Microscale electroporation: challenges and perspectives for clinical applications

Won Gu Lee,*[ab] Utkan Demirci[ab] and Ali Khademhosseini*[ab]

Received 29th October 2008, Accepted 16th January 2009
First published as an Advance Article on the web 29th January 2009
DOI: 10.1039/b819201d

Microscale engineering plays a significant role in developing tools for biological applications by miniaturizing devices and providing controllable microenvironments for *in vitro* cell research. Miniaturized devices offer numerous benefits in comparison to their macroscale counterparts, such as lower use of expensive reagents, biomimetic environments, and the ability to manipulate single cells. Microscale electroporation is one of the main beneficiaries of microscale engineering as it provides spatial and temporal control of various electrical parameters. Microscale electroporation devices can be used to reduce limitations associated with the conventional electroporation approaches such as variations in the local pH, electric field distortion, sample contamination, and the difficulties in transfecting and maintaining the viability of desired cell types. Here, we present an overview of recent advances of the microscale electroporation methods and their applications in biology, as well as current challenges for its use for clinical applications. We categorize microscale electroporation into microchannel and microcapillary electroporation. Microchannel-based electroporation can be used for transfecting cells within microchannels under dynamic flow conditions in a controlled and high-throughput fashion. In contrast, microcapillary-based electroporation can be used for transfecting cells within controlled reaction chambers under static flow conditions. Using these categories we examine the use of microscale electroporation for clinical applications related to HIV-1, stem cells, cancer and other diseases and discuss the challenges in further advancing this technology for use in clinical medicine and biology.

1. Introduction

It is becoming increasingly evident that biology can be further enhanced through the use of microscale technologies.[1] Microscale technologies have been used to enhance a wide variety of multidisciplinary fields by miniaturizing devices for life science and biotechnology applications.[2–4] Similarly, microscale engineering can be used to engineer living tissues through bottom-up approaches by assembly of microscale tissues.[5,6] In addition, microscale engineering also provides researchers with increased environmental control by using optical, chemical, physical, and electrical forces.[7–10] Specifically, the use of electrical parameters in microscale environments can be used to successfully eliminate many potential adverse effects associated with macroscale environments such as local pH variation near electrodes, electric field distortion, sample contamination, as well as low cell viability.[11,12] Therefore, scaling down the electrical systems can be used to enhance electroporation without a significant trade-off for biological applications between transfection efficiency and cell viability (Fig. 1).[12] The ability to spatially and temporally control electrical parameters in microenvironments at the single-cell level is potentially beneficial for research areas such as electro-immobilization, manipulation, electroporation, and electrolysis of cells.[11–13]

Electroporation is a powerful tool for gene transfection that uses electric shock. Electroporation is useful for cell and gene

[a] *Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115. E-mail: alik@mit.edu. E-mail: termylee@mit.edu; Fax: +1 617 768 8477; Tel: +1 617 768 8395*
[b] *Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

Insight, innovation, integration

Microscale electroporation is a powerful approach for reducing the limitations associated with conventional macroscale electroporation methods: (i) variations in the local pH, (ii) electric field distortion, (iii) sample contamination, and (iv) difficulties in transfecting and maintaining the viability of desired cell types. In this review, we divide this approach into two categories: microchannel and microcapillary electroporation. However, these two approaches can be used complementarily to promote cell electroporation for clinical applications. We review recent advances of the microscale electroporation methods and their applications in biology as well as current challenges for its use for clinical applications in: (i) HIV, (ii) stem cells and tissue regeneration, (iii) cancer, and (iv) other disease studies.

