Redox epigenetic-modulation (ReMod) of PD-L1 expression by cold atmospheric microplasma

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Introduction

Programmed death-ligand 1 (PD-L1) modulates T-cell activity at the tumor–immune interface and functions as a key immune checkpoint molecule. Here, we define Redox Epigenetic-Modulation (Re-Mod) as a framework in which plasma-generated redox signals act as upstream drivers of epigenetic regulation. Cold atmospheric microplasma (CAM) produces a mixture of reactive oxygen and nitrogen species (RONS), together with UV photons and low energy electrons, under ambient conditions [1]. In this study we applied CAM to human breast cancer cells (MCF-7) for short duration (30s, 60s, 180s) to induce graded redox perturbations. We then evaluated how these mild plasma induced changes affect the expression of immune-related surface molecules specially programmed death-ligand 1 (PD-L1) and major histocompatibility complex 1 (MHC 1 molecules- HLA-A/B) – through redox-sensitive signaling pathways involving STAT2. These findings suggest that PD-L1 regulation through Re-Mod represents a novel axis of immune checkpoint control, with potential implications for combining plasma-based redox modulation with checkpoint blockade or immune cell therapies.

Objectives

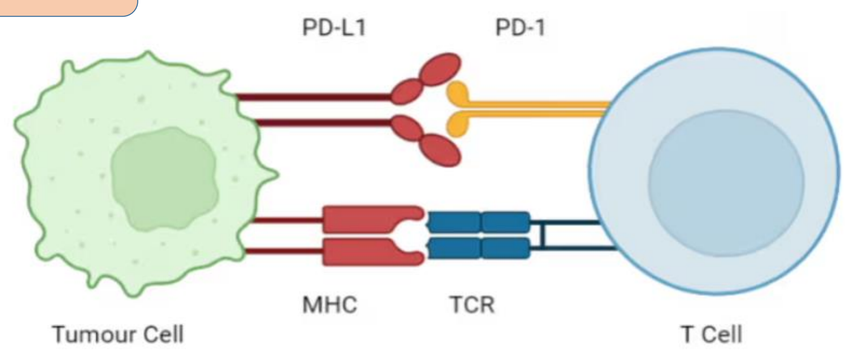


Figure 1: PD-1 & PD-L1 interaction between T-cells and tumor cell.

The PD-1 receptor on the T cell binds to PD-L1 on the cancer cell, sending an inhibitory signal that decreases T cell activation, proliferation, and cytokine production. This interaction prevents the T cell from killing the tumor cell, acting as a "brake" on the immune response. When the TCR binds to the tumor-MHC complex, it initiates a signaling cascade within the T cell.

- To elucidate whether sub-lethal N_2 CAM modulates PD-L1 expression in MCF-7 cells through redox signaling pathways.
- To observe the PD-L1 expression of immune-regulatory molecules (HLA-A, HLA-B, & STAT2) in MCF-7 cells after applying low-dose of CAM.

Methodology

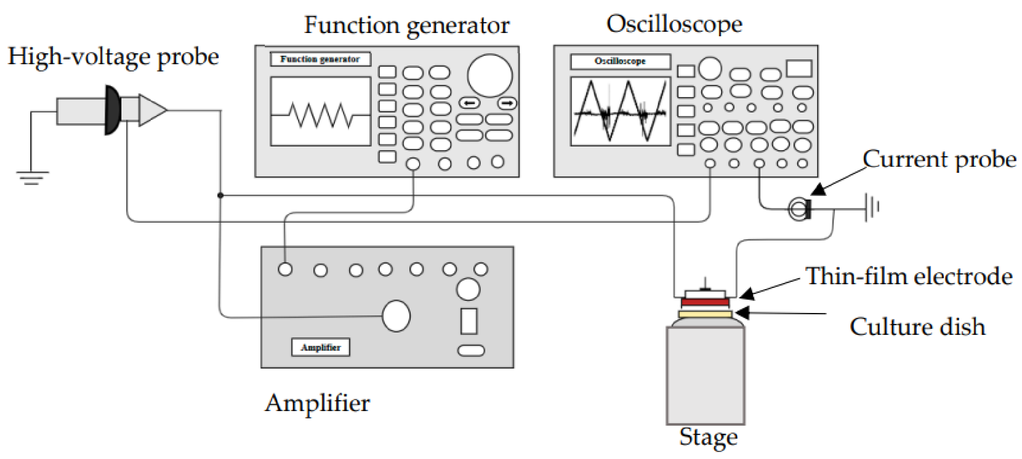


Figure 2: Schematic diagram of the experimental set up for CAM treatment [2].

