

Continuous Flow Electroporation

Electroporation is a method used to introduce nucleic acids into cells or tissues. Recent progress in this field has made this method applicable to a wide range of cells. The current commercial electroporation devices are easy to use and beneficial for many cell lines where non-viral gene transfer is required. However, the recommended cell population used in current the electroporation device is around 10^5 – 10^6 . For clinical applications, there is a need to transfect a large number of cells (e.g. $>10^9$ cells) with high transfection efficiency and cell viability. A continuous electroporation device that could provide high cell throughput with a mild and uniform electro pulse would be highly desirable. Two novel micro/nanofluidic electroporation methods have been developed for both *in vitro* and *ex vivo* delivery of oligonucleotides and plasmid DNAs. Mouse embryonic stem cells and a hard-to-transfect acute leukemia cell line (e.g., K562) are used as model systems.

Continuous Flow Electroporation (CFE) ^(1,2)

The schematic of the CFE process is described in Figure 1. Cells mixed with therapeutic materials flow through the converging microchannels from the large end to the small end under pressure provided by a syringe pump. A low AC or DC electric field is then applied to produce the required high electric field strength near the small end. When cells sequentially pass through the throat region of the converging microchannels, they experience acceleration and a highly focused electric field there, similar to a single electric pulse in the bulk electroporation process. Because of its continuous operation and the confinement of the microchannels, every cell will experience the same level of electroporation and, consequently, the delivery of therapeutic materials can be accomplished uniformly for a large number of cells. If an array of 10^5 converging microchannels is used, this CFE setup could easily reach the targeted production of $\sim 10^9$ cells in one minute, assuming each cell needs 10 ms to pass through one channel (the total cell number = $10^5 \times 60$ (s/min) $\times 10^3$

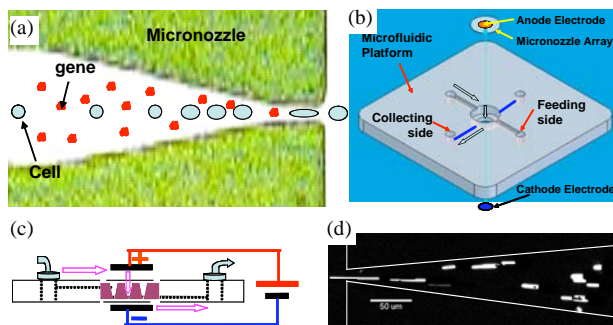


Figure 1. Schematic of CFE process. (a) Flow-through cells and genes through micronozzles; (b) Microfluidic platform design and integration; (c) 3D construct and (d) cross-section view. Preliminary result is given in (d) using 3 mm PS beads to mimic sequential pass through a 20 mm converging channel. The channel edges are outlined for clarity.

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(ms/s) /10 (ms/cell) = 6×10^8 cells). In this case, conventional optic fibers with a diameter of 125 μm are assembled into a large bundle using cladding materials. A sacrificial template imprinting (STI) method will be used to produce the nozzle array, with the small end having a diameter around 20-30 μm. Fluidic connectors are bonded onto the inlet/outlet of the plastic chip. A programmable syringe pump is connected to a loaded sterilized syringe (1-3 ml), pushing the cell and the medium through to the nozzle array.