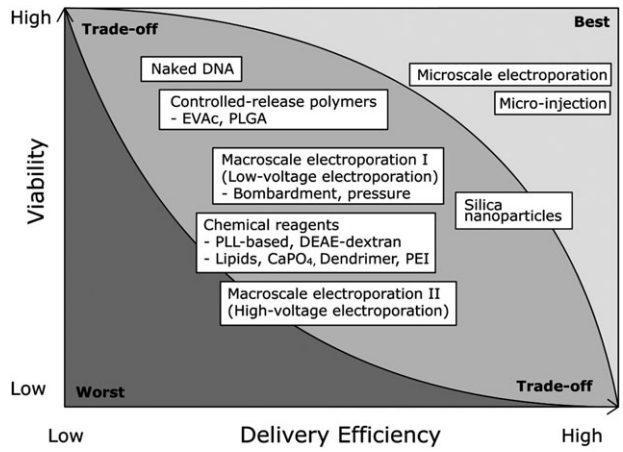

Fig. 1 Comparison of delivery efficiency *versus* viability in various gene transfection methods. Note the classification was made by updating recent advances of this field as outlined in the review paper published in *Nature Biotechnology*, 2000.[25]

studies such as developmental biology,[14,15] cardiac electrophysiology,[16] DNA migration,[17] formulation of DNA delivery,[18] and gene insertion into cultured mammalian embryos.[19] Most researchers in the electroporation field consider its future applications as a gene therapy tool for clinical applications[20,21] such as DNA immunization,[21] DNA electrotransfer (electroporation-mediated gene therapy),[22,23] and vaccination.[24] To address potential problems of these macroscale methods, however, the ability to safely and efficiently transfer exogenous materials into living cells is necessary.[25] More recently, microscale electroporation methods have been successfully developed with enhanced transfection efficiency and cell viability.[12,26] For example, a microscale electroporation device that uses a microcapillary as its reaction chamber, has been used for numerous biological applications.[27–50] (A more detailed description on this literature is given later.)

In this review, we focus on recent efforts in the field of microscale electroporation. First, we explain the benefits of microscale electroporation in comparison to its counterparts. We then discuss the recent advances in the microscale electroporation with respect to the use of microchannel and microcapillary electroporation. Finally, we address current challenges and perspectives of microscale electroporation for clinical applications. Specifically, key applications of microscale electroporation and recently published literature (2007–2008), are highlighted with regards to: (i) HIV-1, (ii) stem cells and tissues, (iii) cancer cells, and (iv) other disease studies. Throughout this review, we also provide a broad overview of the latest achievements of the microscale electroporation field as it relates to the cellular and genetics research.

2. Microscale electroporation: a paradigm shift in electroporation

In the beginning of the millennium, Dan Luo and Mark Saltzman raised an issue through their review paper that future DNA delivery systems should fully accommodate all DNA delivery processes to transfer target DNA molecules into the nucleus in a safe and effective manner.[25] They aimed to categorize the conventional DNA transfection methods for their delivery efficiency and toxicity. Importantly, they suggested that a novel combination method should be developed with better DNA delivery efficiency and viability by integrating mechanical, electrical, and chemical methods.[25] Microscale electroporation is a technique that can be used for this integrative approach.

Generally, electroporation (hereafter, called 'macroscale electroporation') is a nonviral transfection method that can electrically deliver exogenous genes or engineered nanomaterials into biological samples within the large volume chamber (*e.g.*, a few cubic centimeters) by applying electric field. Specifically, macroscale electroporation has a number of problems,[9,10,12] which include (i) relatively low cell viability (typically, 20–50%) and transfection rate (less than 50% in mammalian cell lines and much less in hard-to-transfect cells), (ii) the need for empirical optimization of cell-specific protocols (electrical and physicochemical factors), (iii) a sensitive and complicated experimental process, (iv) partial understanding of its theoretical mechanism, and (v) cell death due to excessive electrical energy. In contrast, microscale electroporation has several advantages.[9,10,12,51–54] These include: (i) *in situ* visualization of molecular uptake, (ii) real-time monitoring of intracellular response to the external electric pulses, (iii) single cell electroporation, (iv) formation of more symmetrical and uniform electric fields, and (v) more rapid optimization of DNA or RNA transfection protocols. In particular, microscale electroporation can be useful for transfecting cells with better cell viability by minimizing adverse effects due to macroscale environment itself. Table 1 shows a brief summary of advantages of microscale electroporation in comparison to its counterparts. For more detailed information, readers are directed to another review[55] and previous studies on the development of microchannel and microcapillary electroporation approaches.[10,12,51] In this review, we discuss the delivery efficiency and viability of various gene transfection methods in comparison to microscale electroporation by updating recent advances in this field (Fig. 1).

