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ORIGINAL ARTICLE

Blood Coagulation and Living Tissue Sterilization by Floating-Electrode Dielectric Barrier Discharge in Air

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Abstract Thermal plasma discharges have been widely used in the past for treatment of living human and animal tissue. However, extensive thermal damage and tissue desiccation occurs due to extreme temperatures. Some solutions have been offered where the temperature is lowered by short current pulses, addition of noble gases, or significant decrease in the size of treatment electrodes. We propose a method of direct treatment of living tissue that occurs at room temperature and pressure without visible or microscopic tissue damage. The presented Floating-Electrode Dielectric Barrier Discharge plasma is proven electrically safe to human subjects and our results show no gross (visual) or histological (microscopic) damage to skin samples in minutes, complete tissue sterilization from skin flora in seconds, and blood clot formation in seconds of electric plasma treatment. We also observe significant hastening of blood clot formation via electric plasma induced catalysis of "natural" processes occurring in human blood. A model describing these processes is offered.

Keywords Non-thermal plasma · Sterilization · Disinfection · Blood coagulation · Blood coagulation model · Blood clotting · Wound healing

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1. Introduction

The term "plasma," commonly employed in physical sciences for ionized medium, originally stems from the biological and medical fields[1]. Despite this historical connection, electrical plasma (e-plasma, electrical or gaseous discharge) is typically associated with high energy physics or low pressure processes employed in the semiconductor industry, while being rarely used in medical applications directly. The few known uses of e-plasma in medicine are based mainly on high temperature generated by conventional thermal discharges. One good example of this is the Argon Beam or Argon Plasma Coagulator (APC) developed mainly to cauterize wounds [1]. This device generates e-plasma in flowing argon through ionization by high frequency ($\geq$ 350 kHz) electrical discharge [2]. The flow of argon takes e-plasma outside the ionization tube creating a jet that impinges onto the tissue. High temperature of the e-plasma ($\sim$ 10,000 K) leads to rapid cauterization and tissue desiccation. The procedure is painful and significant thermal tissue damage (up to 7 mm deep) results in prolonged healing [1–3]. In general, today's medical community is shifting the preference toward non-equilibrium room temperature discharges where thermal damage is minimized or eliminated. Main idea of the presented work is to introduce direct e-plasma treatment of living tissue where sterilization occurs without any visible or microscopic damage and blood is coagulated in an open wound without damage of the surrounding tissue.

Initial steps in elimination of thermal damage by e-plasma have been made earlier. The most obvious is, of course, pouring saline[2] over the arc to cool it off and prevent any significant tissue desiccation [2]. A more refined e-plasma-based surgical tool that has recently been reported is the Pulsed Electron Avalanche Knife (PEAK). In this device, thermal damage to the tissue is reduced by keeping the current pulses short (microseconds) and the electrode thin (microns). Resulting streamers (micro-sparks) of e-plasma, forming under the micro-wire, deposit significant energy into the tissue rupturing it quickly without causing destruction of surrounding areas. Precision cutting has been demonstrated in this way [4, 5]. Further advances in the non-thermal direction have been made by further size reduction of the needle tip and introduction of noble gases, where discharge power can be significantly lower. A discharge capable of a much gentler, non-thermal interaction with tissue, "*plasma needle*", has been proposed [6]. It involves a "glow" discharge igniting at the end of a sharp pin in flowing helium upon application of a radio frequency ($\sim$ 13 MHz) electromagnetic excitation. This discharge operates at near room temperature, dissipating milli Watts in several cubic millimeters. Suggested applications include treatment of dental cavities and skin disorders. This e-plasma has been demonstrated to destroy cells and bacteria in a highly localized fashion without disturbing the nearby tissue [6]. While the localized and non-thermal nature of a *plasma needle* can be beneficial, its power and temperature will be still high if the device is scaled up owing to the thermal nature of atmospheric pressure RF discharges. The challenges offered by biology and medicine still require reasonable sizes and room-temperature[3] discharges which we address in our work.

In this paper we report a new non-thermal, room temperature e-plasma discharge operating in open air which is safe for treatment of living animal or human tissue (Fig. 1). This

[1] This term is often attributed to Irwing Langmuir who used it to capture life-like appearance of electric discharge in gas phase and its similarity to electrolyte fluids, like blood, which consist of many ions, charged and neutral molecules, and dipoles.

[2] "Saline" is a solution of 0.9% weight/volume of sodium chloride in sterile purified water.

[3] Herein by "room temperature" we mean biologically compatible temperature range of 22 to 36°C, depending on the surface or tissue.

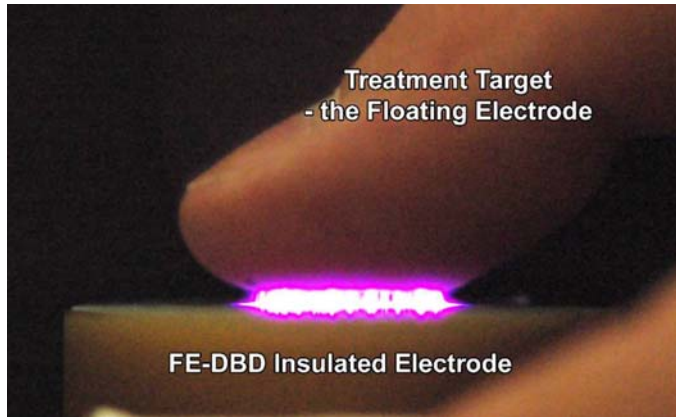


Fig. 1 Electrically safe non-thermal e-plasma for treatment of living tissue without causing damage

approach allows for novel treatment of biological and medical surfaces where no tissue damage is observed while some biological processes are initiated and/or catalyzed. Additionally we offer a model describing the influence of this e-plasma on concentrations of various ions in blood plasma.[4] The principle of operation of the proposed e-plasma is similar to Dielectric Barrier Discharges (DBD) introduced by Siemens in the middle of 19th century [7, 8]. It occurs at atmospheric pressure in air when sufficiently high voltage of continuous waveform or pulses of short duration are applied between two insulated electrodes. The presence of the insulator between the electrodes prevents the build-up of high current. As a result, the discharge creates e-plasma without substantial heating of the gas, thus offering no medium limitations (i.e. need for lower pressure or noble gases). We found that it is possible to replace one of the electrodes by an object with high capacity for charge storage—a "floating electrode"(FE). Living tissue of animal or human body with its high water content and a relatively high dielectric constant has the required high capacity for charge storage [9, 10] and, therefore, can easily be employed as the FE of the DBD e-plasma. In this case the FE-DBD e-plasma is created in the gap between the living tissue and the other insulated electrode. While the current in the gaseous discharge gap is mainly due to motion of charge carriers (electrons and ions), it continues mostly in the form of displacement current through the tissue. Moreover, most of the energy due to the e-plasma current is dissipated in the gap, just like in the case of a conventional DBD. Thus, in the FE-DBD we obtain non-thermal e-plasma that remains at room temperature throughout the operation of the system while active species, radicals, ultraviolet radiation, and sub-millimeter scale temperature fluctuations offer "synergetic" effect in tissue treatment. Moreover, the e-plasma generated in this way can be applied directly to a living human tissue without thermal or chemical damage [11–13]. In addition to sterilization of tissue, we demonstrate that the FE-DBD e-plasma rapidly coagulates blood. Rather than a physical influence, we observe that e-plasma catalyses the natural blood coagulation processes as was confirmed by experimental data and a model based on e-plasma influence on ion concentrations in blood plasma.

