

TREATMENT OF ENDOTHELIAL CELLS BY NON-THERMAL ATMOSPHERIC PRESSURE DIELECTRIC BARRIER DISCHARGE PLASMA

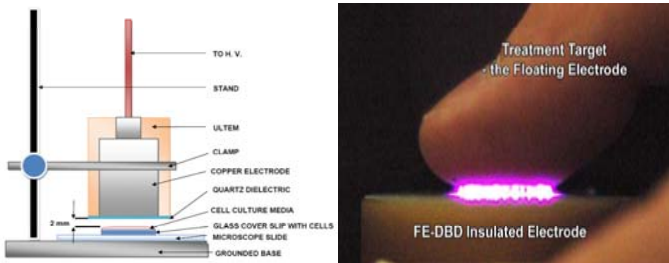
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INTRODUCTION

- Non-thermal atmospheric pressure dielectric barrier discharge plasma (Non-thermal plasma) has emerged as a promising new tool in medicine.
- Non-thermal plasma has enabled a number of new medical applications including blood coagulation, induction of apoptosis, modulation of cell attachment and sterilization of living tissues without damage.
- 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



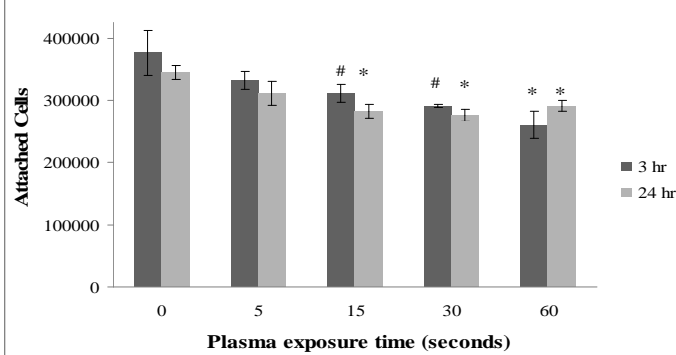
NON-THERMAL DBD PLASMA

- 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.
- The discharge creates an electrically safe plasma without substantial heating of the gas. This approach allows the electrical charges in the plasma to initiate or catalyze biological processes without thermal damage of biological samples

EXPERIMENTAL METHODS

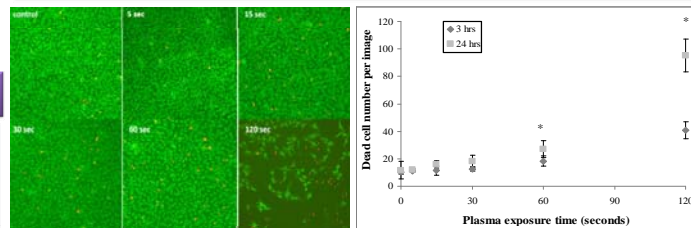
- Endothelial cells on glass cover slips, covered with 100 μ l of media (to prevent drying) were exposed to low power plasma at exposure times ranging from 5 to 120 seconds. Following treatment cover slips were returned to the incubator in 12-well plates after adding 1.5 ml of supplemented media.
- Non-thermal plasma endothelial cell cytotoxicity was measured via cell counts and a Live/Dead assay. For cell counts, at 3 and 24 hours following plasma treatment, attached (live) cells were trypsinized and counted using a Coulter counter. Cell viability was assessed with a Live/Dead Viability/Cytotoxicity Assay
- Endothelial cell membrane damage was assessed by measuring release of Lactate Dehydrogenase (LDH). 0.5 ml of media was taken from each sample at 0 to 24 hours after plasma treatment. LDH was quantified in the collected media samples using the Cytotox-ONE Homogeneous Membrane Integrity Assay
- To determine effect on endothelial cell proliferation, 10,000 cells seeded on 18 mm diameter were exposed to low power plasma, transferred to new 12-well dishes, and incubated for an additional 5 days. Cell number was quantified on days 1 and 5 by trypsinizing attached cells and counting them with a Coulter counter. Fold growth was measured by taking ratio of cell number on day 5 to those on day 1
- For measuring release of FGF a 125 μ l of media was taken from each sample at 0 to 24 hours after plasma treatment. FGF-2 levels in the media were quantified by FGF-2 ELISA.

