Molecular characterization of tight junction proteins of bEnd.3 cells after plasma irradiation

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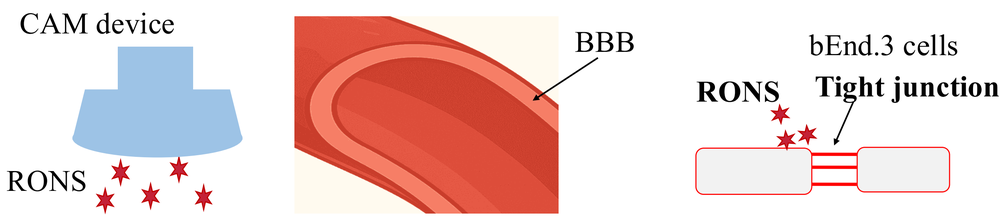
1. Introduction

The blood–brain barrier (BBB) is a critical defense system that restricts the entry of most drugs, composed of endothelial cells, pericytes, and astrocytes. Tight junctions between endothelial cells are particularly important, maintaining barrier resistance and regulating the passage of substances to protect the brain's internal environment [1]. In our recent work (Alam et al., 2024), we showed that reactive oxygen and nitrogen species (RONS) generated by cold atmospheric microplasma (CAM) enhances drug delivery across bEnd.3 cells by transiently altering barrier properties [2]. Building on this, the present study examines the molecular kinetics of this effect by investigating how CAM (thin film electrode, 4 kV) modulates claudin-5 expression. We hypothesize that CAM induces a transient downregulation of claudin-5, followed by recovery, reflecting an adaptive oxidative stress response.

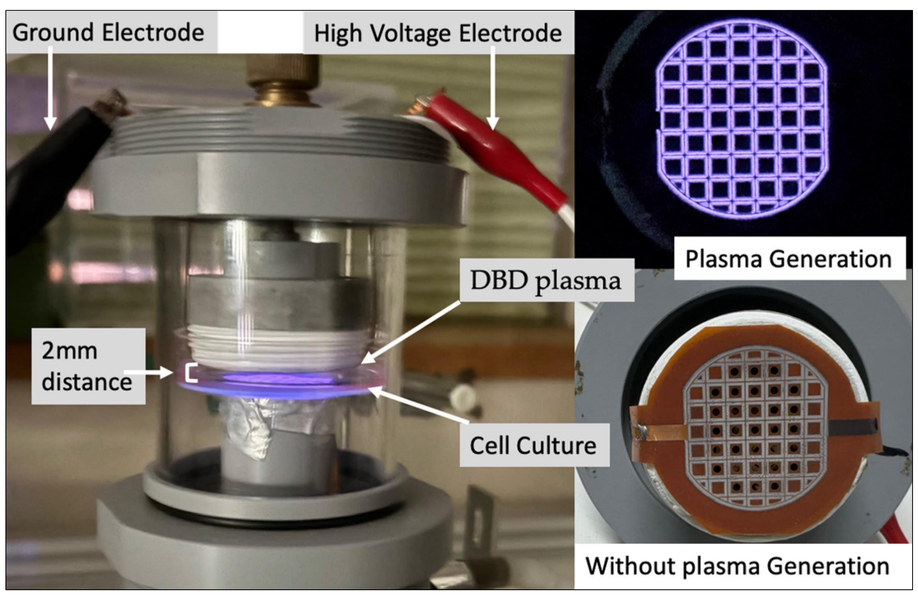
2. Objectives



To observe the expression level of tight junction protein in brain endothelial cells (bEnd.3) treated by CAM-generated RONS.

3. Experimental Procedure

- Immortalized brain endothelial cell (bEnd.3) was cultured in DMEM medium, and dielectric barrier discharge (DBD) plasma was applied in cell culture at $4kV_{p-p}$ with 5kHz frequency for 2 minutes and then incubated for 1, 3, 6, 9, or 12 h.
- **qPCR:** Total RNA was extracted (Promega kit), converted to cDNA, and claudin-5 expression was quantified on a QuantStudio 3 system with β-actin as control.
- **Immunocytochemistry:** Fixed cells were stained with rabbit anti-mouse claudin-5, and Texas Red-X secondary antibody, and fluorescence images were captured using a Keyence fluorescence microscope to evaluate claudin-5 localization.



