30th Annual International IEEE EMBS Conference
Vancouver, British Columbia, Canada, August 20-24, 2008

Non-Thermal Dielectric Barrier Discharge Plasma Treatment of Endothelial Cells

Sameer U. Kalghatgi, Member IEEE, Gregory Fridman, Alexander Fridman, Gary Friedman and Alisa Morss Clyne

Abstract—**Non-thermal dielectric barrier discharge plasma is now being widely developed for various medical applications such as skin sterilization, blood coagulation, induction of apoptosis in malignant tissues, and wound healing among others. In this paper, we investigate the toxicity of non-thermal plasma treatment on endothelial cells, which line all blood contacting surfaces in the body. Our initial results indicate that low power non-thermal plasma is relatively non-toxic to endothelial cells at short exposure times up to 30 s, while non-thermal plasma treatment at longer exposure times is cytotoxic. Non-thermal plasma at shorter exposure times may induce proliferation in the cells.**

I. Introduction

Non-thermal atmospheric pressure dielectric barrier discharge plasma has emerged as a novel tool in medicine. Compared to the effects of the more conventional thermal plasma [1], non-thermal plasma is selective in its treatment since it does not burn tissue. This enables many new medical applications including sterilization of living tissue without damage [2], blood coagulation [2] induction of apoptosis in malignant tissues [4], [5] modulation of cell attachment [6], and wound healing [9]

The operating principle of the plasma discharge used in this work is similar to the Dielectric Barrier Discharge (DBD) introduced by Siemens in the middle of 19th century [11]. DBD occurs at atmospheric pressure in air or other gases when sufficiently high voltage of sinusoidal waveform or pulses of short duration are applied between two electrodes, with at least one of them being insulated [12]. The presence of an insulator between the electrodes prevents the build-up of high current. As a result, the discharge creates an electrically safe plasma without substantial heating of the gas (Fig. 1). This approach allows the electrical charges in the plasma to initiate or catalyze biological processes without thermal damage of biological samples [10].

Manuscript received April 7 2008.
Sameer Kalghatgi & Gary Friedman are in the Department of Electrical and Computer Engineering, Drexel University, Philadelphia, PA 19104, USA (phone: 215-895-2909; e-mail: sameer.kalghatgi@drexel.edu, gary@cbis.ece.drexel.edu).

Gregory Fridman is in School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104, USA (e-mail: greg.fridman@drexel.edu).

Alisa Morss Clyne is in the Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA 19104, USA (e-mail: asm67@drexel.edu)

Alexander Fridman is in the Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA 19104, USA (e-mail: af55@drexel.edu).

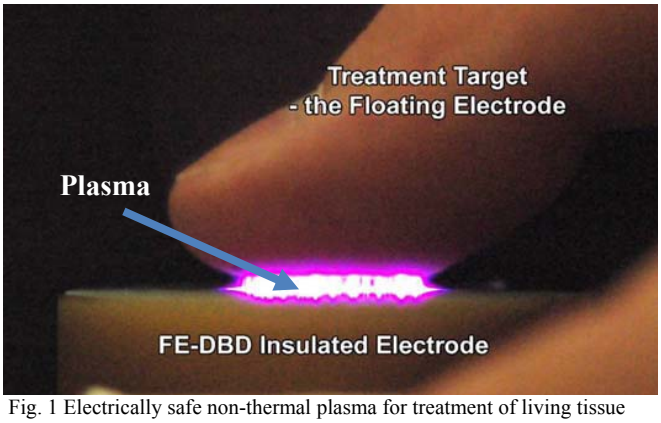


Fig. 1 Electrically safe non-thermal plasma for treatment of living tissue

In this paper, we investigate the toxicity and potential benefits of direct non-thermal plasma treatment of endothelial cells, which line all blood contacting surfaces in the body. Endothelial cells play a guiding role in angiogenesis, the growth of new blood vessels from existing vessels. In varied disease conditions, healing may result from promoting or inhibiting angiogenesis. Our specific hypothesis is that plasma treatment properties can be varied to grow or regress blood vessels. This research has further application to the effect of non-thermal plasma on the vasculature, which is exposed during plasma treatment of many tissues. The direct plasma treatment of other cell types is described in detail in prior work [10].

