waves injection or voltage application to dissociate the gaseous media into electrons, ions, free radicals, photons and atoms in metastable state (Nehra *et al*., 2008), Fig. 2 summarized the types of species in plasma.

The plasma is divided according to its temperature to two main parts: thermal, non-thermal or cold. Thermoplastic plasma is the one in which electrons and heavy particles (the ions neutral particles) have the same temperature. Non-thermal plasma is where the electron temperature is much higher than the temperature of the heavy particles. This type of plasma has a temperature of <40°C. There are many ways to produce non-thermal plasma including Dielectric Barrier Discharge (DBD), corona discharge, Atmospheric Pressure Plasma Jet (APPJ) and plasma needle.

The discharge system of the dielectric isolator FE-DBD for discharge includes when the electrode energy is approaching the surface to be processed at a distance (discharge gap) <3 mm (Fridman *et al*., 2007). The system of discharge with the barrier of the electrostatic barrier of the party to produce cold plasma has wide applications can be summarized in the following areas: its effective effect in the treatment of many diseases of cancer (Laroussi *et al*., 1999).

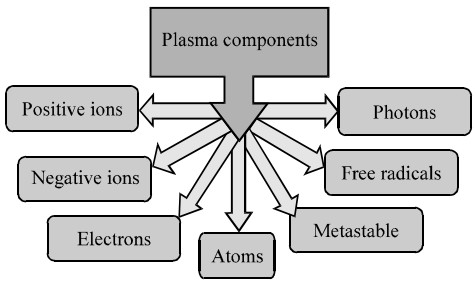

Fig. 1: Plasma components (Nehra *et al*., 2008)

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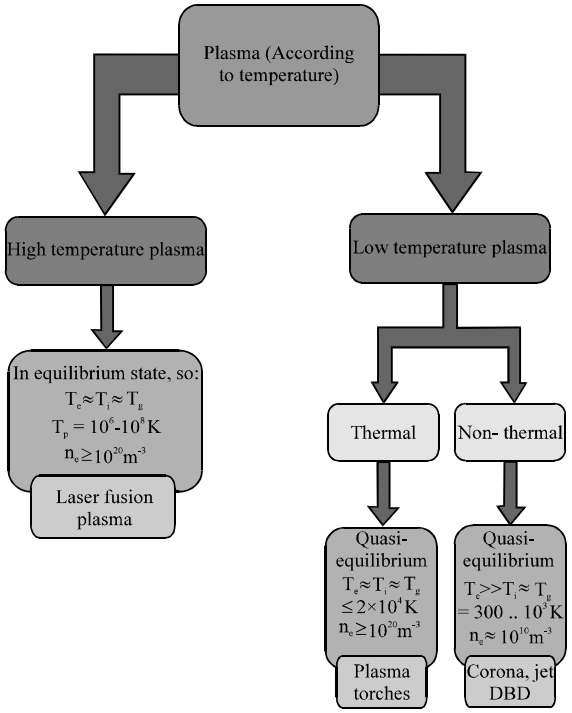

Fig. 2: Plasma classification according to its temperature

MATERIALS AND METHODS

FE-DBD probes: Two types of probes were used according to the use of the system as the following. probes of small diameters, consists of two basic parts.

The base: A plastic vial of length 10 cm and diameter 4 cm with changeable cap which is used as a base for the external probe and an isolated metal wire passes through it which ends with a spring. The other side of this spring is connected to a conductive metal piece that penetrate a circular insulation barrier of diameter equal to the inner diameter of the vial. Both of spring and barrier are fixed in the outer entry of the vial such that the conductive metal directed outside the vial to represent the high voltage electrode as shown in Fig. 3.

The external probe of floating electrode: Probe diameter ranges between 2-5 mm. This probe consists of metal rod (stainless steel) covered by an insulator (Pyrex glass) of thickness 1 mm as shown in Fig. 3, fixed on base-installable cap of the vial, so that, the probe is passing through the cap to connect a metal slice. When the probe is tightened to the base, the metal tip would be connected to the external side of the base to generate a complete electrical circuit. The poles were designed in various diameters 2-5 mm as shown in Fig. 3 and 4.