3. Recent advances of microscale electroporation

In this section, we focus on recent advances of the microscale electroporation methods, especially for single cell electroporation and controlled electrical microenvironments. To examine the previous microscale electroporation studies (before 2007), readers are directed to another review,[55] entitled ''Electroporation of cells in microfluidic devices'', which is divided into three parts based on the application: (i) analyzing cellular properties or intracellular contents, (ii) transfecting cells, and (iii) inactivating cells. Here, we divide microscale electroporation into two main methods: microchannel and microcapillary electroporation (Table 2). The former can be defined as a microscale electroporation that uses simple or complex microchannels under various flow conditions in a controlled manner. The latter can be defined as a microscale electroporation that uses microcapillaries as its reaction chamber without flow. Specifically, the microscale reaction chamber can be useful for reducing adverse effects for single

Table 1 Advantages of microscale electroporation in comparison to its counterparts

Properties	Conventional electroporation	Microscale electroporation	Key remarks
Efficiency			
Transfection	+	+ + +	Hard-to-transfect
Viability	+	+ + +	Cells[12,26]
Electrical			
E-field form	+ (Distorted at edge)	+ + + (Uniform)	Symmetrical[12]
Electrode surface	+ (Large reaction area)	+ + + (Smaller area)	Size & array[12]
Electrode material	+ (Aluminum)	+ + + (Platinum or gold)	Backflow[9]
Electro-osmosis	N.A.	Possible in microchannel	—
Mechanical			
Microfluidics	N.A.	Highly available	Microflow[9]
Heat dissipation	+ (Little)	+ + + (High)	—
Chamber size	+ (Large)	+ + + (Small)	—
Sedimentation	+ (Too many cells)	+ + + (In microcapillary)	—
Siphon's effect	N.A.	Possible in microchannel	—
Chemical			
Metal ions	+ (Al^{3+} dissolved)	+ + + (No ions dissolved)	Cell lysis[86]
Oxide formation	+ (Al oxide formation)	+ + + (No oxide)	Electrolysis[12]
Water dissociation	+ (Highly)	+ + + (Little)	—
pH variation	+ (Severely occurs)	+ + + (Little occurs)	—
Optical			
Visualization	+ (Difficult)	+ + + (In microchannel)	Uptake imaging[9]
Monitoring	+ (Difficult)	+ + + (In microchannel)	Real-time[11]
Physiological			
Sample volume	Large volume required	Smaller volume required	—
Sample delivery	+ (Necessary)	+ + + (Easy or unnecessary)	—
Buffer contents	+ (PBS or complex)	+ + + (Simple)	—
Contamination	+ (Very easily at electrode)	+ + + (Hardly)	—
Cell applications			
Stem cells	+	+ + +	Blood cells
Primary cells	+	+ + +	Single cell level[11]
Suspension cells	+	+ + +	—
Single cell analysis	+	+ + + (In microchannel)	—
Others			
Processing time	+ (Long)	+ + + (Short)	Short time[12,68]
Throughput	+	+ + + (Available in automation)	High-throughput
Integration	N.A.	Possible in microchannel	User-friendly[12]
Convenience	+ (Sample handling)	+ + + (In microcapillary)	—
Disposability	+ (Difficult)	+ + + (Highly available)	Device & test
Cost	+ (Expensive)	+ + (Relatively cheaper)	—