II. Materials, Methods and Experimental Set Up

A. Non-thermal plasma generation

Non-thermal atmospheric pressure dielectric barrier discharge plasma was produced using an experimental setup similar to one previously described and schematically illustrated in Figure 2 [2]. The non-thermal plasma was generated by applying alternating polarity pulsed (200 Hz – 1 kHz) voltage of ~20 kV magnitude (peak to peak) between the insulated high voltage electrode and the sample undergoing treatment using a variable voltage and frequency power supply (Quinta, Russia). 1 mm thick, polished clear fused quartz was used as an insulating dielectric barrier covering the 2.5 cm diameter copper electrode. The discharge gap between the bottom of the quartz and the treated sample surface was fixed at 2 mm. Discharge power density was measured to be roughly 0.25 Watts/cm^2 (at 200Hz) and 1.5 Watts/cm^2 (at 1 kHz) using both electrical

Authorized licensed use limited to: Drexel University. Downloaded on November 20,2026 at 18:16:30 UTC from IEEE Xplore. Restrictions apply.

characterization and a specially designed calorimetric system [13].

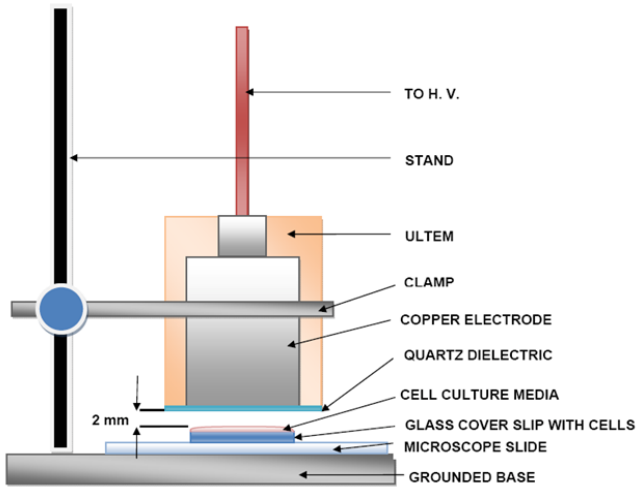


Fig. 2. Schematic of the experimental setup showing the high voltage electrode and the sample holder

B. Endothelial cell culture

Porcine aortic endothelial cells (PAEC) were maintained in low glucose Dulbecco's Modified Eagle's Medium (DMEM) (Cellgro, Mediatech, VA, USA) supplemented with 5% fetal bovine serum (Hyclone, UT, USA), 1% L-glutamine, and 1% penicillin-streptomycin (GIBCO, Invitrogen, CA, USA). Media was changed every two days. For each assay, cells were washed with phosphate buffered saline, harvested with 0.1% trypsin (GIBCO, Invitrogen, CA, USA), and seeded near confluence (4 x 10^5 cells/well) on 18 mm diameter glass cover slips (VWR, PA, USA) in 12-well plates (Corning Costar, NY, USA). Cells were cultured for 24 hours prior to plasma treatment in 1.5 ml supplemented media in a 37°C, 5% CO_2 incubator to allow full attachment and spreading.

C. Plasma treatment of attached cells

Endothelial cells on glass cover slips were exposed to low power plasma at exposure times ranging from 5 to 120 seconds. Briefly, each cover slip was removed from the 12-well plate and placed on a microscope slide, which was then positioned on the grounded base of the plasma device. 100 µl of serum free media was added to the glass cover slip before plasma treatment to prevent sample drying. Following plasma treatment, the cover slip was immediately placed in a new 12-well plate, 1.5 ml of supplemented media was added, and the samples were returned to the incubator.