[4] "Blood" consists of two major parts: blood cells and blood plasma. *Blood cells* are red blood cells, platelets, and white blood cells (lymphocytes, monocytes, neutrophils, eosinophils, and basophils). *Blood plasma* consists of water, ions (calcium, magnesium, sodium, etc.), large organic molecules (amino acids, glucose, lipids, nitrogenous waste, and proteins, like albumins, globulins, clotting factors and fibrinogen), and some trace elements and vitamins. Herein we use "blood" as whole blood, and "blood plasma" as defined.

2. Materials, Methods, and Experimental Setup

Varying frequency and voltage power supply for generation of FE-DBD e-plasma was based on a system consisting of a wave-form generator, amplifier, and a transformer. A wave-form generator (CFG253/280, Tektronix, Inc.; Richardson, TX) was used for generation of 0–22.5 V rms sine, square, and triangular waves. Signal was then amplified (PowerTron 250A amplifier, 0–22.5 V rms, Industrial Test Equipment Co. Inc., Port Washington, NY) and stepped up to high voltage (Transformer, 22.5 V rms primary and 20 kV secondary, Industrial Test Equipment Co. Inc., Port Washington, NY) to achieve desirable high voltage signal. This system was bulky and later a custom, more compact varying frequency and voltage power supply was constructed in cooperation with Quinta, LTD (Moscow, Russia).[5] Electric discharge generated by this power supply is sufficiently uniform (Fig. 2) for treatment of tissue and blood, where micro-patterns created by this and similar discharges are of no great importance [14].

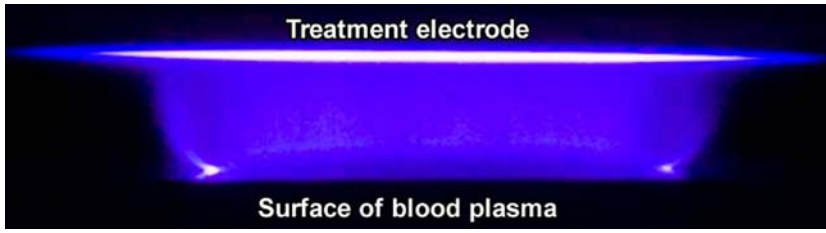


Fig. 2 FE-DBD treatment of blood—sufficiently uniform for biological purposes

E-plasma was generated between the insulated high voltage electrode and the sample (FE) undergoing treatment. One millimeter thick polished clear fused quartz (Technical Glass Products, Painesville, OH), was used as an insulating dielectric barrier. Three electrodes were constructed for treatment of various shapes and configurations of samples (Fig. 3). Round electrode (25.4 mm diameter) was used for treatment of samples where precise control of distance from the electrode to the sample was desired for increased repeatability of experimental results. Roller and wand electrodes were used as hand-held electrodes for treatment of varying shapes of samples where portability or large area of the electrode was desired over the precision of treatment.

For power analysis of FE-DBD e-plasma in continuous or pulsed mode (Fig. 4—principal schematic, and Fig. 5—signal output) we measure current passing through e-plasma and the voltage drop in the gap. For current analysis we utilized a magnetic core current probe (1 V/A $+1/-0\%$ Sensitivity, 10 ns usable rise time, 35 MHz bandwidth, Model 4100 Pearson Current Monitor, Pearson Electronics, Palo Alto, CA). Voltage was measured using a wide bandwidth voltage probe (PVM-4 1000:1, North Start High Voltage, Marana, AZ). Signals were acquired and recorded by a Digital Phosphor Oscilloscope (500 MHz bandwidth, 5×10^9 samples/s, TDS5052B, Tektronix, Inc, Richardson, TX) (Fig. 4). Acquired data was then integrated using MATLAB code[6] (MATLAB Release 14, Mathworks, Inc. Natick, MA).

For all tests discussed in this paper, round electrode fixed by micro-positioners at 2.7 mm from the treated sample for blood samples and 1.5 mm for tissue and agar samples was

[5] For further information on power supply operation and/or construction please contact the corresponding author.

[6] For further information on and/or a copy of MATLAB code please contact the corresponding author.

