# Cold Plasma Technology assisted Neuroblastoma Cancer Treatment

# Abstract

Neuroblastoma is a very rare type of cancerous tumor that normally affects children. Each year, 800 new cases are diagnosed in the United States. Neuroblastoma accounts for 50 percent of all cancers in infants, making it the most common tumor in infants younger than 1 year. Neuroblastoma tumors generally develop in the adrenal glands and can spread to other areas including the chest, the spine or spinal cord regions and the abdomen. Application of cold plasma technology for leukemia cancer treatment has been researched and demonstrated .This research study investigates the effect of our newly developed cold plasma technology on neuroblastoma cancer cells and also develop an optimized plasma dosages for feasible cancer treatment. In this research, Neuro2a cells that are murine neuroblastoma line derived from AJ mice is cultured in DMEM media. Triplicate samples of 104 neuroblastoma cells were plated and treated with cold plasma technology. Post-treated cells were read at zero-hour, 24-hour and 48-hour time periods after treatment and the results of the study will be presented.

# Introduction

Plasma, the fourth state of matter, is a partially ionized gas consisting of heterogenous mixture of various ions, free radicals, UV photons, electrons and reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [7,8]. Generally, plasma is generated in high temperature laboratory conditions, however, recent progress in atmospheric plasma led to the creation of cold plasmas with ion temperature close to room temperature [4], called “non-thermal” or “cold atmospheric plasma” [10]. Cold Atmospheric Plasma (CAP) is produced by applying pressure and high voltage electric field to compressed gas [9,10]. The ability to generate plasma at room temperature and its unique heterogenous composition allows scientists to interfere with tumor cells growth and their mechanism of action. CAP exposure has proven to have damaging effects on polypeptides, DNA, and enzyme proteins involved many biochemical activities [5]. These activities include redox signaling, pathways involving cell-fate determining protein kinases and transcription factors [8]. Due these remarkable capabilities of CAP, recent investigation of cold plasma focuses on its anti-cancer properties and how it can be utilized in cancer treatments.

Previous research has indicated cancer cells to be particularly sensitive to ROS, a product of CAP. Effects of ROS on cancerous cells included DNA damage, cellular proliferation and apoptosis (programmed cell death) [5,7,10]. It has also been observed that plasma induces viable cells to produce their own ROS/RNS. Due to its deteriorating effects on cancerous cells, its effects on normal, healthy cells have come into question. Therefore, it is important to note that non-cancerous cells seem to tolerate CAP and some are even resistive to it [4]. The effect

of plasma on healthy cell is minimal and well tolerated. Media pH level remain unchanged after treatment and thermal effects are negligible [4].

In this research, CAP was applied to neuroblastoma cells. Neuroblastoma (NB), an embryonic malignancy, originates in early childhood from neural cells and is one of the most common neoplasms in children, accounting for nearly 50% of solid tumors in children of 1-4 years of age. Neuroblastoma is genetically, clinically, morphologically and biologically an extremely heterogeneous tumor[2,3]. This makes choosing an adequate therapeutic treatment difficult. CAP has proven to kill the viable cancer cells by inducing apoptosis and limiting tumor migration rate [4].

## Materials and Methodology

### Cold Atmospheric Plasma

The CAP device utilized for this experiment was previously described [9] and a schematic block diagram of the system is shown below in figure 1. Detailed explanation of CAP production and application of using this system has also been previously mentioned [9].

### Murine Neuroblastoma Cells and Cell Culture

Neuro2a cells, neuroblast cell type of the brain tissue associated with disease neuroblastoma, were purchased from American Type Culture Collection and cultured in D10 media of DMEM medium containing 10% Foetal bovine, 1% Streptomycin/Penicillin, 1% L-glutamate. Cells were thawed, incubated and then subcultured. Culture method instruction provided on ATCC website were followed and subculture was cultivated in 5:1 ratio of growth medium and cells, respectively.

