Adaptation of Operational Parameters of Cold Atmospheric Plasma for Cancer Treatment

Eda Gjika1, Sonali Pal-Ghosh2, Anna Tang1, Megan Kirschner1, Zhitong Chen1, Gauri Tadvalkar2, Jerome. Canady3, Mary Ann Stepp3, Michael Keidar4

1 Department of Mechanical and Aerospace Engineering, George Washington University, Washington, DC 20052, USA.
2 Department of Anatomy and Regenerative Biology, George Washington School of Medicine, Washington DC 20052.
3 Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma Park, MD 20912, USA.

1. STUDY MOTIVATION

The application of cold atmospheric plasma (CAP) in the treatment of cancer cells has attracted a great deal of attention in the past two decades. CAP has been introduced as a selective therapy with an affinity of inducing cell death in cancer cells while leaving normal cells unharmed. Successful application of CAP has been reported for in vitro treatment of approximately 20 types of cancers.

Fig. 1. Selective treatment of glioblastoma.

Findings from several studies revealed that different types of tumors exhibit different responses to treatment when exposed to the same CAP conditions. Therefore, the development of an Adaptive CAP Platform concept for enhancing selective destruction of cancer cells independent of cancer type or CAP treatment condition becomes imperative.

In vitro proof-of-principle investigation:
1. Identify method for real time monitoring of cell death.
2. CAP mechanism of action.
3. Response to CAP exposure in different cancers (Glioblastoma (U87) and Breast Cancer (MDA-MB-231).
4. Role of two CAP operational parameters:
   a) Treatment duration in the modification of overall plasma action.
   b) Discharge voltage in the adjustment of plasma composition.

Fig. 2. Adaptive CAP Platform for enhanced therapy independent of cancer type.

School of Engineering & Applied Science

THE GEORGE WASHINGTON UNIVERSITY

2. ROS & RNS CONCENTRATION

The effectiveness of CAP therapy has been linked to CAP generated reactive oxygen species (ROS) such as hydrogen peroxide. Regulating ROS consumption may serve as trigger of cell signaling related to the initiation of apoptosis – controlled cell death.

a) CAP generates substantially higher hydrogen peroxide concentration with increasing exposure time and discharge voltage.

b) Hydrogen peroxide concentration in media decreases over time and the rate of consumption decreases.

Fig. 3. Concentration of CAP generated extracellular H2O2 in cell culture media measured immediately after treatment.

3. CELL RESPONSE IN REAL TIME

Response to treatment can be monitored in real time with Real-Time-GLO MT Cell Viability Assay (Promega) in a continuous read out method. CAP reduces cell viability as a function of:
1. Treatment duration (Figure 4)
2. Plasma discharge voltage (Figure 5)

Cell response to CAP treatment is detectable in real time.

Fig. 4. Cancer cell viability after different CAP exposures.

Fig. 5. Cancer cell viability after treatment with different CAP discharge voltages.

4. CAP EFFECT ON CELLULAR PROCESSES

4.1. APOPTOSIS

CAP induces controlled cell death (apoptosis) with plasma treated cancer cells showing a higher apoptotic activity than untreated control for the majority of the post treatment time points. The trend in the rate of apoptosis varies per cell line within the first hour after CAP exposure.

Fig. 6. Effect of CAP treatment on MDA-MB-231 cell death.

4.2. PROTEIN SYNTHESIS

CAP influences protein synthesis deregulation which impacts cancer development and growth. The impact of CAP on the rate of protein synthesis was assessed with a Click-IT Plus OPP assay by incorporating OPP as a proxy for total Protein synthesis.

CAP treatment results in a decrease of green fluorescence compared to untreated controls.

Fig. 7. CAP reduces rate of protein synthesis in cancer cells.

4.3. MITOCHONDRIAL MEMBRANE POTENTIAL

Loss of mitochondrial membrane potential has been reported as an essential event that commits cells to undergo apoptosis. Glioblastoma and breast cancer cells data reveal a statistically significant reduction in mitochondrial membrane potential at 6, 12 and 24 hours after treatment associating the inability to retain mitochondrial membrane integrity to CAP exposure.

Fig. 8. CAP treatment impacts membrane integrity of mitochondria.

5. SUMMARY

- Instantaneous CAP response can be effectively monitored by Real-Time-GLO Assay with results interpreted as cell viability.
- CAP reduces cell viability as a function of discharge voltage and treatment time.
- Regulating ROS secretion or consumption may serve as a trigger of cell signaling related to the initiation of apoptosis.
- CAP decreases cell viability and induces apoptosis as measured by a loss of mitochondrial potential and deregulation of OPP expression – indicative of the rate of protein synthesis.

Reference


Fig. 8. CAP treatment impacts membrane integrity of mitochondria.