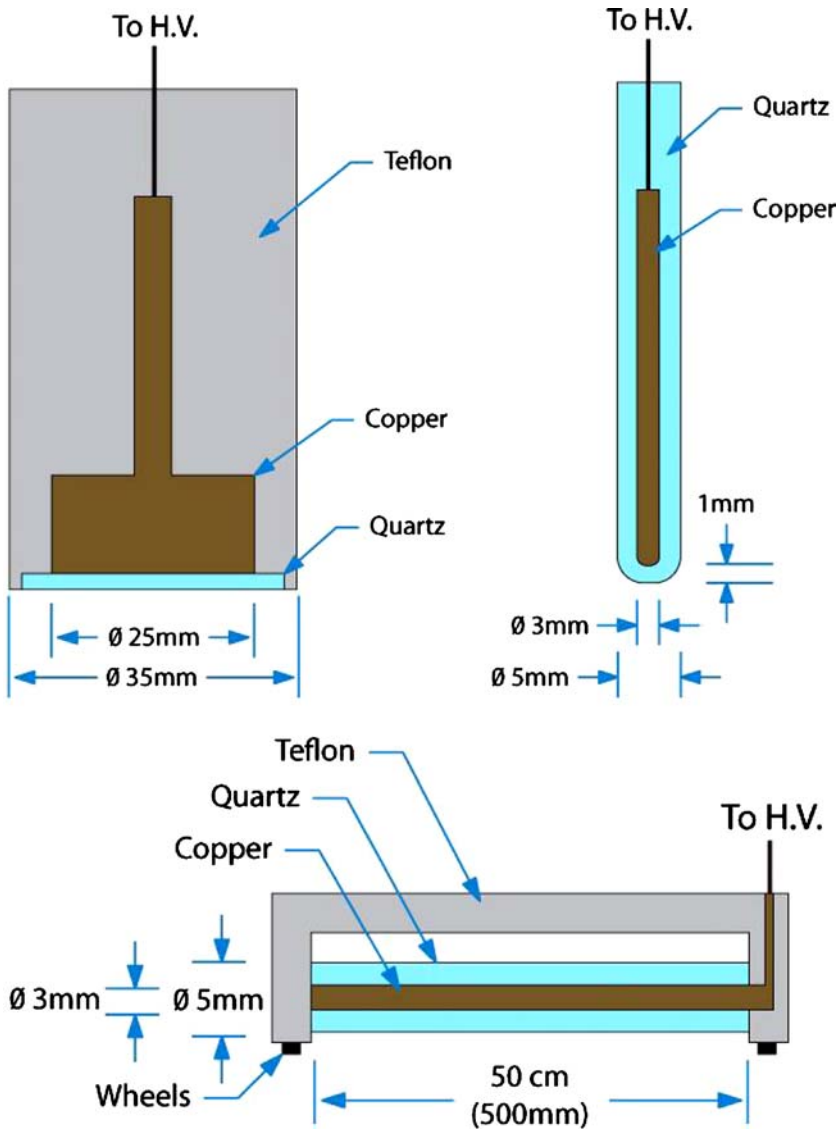


Fig. 3 FE-DBD treatment electrodes: "round" (top left), "wand" (top right), and "roller" (bottom)

utilized. Distances of 2.7 and 1.5 mm were chosen based on maximum power input into plasma—capacitive power match to the transformer is best at 2.7 mm for liquid samples and 1.5 mm for tissue and agar samples, based on our electrode setup and our power supply matching network construction. In the future we will address the power matching issues by constructing a new power supply with lower dependence on the surface being treated.

For treatment of 500 μL blood samples a special setup was constructed. This setup allows for precise control over the distance from the top of the treated sample to the dielectric barrier. Volume of 500 μL was chosen as it is the minimum volume acceptable for testing by Drexel University College of Medicine Hematology (DUCOM) Lab. To achieve precise volume, a hole of 3.7 mm deep was cut by a 25.4 mm ball mill and then polished to eliminate any sharp

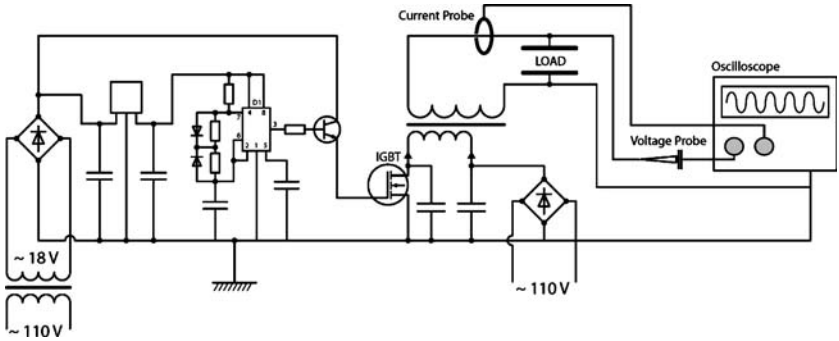


Fig. 4 FE-DBD power supply schematic and power analysis setup schematic

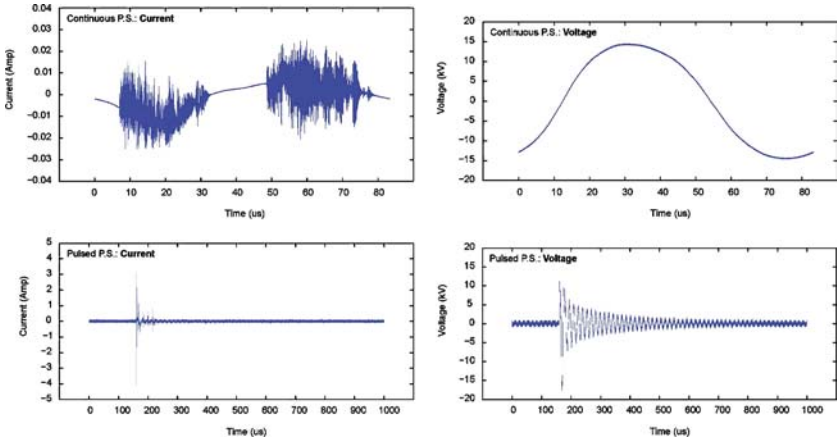


Fig. 5 Characteristic current and voltage signals per period for continuous wave and pulsed power supplies

edges. For e-plasma treatment of different volumes of blood plasma we have constructed a set of four electrodes of different volumes: 0.5, 1, 1.5, and 2 mL. A 19.1 mm tall acrylic plate was used as a base and stainless steel rods were inserted into a 12.7 mm through-hole. For 0.5, 1, 1.5, and 2 mL volumes 15.8, 11.9, 7.9 and 4.1 mm tall stainless steel rods were used respectively. Treatment of tissue samples was accomplished either by fixing a sample of skin on stainless steel vacuum plate or by holding the electrode by hand over an organ (for hand-held treatment, the electrode was enclosed by a "jacket" to allow for precise distance control). During treatment of agar plates the electrode and the plate were held in place by micro-positioners (Fig. 6).

Blood plasma samples were analyzed at the DUCOM Hematology lab utilizing the STA Compact® (Diagnostica Stago, Parsippany, NJ) analyzer for prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT). Samples were obtained from healthy blood donors and patients with clotting difficulties at the Hahnemann University Hospital (DUCOM Institutional Review Board (IRB) protocol #03295-01A1, 2005 and #1610, 2006). Upon receipt, cells were separated from blood plasma by centrifugation and blood plasma frozen (−80°C) for later experimentation. The thawing procedure consisted of storing the frozen sample in the refrigerator (+5°C) for 1 h and then in cold water (+10°C) for 30 min. Immediately after thawing, the sample was treated by e-plasma and brought to