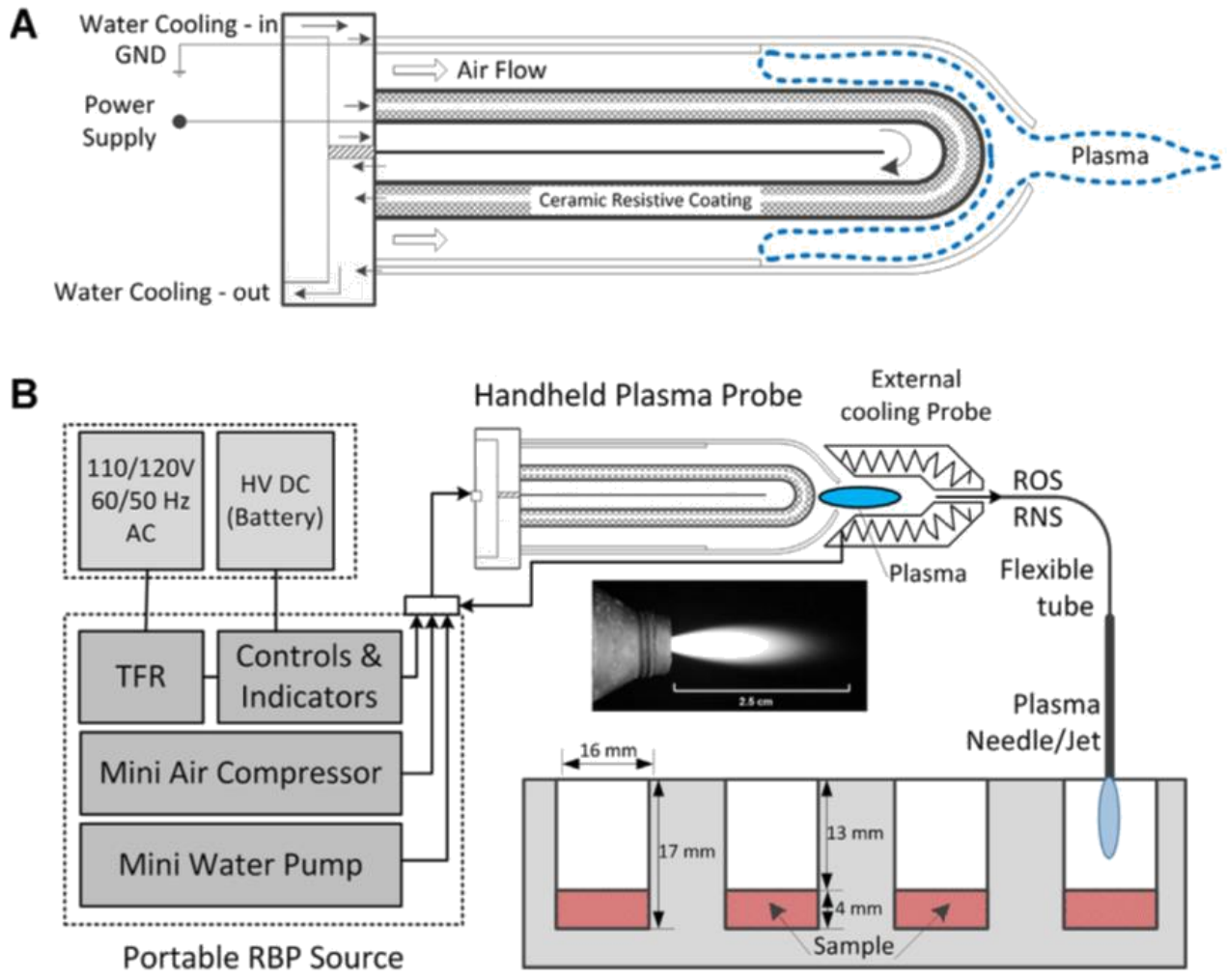


**Figure 1: A:** Air (the operating gas) is passed through the cylindrical electrode gap and gets ionized by exciting the electrodes using a controlled input voltage, thus producing plasma. **B:** The modification of the system allows for the plasma to pass through an additional cooling configuration and reactive nitrogen species are delivered to the samples for treatment via the plasma needle. Samples suspended in the 24-well micro-plate are separated from the plasma needle by a 13 mm gap.

**Sample Assay and Data Collection**

Cells were plated in 96 well plated in 100ul of D10 media. MTT reagent was used to indicated cell metabolic activity after plasma exposure. Reagent was prepared by mixing 5 mg of Thiazolyl Blur Tetrazolium Bromide in 1 ml of phosphate-buffered saline; both items

purchased from Sigma-Aldrich. After CAP treatment, cells were analyzed immediately to obtain zero-hour exposure result and incubated for 24-hour or 48-hour then analyzed again. Number of viable cells were manually counted using polarized microscope. Upon completion of the experiment, cells were lysed and discarded as biohazard.

