

Investigation of cell transfection method by electro-mechanical poration

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<https://hdl.handle.net/2324/7396220>

出版情報 : Kyushu University, 2025, 博士 (工学) , 課程博士

バージョン :

権利関係 : Public access to the fulltext file is restricted for unavoidable reason (3)



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論 文 名 : Investigation of cell transfection method by electro-mechanical poration

(電気・機械的穿孔法による細胞トランスフェクション法に関する研究)

区 分 : 甲

論 文 内 容 の 要 旨

This thesis presents the development of a novel low-cytotoxicity physical transfection technique termed electro-mechanical poration. This method utilizes an electrically induced microbubble generator to convert electrical energy from a power source into combined electrical and mechanical stimulation applied to cells suspended in solution. Upon exposure to this dual-mode stimulation, cells undergo transient membrane poration, allowing the delivery of exogenous nucleic acids into the cytoplasm. The dual-mode approach is designed to overcome the limitations of traditional physical transfection methods, particularly in terms of compatibility with various cell lines and cytotoxicity. This work establishes the theoretical framework, experimental system, and protocols for electro-mechanical poration. Its effectiveness is demonstrated across a variety of mammalian cell lines and genetic cargos, including large artificial chromosomes. Comparative studies with conventional electroporation confirm that the proposed method achieves transfection with significantly lower cytotoxicity. These findings support the potential of electro-mechanical poration as a promising alternative physical transfection strategy for future biomedical applications.

Chapter 1 introduces and compares conventional viral, chemical, and physical transfection methods, emphasizing the limitations of traditional physical approaches, such as high cellular toxicity and poor adaptability to large genetic materials. It then explains the principle of electrically induced microbubbles, which serve as the basis for the method developed in this study. Finally, it outlines the structure and objective of the dissertation: to develop a low-cytotoxicity physical transfection strategy applicable to a wide range of cell lines.

Chapter 2 established the theoretical framework of electro-mechanical poration and investigated the mechanical and electrical stimulation applied to cells through experiments and finite element simulations. Cells were subjected to pressure drag applied by the flow field and periodic electric pulses delivered by the actuator. Quantitative relationships were developed between mechanical stimulation, electrical stimulation, and key cellular outcomes, including viscosity, transfection efficiency, poration probability, and cell viability. Transfection experiments were conducted using Rat osteosarcoma cells (UMR-106), resulting in a transfection efficiency (T.E.) of 25%, cell death rate (D.R.): 9%.

Chapter 3 evaluated the transfection efficiency (T.E.) and death rate (D.R.) of electro-mechanical poration across six mammalian cell lines, including both adherent and suspension cells. The results of adherent cell lines showed as follows: Rat osteosarcoma cell (UMR-106) (T.E. 25%, D.R. 9%), Mouse embryonic fibroblast (NIH/3T3) (T.E. 22%, D.R. 10%), Human fibrosarcoma cell (HT-1080) (T.E. 21%, D.R. 7%), Mouse embryonic stem cell (mES) (T.E. 18%, D.R. 30%). Also, the results of suspension cell lines showed as follows: Human natural killer leukemia cell (KHYG-1) (T.E. 4%, D.R. 12%), Human T-cell leukemia (Jurkat) (T.E. 2%, D.R. 22%). Potential mechanisms behind the transfection efficiency difference between adherent and suspension cells were discussed. The chapter also presented systematic optimization of electrical (pulse sets of applied voltage), mechanical (shear viscosity), and biological (medium composition) parameters. The results demonstrated that each factor significantly affects transfection efficiency and cell viability. Furthermore, the chapter explored the role of actin cortex dynamics in poration by applying cytochalasin D and observing cytoskeletal remodeling. A correlation between cytoskeletal softness and improved poration efficiency was established, providing deeper insight into the underlying mechanisms. The effect of viscosity on transfection was also examined, revealing that increased viscosity enhanced transfection efficiency by amplifying shear and pressure gradients in the microbubble-induced flow field, leading to stronger mechanical stimulation.

Chapter 4 expanded the application scope of electro-mechanical poration. It demonstrated the successful stable transfection of large DNA constructs—artificial chromosomes up to 128 kbp—into HT-1080 and UMR-106 cells. The method was then compared with conventional electroporation, revealing that electro-mechanical poration caused lower cytotoxicity and better preservation of cell morphology. Finite element modeling confirmed that the electrical stimulation applied to cells during electro-mechanical poration was significantly lower. Finally, a needle-free tissue poration injector based on microbubble dynamics was introduced, and a future direction was proposed to integrate cell transfection into localized, minimally invasive therapeutic strategies.

The final chapter (**Chapter 5**) summarized the development and validation of the electro-mechanical poration technique. Electro-mechanical poration applied pressure drag ranging from 0 to 0.96 μN to the cells which delivered momentum of 1.9×10^2 to $4.1 \times 10^2 \mu\text{N}\cdot\mu\text{s}$ within 1 ms. This transient mechanical stress temporarily disrupted the actin cortex, weakening its support of the membrane and promoting membrane poration. Also, the applied electric field of 2.0×10^5 to $3.2 \times 10^5 \text{ V/m}$ was confirmed to be sufficient transmembrane potential to cause poration. Through the combined effect of cytoskeletal weakening and transmembrane potential generation, cells underwent membrane poration and are successfully delivered foreign nucleic acids, even under relatively low stimulation. Theoretical modeling, experimental verification, and comparison with existing technologies affirmed its potential as a novel low cytotoxicity, high compatibility physical transfection platform. Future directions include optimizing parameters for suspension cells and verifying the system in animal models to realize on-demand in vivo transfection.