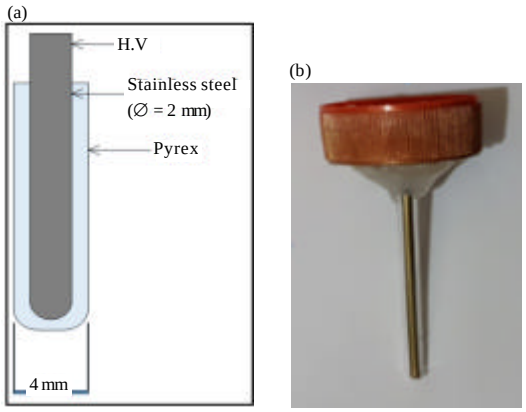

Fig. 3a, b): Small pole of proposed FE-DBD

Large probes of diameters 10-20 mm (used for *in vitro* experiments) which is made of copper cylinder of length 150 mm with a spindle of diameter 2 mm, the spindle was ended by floating base of diameter 20 mm and the other end of spindle is connected to High Voltage (HV) terminal. The floating base was covered by insulator (fiber) and a layer of Pyrex glass of thickness 1mm placed on the upper side (cold plasma generation side) of the system as shown in Fig. 5. Both types of probes are connected to high voltage source to represent one of the electrodes of the proposed FE-DBD system while the other electrode is represented by the live tissue. When the probe approaches near the tissue with a distance <3 mm it would start discharging and generates the plasma between the probe and the tissue (Fig. 6).

HV source: The HV source was designed, especially for the proposed system it can provide a variable voltage (0-25 kV) and variable frequency (0-30 kHz) as shown in Fig. 7 and 8. The circuit is based on a special kind of transformer called Flyback Transformer (FBT) or Line Output Transformer (LOPT). This type of transformers is able to raise the voltage to several thousand volts such type of circuits is fed by continuous voltage equal to 12 V. This circuit depends on an Integrated Circuit (IC) which is called timer 555 and represented by oscillator and timer, the oscillation time changed from few to thousands of pulses per second. The output voltage is variable up to 25 kV and its frequency varies up to 30 kHz. The output HV is measured by high voltage probe of type (Mode P650A by Tektronix), the attention of the probe was 1:1000 (Fig. 9).

***In vitro* cytotoxicity assay:** A cold plasma product can be tested by this system by a normal tissue it is found that it do not cause any toxicity to the normal tissue. A