## Results

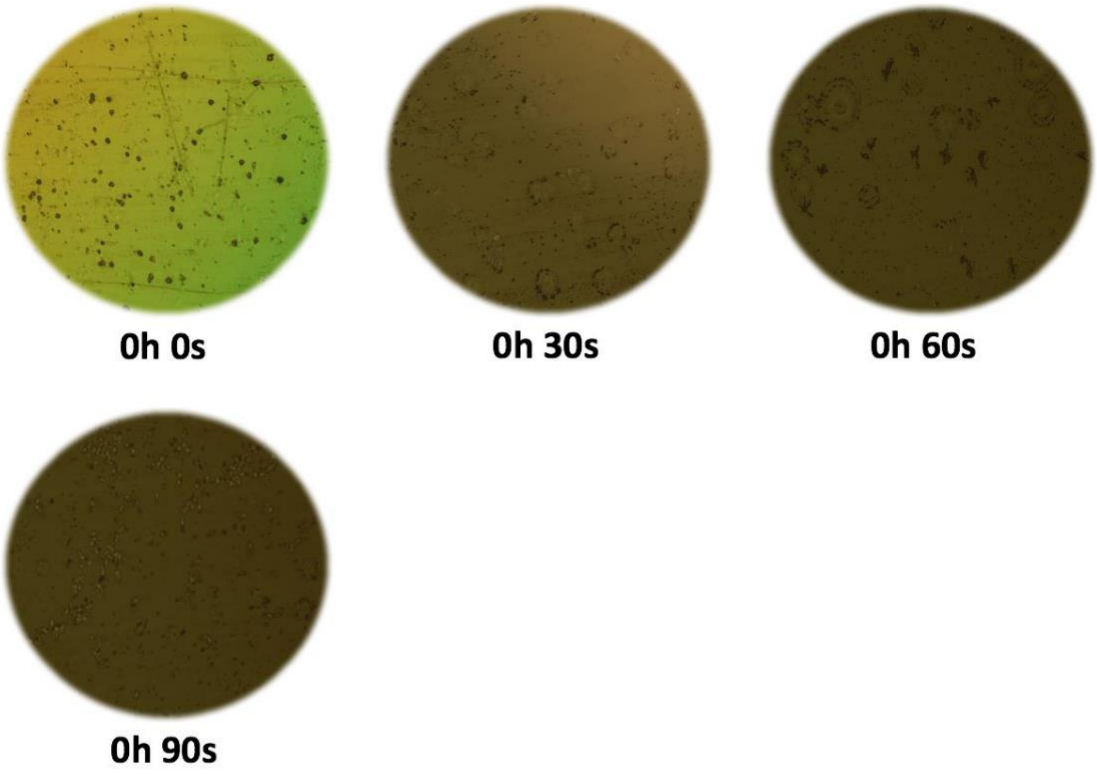


**Figure 2:** microscope pictures for 0s, 30s, 60s and 90s at 0 hours after treatment. There is a drastic change in cells at 0s and at 90s; at 0s most cells are healthy and alive while at 90s most cells are ruptured and dead.