D. Non-thermal plasma cytoxicity assessment

Non-thermal plasma endothelial cell cytotoxicity was measured via cell counts and a Live/Dead assay. For cell counts, PAEC were plasma treated as described. At 3 and 24 hours following plasma treatment, attached (live) cells were trypsinized and counted using a Coulter counter (Beckman Coulter, CA, USA). For the Live/Dead assay, PAEC were seeded 24 hours before plasma treatment. At 3 and 24 hours post treatment, cell viability was assessed with a Live/Dead Viability/Cytotoxicity Assay (Molecular Probes, Invitrogen, CA). Cells were labeled with 1 µM ethidium homodimer and 0.25 µM calcein by adding 250 µl of the Et-HD/Calcein solution and incubating at room temperature for 45 minutes according to the manufacturer's instructions. This assay is based on the principle that live cells convert nonfluorescent, cell-permeant, calcein acetoxymethyl ester to FITC (fluorescein isothiocyanate) fluorescent calcein via intracellular esterase activity. Concurrently, ethidium homodimer enters dead cells and binds nucleic acids because of increased permeability from membrane damage, causing dead cell chromatin to fluoresce TRITC (Tetramethyl Rhodamine Iso-Thiocyanate) red. Plasma treated cells were imaged with a fluorescent microscope (Olympus, USA) using a 10x objective. Fluorescent images were captured digitally with a high performance CCD camera (SPOT microscope digital camera, Diagnostic Instruments, MI, USA). Red and green images were combined using Spot Advanced microscope digital imaging software (Diagnostic Instruments, MI, USA). The number of dead cells was manually counted in Adobe Photoshop (Adobe, CA, USA) in composite images of five distinct areas from each sample.

E. Endothelial cell membrane damage

Endothelial cell membrane damage following non-thermal plasma treatment was quantified by measuring release of the cytoplasmic enzyme lactase dehydrogenase (LDH). For the LDH assay, serum free DMEM with 4 g/L glucose without sodium pyruvate and phenol red was used, since sodium pyruvate interferes with LDH measurement. Cover slips were removed from 12-well plates and 100 µl of sodium pyruvate-free media was added. Cells were treated with plasma for 60 s and transferred to new 12-well plates. 1.5 ml of sodium pyruvate-free, serum free media was added to each coverslip. 10 ng/ml of tumor necrosis factor-α (TNFα) was added to cells as a positive control. 0.5 ml of the medium was collected at 2, 4, 6, 8, 12 and 24 h after plasma treatment. LDH was quantified in the collected media samples using the Cytotox-ONE Homogeneous Membrane Integrity Assay (Promega, WI, USA) according to the manufacturer's instructions. LDH activity was measured at 560/590 nm in a microplate reader (SpectraFluor Plus, Tecan, Switzerland).

F. Endothelial cell proliferation

To analyze the effect of non-thermal plasma on endothelial cell proliferation, 10,000 cells were seeded on 18 mm diameter cover slips in 12-well plates. Cells were exposed to low power plasma, transferred to new 12-well dishes, and incubated for an additional 5 days. Cell media was changed on days 2 and 4. Cell number was quantified on days 1 and 5 following plasma treatment by trypsinizing attached cells and counting them with a Coulter counter. Fold growth was determined by taking the ratio of cell number on day five to day one.

G. Statistical Analysis

Comparisons between two samples were analyzed by unpaired Student's t-test, and comparisons between more

Authorized licensed use limited to: Drexel University. Downloaded on November 20,2026 at 18:16:30 UTC from IEEE Xplore. Restrictions apply.

than two groups were analyzed by ANOVA using Prism software (GraphPad, CA, USA).

III. Results

A. Endothelial cell viability with plasma treatment

Non-thermal plasma (0.25 W/cm^2) was relatively non-toxic to endothelial cells at exposure times up to 60 seconds. While the number of live, attached cells did decrease as plasma exposure time increased, at both three and 24 hours after plasma treatment, more than 70% of endothelial cells remained viable (Figure 3). There was no significant difference between cell viability at 3 and 24 hours following plasma treatment, suggesting no long term plasma toxicity effects on endothelial cells.