The symbols indicate + + +: high or excellent; + +: medium or good; +: low or poor, respectively. The water dissociation can generate gases of oxygen and hydrogen at the surface of the electrodes. The metal oxides can make the pH level of media decreased locally. The heat generation can bring very harmful effects to cell viability during electroporation. N.A.: not available.

cell electroporation. Although two categories (microchannel and microcapillary) help to encompass a number of different microscale electroporation approaches, there might exist still another significant category. For example, for microchannel electroporation, there are many reports utilizing microscale electroporation where adherent cell cultures are electroporated *in situ*, by electrodes that might be microfabricated structures such as planar MEAs, inter-digitated electrodes or 3D electrodes (Table 2). In particular, the cell culture in microscale electroporation might be affected by dynamic flow in microchannels. In this review, however, we aimed to define this category of microchannel electroporation in a broader range, *i.e.*, to the use of microfluidic environments in manipulating, trapping, and transfecting cells during the entire electroporation process within microchannels.

3.1 Microchannel-based electroporation

Microchannel-based electroporation (hereafter called 'microchannel electroporation') can be used for transfecting cells within microchannels. Specifically, microfluidics can be used to manipulate and position biological objects flowing within microchannels. For example, microchannel electroporation can be useful for single cell electroporation such as to genetically modify stem cells.[56] This approach can also be used to transfer electromediated drugs and genes into living cells without an external pulse generator.[57] Therefore, microchannel electroporation can be used to conduct on-chip platform for screening drug candidates and target genes in a high-throughput manner.[57] On the other hand, the electric pulse generator has been widely used as a standard laboratory electroporator which consists of large capacitors and provides

Table 2 Latest achievements of the microscale electroporation methods in advanced gene and cell research (2007–2008)

Methods	Species	Cell type	Specific aims and applications	Future perspective	References
Microchannel-based	Human	HeLa	Finding of an impulsive, electropulsation-driven backflow in microchannels*	Functional gene delivery	9
	Human	MSC	Gene transfer and protein dynamics in stem cells using SCE*	Controlled cell therapeutics	56
	Mouse	NIH3T3	Spatially controlled transfection of nucleic acid payloads into cells in a culture	HTS drug discovery	54
	Hamster	CHO	Delivery of small molecules and genes into cells using a common DC power supply	HTS of drugs & genes	57
	Human	B cell	Detection of kinase translocation using microfluidic electroporative flow cytometry	Drug discovery & tumor diagnosis	58
	Human	A431	Lab-on-a-chip technologies for proteomic analysis from isolated cells under CLSM	LOC integrative electroporation	61
	Human	HeLa	Electrophoresis-assisted SCE for efficient intracellular delivery*	Drug delivery systems	26
	Chicken	B cells	Selective release of intracellular molecules at the single-cell level*	HTS intracellular probing	62
	Human	HeLa	SCE arrays with real-time monitoring and feedback control*	Drug discovery & gene delivery	11
	Hamster	CHO	Microfluidic cell electroporation using a mechanical valve	LSI system for HTS	64
	Human	K562	Continuous low-voltage dc electroporation with polyelectrolytic salt bridges	Disposable integrated system	65
	Human	B. p. cells	An integrated microfluidic system for combined with DEP for electroporation	Pathogen detection	59
	Human	U937	Gene delivery after DEP positioning of cells in a non-uniform electric field (FEM)	DEP integrative electroporation	60
	Hamster	CHO	Rapid optimization by multi-channel electroporation for gene transfection	Rapid protocol optimization	10
	Mouse	NIH3T3	Transfection of exogenous molecules with spatio-temporal control (FEM)	Spatio-temporal control	67
	Human	HEK293	Gene transfer device utilizing micron-spiked electrodes	Human genome analysis	66
	Human	HeLa	Micro electroporation and resealing dynamics for human cancer cells (EIS)	Cell membrane recovery dynamics	63
	Fish	Embryo	Transfection of zebrafish embryos by quantum dots and GFP genes for evaluation	Gene therapy	69
	Human	HeLa	Determination of the optimal physical parameters in the uptake of biomolecules	Shorter experimental time	68
Microcapillary-based	Human	hMSC	Electroporation method using a capillary and wire-type electrode	New electroporation platform	12
	Human	A549	Numerical calculations of SCE with an electrolyte-filled capillary* (EFC, FEM)	Tumor treatment	70
	Human	A549	Effect of cell size and shape on SCE* (EFC, FEM)	Success of electroporation-TMP	71
	Hamster	CHO	Scanning electroporation of selected areas of adherent cell cultures (EFC, FEM)	Success for hard-to-transfect cells	72
	Human	T cells	Targeting the carbohydrates on HIV-1	HIV vaccine development	35
	Human	T cells	Route of HIV-1 transmission with membrane nano-tubes	HIV-1 transmission mechanism	27
	Human	bmMSC	Role of hypoxia-inducible factor-1alpha in matrix metalloproteinase-1 activity	Pathophysiology in bone diseases	28
	Mouse	astrocytes	Neuronal stem cells control in murine astrocytes (Role of Pax6)	Astrocyte generation mechanism	29
	Human	PK8	Induction of hepatocyte growth factor activator gene expression	Treatment of pancreatic cancer	32
	Human	SW480	TMPRSS4 promotes invasion, migration and metastasis of human tumor cells	Cancer therapeutics	37
	Mouse	3T3-L1	Mediation of insulin-dependent regulation of gene expression	Lipid and glucose metabolism	40
	Human	U937	Differential contribution of the CysLTR1 gene with aspirin hypersensitivity	Aspirin-related allergic diseases	47
	Human	HCT116	Analysis of KRAP expression and localization, and genes regulated by KRAP	KRAP functions in cancer cells	49