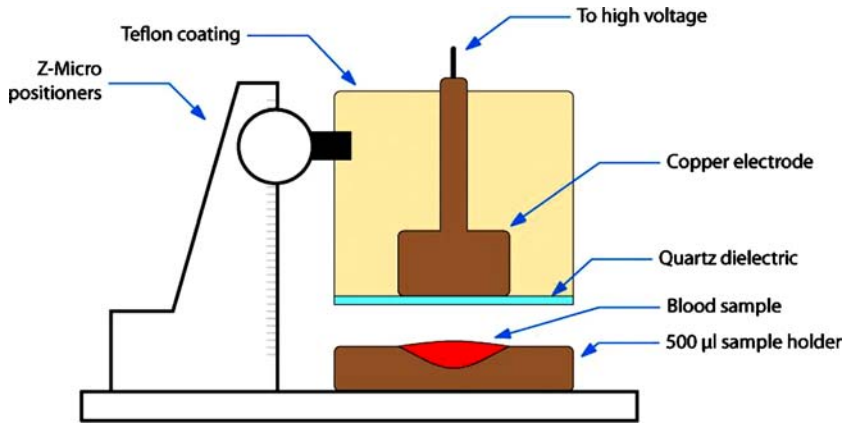


Fig. 6 FE-DBD treatment of blood plasma samples: an experimental setup schematic

the Hematology lab. PT, aPTT, and TT measurements were obtained and processed by a trained technician at the Hematology lab. Tissue samples were obtained from cadavers, explanted organs, and discarded tissue samples (DUCOM IRB protocol #03519-01, 2005). The samples were swabbed using BD BBL™ CultureSwab™ (Becton, Dickinson and Company, Sparks, MD) and the swabs submitted for analysis to the Graduate Hospital Microbiology lab (Philadelphia, PA) where they were plated and subsequently analyzed by a trained technician. Every tissue sample was swabbed before (control) and after e-plasma treatment to assess e-plasma sterilization efficiency. Bacteria for quantitative analysis of sterilization were obtained by transferring some of skin flora[7] from a patient with normal skin flora (as analyzed by our Microbiology lab) onto a blood agar plate (Trypticase™ Soy Agar with 5% Sheep Blood; Cardinal Health, Dublin, OH) [15, 16]. After 24 h at 37°C in air incubator (Fisher Scientific, Pittsburgh, PA) the grown colonies were transferred from agar surface into a sterile container and diluted with purified sterile water. Sixty samples were prepared from the original broth and frozen (−80°C) for later experimentation. The thawing procedure consisted of 30 min in cold water (+10°C). Initial concentration of colony forming units (cfu) was obtained by performing dilution assays of the samples.[8] For experimentation, thawed samples were diluted to desired concentration and either a 20 μl or 1 mL were pipetted onto agar for treatment. One milliliter samples were pipetted onto agar and left to dry for 3 h in a class I biological safety hood (Fisher Scientific, Pittsburgh, PA). Twenty microliter samples were left to dry for 5 min prior to e-plasma treatment and were spread over the agar plate by a sterile swab after treatment (Table 1).

Human tissue samples and organs used in our studies were obtained from cadavers, explanted organs (placenta), and discarded tissue samples from abdomen (stomach), wound tissue (gangrene, etc), foreskin (from circumcisions in infants and adults), and plastic sur-

[7] *Streptococcus* is a spherical gram-positive bacterium occurring in pairs or chains; they cause, for example, scarlet fever and tonsillitis. *Staphylococcus* is a spherical gram-positive bacterium that tends to form irregular colonies; some cause boils or septicemia or infections. *Yeast* is a fungus, commonly species Candida present on skin for an artificial assemblage of higher fungi which have temporarily or permanently abandoned the use of hyphal thalli; they are unicellular, and vegetative reproduction is generally by budding or fission. These three are omnipresent everywhere around us and are in abundance on human skin—they are commonly referred to as "skin flora."

[8] Here cfu counts of the samples were obtained after thawing them; cfu concentration was not obtained for samples prior to freezing—we did not include bacteria that did not survive the thawing procedure.

Table 1 Skin flora sterilization

Original Concentration (cfu)	5 s of FE-DBD (cfu)	10 s of FE-DBD (cfu)	15 s of FE-DBD (cfu)
10^9	850 ± 183	9 ± 3	0 ± 4
10^8	22 ± 5	2 ± 5	0 ± 0
10^7	2 ± 6	0 ± 0	0 ± 0

gery discards (face, breast, etc). Discarded tissue samples were those that neither the patient nor the hospital needed for any further analysis. We obtained these samples after they were de-identified and before they were discarded (DUCOM IRB) protocols #03519-01, 2005 and #03295-01A1, 2005).

3. Floating Electrode DBD (FE-DBD)

Principle of operation of FE-DBD e-plasma can be explained with the help of a relatively simple model. Let us model the insulated electrode as a sphere of diameter D_{el}, while the object whose surface is being treated is modeled as a sphere of diameter D_{ob}. In the absence of the object the electrode capacitance with respect to the far away (located at infinity) ground is given by the well-known formula $C_{\mathrm{el}} = 2\pi\varepsilon_0 D_{\mathrm{el}}$ (where ε_0 is permittivity of free space). Now, if the object being treated has a relatively high dielectric constant (like that of water), it effectively expels most of the electric field from its interior when it is brought close to the electrode. From that point of view this object behaves like a good conductor and, therefore, its capacitance with respect to the far away ground can also be modeled by $C_{\mathrm{og}} = 2\pi\varepsilon_0 D_{\mathrm{ob}}$. The region between the object and the electrode can be modeled roughly as a parallel plate capacitor with the value $C_{\mathrm{gap}} = \frac{\pi\varepsilon_0 D_{\mathrm{el}}^2}{2g}$ (where g is gap distance) if the gap is significantly smaller than the electrode diameter. Note that $\frac{C_{\mathrm{gap}}}{C_{\mathrm{og}}} = \frac{D_{\mathrm{el}}^2}{4gD_{\mathrm{ob}}}$ is significantly smaller than 1 for the typical choices of characteristic sizes.

In the absence of any conduction current the electrical models of the electrode by itself and the electrode near the treated object are well approximated by the circuits in Fig. 7a and b. When the electrode is well removed from the ground, the magnitude of the applied voltage V is insufficient to create electric field strong enough to cause the breakdown and discharge. However, when the object with a high dielectric constant is sufficiently close to the electrode, most of the applied voltage appears now across the gap. This is because the capacitance of the object with respect to ground is much larger than the gap capacitance and

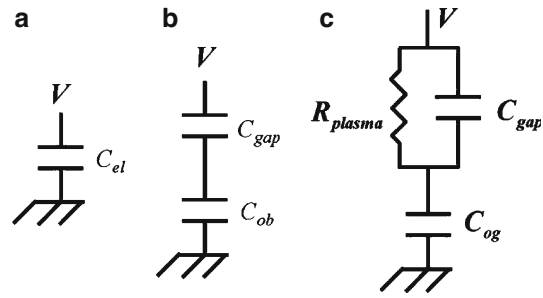


Fig. 7 Simplified electrical schematic of (**a**) electrode itself, (**b**) electrode near the treated object, and (**c**) e-plasma discharge on the treated object

voltage divides across these capacitors proportionally to the inverse of their size. This results in a strong electric field in the gap which can now lead to breakdown and discharge.

The electrical circuit model can be further refined by taking into account non-linear resistance and capacitance of the plasma created in the gap. The resulting circuit refinement is shown in Fig. 7c. The refined circuit does not change the main conclusion that most of the applied voltage appears across the plasma gap.