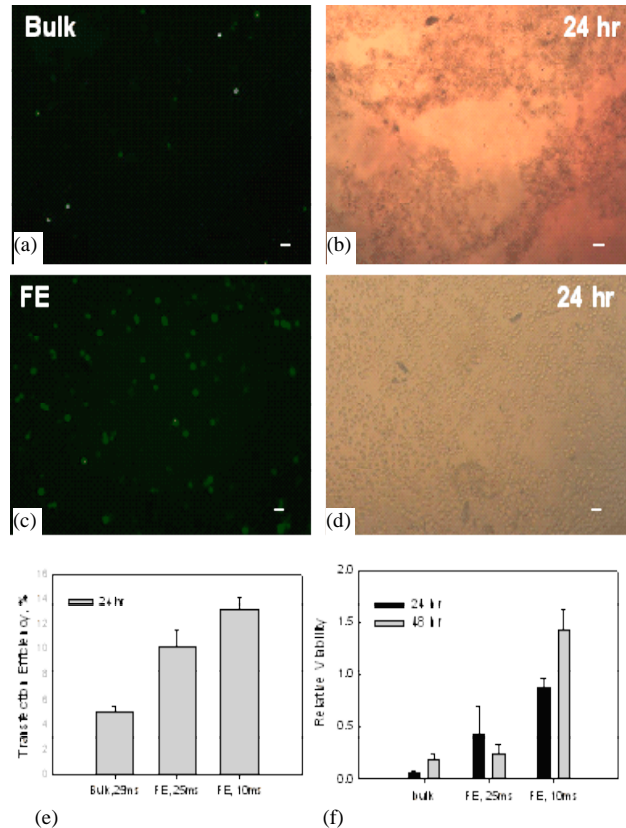


Figure 2. Plasmid DNA (pWizGFP) transfection in K562 Acute Leukemia Cell (AML) line by conventional electroporation (a, fluorescence, b, bright field images) and by continuous flow electroporation (c, fluorescence, d, bright field images). The scale bars present 100 μm. Quantitative measurement of transgene expression is done by flow cytometry (e) and the cell viability by MTS assay (f).

The concept was demonstrated using pWizGFP plasmid and K562 (a hard-to-transfect Acute Leukemia Cell line) as models. After applying a programmed electric field, treated cells were cultured in fresh medium, samples were collected and gene expression was measured 24-48 hours afterwards. As shown in Figures 2a, 2c and 2e, the continuous flow electroporation shows significant improvement of transgene expression compared with current electroporation techniques. Cell lysis during poration was highly avoided with no

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observable cell debris, which was serious when using conventional technologies (Figs. 2b, 2d). Quantitative results from the MTS assay confirmed our observation of cell conditions, with the cell viability at more than 50% or even higher with continuous flow electroporation (Fig. 2f). Similar results were also observed with pWizGFP transfection in mouse Embryonic Stem cells.⁽³⁾

Continuous Flow Electroporation with Microcapsules⁽⁴⁾

To gain the advantages of electric field localization and cell/gene confinement in the Membrane Sandwich Electroporation (MSE) process, cells and therapeutic materials are encapsulated in nanoporous microcapsules in the CFE process. Essentially, this is to convert the two-dimensional membrane-(cell/therapeutic materials)-membrane structure into a three-dimensional analogy. Cell culture in microcapsules actually works better than conventional methods because of the *in vivo*-like microenvironment and immunoprotection provided by the microcapsules.

Microencapsulation encloses cells within a hollow sphere with a diameter that can be controlled in the range of 200-1500 μm and a semi-permeable membrane with a surface pore size of 5-200 nm. The basic principle of microencapsulation has emerged as a promising therapeutic strategy to treat a wide range of diseases from endocrine disorders and central nervous system diseases. These pores allow for the bidirectional diffusion of nutrients, oxygen, secreted therapeutic product, and waste, but can prevent the penetration of high molecular weight substances from the microcapsule, such as antibodies and immunocytes. A variety of polymers, such as chitosan, polyacrylates, alginate, polyamino acids, and polyamides, have been used to produce microcapsules⁽²⁾. Among them, the alginate-polylysine-alginate (APA) microcapsule is one of the most widely studied microcapsules for its good biocompatibility in cell culture. The procedure to fabricate APA microcapsules includes the formation of calcium alginate beads, adsorption of positively charged polylysine to form an alginate-polylysine membrane,