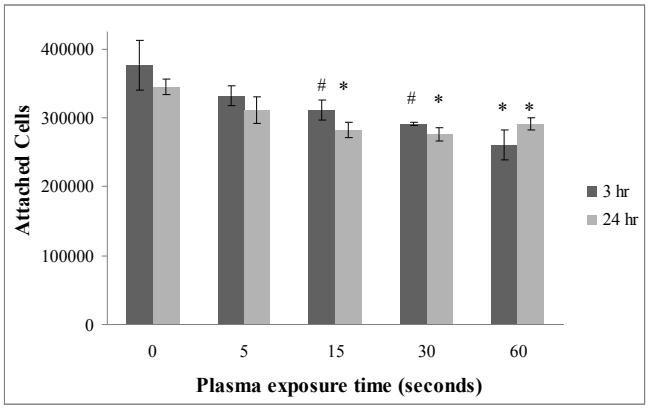
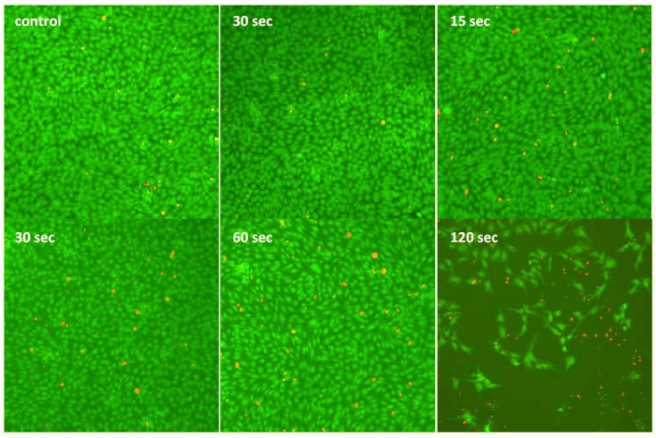


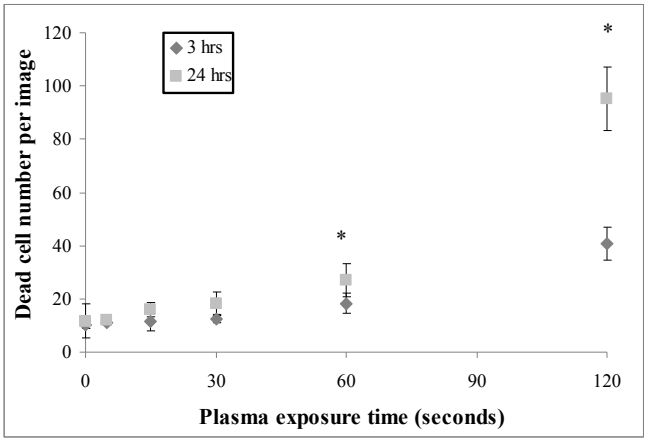
Fig. 3. The number of live, attached cells decreases as plasma exposure time increases up to 60 s ($p < 0.01$ by ANOVA) at 3 and 24 hours post-exposure. * $p < 0.01$ as compared to 0 s plasma treatment control.

A Live/Dead assay was used to confirm cell count results. As shown in Figure 4A, cells treated with plasma for short exposure times (5, 15, and 30 s) showed few dead cells (red), validating that low power plasma treatment is relatively non-toxic at short exposure times. At longer plasma exposure times (60 and 120 s), a significant number of dead cells were evident. Particularly at 120 s, very few live cells (green) were seen. This extensive cytotoxicity is likely related to sample drying under extended plasma treatment. Therefore, 120 s of plasma exposure was not used for subsequent assays.

Dead cells in five distinct areas of each sample, similar to those shown in Figure 4A, were quantified and plotted in Figure 4B. Again, the number of dead cells increased as we increased the plasma exposure time ($p < 0.01$ by ANOVA). At 60 s, there were nearly two fold the number of dead cells as compared to control. There was no significant difference between dead cells measured at either 3 or 24 hours following plasma exposure, further confirming that plasma toxicity effects occur shortly following plasma treatment.



(A)



(B)

Fig. 4. Endothelial cell death increased with plasma exposure time, as measured by Live/Dead assay. (A) Fluorescent images, and (B) quantitation of five areas of each sample. * $p < 0.01$ as compared to control (0 s) .

