Exposure Time-Dependent Dynamic Effects of Non-Thermal Plasma

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Abstract: We investigated the temporal, dynamic effects of non-thermal plasma (NTP) on a human keratinocyte cell line *in vitro* to identify correlative relationships between plasma effectors and cellular responses. NTP exposure resulted in increased mitochondrial superoxide and nitric oxide production, and extra cellular calcium (Ca^{2+}) release in an exposure time-dependent manner. Optimal NTP exposure enhanced cellular proliferation, important for promoting scratch closure.

1. Introduction

The transient application of NTP exposes cells to reactive oxygen and nitrogen species (RONS) and changes the redox status of cells in tissues immediately [1,2]. However, clinically relevant biological changes occur hours or days later. Therefore, it is difficult to determine if the "dose" of NTP was adequate to achieve desired clinical outcomes during NTP application [3,4]. To partially address this limitation, we investigated the relationship between applied doses (as defined by duration of exposure) and NTP effectors with biological outcomes in an *in vitro* scratch closure model. Measurement of NTP produced RONS was correlated with observed immediate and late molecular changes in cells and their efficacy in a scratch wound closure model. This is the first step toward developing approaches to establish a definition of dose for plasma medicine.

2. Methods

NTP was generated using a volume dielectric barrier discharge (vDBD) driven by a microsecond pulsed power supply. Scratched human HaCaT keratinocyte cultures were exposed for 5, 10 or 15 seconds in a 24-well plate. Hydrogen Peroxide (H_2O_2), and Nitric Oxide (NO) were measured electrochemically, immediately after NTP exposure. To characterize early changes in cells, mitochondrial superoxide, and mitochondrial NO were measured immediately after NTP exposure using commercial kits. Extracellular calcium (Ca^{2+}) release from cells was measured electrochemically immediately after NTP exposure. Toxicity of NTP was determined by total live cell count 24 hours after NTP exposure. The expression of the proliferative marker Ki 67, as measured by antibody staining, was used to determine the late effect of NTP on cell function that closely reflects ability to close a scratch wound 24 hours after NTP exposure. Finally, efficacy of the different NTP exposure duration for scratch closure was established by image analysis over a period of 24 hours. All analysis was unbiased and performed using an automated imaging system and its associated software. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, using GraphPad Prism.

3. Results and Discussion

The amount of H_2O_2 and NO produced in the medium increased with increasing NTP exposure time. This correlated with the early changes in the mitochondrial superoxide produced in cells (Fig 1 A). A corresponding increase in mitochondrial NO indicated that cells undergo oxidative stress in an NTP exposure time-dependent manner. Ca^{2+} release from cells also trended with exposure time. These early changes correlate with live cell counts in culture (Fig 1 B)., i.e., 15 second exposure resulted in the highest cytotoxicity. However, maximum cell proliferation, as indicated by Ki67 expression, resulted from 10 second NTP exposure and promoted scratch closure. Our results indicate that the optimum dose of NTP enhances cell proliferation resulting in healing of wounds.

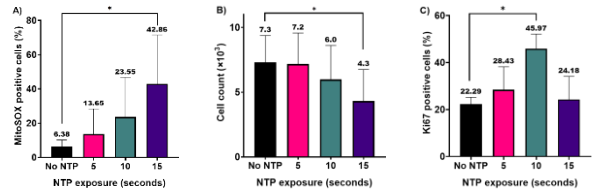

Figure 1: A) Mitochondrial superoxide production immediately after NTP B) Total cell count 24 hr. after NTP exposure; C) Proliferative (Ki 67 Positive) cells (%) at 24 hr. after NTP exposure.

4. Conclusion

Correlative relationships between plasma effectors and the dynamic, early, and late cellular responses can be used for predictive dose for specific biological applications.

Acknowledgement

This research was supported by the National Institutes of Health (Award Number 5R01EB029705).

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