The asterisk (*) indicates single cell level study. [Terms] SCE: single cell electroporation; CLSM: confocal laser scanning microscopy; MEA: microelectrode array; LOC: lab-on-a-chip; HTS: high-throughput screening; LSI: large-scale integration; FEM: finite element modeling; EIS: electrochemical impedance spectroscopy; DEP: dielectrophoresis; EFC: electrolyte-filled capillary; TMP: transmembrane potential; KRAP: Ki-ras-induced actin-interacting protein. [Cell lines] MSC: mesenchymal stem cells; NIH3T3: mouse embryonic fibroblast cells; CHO: Chinese hamster ovary; A431: human epithelial carcinoma cells; K562: human chronic leukemia cells; A549: human lung carcinoma cells; B. p.: Bordetella pertussis; HEK293: human embryonic kidney cells; U937: human monocytic cells; bmMSC: bone marrow-derived mesenchymal stem cells; PK8: pancreatic cancer cells; SW480: colon carcinoma cells; 3T3-L1: preadipocytes; HCT116: human colon cancer cells.

high voltage pulses easily. Also, this device is potentially beneficial for enhancing microscale electroporation consistently with its own advantages: (i) readily-available PC interface (*e.g.*, data acquisition cards), (ii) setup simplification, (iii) increased flexibility, (iii) larger integration capability, and (iv) low cost in use. These features might be big advantages for upgrading microscale electroporation in a wider range of biological applications.

Microchannel electroporation can be useful for high-throughput applications in electroporation-based drug-discovery and delivery research.[54] In particular, this technology can be integrated with other research tools at the single-cell level. For example, directed localization of kinases within cells is generally essential for their activation in signal transduction. Since these events can be detected by imaging a low number of cells and sub-cellular fractionation, however, they require the high-throughput probing of entire cell population to obtain information at the single-cell level. Thus, a microfluidic electroporative flow cytometry can be used to perform this detection in a high-throughput manner.[58] After sample preconcentration, genetic amplification and detection is necessary for pathogen detection. Dielectrophoresis (DEP) can be useful for manipulation, isolation, preconcentration and lysis of such samples as well as electroporation in microchannels.[59,60] In addition, the DEP can be combined with saw-tooth microelectrodes to enhance electroporation in a controlled manner. Also, the overall process of protein release can be monitored by confocal fluorescence microscopy.[61] To use relatively low voltages in single-cell electroporation, electrophoresis can be used to enhance delivery of compounds, followed by electroporation. For example, this can be used to assist electroporative uptake of impermanent molecules such as Calcein (MW: 622 Da) and Oregon Green Dextran (MW: 70 000 Da) in microchannels. In particular, subsequent to electroporation delivery efficiency can be enhanced more than an order of magnitude compared to diffusion alone.[26]