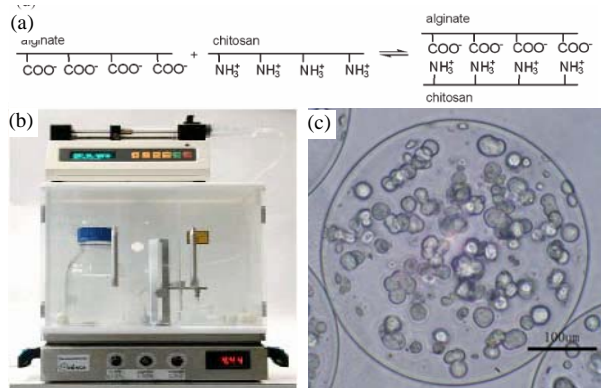


Figure 3. Microencapsulation process: (a) membrane materials of ACA, (b) Nisco Encapsulation Unit, and (c) an APA microcapsule with K562 cells. lee.31@osu.edu

alginate matrix. Figure 3 shows the microcapsulation device and an APA microcapsule with K562 cells inside.

Since there is no need for a large number of nozzles, we fabricate tapered 2D channels with a size around 100 μm at the small end and 500 μm at the large end. To avoid a sediment build-up of microcapsules in the syringe, microcapsules are pre-injected into the large leading channel. Then a programmable syringe pump is connected to push the medium and the microcapsules forward to the converging zones. Since the materials used for microcapsules (e.g. alginate) are elastic enough to deform when passing through the small end (e.g., 100 μm) of the converging microchannel, they can be squeezed to form a sandwich-like configuration (Fig. 4). The sequential pass ensures that every microcapsule will experience the same electric pulse in the throat region. After electroporation, microcapsules are broken with needles or high shear flow and cells are collected and cultured for further transgene expression and cell viability studies.

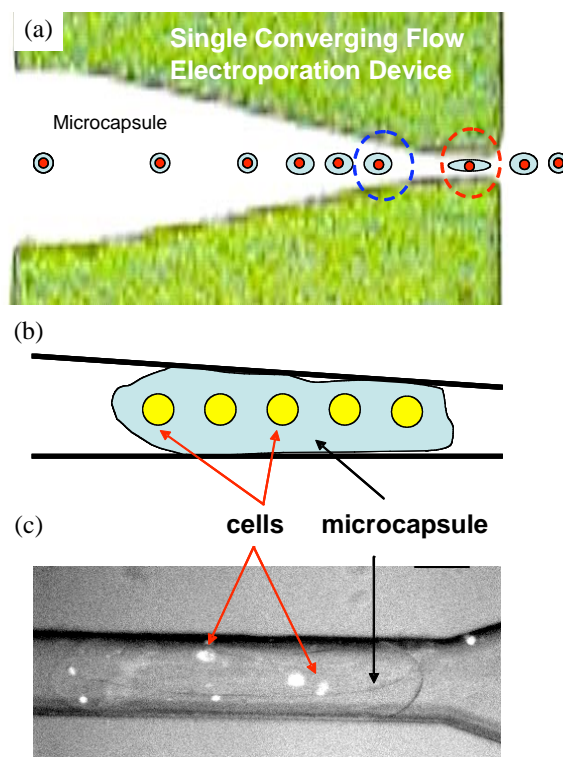


Figure 4. (a) Schematic of Converging Flow Electroporation (CFE) with microcapsules, and deformation of microcapsule at the throat region (b) schematically and (c) experimentally.

Publications

1. S. Wang, X. Zhang, C.G. Koh and L.J. Lee, "Microfluidic Continuous Flow Electroporation: Design and Gene Transfection Study", (submitted).
2. X. Zhang, W. Wang, Y. Xie, Y. Zhang, X. Wang, X. Guo, X. Ma. *Appl Biochem Biotechnol.* **134**, 61 (2006).
3. S. Wang, X. Zhang and L.J. Lee, "Gene Delivery to Embryonic Stem Cells by Continuous Flow Electroporation", (to be submitted).
4. X. Zhang, S. Wang and L.J. Lee, "Gene Delivery to Leukemic Cells by Continuous Flow Electroporation", (to be submitted).