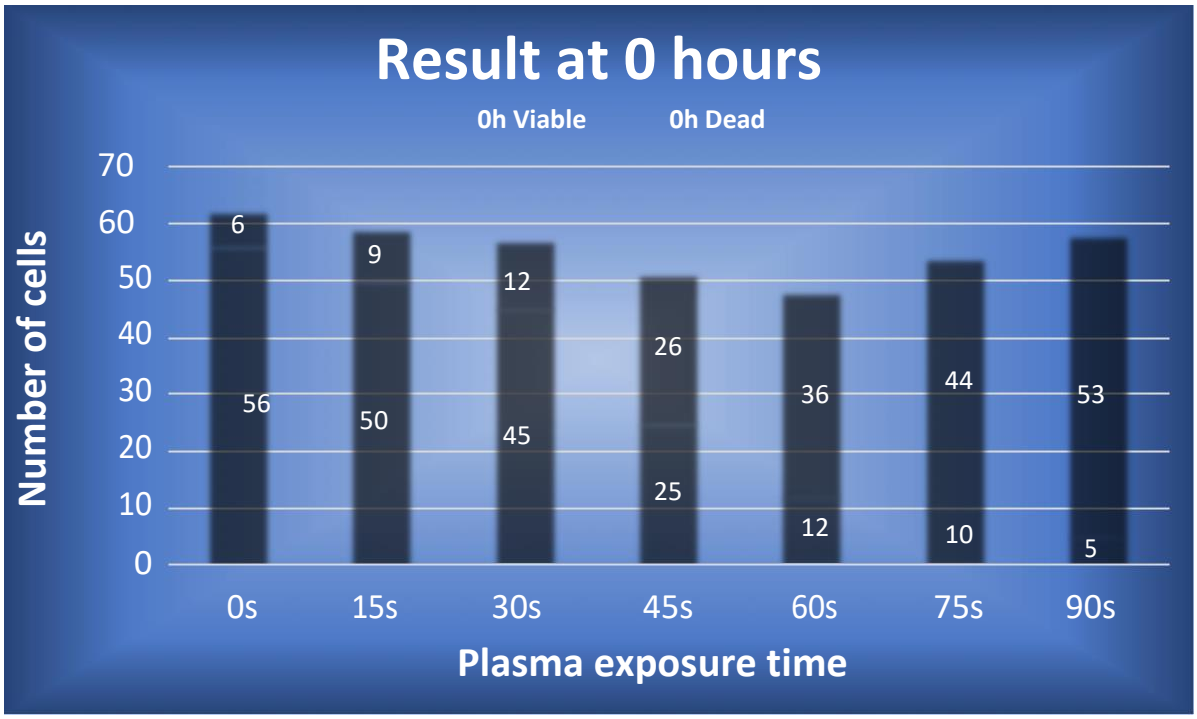


**Figure 3:** amount of viable and dead cells right after the plasma treatment.

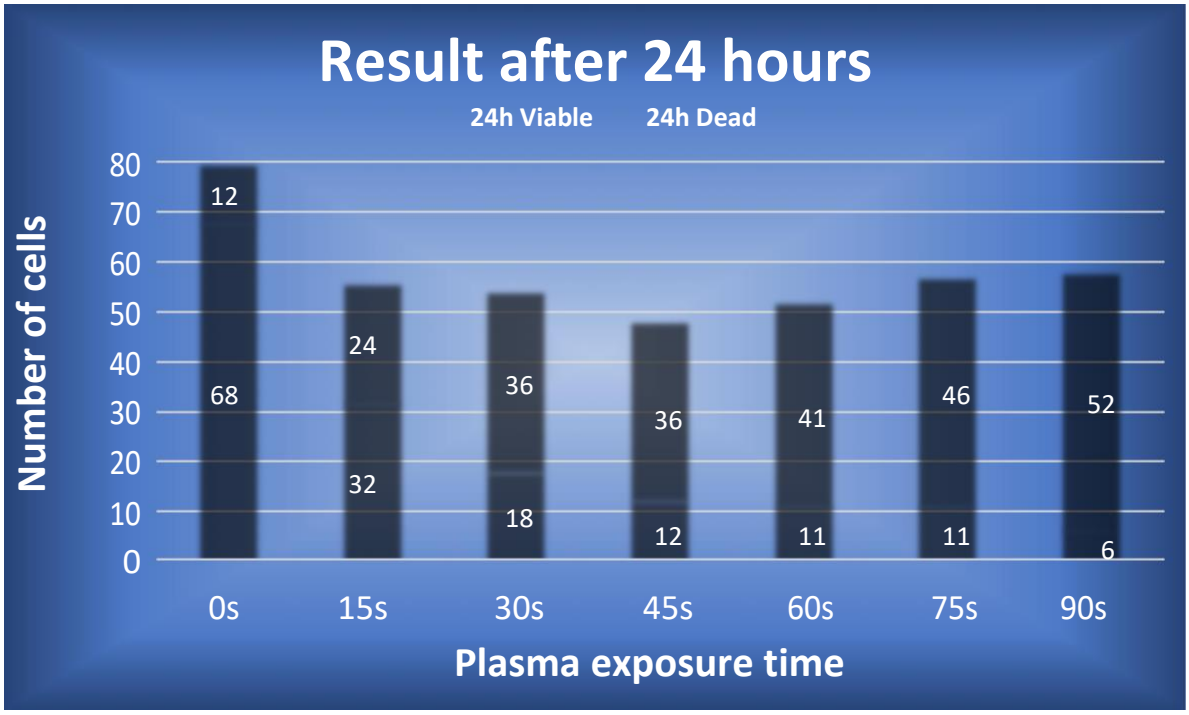


**Figure 4:** amount of viable and dead cells 24 hours after the plasma treatment.

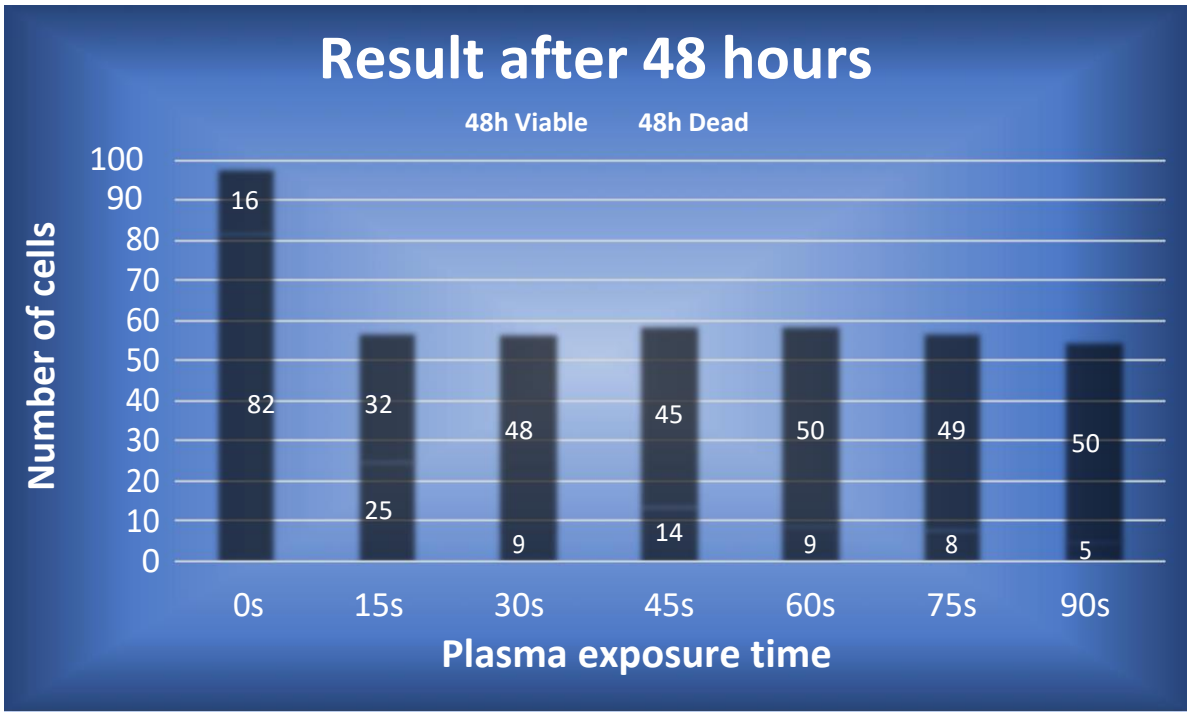


**Figure 5:** amount of viable and dead cells 48 hours after the plasma treatment.

## Conclusion

Based on the outcomes of the research, it can be concluded that Cold Atmospheric Plasma (CAP) does prove to be effective in treating neuroblastoma cancer cells. CAP decreases metabolic activity, induces apoptosis and dramatically reduces the number of viable cancer cells [10]. Current hypothesis regarding the mechanism of action of CAP therapy for cancer suggests that ROS is primarily responsible the effect of CAP [8]. As expected, the amount of viable Neuro2a cells decreased with increasing plasma exposure time. At the same time, the ratio of viable to dead cells for 24 hours and 48 hours after treatment was, for the most part, quite consistent. Although the ratio of viable cells to dead cells was slightly higher for 48 hours,

it was not significantly higher to conclude that the outcome after 48 hours of treatment results is better data than 24 hours. More trials are required to make that conclusion.

## Acknowledgement

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