Single cell analysis of intracellular contents is beneficial for probing the heterogeneity of a cell population. Microchannel electroporation can be useful to release intracellular molecules without completely disrupting the cell membrane.[62] This approach can provide a high-throughput method for probing different intracellular molecules for single cells by localizing the electroporation to a specific region of the cell membrane. Microchannel electroporation can be used to improve the overall process of intracellular delivery of drug compounds, RNA, or DNA in a rapid and controlled manner. For example, a feedback-controlled bench-top system can be used to manipulate disposable microfluidic devices for manipulation, electroporation and real-time monitoring of single cells. This approach can be used to provide controlled parallel pressure and electrical application for individual cells in a reproducible manner.[11] Probing impedance of cell membrane is necessary to fully understand resealing dynamics of the membrane in a nonlinear current response. For example, dynamic process of membrane permeability of human cancer cells can be measured in microchannel during electroporation by combining electrochemical impedance spectroscopy (EIS) with microchannel electroporation.[63]

Electroporation can be enhanced by modifying the channel geometries. For example, polydimethylsiloxane (PDMS) mechanical valves can be used to generate electropulsation in simple microchannels and large-scale integrated (LSI) microfluidic systems.[64] This reduces the cost and complexity of the electroporation devices by eliminating the use of a pulse generator and microfabricated electrodes. To eliminate the formation of bubbles, a continuous low DC voltage can be applied to drive microchannel electroporation in the microstructures of polydiallyldimethyl-ammonium chloride (pDADMAC).[65] The microchannel can be equivalently used as an electrical circuit model to define microscale environments. Thus, it can provide multiple electric field gradients in a single microchip with different channel lengths. Several electrical conditions, *e.g.*, electric field can be made at a single voltage applied.[10] Microchannel electroporation can be combined by various microstructures with physically modified geometries such as microscale spike structures,[66] microelectrode arrays (MEA),[67] three-dimensional (3D) microelectrodes,[68] and other MEMS designs.[69]

3.2 Microcapillary-based electroporation

Microcapillary-based electroporation (hereafter called, 'microcapillary electroporation') can be used for transfecting cells within controlled reaction chambers. Microcapillary electroporation can be divided into two main categories, depending on its applications: individual and cell population electroporation. The first approach is based on an electrolyte-filled capillary (EFC) modeled with a finite element method, and can be useful for selectively treating the targeted cell without affecting its neighbors. Thus, it can be used to focus an electric field for single-cell electroporation.[70,71] This process can be computer-controlled by scanning the cell culture and locally delivering both an electric field and reagent to the specific area of individual cells without affecting the surrounding cells.[72] Specifically, this approach can be useful for directing the size and shape of the electroporated area of cells combined with the scanning patterns. A new theoretical model and its numerical simulations can also be achieved by the finite element modeling (FEM) analyses.[70–72]