B. Endothelial cell membrane damage

Endothelial cell membrane damage was assessed by LDH release. Cells were treated with non-thermal plasma for 60 seconds, and LDH was measured in the media up to 24 hours following plasma treatment. The positive control measurement was also taken at 24 hours. Media LDH levels increased up to 24 hours post treatment ($p < 0.01$ by ANOVA). LDH release was significant by 4 hours after plasma treatment and continued to rise throughout the first 24 hours. The released LDH from plasma treatment was comparable to the TNFα positive control.

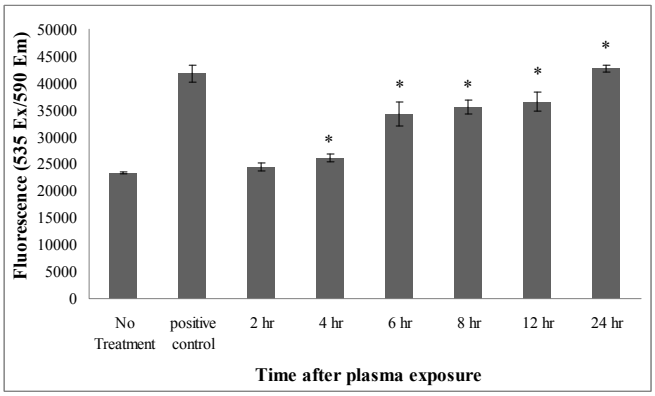


Fig. 5. Endothelial cell LDH release increases up to 24 hours post plasma exposure. * $p < 0.01$ as compared to untreated cells.

Authorized licensed use limited to: Drexel University. Downloaded on November 20,2026 at 18:16:30 UTC from IEEE Xplore. Restrictions apply.

C. Endothelial cell long term response to plasma treatment

Endothelial cells demonstrated enhanced proliferation following plasma treatment. Five days after plasma treatment, control endothelial cells that were not plasma treated experienced roughly 5 fold growth when compared to one day after plasma treatment in media with 5% serum. Cells treated with plasma showed greater proliferation than control up to 30 seconds of plasma treatment. At the 30 second treatment peak, endothelial cells demonstrated twice as much proliferation as untreated controls. Although the overall number of attached cells in samples treated for 30 s was less than control one day after plasma treatment, the remaining cells proliferated significantly faster than control. However, increased plasma treatment times beyond 30 seconds resulted in decreased cell proliferation.

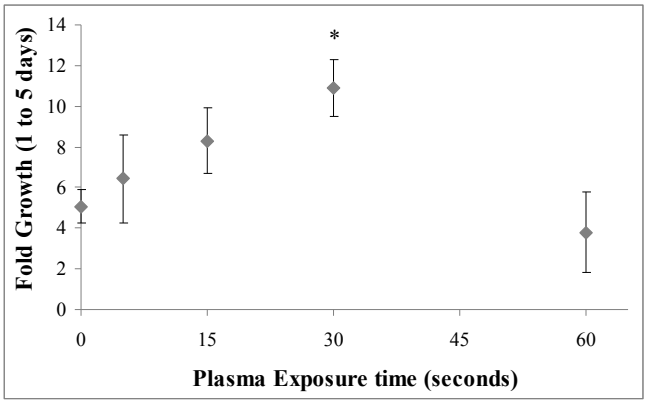


Fig. 6. Endothelial cell fold growth is enhanced in non-thermal plasma treated cells 5 days after treatment. * $p < 0.01$ as compared to control.

IV. Discussion

We have demonstrated that non-thermal plasma treatment is relatively non-toxic to endothelial cells. More than 70% of cells survive plasma treatment without compromising long term viability. These results are promising since our treatment model – an endothelial cell monolayer on a glass substrate covered with a thin media film – is significantly more severe than what would be experienced by cells either *in vivo* or as part of three-dimensional *in vitro* models. We believe that plasma treatment will prove even less toxic to endothelial cells within a physiologic tissue.

Our preliminary data suggest that low levels of non-thermal plasma treatment can induce endothelial cell proliferation. One potential mechanism for this proliferative effect is the release of growth factors such as fibroblast growth factor-2 (FGF2), a potent angiogenic factor. Plasma treated endothelial cells experience membrane damage and release of intracellular contents such as LDH. FGF2 is only known to be released during cell membrane damage, therefore it is possible that plasma induces FGF2 release. This FGF2 would then bind to endothelial cells that survive the initial plasma treatment and induce proliferation. Further experimentation is needed to confirm this hypothesis.

