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Drug Delivery in Brain Endothelial Cells by Cold Atmospheric Microplasma

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ABSTRACT

Brain microvascular diseases, including ischemic stroke and cerebral microangiopathies, are difficult to treat due to the protective function of the blood-brain barrier (BBB), which limits drug access to the brain. To improve therapeutic outcomes, innovative strategies are needed to enhance drug delivery across brain endothelial cells [1]. One such approach is cold atmospheric micro-plasma (CAM), a non-thermal plasma operating near room temperature, making it suitable for biological use. CAM produces reactive oxygen and nitrogen species (RONS), which can transiently disrupt cell membranes, allowing for increased drug entry without causing cell damage [2].

This study explored CAM's potential to enhance the uptake of fluorescein isothiocyanate-dextran (FITC-dextran, 150 kDa), a model drug, in brain endothelial cells (bEND3), and evaluated its effect on cell viability. The bEND3 cells, which model the BBB in vitro, were cultured under standard conditions. CAM was generated using a thin-film electrode at 5 kHz, with peak-to-peak voltages of 3.0, 3.5, 4.0, and 4.5 kV. Cells were exposed to CAM for 2 minutes at a 2 mm distance from the electrode, followed by immediate FITC-dextran treatment. Fluorescence microscopy and a microplate reader were used to measure drug uptake, while trypan blue staining assessed cell viability.

Results showed that CAM significantly improved FITC-dextran uptake in bEND3 cells. Higher voltage increased molecular absorption, as seen in elevated fluorescence, but also reduced cell viability. The best balance between drug uptake and cell health was observed at 3.5 and 4.0 kV, where cells remained largely viable while still showing enhanced drug entry.

These findings support CAM as a promising, non-invasive strategy for delivering large molecules across the BBB. The RONS generated by CAM temporarily increase membrane permeability under controlled conditions, improving drug delivery without permanent cell damage.

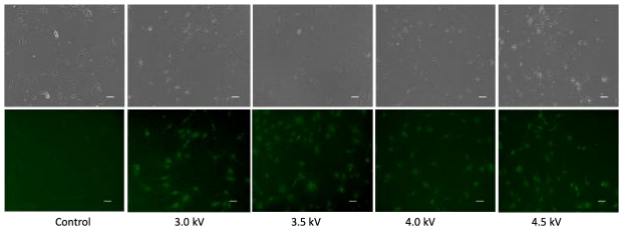

Fig 1: Fluorescence intensity of FD-150 uptaken by bEND.3 cells after plasma irradiations. Microscopic image (Scale bar 50μm).

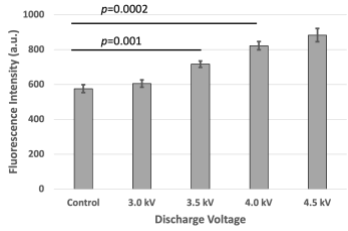

Fig 2: Fluorescence intensity from microplate reader ($p < 0.05$).

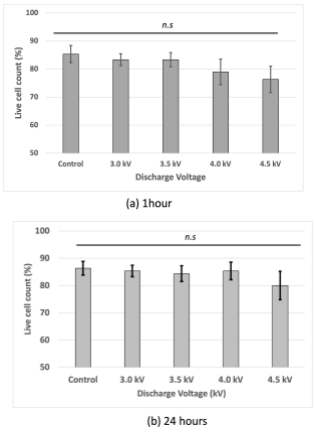

Fig 3: Cell viability of bEND.3 cells after (a) 1 hours incubation and (b) 24 hours incubation following plasma irradiations using trypan blue with hemocytometer.

References

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