Another approach is based on a tip-type microcapillary electroporation for providing higher cell transfection and then faster sample handling by integrating this capillary tip with a pipette that has been widely used in the laboratory setting.[12] This approach can be used to develop a novel electroporation platform with enhanced transfection efficiency and cell viability for a wide range of mammalian cells such as general cell lines, primary cells, and stem cells. Briefly, a common problem in macroscale electroporation is that it results in a lower cell viability than the other transfection methods (Fig. 1). Since microcapillary electroporation has a small electrode surface area, it can be used to considerably reduce cell death during electroporation. Specifically, it can be useful for minimizing variations in local pH level during electroporation. The overall process of electroporation can also be reduced to 15 min under different conditions. Importantly, this method can be useful for enhanced transfection efficiency (about 80% in cell lines and more than 50% in stem or primary cells) and viability (approximately 70–80%) (Fig. 2).[12] Furthermore, this can be potentially beneficial for studying infectious diseases and

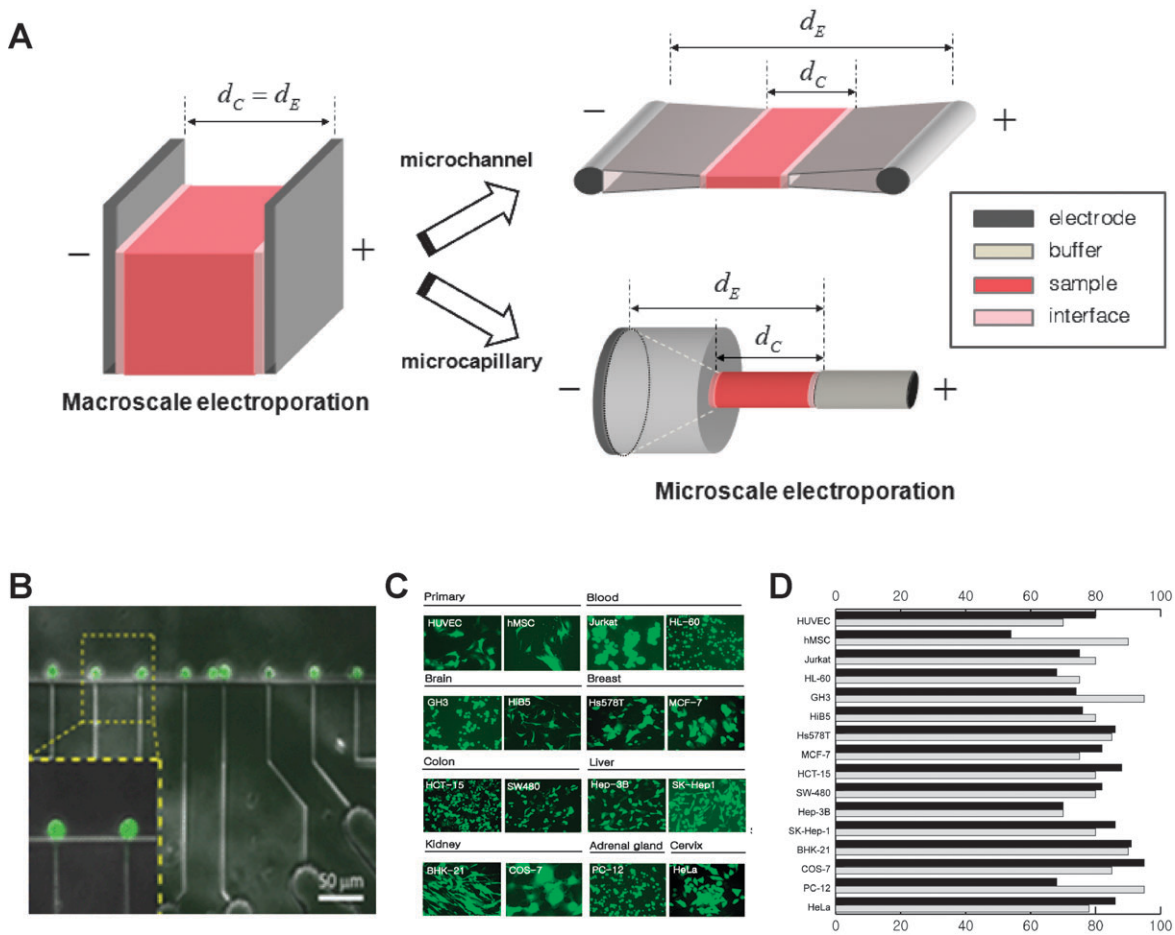

Fig. 2 Schematic illustration for electroporation and representative examples for recent advances for microscale electroporation. (A) Schematic of cuvette-type macroscale electroporation (left) and microchannel and microcapillary electroporation (right). Note d_C is the distance of chamber effectively exposed to electric field, and d_E is the distance between electrodes. (For more detailed geometry of microchannel electroporation, please see ref. 55) (B) Microchannel electroporation of an array of HeLa cells trapped at the single-cell level by using a feedback-controlled microfluidic system (overlay of bright field and FITC). Reprinted by permission (ref. 11) (C) Results for microcapillary electroporation of different cell types with pEGFP by using a capillary and wire-electrode system (fluorescent images), and (D) Summary of cell transfection efficiency (dark gray) and cell viability (light gray) obtained by microcapillary electroporation. Reprinted by permission (ref. 12).

generating diagnostic devices because of its disposable reaction chamber.[27,35]

4. Current challenges and perspectives of microscale electroporation

In this section, we provide a broad overview of the latest achievements of microscale electroporation and its remaining challenges for widespread clinical use. Microchannel electroporation shows great promise for on-chip integration and visualization of cell electroporation at the single-cell level.[9,10,12,51] However, it has difficulties in manipulating the transfected cells from the channels and in delivering those cells into post-electroporation steps. On the other hand, microcapillary electroporation can be used to address those problems and to integrate its platform with a user-friendly pipette. But, it has difficulties in combining its function with other techniques for direct visualization of its mechanism. Therefore, these two approaches can be complementarily used to promote cell electroporation.