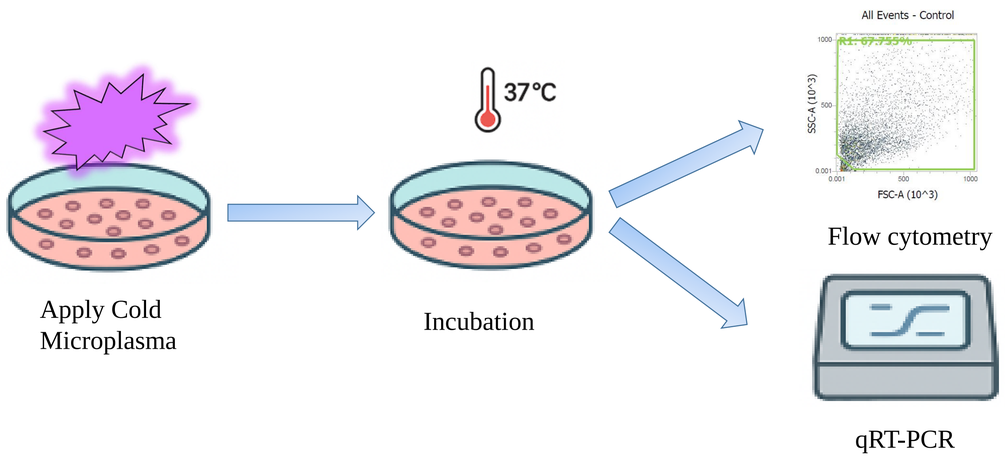


Figure 3: Cell viability assessed by flow cytometry & relative gene expression evaluated by qRT-PCR.

Plasma parameters:
Frequency- 1 kHz ; Voltage- 3 kV (pk–pk); Gas- N_2; 3 L min^{-1}; Wave form- Ramp, Distance- 3 mm
Treatment Time: 30 s, 60 s, and 180 s; **Incubation time:** 1 hour
Propidium Iodide (PI) dye used to observe cell viability.
Function is to label dead or dying cells by staining their DNA red.
Data analysis was done using flow cytometry & qRT-PCR.

Results

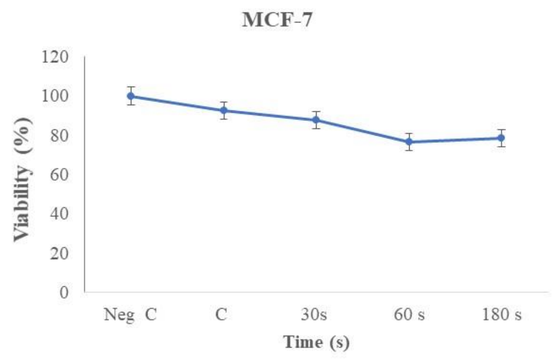


Figure 4: Cell viability of MCF-7 cells after cold microplasma treatment assessed by flow cytometry using PI staining. Negative control (Neg C) represents cells unstained control, control (C) includes PI-positive dead cells. **n = 3, mean ± SD**

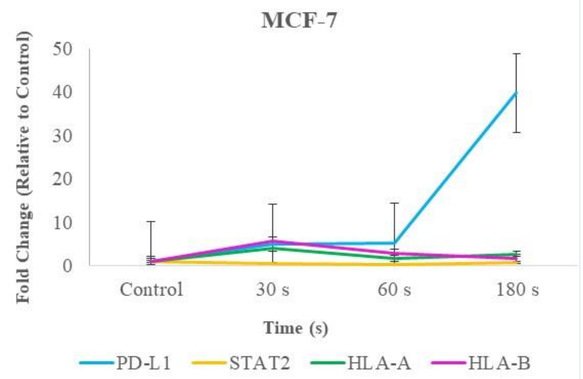
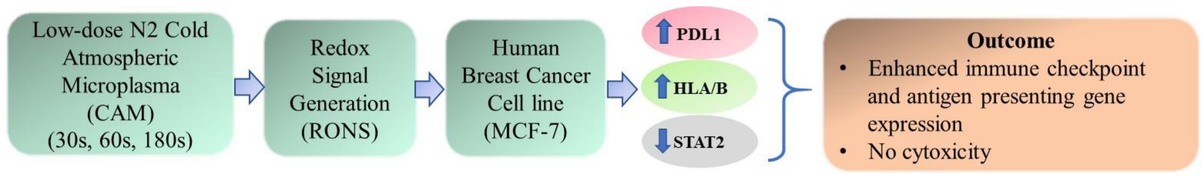


Figure 5: Fold change in genes (PD-L1, STAT2, HLA-A, and HLA-B) expression in MCF-7 cells normalized to GAPDH and relative to untreated control cells. **n = 3, mean ± SD**

Discussion



- CAM produced exposure-dependent changes in **immune-checkpoint related molecule (PD-L1)** and signaling genes in MCF-7 cells.
- **HLA-A and HLA-B** exhibited **higher expression** at shorter exposure times (30s), indicates enhanced antigen-presenting capacity, which may contribute to improved immune recognition of tumor cells.
- **STAT2 decreasing** trend at the mRNA level.
- A **transient, non-cytotoxic deceleration** of the **cell cycle** was observed (data not shown due to space); reversibility will be tested.

Conclusion

- Low-dose plasma can modulate **immune-checkpoint related molecule and immune-related genes**, suggesting a controllable immunomodulatory strategy.
- All effective conditions were within a sub-cytotoxic window **(viability maintained).**
- Mild plasma exposure can **fine-tune tumor immunogenicity** while preserving cell viability.

References

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[2] Begum F, Kristof J, Alam MJ, Sadiq AH, Hasan M, Soichiro K, Shimizu K. Exploring the Role of Microplasma for Controlling Cellular Senescence in *Saccharomyces cerevisiae*. Molecules. 2025; 30(9):1970.