At about 10 kHz, the following circuit parameters typical for our experiments can be estimated assuming that body diameter is roughly 1 m, the electrode diameter (illustrated in Fig. 3) is about 25 mm, and the gap is about 1 mm.

$$C_{\text{gap}} = \frac{\pi D_{\text{el}}^2 \varepsilon_0}{4g} \approx 4 \times 10^{-12} F = 4\,\text{pF}, \quad \frac{1}{\omega C_{\text{gap}}} \approx 4.2\text{M}\Omega,\ R_{\text{plasma}} = \frac{V^2}{\text{Power}} \approx 5 - 10\ \text{M}\Omega$$

$$C_{\text{og}} = 2\pi \varepsilon_0 D_{\text{body}} \approx 50 \times 10^{-12} F = 50\,\text{pF}, \quad \frac{1}{\omega C_{\text{og}}} \approx 300\,k\Omega$$

Safety of the object being treated by plasma is ensured because current the power supply delivers under 5 mA. Although such currents may cause some mild discomfort, they do not cause muscle or cardiovascular malfunction in humans and, therefore, are deemed safe by the US Occupational Safety and Health Administration (OSHA), for example [17].

4. Blood Coagulation

FE-DBD was experimentally confirmed to significantly hasten blood coagulation. Visually, a drop of blood drawn from a healthy donor and left on a stainless steel surface coagulates on its own in about 15 min, while a similar drop treated for 15 s by FE-DBD e-plasma coagulates in under 1 min (Fig. 8). FE-DBD treatment of cuts on organs leads to similar results where blood is coagulated without any visible or microscopic tissue damage. Figure 9 shows a human spleen treated by FE-DBD for 30 s—blood is coagulated and tissue surrounding the treatment area looks "cooked", however the temperature of the cut remains at room temperature (even after 5 min of FE-DBD treatment) and the wound remains wet, which will, in turn, decrease healing time. Additionally we observe a significant change in blood plasma protein concentrations after treatment by e-plasma of blood plasma samples from healthy

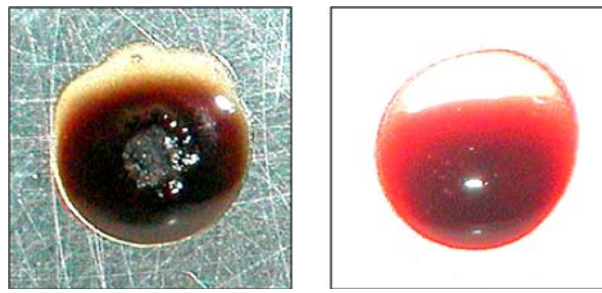


Fig. 8 Blood drop treated by FE-DBD: 15 s of FE-DBD (left) and control (right); photo was taken 1 min after the drops were placed on brushed stainless steel substrate

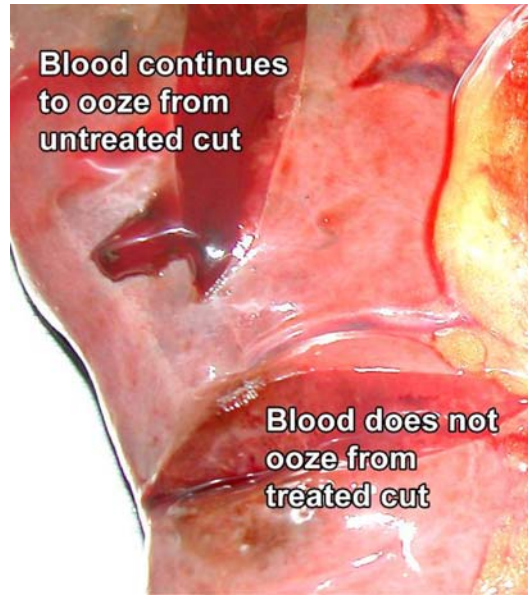


Fig. 9 Thirty seconds of FE-DBD treatment of human spleen: blood coagulates without tissue damage. Top cut: blood continues to ooze from an untreated area; bottom cut: blood coagulates while the wound remains wet

patients, patients with Hemophilia, and blood samples with various anti-coagulants.[9] For analysis of blood plasma we have employed a set of standard test procedures accepted in a hospital setting as determining of the blood coagulation rate: aPTT, PT, and TT. These tests were chosen as they are the most clinically relevant and are used commonly in the hospital to collectively test for the most common clotting pathologies [18]. The PT measures the clotting time from the activation of factor VII through the formation of fibrin clot [19]. This test measures the integrity of the "Tissue Factor" pathway of coagulation, whereas the aPTT measures the integrity of the "Contact Activation" pathway of coagulation [20]. The TT test is a measure of the rate of conversion of fibrinogen to fibrin when thrombin has been introduced—it measures hemostatically active fibrinogen [20, 21].

FE-DBD e-plasma with a high-enough dosage would eventually coagulate any blood sample; however, we observe a significant difference in the results obtained even at low doses (few seconds up to few minutes at $\sim$1 W/cm^2) from treatment of blood plasma samples from patients with Hemophilia and healthy donors. This observation led us to a model of influence of e-plasma on natural processes occurring in blood plasma undergoing treatment. As normal blood is coagulating, many biologically complex processes take place. These processes have been studied extensively and a good understanding of the entire process has been achieved [20]. To simplify the analysis of FE-DBD e-plasma and its influence on blood coagulation time we separated blood plasma from blood cells. We then subjected these samples to low doses of FE-DBD e-plasma and analyzed a few major blood proteins (coagulation factors). We hypothesize that low doses of FE-DBD e-plasma promote and/or speed up the natural coagulation process. We observe a thin transparent film formation on the surface of the sample starting from $\sim$30 s of treatment. PT and aPTT tests of the samples subjected to less than 30 s of treatment show no change on them and the blood plasma remains visually intact (Fig. 10).

[9] Anti-coagulants, like sodium heparin or sodium citrate, are designed to bind various proteins in the coagulation cascade thus controlling coagulation rate or preventing it altogether.

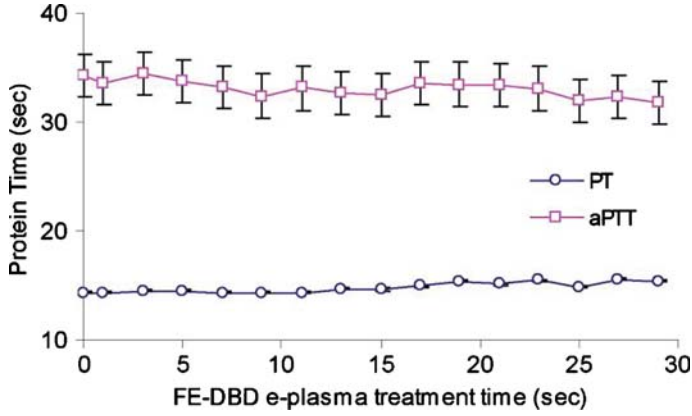


Fig. 10 Blood plasma PT and aPTT behavior prior to film formation: aPTT time (top) and PT time (bottom)

While normal blood plasma is not much influenced by FE-DBD in under 30 s, subjected to higher dose of FE-DBD its protein and enzyme behavior changes significantly (Fig. 11).

