Microplasma Facilitates Macromolecule Uptake in Leukemia Cells Through Membrane Reorganization and Endocytic Gene Upregulation

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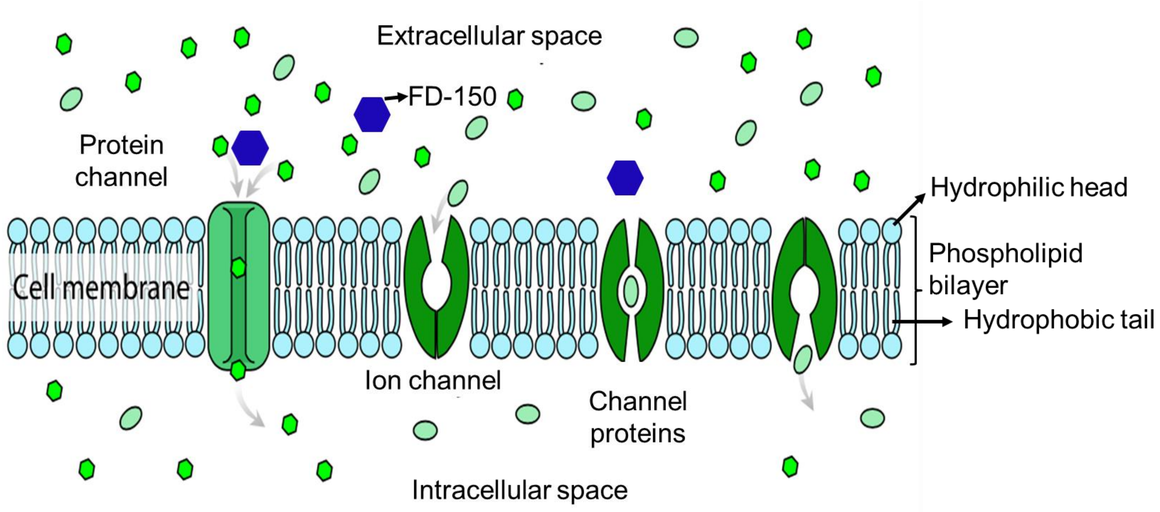
1. Introduction

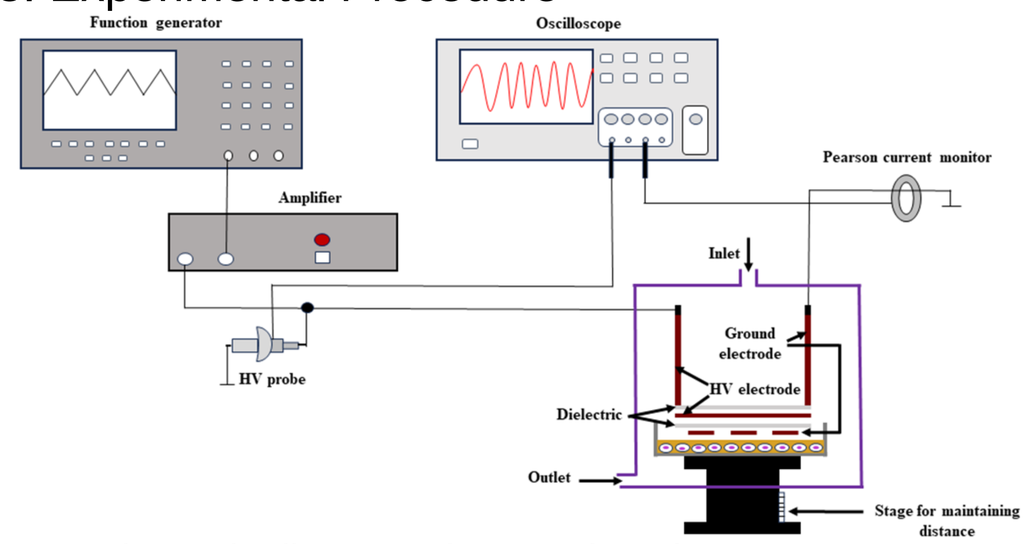
Cell membranes act as selective barriers, making the delivery of large therapeutic molecules challenging. Non-thermal microplasma can transiently modulate membrane properties through reactive oxygen and nitrogen species (RONS), enhancing cellular uptake. In this study, we investigated the effects of air microplasma on FD-150 (150 kDa) uptake in HL-60 cells. Plasma treatment increased FD-150 internalization, altered membrane potential, and disordered membrane lipids, indicating enhanced permeability. Importantly, qRT-PCR data showed a 2.9- to 4.9-fold upregulation in Clathrin heavy chain (CLTC) expression following plasma treatment. One probable mechanism for CLTC upregulation involves redox-sensitive activation of transcription factors, such as NF-κB, which regulate genes involved in vesicular transport and membrane trafficking [1]. Together, these findings reveal that microplasma facilitates macromolecule delivery by modifying both membrane biophysics and gene expression, providing valuable insight for plasma-based therapeutic strategies.

