Bio-Medical Applications of Non-Thermal Atmospheric Pressure Plasma

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Keywords: electric plasma, non-thermal plasma, atmospheric pressure discharges, sterilization, disinfection, blood coagulation, wound treatment, wound healing.

Non-Thermal Floating Electrode Dielectric Barrier Discharge (FE-DBD) Plasma in Direct Contact with Living Tissue

The focus of this work is direct application of non-thermal atmospheric pressure plasma to human tissue, where this tissue serves as a second active electrode. Such use has significant advantages over the existing technologies. Ultra Violet (UV) radiation, especially high energy Vacuum UV (VUV), does not have good penetration depth in air and VUV exists only in the discharge area. Short-lived active species and radicals (like OH, NO, $O_2(1\Delta_g^+)$ and other electronically excited species, atomic Oxygen, etc.) do not, generally, exist outside of the discharge gap, either. Electron and ion bombardment of the surface has been linked to very efficient sterilization rates [1-5] and that also only exists in plasma. We observe experimentally a difference of a few times up to a few orders of magnitude improvement in bacteria inactivaon rates under direct plasma treatment as compared to a "jet" where we blow out plasma and only treat the surface with the afterglow. Direct plasmas were not yet used in medicine for fear of application of 35,000 Volts directly to a human body. However, in the presented setup no voltage is applied directly to the body and most of the power is deposited in the discharge itself, leaving the living tissue undergoing treatment unharmed (Figure 1) [6].

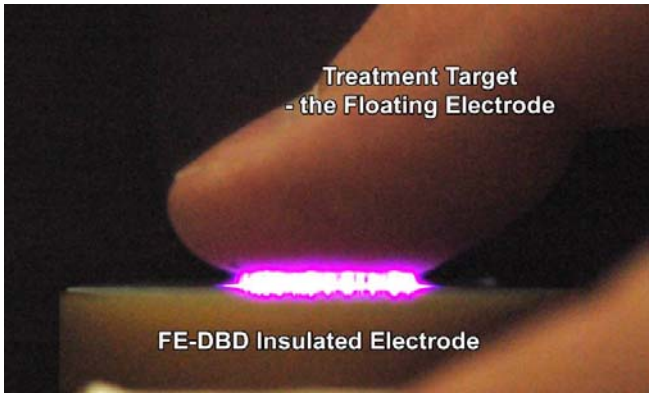


Figure 1. Direct Application of FE-DBD electric plasma to living tissue. Here tissue serves as an active electrode.

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Thermal plasmas today enjoy a wide array of applications in engineering, chemical, biological, medical, and other fields [3, 7-12]. Both thermal and non-thermal and high and low pressure systems are widely used in many industries. There is, however, an apparent lack of applications of non-thermal atmospheric pressure discharges in medicine and biology. Low temperature and cold "jets" are beginning to gain popularity, however their abilities are somewhat limited since plasma is not directly applied and only afterglow products are used in those treatments [4, 9, 13-15]. Herein, examples are given of direct plasma application, where treated tissue serves as a second active electrode, for treatment of tissues, blood, cell lines, and living animals. Direct plasma application to living tissue (Figure 1) is compared with indirect application of afterglow (or "jet"). Some of the potential applications of such treatments are also discussed. Additionally, safety of such treatments to human subjects is addressed.

FE-DBD Plasma in Wound Treatment: Living Tissue Sterilization

A set of experiments was performed to evaluate effectiveness and safety of the proposed method. Main idea of these experiments is to investigate efficacy of direct application of non-thermal plasma in wound healing. By direct application we mean electric plasma in contact with the treated surface (Figure 1) where the surface serves as a second, active electrode. Electrical schematic of such a system was presented by us before [6] and we have shown that in this setup, plasma application is safe due to quartz dielectric on the energized electrode and high dielectric constant of the human[†] [16].



Figure 2. Animal treated for 40 seconds by FE-DBD, two weeks after treatment shows no signs of distress. Skin appears normal visually and no damage is observed histologically.

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 [6, 7, 17, 18]. Non-damaging application of FE-DBD plasma was already reported by our group (see [6]); however, we are able to safely apply the treatment to living tissue of a living animal as well. A tissue toxicity study has been conducted[‡] and we have shown that doses required for sterilization (few seconds) are significantly lower than doses required to damage tissue. Additionally, animals survive the treatment well and show no signs of distress two weeks after treatment; is short, once these mice wake up from anesthesia they act like nothing happened (Figure 2).

[†] Additionally, safety of this system is maintained by limiting current to below 10 mAmp, which is considered safe by the Occupational Health and Safety Administration.

[‡] This study was performed under the Drexel University College of Medicine Institutional Animal Care and Use Committee (IACUC) protocol #15975-01.