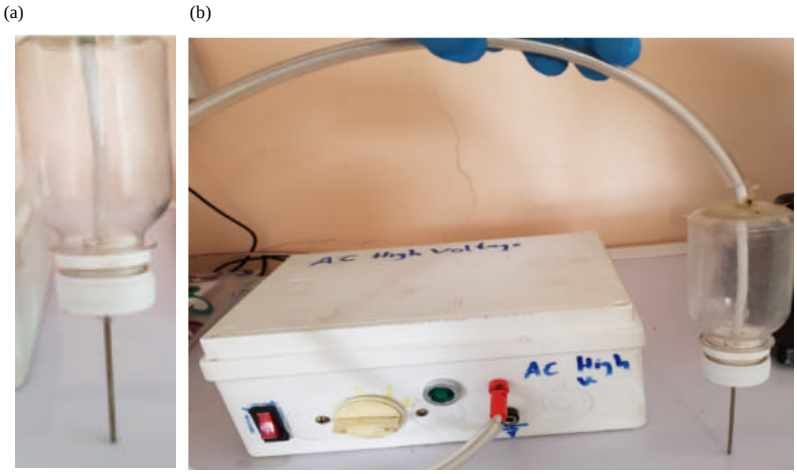

Fig. 4: Proposed FE-DBD system of small poles

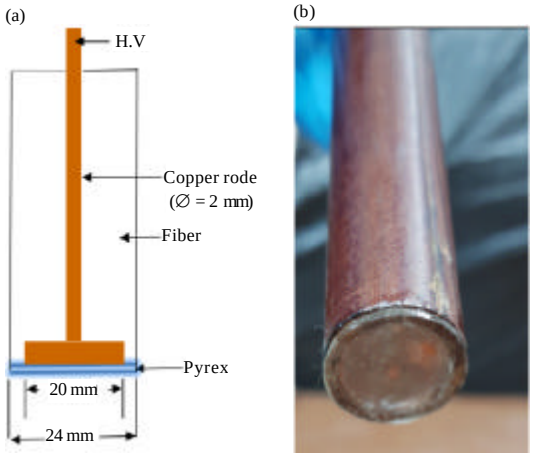

Fig. 5: Large probe of proposed FE-DBD

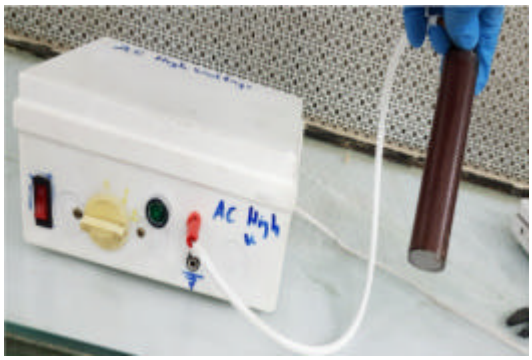

Fig. 6: Proposed FE-DBD system of large poles

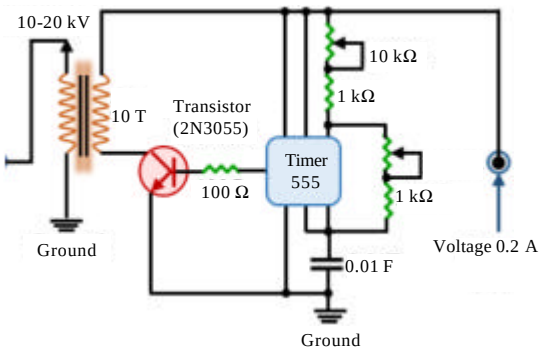

Fig. 7: The used electrical circuit for high voltage source

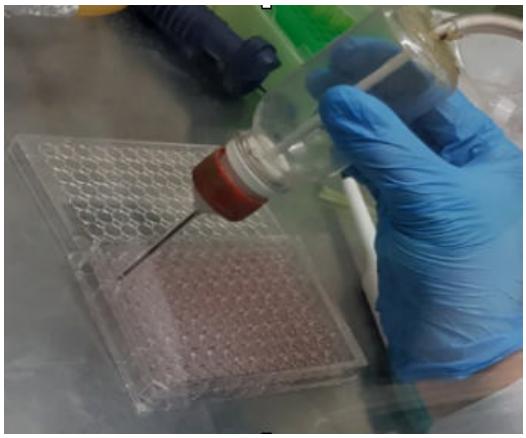

Fig. 8: CAP exposure tissue culture

laboratory study was carried out on tissue culture in an sterilized environment using tissue for tissue culture a microplate of 96 (12×8) well was used, each well was used to seed 10, 000 normal cells to be incubated at 37°C for 24 h until monolayer was achieved (inverted microscope is used to confirm monolayer formation).

Fig. 9: *In vivo* study show: a) The exposure of cold plasma and b) Gross examination of the exposed mice showed no vesible lesion in all internal organ

Cells were exposed to CAP using proposed plasma generator system with small diameter probe for three different intervals 5, 10 and 15 sec and some cells were left without treatment as a control. The described steps were done in triplets and re-incubated at 37°C for different intervals, 24, 48 and 72 h. After incubation, the growth medium was decanted off. These steps were repeated three times to confirm the veracity. Crystal violet assay is added (100 μL/well) for capturing (Fig. 10) then incubated for 20 min at 37°C after that, the microplate is washed by water to be ready for capturing. Then, microplate reader device is used to read the three experiments to calculate the percentage of live cells (inhibition rate). The calculated mean of inhibition percentages of 24, 48 and 72 h were recorded separately for results analysis.

***In vivo* toxicity study:** A study was to ascertain if cold plasma treatment would cause any damage, liver, kidney and spleen tissue or to the animal itself (mice female). To ascertain this effect, 20 mice were divided into 4 groups of three animals in each group. The first group was a control group which did not receive any treatment and the other 3 groups remained as experimental groups. Was exposed the cold plasma. Histology analysis also, showed no macroscopic damage was induced to this tissues by plasma treatment (Fig. 11-13).

The characteristics and results: Different probes are made according the type of application all of them elaborate under the same principle; Metal pole covered by insulation material and high voltage source. The system has lightweight with a small volume of dimensions (10×15×25 cm) which easy to use and carry to hospitals, medical clinics or research center. High efficiency in cancer cells treatment with short duration. It is possible to construct a very smaller probe that can be used for endoscopy. The system is able to change the intensity of the cold plasma output of five different values by the main switch power capacity installed on the interface of the device which has 5 choices (1, 2, ..., 5) where the capacity of the plasma varies according to each choice as shown in Table 1 and 2.