2. Goal

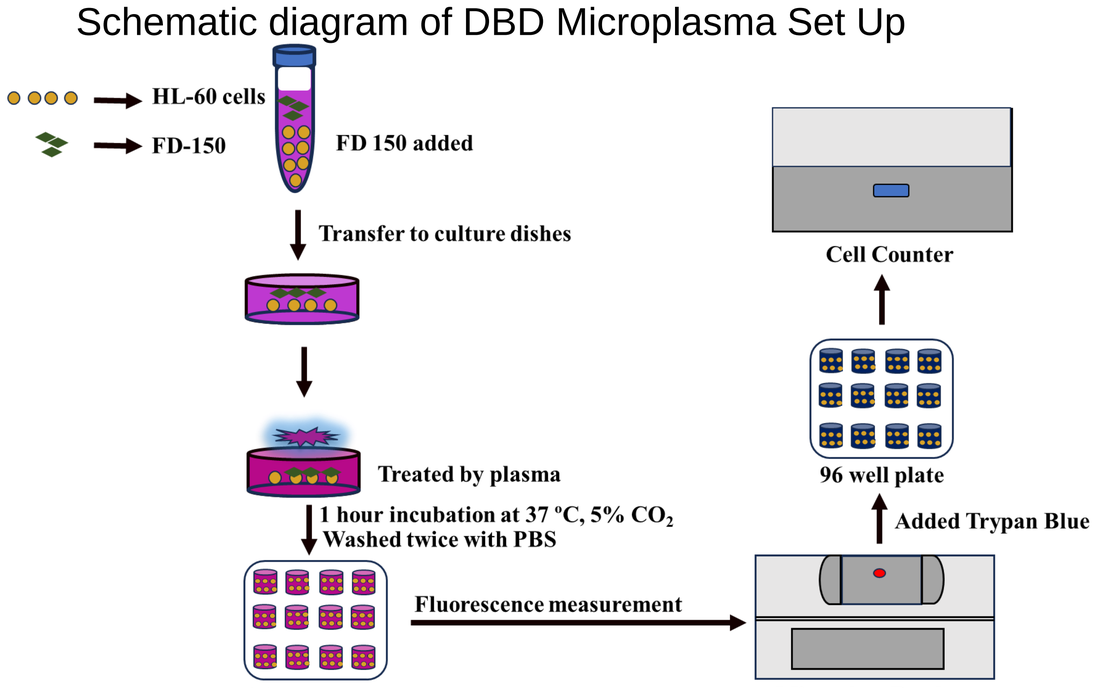


FD-150 cannot cross the cell membrane directly. Therefore, we used CAM to modulate membrane properties and stimulate endocytosis, enabling FD-150 internalization.