Cold Atmospheric Plasma Set Up

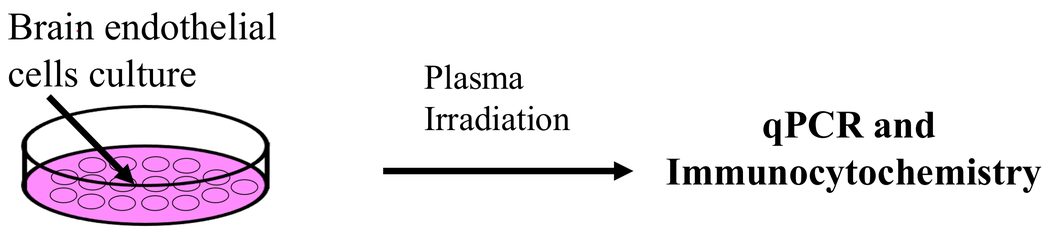


Figure: Experimental Procedure

4. Results

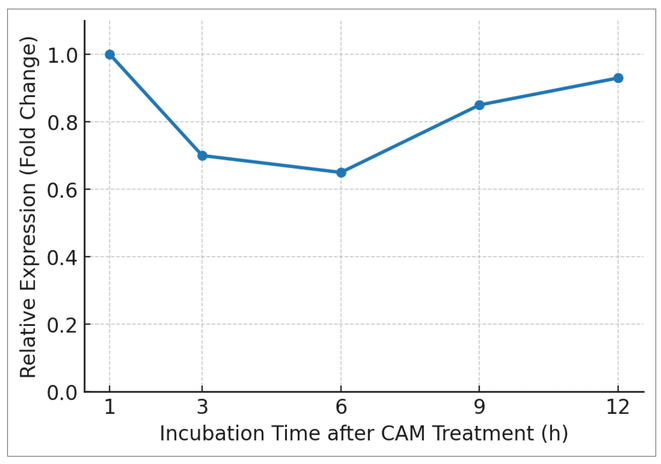
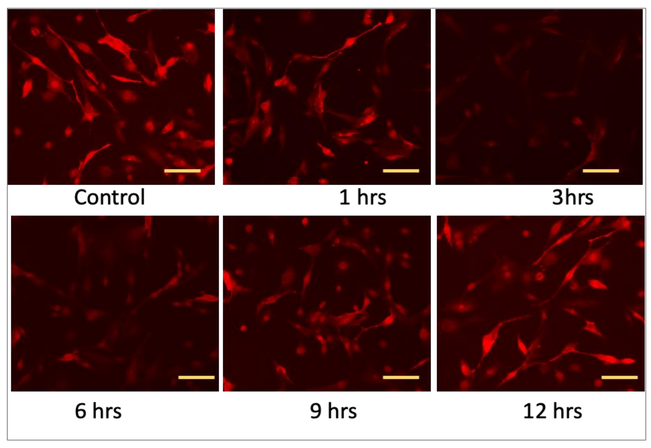


Figure: Relative claudin-5 expression in bEnd.3 cells following CAM treatment (4 kV). qPCR analysis shows no change at 1 h, significant downregulation at 3–6 h, and gradual recovery at 9–12 h, indicating a transient and reversible effect of plasma exposure.



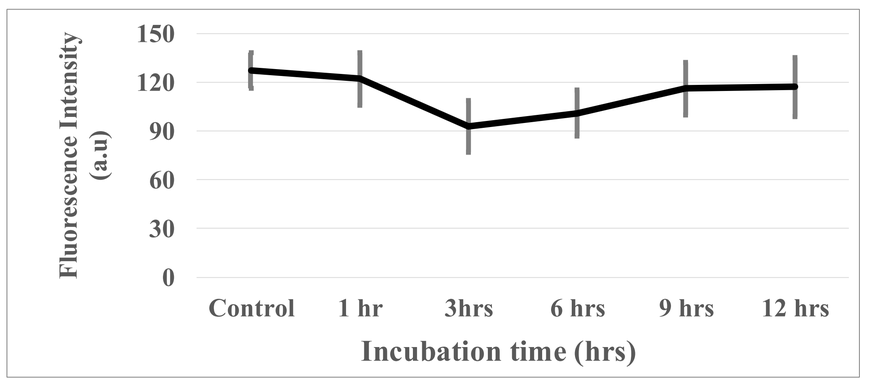


Figure: Top: Immunofluorescence images of claudin-5 in bEnd.3 cells after CAM treatment (4 kV). Strong junctional staining was observed at 1 h, reduced intensity at 3–6 h, and partial recovery at 9–12 h, confirming the transient modulation of claudin-5 expression. (Magnification= 10x, Scale bar = 50 μm). **Bottom**: Fluorescence Intensity extracted by imageJ software.

5. Discussion

- CAM treatment at 4 kV caused time-dependent modulation of claudin-5 in bEnd.3 cells.
- No change at 1 h suggests effects are due to gradual ROS signaling, not immediate transcriptional repression.
- Downregulation at 3–6 h may involve oxidative stress pathways such as MAPK or NF-κB.
- Recovery at 9–12 h indicates possible activation of adaptive responses, likely via antioxidant signaling (e.g., Nrf2).

6. Conclusions

- Our findings provide novel evidence that cold atmospheric microplasma modulates claudin-5 expression in bEnd.3 cells in a time-dependent and reversible manner.
- This transient modulation suggests a window of enhanced endothelial permeability that could be harnessed for therapeutic delivery of large molecules e.g. DNA, RNA and antibodies etc.

7. References

1. Wu D., *et al*. *Signal Transduct Target Ther*. 2023;8:217.
2. Alam M.J., *et al*. *Macromol*. 2024;4:597–609.