ATTACHED CELLS VS PLASMA TREATMENT



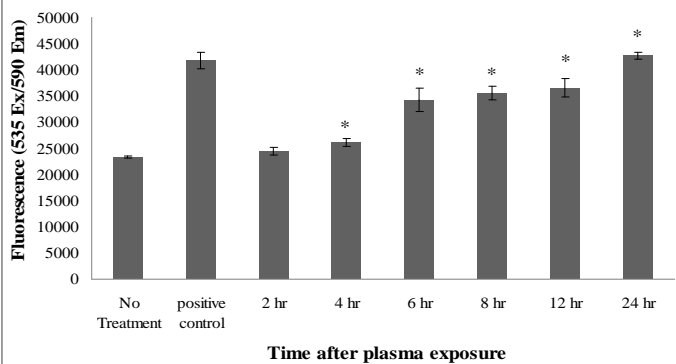
The number of live, attached cells decreases as plasma exposure time increases up to 60 s ($p < 0.01$ by ANOVA) at 3 and 24 hours post-exposure. * $p < 0.01$ as compared to 0 s treated control. # $p < 0.05$ as compared to 0 s treated control

LIVE/DEAD VIABILITY/CYTOTOXICITY ASSAY



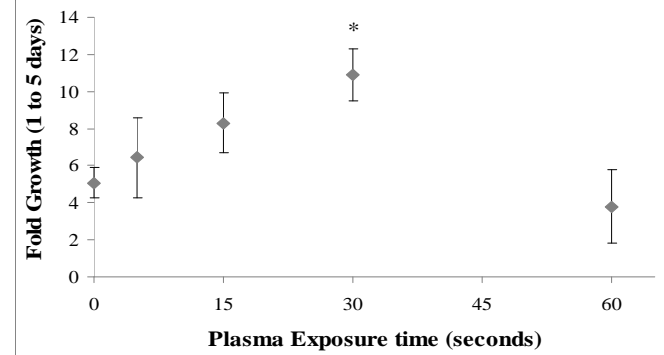
Endothelial cell death increased with plasma exposure time, as measured by Live/Dead assay. Fluorescent images, and quantization of five areas of each sample. * $p < 0.01$ as compared to control (0 s)

ENDOTHELIAL CELL MEMBRANE DAMAGE



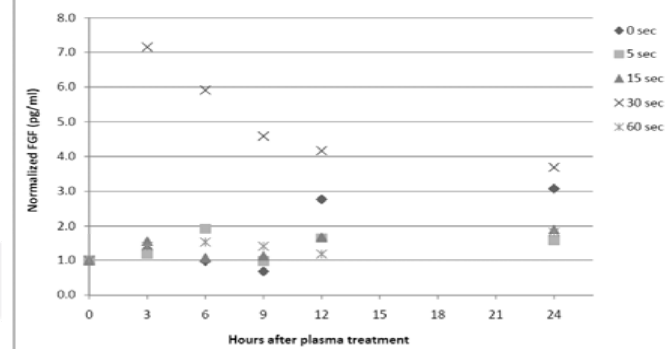
Endothelial cell LDH release increases up to 24 hours post plasma exposure. * $p < 0.01$ as compared to untreated cells

PROLIFERATION ASSAY



Endothelial cell fold growth is enhanced in 30 s low power non-thermal plasma treated cells 5 days after treatment. * $p < 0.01$ as compared to control.

FGF ELISA



Endothelial cells exposed to 30 s of low power non-thermal plasma release significant levels of FGF at 3 and 6 hours after plasma treatment as compared to 0 s treated control

CONCLUSIONS AND FUTURE WORK

- Non-thermal plasma is relatively non-toxic to endothelial cells at short exposure times
- An intriguing finding is the enhancement of cell proliferation following plasma treatment. Angiogenesis could be controlled
- The proliferative mechanism and 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.