Table 1: The different parameter of system

Levels	V_{out} (kV)	I_{out} (mA)	Power (I.V) (W)	Frequency (kHz)
1	13.200	0.50	6.60	70.0
2	17.600	0.62	10.90	71.8
3	23.760	0.70	16.60	73.9
4	27.280	1.10	30.00	74.4
5	33.440	1.70	56.84	76.8

Table 2: Temperature and dose of cold plasma

Electrode diameter (cm)	Electrode area (cm^2)	Plasma dose (P×t/Area) (J/cm^2) t = 5 sec	Plasma dose (P×t/Area) (J/cm^2) t = 10 sec	Plasma temperature (C°)
0.4	0.125	264	528	32.9
0.5	0.196	168	336	33.5
0.6	0.282	117	234	37.4
0.7	0.384	85.9	171	39.2
1	0.785	42	84	42.1
2	3.140	10.5	21	45.0

The experiments were done at Iraqi Center for Cancer and Medical Genetics Research and were analyzed by graph pad prism V 7.0 to estimate the behavior of the proposed CAP system *in vitro* experiments, the cytotoxicity was studied normal cell line (REF) under different doses of CAP, 5, 10 and 15 sec, REF cells

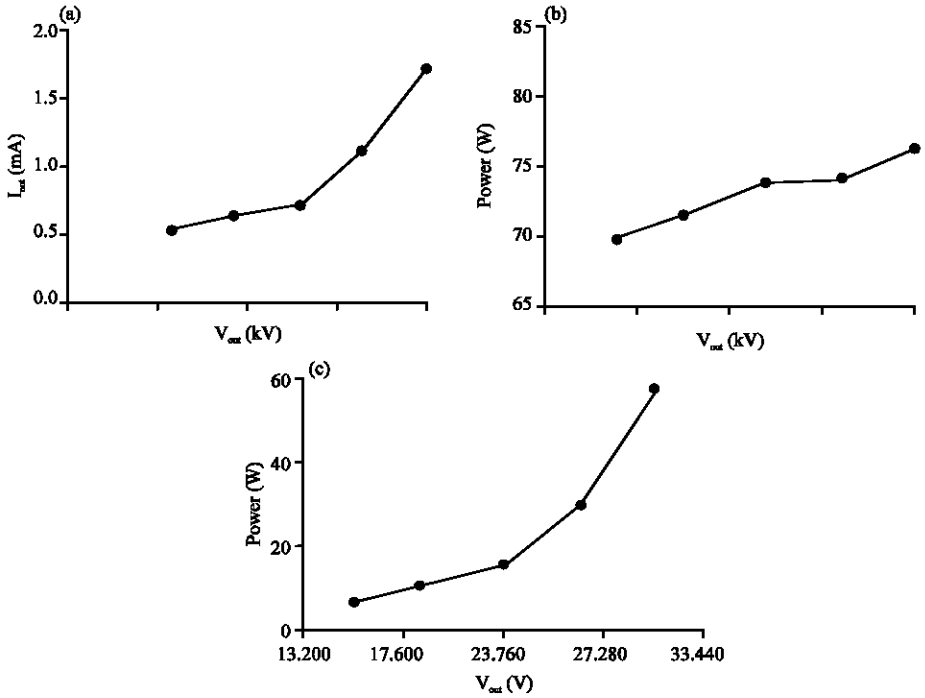

Fig. 10: Proposed system electrical characteristic: a) 1-5 curve; b) Frequency and c) Power