The observed behavior of blood plasma proteins is somewhat counter-intuitive—one would expect PT time, for example, to go down for blood that coagulates faster. PT time is representative of the time required for normal blood plasma to produce sufficient amount of thrombin, thus finalizing the cascade and forming a blood clot [20]; however, in this experiment, we are working with fixed-volume samples and we only have a fixed amount of "building blocks" required for blood enzymes to build fibrin. As fibrin starts to polymerize into a clot, its concentration as a monomer decreases. In other words, a fixed volume of blood portion of which is clotting is depleting itself of proteins required for clotting and thus exhibits longer PT, aPTT, and TT times as is observed (Fig. 11). This is a simple hypothesis to verify by using varying sample volumes at fixed treatment area; and we do observe that for different blood volumes with the same surface area of the treatment we get same rate of film formation but different rate of protein depletion (Fig. 12—setup, Fig. 13—results).

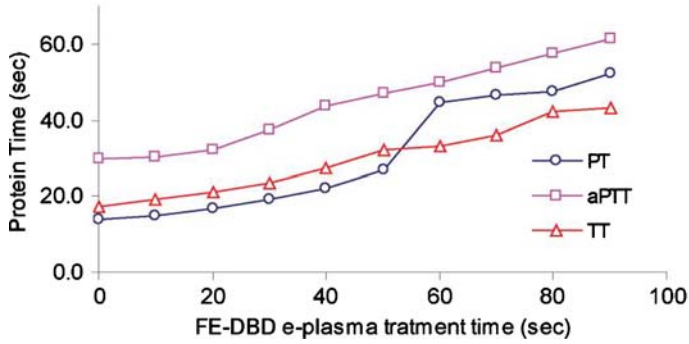


Fig. 11 Blood plasma behavior at higher FE-DBD doses: PT, aPTT, and TT times

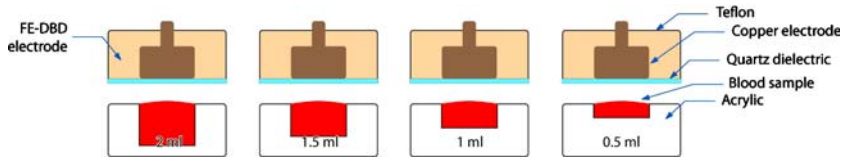


Fig. 12 Setup schematic for blood samples of different volumes with the same surface are of FE-DBD treatment

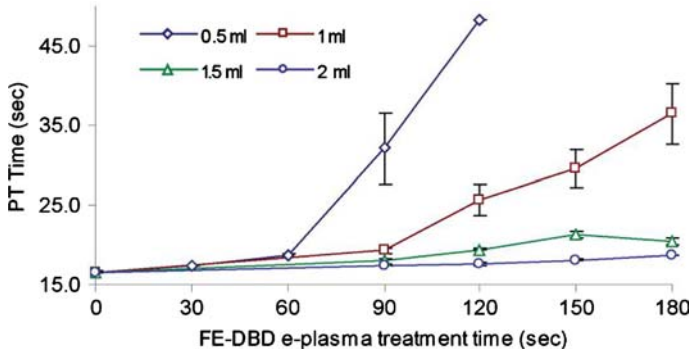


Fig. 13 PT times for blood samples of different volumes with the same surface area of FE-DBD treatment

5. Model of FE-DBD Influence on Blood Coagulation

We observe that FE-DBD e-plasma is able to significantly hasten blood clot formation and influence normal blood protein concentrations. Here, a better understanding of influence of FE-DBD is needed and we propose a first step in this direction—an explanation of influence of FE-DBD on ion concentrations in blood plasma. The presented model compares normal blood coagulation rate with blood undergoing e-plasma treatment by assuming linear relationship between ion concentrations in blood plasma and positive radical production by e-plasma at the surface of blood. Of note in the standard coagulation cascade is dependence of most reactions on calcium ion concentration (Fig. 14) [22, 23]. E-plasma is effective in increase of Ca^{2+} concentration through a redox (reduction/oxidation) mechanism $\left[Ca^{2+}R^{2-}\right] + H^{+}_{(H_2O)} \overset{^{k}Ca|^{k}_{-Ca}}{\longleftrightarrow} \left[H^{+}R^{2-}\right]^{-}_{(H_2O)} + Ca^{2+}_{(H_2O)}$ provided by hydrogen ions generated in air plasma through a sequence of ion-molecular processes [24] (here R represents calcium-binding protein complexes, like S100A7, for example [25]). Though the reaction is reversible, we will neglect contribution of reverse reaction (k_{-Ca}) due to abundance of H^+ ions generated by e-plasma in the following mechanism (here $X_{(H_2O)}$ signifies ion X dissolved in water): $N_2^+ + H_2O \rightarrow N_2 + H_2O^+_{(H_2O)}$ and then $H_2O^+_{(H_2O)} + H_2O \rightarrow H^+_{(H_2O)} + OH$. These reactions are nearly instantaneous and we can assume every N_2^+ ion, produced by e-plasma, to yield an H^+ ion in the solution [24]. Detailed modeling of e-plasma-chemical processes in air gives flux of N_2^+ ions in FE-DBD e-plasma onto the surface of the blood as a function of discharge power [24]. For our experimental conditions we can estimate calcium ion production rate of 6×10^{16} ions/s. Of note is that OH radical generated in the above reaction is then lost in reaction with one of the phospholipids (PL), abundant in blood plasma (PL form cell membranes, for example): $OH + Pl{\bullet}H \xrightarrow{k_{pl1}} H_2O + Pl$. Phospholipid radicals react

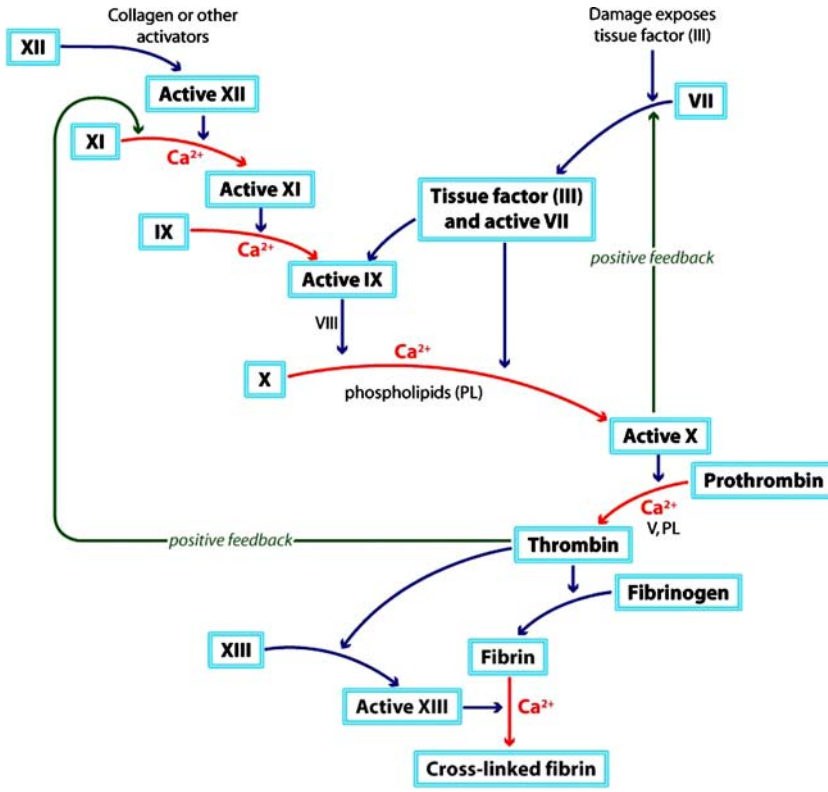


Fig. 14 Simplified coagulation cascade (adopted from D.U. Silverthorn [28])