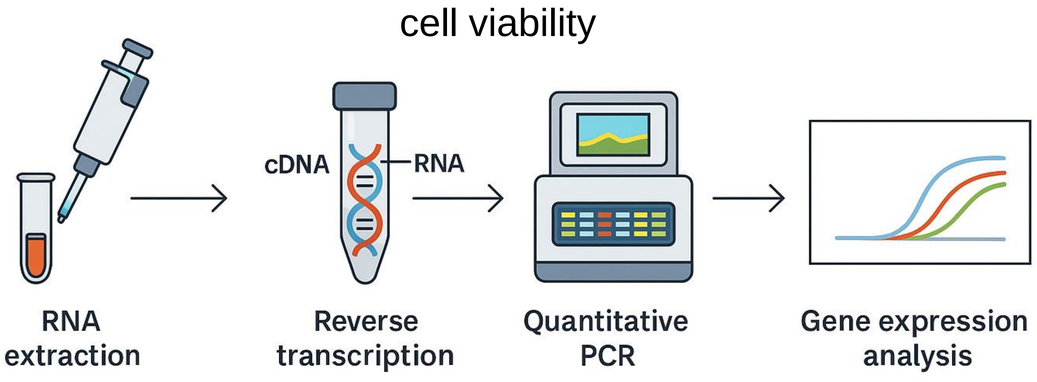
3. Experimental Procedure



Schematic diagram of DBD Microplasma Set Up

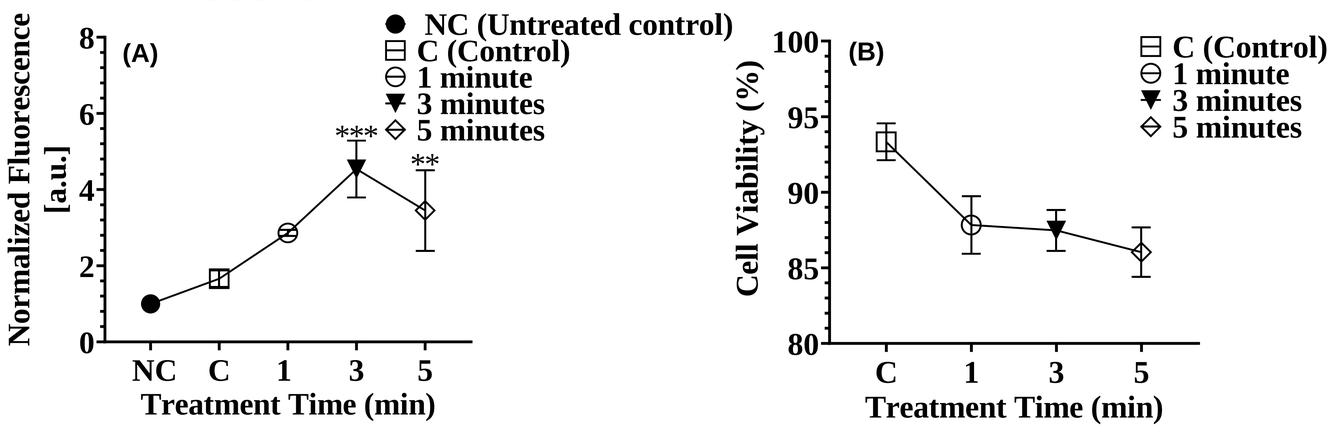


Experimental procedure for evaluating fluorescence intensity and cell viability

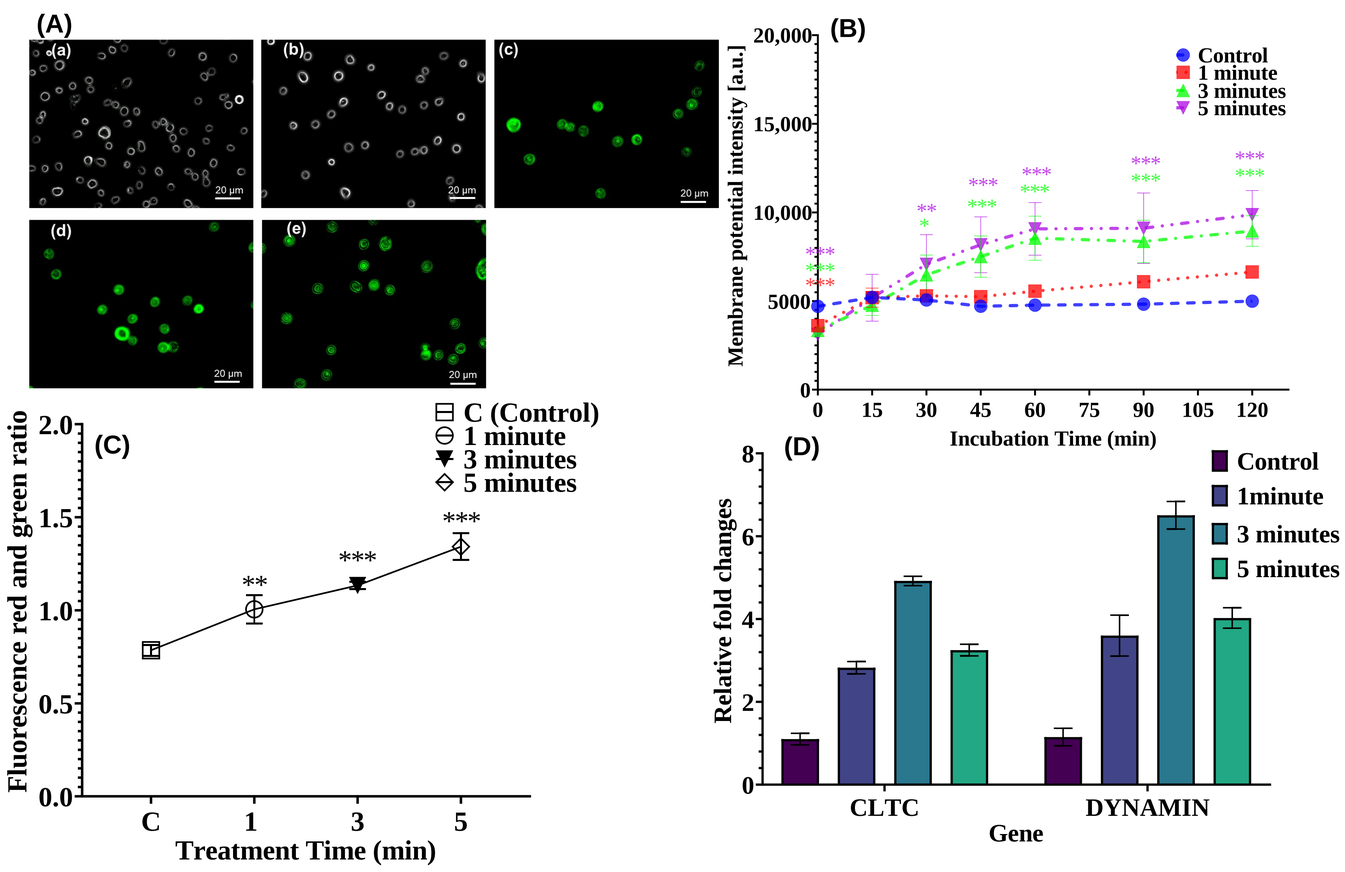


Gene expression evaluation by qRT-PCR

4. Results



(A) Fluorescence intensity of FD-150 uptake in HL-60 cells after microplasma irradiation.
(B) Viability of HL-60 cells following microplasma irradiation.



(A) FD-150 uptake in HL-60 cells: (a) NC (no FD-150), (b) Control, (c) 1 min, (d) 3 min, and (e) 5 min microplasma treatment.**(B)** Changes in cell membrane potential after microplasma exposure.**(C)** Fluorescence red/green ratio indicating membrane lipid disorder and permeability.**(D)** Relative fold change in Clathrin (CLTC) and Dynamin genes expression.

5. Discussion

Transient membrane reorganization may aid small cargo entry, but the large size of FD-150 (18 nm [2], 150 kDa) makes **clathrin-mediated endocytosis (CME)** the dominant uptake pathway. CME generates ~100 nm [3] vesicles and involves Clathrin and Dynamin, both of which showed increased transcript levels in our study. While caveolae-mediated endocytosis may contribute, a 2.9-4.9 folds increase expression of CLTC suggest CME as the primary mechanism of FD-150 internalization.

6. Conclusion

- Microplasma discharge enhanced FD-150 uptake in HL-60 cells by inducing membrane depolarization and lipid disorder.
- These membrane alterations may also help initiate endocytosis.
- Upregulation of Clathrin and Dynamin indicates activation of endocytic pathways, with clathrin-mediated endocytosis likely the primary route for macromolecule internalization.
- This mechanism may be harnessed to deliver large therapeutics, such as antibodies or nucleic acids, for the treatment of cancer and other diseases.

7. References

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[2]. Wen, He, Jinsong Hao, and S. Kevin Li. "Characterization of human sclera barrier properties for transscleral delivery of bevacizumab and ranibizumab." Journal of pharmaceutical sciences 102.3 (2013): 892-903.
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