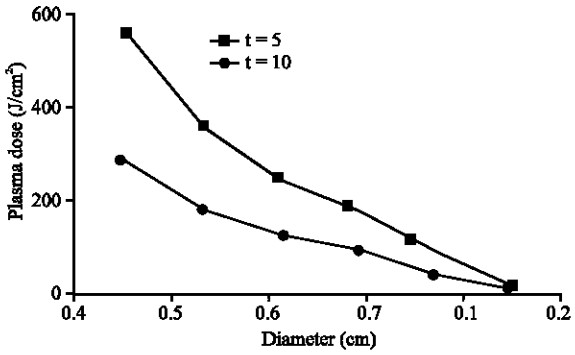

Fig. 11: Plasma dose according to probe diameter

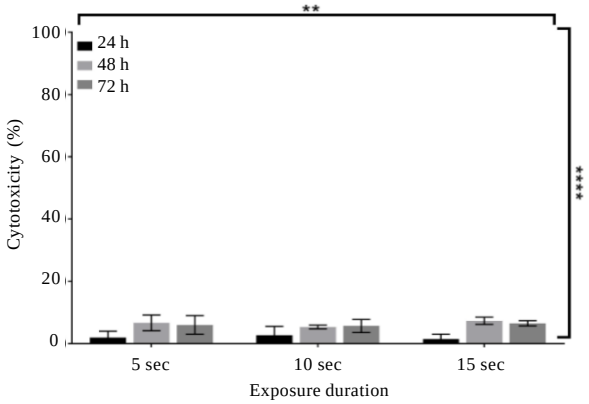

Fig. 12: Cytotoxicity of normal cells (ref) to different exposure

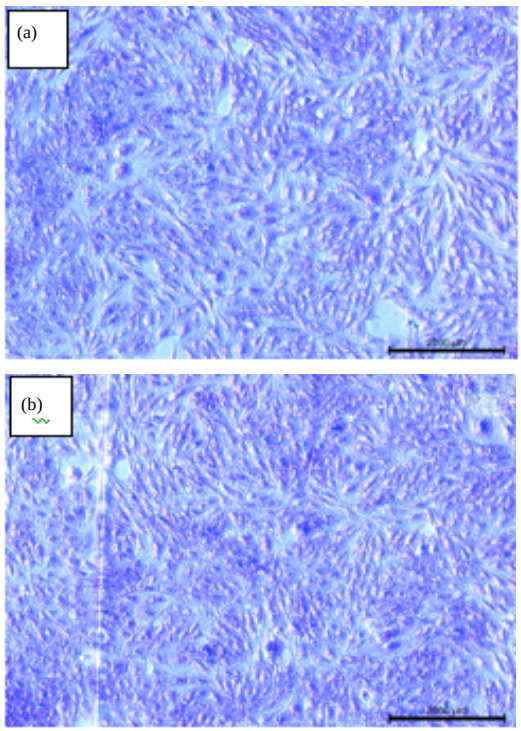

Fig. 13: Normal cells: a) Control cells under inverted microscope and b) CAP-treated under inverted microscope (Both treated and untreated showed no morphological changes)

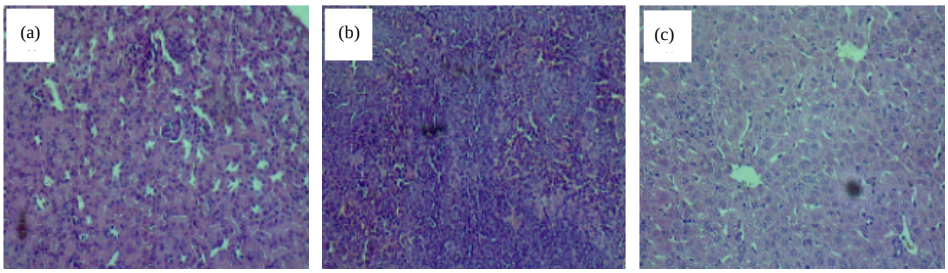

Fig. 14: Histological sections in the internal organs of group control (20×); a) Normal structure of kidney; b) Normal structure white pulp and red palp and c) Normal hepatic parenchyma structure

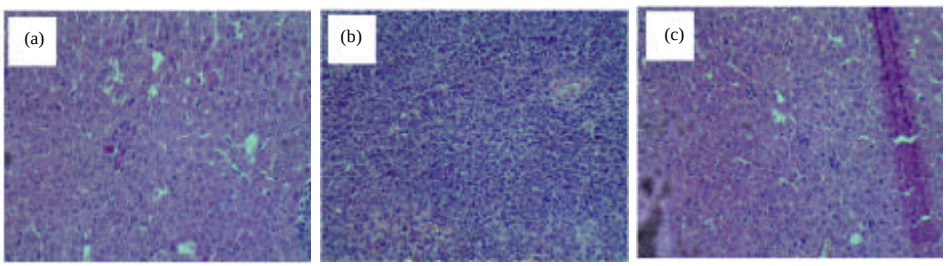

Fig. 15: Histological sections in the internal organs of group plasma (20 sec exposure): a) Congestion and hemorrage glomerly tubler necrosis nerowing of tubler lumen; b) Spleen hemorrage in red palp and c) Hepato size with congestion RBC and patchy hemrrage and inflomatry lempho size

exhibited very little or no cytotoxicity, Fig. 12 shows the behavior of cytotoxicity. Histopathological examination for organ study was taken in consideration and was captured using different magnification factors as show in Fig. 14 and 15. Gross examination Fig. 15 reveded no pathological changes to the internal organs, histological examination to the treated animals showed no considerable signes of toxicity in liver, spleen while kidney showed congestion and hemorrage and tubular necrosis associated with high dosses in Fig. 15a-c.

COCLUSION

In the field of environment (Janga *et al*., 2013; Garcia-Alcantara *et al*., 2013) Medical applications in the treatment of dermatology and dentistry (Fridman *et al*., 2008; Chiper *et al*., 2011). Its effective effect in the purification of bacteria (Chiang *et al*.,2010; Wagner *et al*., 2003). Use in wound treatment (Eliasson *et al*., 1987) and proved its effectiveness on blood clotting (Kogelschatz, 2003).

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