with oxygen-containing lipids and PL forming peroxide bonds: $\mathrm{Pl}\bullet + \mathrm{PlO_2} \xrightarrow{k_{pl2}} \mathrm{Pl–O–O–Pl}$ [26, 27]. Water formation reactions between OH radicals and lipids are usually irreversible and we can neglect the contribution of k_{pl1} and k_{pl2} [26, 27]. The ability of FE-DBD plasma to generate sufficient amount of Hydrogen ions was confirmed in an experiment where water and PBS (Phosphate Buffered Saline) samples are treated by this e-plasma. pH changes down to 6 in 30 s of treatment, even in the buffer solution [13].

Lawson et al. propose a set of 12 reactions that govern the blood coagulation cascade of which calcium ion mediates eight [29, 30]. However, Lawson assumes saturated calcium concentration. Since in our model e-plasma treatment produces calcium ions at a constant rate, we propose that all the reaction rates will be influenced accordingly. Ataullakhanov et al. propose that the relationship is non-linear [22]. However, after careful analysis of their model we claim that assumption of a linear relationship between reaction rate and Ca^{2+} concentration not only would simplify our model, but would prove to be comparably accurate in a first-order approximation we are trying to achieve. Linear approximation (one-to-one ratio) of amount of calcium added to blood to the rate of coagulation fits almost perfectly into Ataullakhanov's experiment; though in reality the ratio is a bit skewed in favor of small additions of calcium suggesting a bigger increase in the rate of fibrin clot formation with even low additions of Ca^{2+} [22]. Thus, we assume that the reaction rates proposed by Lawson et al. and the new reaction rates we are proposing are proportional to each other for every reaction that involves calcium: $k_{new} = k_{old} \cdot [Ca^{2+}]_{actual}/[Ca^{2+}]_{initial}$ (here k_{new} is the new

reaction rate constant calculated based on the initial reaction rate constant k_{old}, and initial calcium ion concentration of 0.001 mol/L [23]). The resulting set of reactions proposes a double decrease in time until prothrombin formation (Fig. 15).

Thrombin is one of the proteins responsible for blood clot formation as it is responsible for conversion of fibrinogen into fibrin monomer (Fig. 14), which in turn polymerizes to form fibrin micro-filaments that entrap platelets and form a clot; thus thrombin can be considered as an initiator of fibrin polymerization and clot formation [23, 29]. Proposed by our model, time needed for blood to initiate formation of thrombin is decreased from ∼30 s for normal blood to ∼15 s for blood undergoing FE-DBD treatment. This result is consistent with reality, however further improvements to the model are desirable—the model is currently lacking e-plasma radical formation and ultra violet (UV) radiation interaction with blood plasma proteins, millimeter-scale evaporations caused by micro-discharges, and blood fluid dynamics (growth and transport of fibrin clot from the surface to bulk of the blood). Future improvements to our model will address these issues.

6. Tissue Sterilization

Conventional electric discharges (both high and low pressure and temperature) are well-known for their ability to sterilize various surfaces [6, 24, 31]. The advantage of FE-DBD system we are presenting is its ability to sterilize living animal or human tissue without any damage to the treated tissue (Fig. 1). Our results confirm that there is no gross (visible) or histological (microscopic) damage to the treated skin and organ samples in as much as 5 min

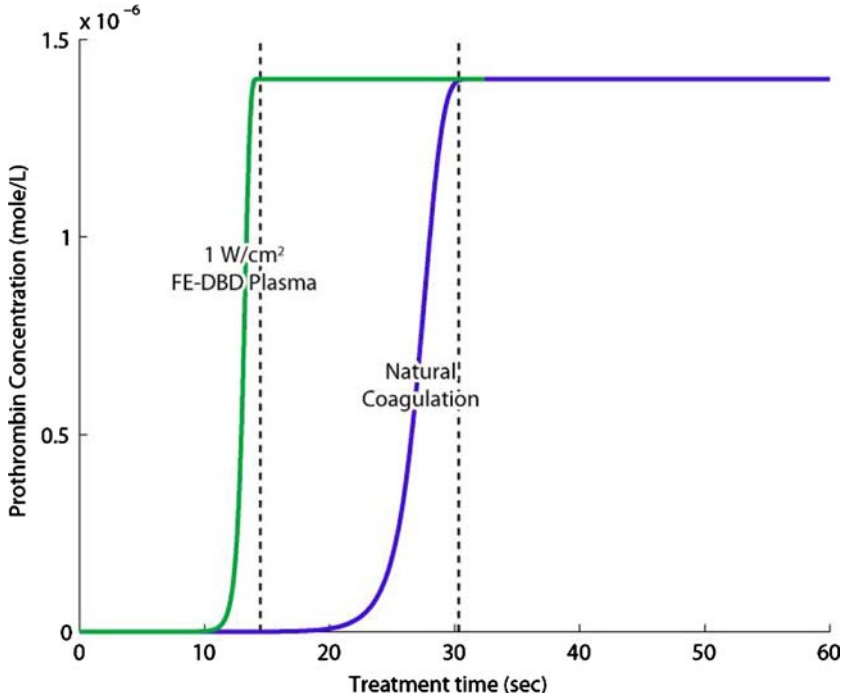


Fig. 15 Prothrombin kinetics: two-fold decrease in clot formation time with e-plasma

of treatment (Fig. 16) while complete hospital-grade sterilization is achieved in less than 6 s of e-plasma treatment. Ultimate use of the FE-DBD system is to treat wounds of human patients, so our sterilization tests were performed by swabbing and culturing bacteria and fungi from human skin samples before and after e-plasma treatment. While we show growth of normal skin flora (mixture of *Streptococcus*, *Staphylococcus*, and *Yeast*) on cultures from swabs taken on control samples, we register no growth on cultures from swabs taken on samples treated by e-plasma for 2–6 s, depending on the level of initial contamination of the skin. Skin samples treated by e-plasma show no visible damage (Fig. 16, top) and histological analysis shows no microscopic damage (Fig. 16, bottom).