The specific mechanism for the plasma effect on endothelial cells is similarly unclear. Non-thermal plasmas produce both charged ions and reactive oxygen species. Both of these could be toxic to cells, induce low levels of cell membrane damage, and potentially change intracellular signaling pathways. Specific plasmas can be created to produce either charged ions or reactive oxygen species in order to elucidate the critical mechanism.

V. Conclusions and Future work

The initial results presented in this paper indicate that non-thermal plasma is relatively non-toxic to endothelial cells at short exposure times, while non-thermal plasma treatment at longer exposure times is cytotoxic. An intriguing finding is the enhancement of cell proliferation following plasma treatment. It is therefore possible that by tuning plasma properties, angiogenesis could be controlled. The proliferative mechanism, as well as the type of cell death (apoptosis or necrosis) incurred due to non-thermal plasma, needs to be evaluated. Low power plasma treatment shows promise for novel therapies focused on promotion or inhibition of endothelial cell mediated angiogenesis.

References

[1]. Vargo, J. J., *Clinical Applications of Argon Plasma Coagulator*, Gastrointestinal Endoscopy, 2004. 59(1): p 81-88

[2]. Fridman, G., et. al., *Blood Coagulation And Living Tissue Sterilization By Floating Electrode Dielectric Barrier Discharge In Air*, Plasma Chemistry and Plasma Processing 2006. 26: p 425-442

[3]. Kalghatgi, S., U., et al; *Mechanism of Blood Coagulation by Non-Thermal Atmospheric Pressure Dielectric Barrier Discharge Plasma,* IEEE Trans. Plasma Sci. 2007 35(5): p 1559-1566.

[4]. Fridman, G., et. al., *Floating Electrode Dielectric Barrier Discharge Plasma in Air Promoting Apoptotic Behavior in Melanoma Skin Cancer Cell Lines*", *Plasma Chemistry and Plasma Processing, 27(2): p163-176.*

[5]. Kieft, I. E., et. al., *Reattachment and Apoptosis after Plasma-Needle Treatment of Cultured Cells,* IEEE Tans. Plasma Sci. 2006 34(4): p 1331-1336

[6]. Stoffels, E., et. al., *Superficial Treatment Of Mammalian Cells Using Plasma Needle*, Journal of Physics D: Applied Physics, 2003. 36: p 2908-2913

[7]. I.E. Kieft, D. Darios, A.J.M. Roks, E. Stoffels-Adamowicz, *Plasma Treatment Of Mammalian Vascular Cells*, IEEE Trans. Plasma Sci., 2005. 33(2): p 771-775

[8]. Stoffels, E., et. al., *Gas Plasma Effects on Living Cells*, Physica Scripta, 2004. T107: p 79-82.

[9]. Dobrynin, D., Ignakhin, V., Gostev, V., and Misyun, F., *Cold Plasma Treatment of Bacterial Cornea Lesions and Eyelid Defeats*

[10]. Fridman, G., et al., ***Comparison of Direct and Indirect Effects of Non-Thermal Atmospheric Pressure Plasma on Bacteria***, *Plasma Process. Polym., 2006, 10.1002/ppap.200600217.*

[11]. Siemens, C. W., *On the Electrical Tests Employed During the Construction of the Malta and Alexandria Telegraph, and on Insulating and Protecting Submarine Cables*. Journal of the Franklin Institute, 1862. 74(3): p. 166-170

[12]. Egli, W., Kogelschatz, U., and Elliason, B., *Modeling of Dielectric Barrier Discharge Chemistry*. Pure and Applied Chemistry, 1994. 66(6): p. 1275-1286.

[13]. Ayan, H., et al., *Heating Effect of Dielectric Barrier Discharges in Sterilization. The 34th IEEE International Conference on Plasma Science, June 17-22, 2007, Albuquerque, New Mexico*.

Authorized licensed use limited to: Drexel University. Downloaded on November 20,2026 at 18:16:30 UTC from IEEE Xplore. Restrictions apply.