Promoting Blood Coagulation with FE-DBD Plasma

In a connected set of experiments we have studied doses required to promote blood clot formation and we observe that FE-DBD plasma is able to coagulate blood at energies significantly lower than those required to "bake" the blood (denature the proteins). The fact that the influence of FE-DBD differs for normal blood, blood on anti-coagulants[§], and blood obtained from patients with clotting disorders (Hemophilia A) leads us to believe that FE-DBD influences some natural processes in blood. We have modeled influence of FE-DBD plasma on ionic balances in blood and obtained convincing results offering initial insight into the mechanism of influence of electric plasma [6].

Skin Flora Sterilization by FE-DBD

In quantitative evaluation of the ability of FE-DBD plasma to sterilize the treated tissue from bacteria we have observed roughly a 6 to 7 log reduction in 5 seconds of treatment (Table 1). This supports our initial results where we have indicated that this plasma treatment achieves complete sterilization in 1 to 3 seconds of treatment[**] [6]. This could also be a promising result for wound healing oriented applications where it could be desirable to sterilize the wound tissue without simultaneously coagulating the blood.

Table 1. Bacterial inactivation rate under FE-DBD plasma treatment.

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

FE-DBD Plasma in Treatment of Human Melanoma, Macrophages, and *Leishmania Major* promastigote cell lines

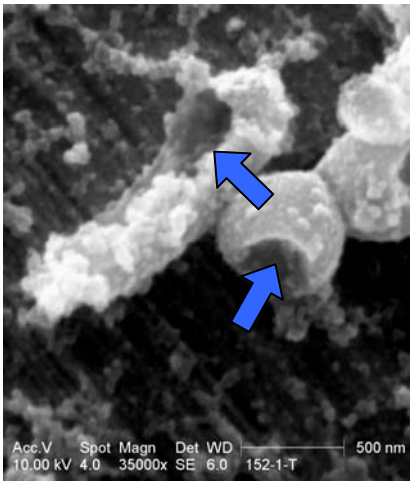


Figure 3. Membrane damage of Streptococcus (rods) and Staphylococcus (spheres) cells.

Such a wide selectivity range of plasma "preferring" to inactivate bacteria over damaging healthy cells could potentially be explained by difference in mechanisms by which bacteria and mammalian cells are inactivated. Eukaryotic and prokaryotic cells differ drastically in size and function. Eukaryotes can have significantly different membrane structure and are generally able to recover from small membrane fractures rapidly; while prokaryotes we are dealing with[††] do not [19]. We note that the observed selectivity could potentially be due to synergetic effect of ion bombardment of the plasma membranes, electric field, and direct treatment by high energy Vacuum Ultra Violet (VUV). This, however, needs to be investigated in

[§] We have tested blood on Heparin and Sodium Citrate as anti-coagulants.
[**] Commonly, total concentration of bacteria on skin does not exceed ~10^5 cfu per ml.
[††] "Skin flora" – a collection, or broth, of common bacteria and fungi always present on skin, like Streptococcus, Staphylococcus, and Yeast.

further detail; we do, however, observe that cells "pop" which is likely to be caused by ion bombardment (Figure 3). We observe this behavior if we compare two cell lines: *Leishmania Major* promastigotes[‡‡] and human Macrophages. We clearly see the difference in inactivation rates where doses required to almost completely inactivate the parasites are significantly less than those required to inactivate macrophages.

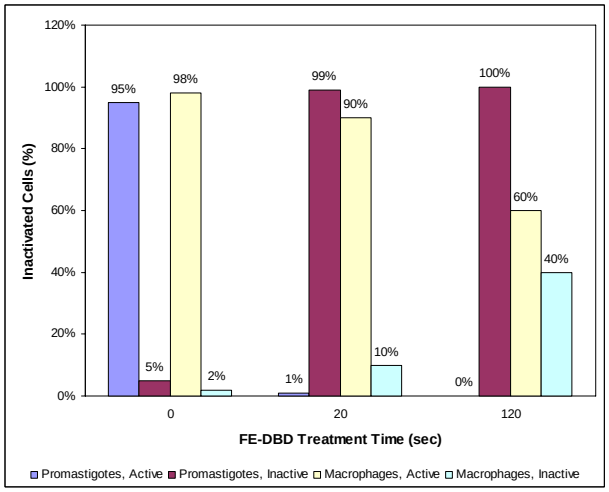


Figure 4. Comparison of active (first) and inactive (second) *L. Major* promastigotes with active (third) and inactive (fourth) Macrophages: Macrophages survive much higher doses of FE-DBD plasma.

Future Applications of FE-DBD *Direct* Plasma Treatment

Fields of medicine and biology appear nearly untapped by non-thermal plasmas where these plasmas are applied directly to living animal or human tissue. Clearly, there is selectivity of electric plasma treatment to certain cell lines over others. This is possible due to significant biochemical and biological difference between these cell lines and we note that we are able to tune the physical phenomenon that is plasma, taming it to biological applications by varying the amount of UV radiation, concentration and energies of charged and active species and radicals, and electric field.

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[‡‡] Or "parasites". They are cylindrical and are about 5 to 10 microns in length and ~ 1 micron in diameter.

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