Proceedings of the ASME 2009 Summer Bioengineering Conference (SBC2009)
June 17-21, Resort at Squaw Creek, Lake Tahoe, CA, USA

SBC2009-XXXXXX

NON-THERMAL ATMOSPHERIC PRESSURE DIELECTRIC BARRIER DISCHARGE PLASMA ENHANCES ENDOTHELIAL CELL PROLIFERATION VIA FIBROBLAST GROWTH FACTOR-2 RELEASE

Sameer Kalghatgi (1), Alexander Fridman (2), Gary Friedman (1), Alisa Morss Clyne (2)

(1)Electrical and Computer Engineering
Drexel University
Philadelphia, PA-19104
USA

(2)Mechanical Engineering and Mechanics
Drexel University
Philadelphia, PA-19104
USA

INTRODUCTION

Cold atmospheric pressure plasma is currently being investigated for a wide range of clinical applications, including skin sterilization, blood coagulation [1, 2], malignant cell apoptosis [1], and wound healing [1]. However, the effect of non-thermal plasma on the vasculature is unclear. Blood vessels are affected during plasma treatment of all tissues, and vessels themselves may be an important potential target for clinical plasma therapy.

We investigated the effect of cold plasma treatment on endothelial cells, which line the inner surface of blood vessels. Endothelial cells play a guiding role in angiogenesis, the growth of new blood vessels from existing vessels. In various disease conditions, healing may result from promoting or blocking angiogenesis.

We hypothesize that plasma treatment can be varied to grow or regress blood vessels. We have previously demonstrated that high dose plasma treatment induces endothelial cell apoptosis, whereas low plasma doses are relatively non-toxic. In this paper, we present enhanced proliferation in low dose plasma treated endothelial cells *in vitro,* as well as mechanisms for the observed effect.

MATERIALS AND METHODS

Cell Culture

Porcine aortic endothelial cells (PAEC) were maintained in low glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin. For plasma treatment, cells were washed with phosphate buffered saline, detached with 0.1% trypsin and seeded near confluence on 18 mm diameter glass cover slips in 12-well plates. 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.

Non-Thermal Plasma Treatment

Non-thermal atmospheric pressure dielectric barrier discharge plasma was produced using an experimental setup similar to one previously described [2]. PAEC on glass cover slips were exposed to low power plasma for 0 to 60 seconds. Following plasma treatment, cover slips were immediately placed in a new 12-well plate, 1.5 ml of supplemented media was added to each well, and the samples were returned to the incubator.

Endothelial Cell Counts and Proliferation

Viable endothelial cell number was determined by counting trypsin-detached cells in a Coulter counter. Endothelial cell proliferation was measured through cell counts either on directly treated cells or through a conditioned media assay. For directly treated cells, fold proliferation was determined by taking the ratio of cell number on day five to day one.

Endothelial Cell Fibroblast Growth Factor-2 Release

Fibroblast growth factor-2 (FGF2) release from plasma treated cells was measured by enzyme linked immunosorbent assay (ELISA) using FGF Elisa Kit (R&D Systems) as per manufacturer instructions. FGF2 effects were blocked using an FGF2 neutralizing antibody (10 μg/ml), which was pre-incubated for 30 min with the conditioned media prior to adding it to cells.

Statistical Analysis

Data are mean ± SD. Statistical significance ($p<0.05$, #) was evaluated using Student's t test (2 groups) and ANOVA (>2 groups).

RESULTS

Cell Counts and Proliferation

Endothelial cell proliferation was enhanced by low dose non-thermal plasma treatment. Cells treated with plasma showed greater viable cell number than control with up to 30 seconds of plasma treatment. With the 30 second treatment, endothelial cells demonstrated twice as many viable cells on day 5 as untreated controls. However, increased plasma treatment times beyond 30 seconds resulted in decreased cell number via induction of apoptosis.

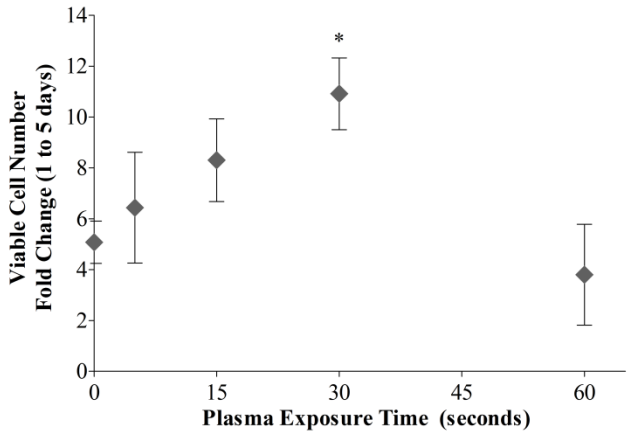

Figure 1 Endothelial cell proliferation is enhanced 5 days after plasma treatment, as measured by viable cell counts.*p < 0.01

Endothelial Cell FGF2 Release

To determine if enhanced proliferation was related to cell growth factor release, we measured FGF2 in cell conditioned medium following 30 s plasma treatment. The media FGF2 level increased up to 3 h after plasma treatment (3.9 J/cm^2, 30 s) and then rapidly decreased up to 24 h after plasma treatment. In contrast, FGF2 media levels for cells treated with 10 ng/ml TNF-α as a positive control rose more slowly but continued to rise up to 24 h.

