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## Effect of Cold Atmospheric Plasma on CT26 Murine Colorectal Carcinoma Cells

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### OPEN ACCESS

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### ABSTRACT

**Introduction:** Colorectal cancer remains a major clinical challenge. Cold atmospheric plasma (CAP) generates reactive oxygen and nitrogen species that selectively damage cancer cells, triggering oxidative stress and cell death.

**Materials and Methods:** A helium CAP jet (AC, 6.8 kV, 12–13 kHz) was used to treat CT26 murine colorectal carcinoma cells. The cells were seeded at  $1 \times 10^5$  cells/mL in 24-well plates and exposed to CAP for 30–240 s at fixed voltage, frequency, gas flow, and 1 cm jet-sample distance. The levels of hydrogen peroxide ( $H_2O_2$ ) in PBS and DMEM/F12 + 10% FBS were quantified calorimetrically using a horseradish peroxidase-based kit. Intracellular reactive oxygen species (ROS) were measured by flow cytometry. Cell viability was assessed using the MTT assay, which measures mitochondrial metabolic activity by reducing tetrazolium to formazan.

**Results and Discussion:** CAP exposure elevated extracellular  $H_2O_2$  over time. In the PBS solution,  $H_2O_2$  levels increased from 36.3  $\mu M$  to 342  $\mu M$  over 30–240 s. In the DMEM/F12 + FBS solution, levels increased from 92  $\mu M$  to 360  $\mu M$  over 240 s. Intracellular ROS levels rose from 61  $\mu M$  (control) to 79  $\mu M$  over 180 s. CAP treatment reduced viability in a duration-dependent manner, decreasing to 82% at 30 s and 18% at 240 s. The  $IC_{50}$  was determined to be between 90 and 120 seconds, which is consistent with CAP's cytotoxic action via oxidative mechanisms. These findings support the use of CAP as a selective antitumor modality for colorectal cancer, motivating the optimization of exposure parameters and media conditions to maximize therapeutic windows.

**Conclusion:** Direct helium CAP produces oxidative stress in CT26 cells by elevating extracellular  $H_2O_2$  and intracellular ROS levels, leading to a reduction in cell viability with an  $IC_{50}$  of approximately 90–120 s.



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**Keywords:** Cold atmospheric plasma, Colorectal cancer, Reactive oxygen and nitrogen species

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