Following the qualitative test of human skin tissue, we carried out investigation of effects of e-plasma on bacterial cultures to quantify the extent of sterilization and determine possible factors responsible. For our samples we cultured a large quantity of bacteria obtained from a swab of cadaver tissue on blood agar. The concentration of 10^9 cfu per milliliter of liquid was chosen as it is roughly 10,000 times greater than that on normal skin [15, 16]. The prepared culture plates were treated by FE-DBD and the plates were then incubated for 24 h. We observe no growth on the areas treated by FE-DBD for a few seconds (Fig. 17) and we are able to quantify the extent of e-plasma sterilization (Table 1) based on treatment dose—with increased dose we are able to sterilize quite far from the treatment electrode (Fig. 18—schematic illustration and Fig. 19—results). Of note is no visible damage to agar even at higher doses—bacteria grows on the treated agar normally, if re-inoculated. Even when we employed a fan to flow air through e-plasma at high rate the "complete inactivation" shifts only slightly and remains practically independent of the flow rate employed. Even though further investigation is necessary, these results suggest that direct treatment by plasma is more potent in bacterial activation than indirect treatment (by a jet, for example). We hypothesize

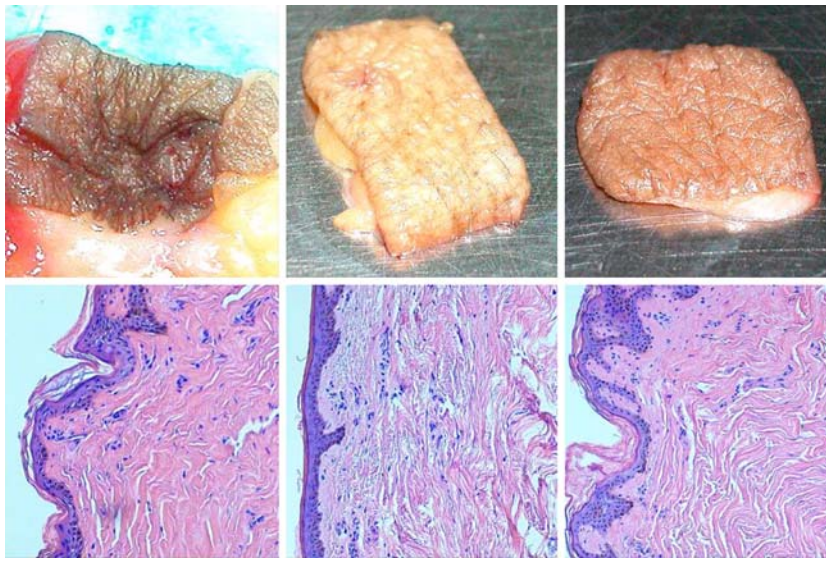


Fig. 16 Photos (top) and tissue histology (bottom) of cadaver skin samples after FE-DBD treatment: control (left), 15 s of treatment (middle), and 5 min of treatment (right)—no visible damage is detected



Fig. 17 Petri dish with blood agar, seeded by $\sim 1.3 \times 10^7$ cfu/cm^2 (10^9 cfu/ml) of skin flora and then treated by FE-DBD plasma for 10 s. While the plasma region diameter is roughly 25 mm, the "inner" circle of inactivated bacteria and fungi diameter is ~35 mm and the "outer" circle where the bacteria is partially inactivated (colonies are visible) is ~54 mm

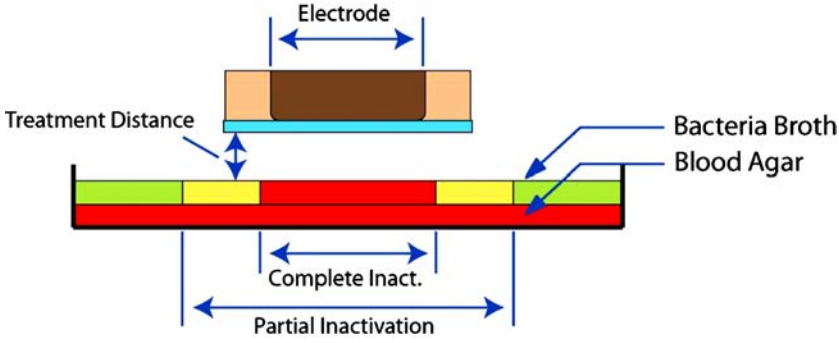


Fig. 18 Schematic illustration of FE-DBD treatment of agar dishes with bacterial broth

that this is due to high concentration of short-lived active species and radicals as well as UV radiation that is unable to penetrate far out of e-plasma region (especially vacuum UV with wavelengths below 180 nm) [32, 33].

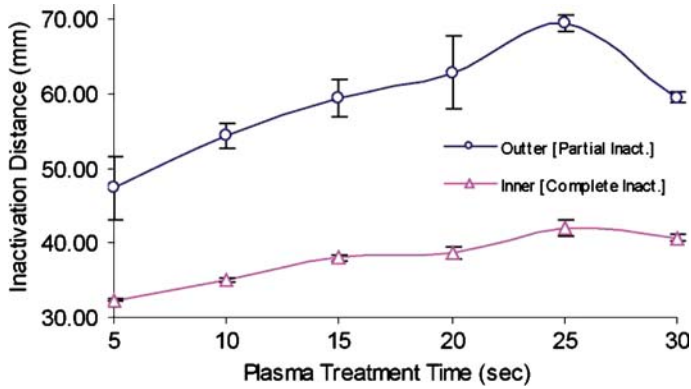


Fig. 19 Dependence of inner circle diameter (bottom) and outer circle diameter (top) of skin flora inactivation on FE-DBD treatment time

7. Conclusions

The purpose of this work is to introduce a new way of living tissue sterilization and blood coagulation that can be applied directly to living animal or human tissue without damage or pain. For this technology we envision a vast array of future applications in a hospital setting, on the battlefield, and in everyone's home. E-plasma sterilization is not only more environmentally friendly than most of today's technologies, but it was also shown to be less destructive to human tissue than the state-of-the-art electrical methods available today.

The proposed method, however, may have certain limitations, and a number of open questions remain. In the presented work we have not addressed toxicity of FE-DBD and its mutagenic capabilities. We are currently investigating FE-DBD toxicity via treatment of living mouse skin surface with escalating doses to find the maximum acceptable dose (DU-COM Institutional Animal Care and Use Committee (IACUC) protocol #15975-01). Our limited observations so far suggest that the maximum non-toxic dose is far greater than that required for complete tissue sterilization and blood coagulation, however the study is not yet complete. Mutagenic capabilities of e-plasma still remain an open question and we are in the process of developing a series of in-vitro cell-level studies on human epithelial cell lines to determine e-plasma influence on DNA (if any).

Future work will address the portability of FE-DBD device, its toxicity to animals and humans, and possible mutagenic potential. Additionally a careful model of e-plasma influence on natural processes in the blood coagulation cascade as well as a model of cell wall rupture by e-plasma will be constructed.

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