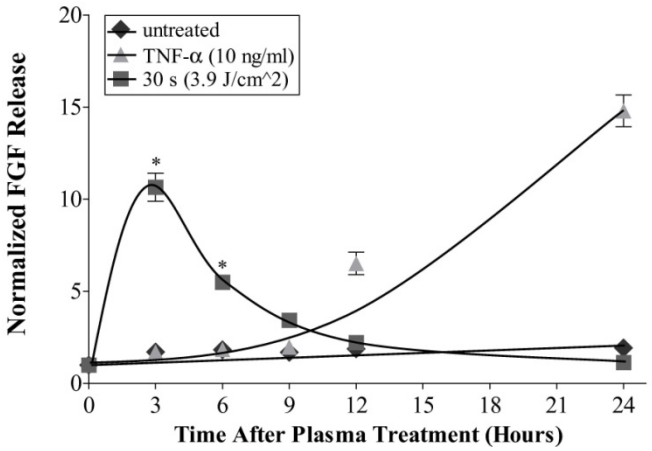

Figure 2 Non-thermal plasma induces FGF2 release from endothelial cells which peaks at 3 h after plasma treatment and subsequently decreases to 24 h after plasma treatment.*p<0.01

Role of FGF-2 release in Plasma Enhanced Proliferation

The specific role of released FGF2 in enhanced endothelial cell proliferation by plasma treatment was investigated using an FGF2 neutralizing antibody to block FGF2 effects. The FGF2 neutralizing antibody had no significant effect on cells exposed to untreated cell conditioned medium. However, the FGF2 neutralizing antibody significantly suppressed proliferation in endothelial cells exposed to plasma-treated cell conditioned medium. The viable cell number for samples incubated in plasma-treated cell conditioned medium with a neutralizing antibody was similar to samples incubated in untreated cell conditioned medium.

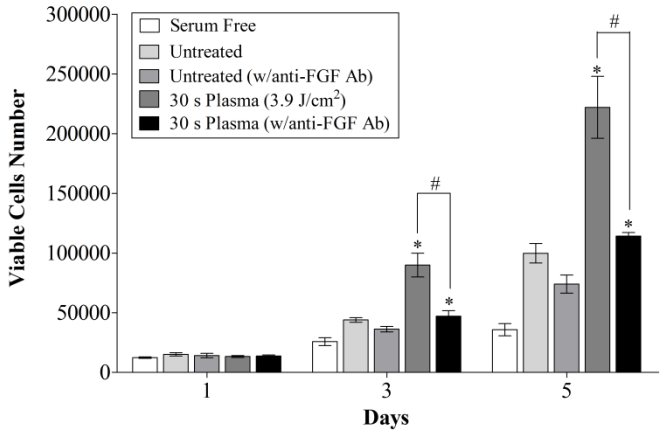

Figure 3 Blocking FGF2 in the conditioned medium by FGF-neutralizing antibody inhibits proliferation of non-thermal plasma treated endothelial cells.*p < 0.01, #p < 0.05

DISCUSSION

Non-thermal plasma is relatively non-toxic to endothelial cells at shorter exposures, while higher doses of plasma induce cell death via apoptosis**.** Our prior data suggest that low dose plasma treatment induces sub-lethal cell membrane damage, since lactase dehydrogenase is released but viable cell number is not significantly decreased with less than 60 second treatment.

We now show that low dose plasma treatment enhances endothelial cell proliferation through rapid release of FGF2. Since FGF2 has no signal sequence for secretion, it is only known to be released with cell membrane damage [3]. Non-thermal plasma produces a large concentration of reactive oxygen species in the extracellular environment (cell medium) covering the cells during treatment. The endothelial cell membrane damage, and the subsequent release of FGF2, may be related to intracellular and extracellular reactive oxygen species produced by active short living and long living neutral plasma components.

CONCLUSIONS

Low dose, non-thermal atmospheric pressure dielectric barrier discharge plasma enhances endothelial cell proliferation via FGF2 release. Our previous work indicates that high dose plasma treatment induces endothelial cell death via apoptosis. These studies suggest that modulation of plasma treatment dose may be able to progress or regress blood vessel growth. Future work includes cell treatment within a three-dimensional model.

REFERENCES

[1] Fridman, G. et. al. Plasma Processes and Polymers, 5(6):p 503-533 (2008).
[2] Kalghatgi, S., et. al., IEEE Trans. Plasma Sci., 35(5): 1559-1566 (2007).
[3] Nugent, M.A. et. al. Int J Biochem Cell Biol 32:115-120.(2000)