Microchannel electroporation can become more powerful when combined with the other techniques. Nevertheless, this technology can be hardly used in a channel format for clinical applications because it requires special technical backgrounds for microfluidic setup and operation. Therefore, the ability to automate the operation with the feedback control is beneficial for enhancing its use in clinical applications (Fig. 2A). Microchannel electroporation can also be useful for studying the electroporation mechanism *in vitro*. To widen its use for clinical applications, however, this technology may require a user-friendly platform that can enhance both transfection efficiency and ease-of-use for clinical standards and applications. Currently, microscale electroporation has different platforms, so it is difficult to bring their biological results into clinical standards and protocols. Those methods may also require standardization for clinical and biological applications. To establish clinical standards or protocols, in particular, the ability to transfect cells more reliably is desired. For example, a microcapillary electroporation device can be combined with a user-friendly pipette. In addition, this device also

enabled us to improve the established protocols with higher cell transfection efficiency and viability (Fig. 2B and C). This has led to a wide variety of clinical and biological advances.[27–50]

To address both efficiency and ease-of-use for microscale electroporation for clinical applications, here we focus on the latest achievements of microcapillary electroporation rather than microchannel electroporation. Specifically, we discuss future perspective for microscale electroporation by specifying emerging examples for clinical and biological applications in: (i) HIV, (ii) stem cells and tissue regeneration, (iii) cancer, and (iv) other disease studies.

4.1 HIV studies

HIV infection affects more than 33 million people worldwide.[73] The HIV virus affects $CD8^+$ and $CD4^+$ T-cell function, which results in loss of host's immunity.[74] Preclinical and clinical studies have used DNA delivery by needle for such HIV infection and vaccine studies, however, these have resulted in weak antibody and T-cell responses. Since the safe and effective delivery of DNA vaccines is still challenging, more efficient techniques are required.[75] Electroporation is a potentially promising approach for the development of DNA vaccines. Even though this technology has higher transfection efficiency, it still requires better cell viability after electroporation. Microcapillary electroporation is one potential approach to achieve desired gene delivery for such an application. For example, to develop an effective HIV vaccine, this approach was used for studying efficient stimulation and transmission of HIV-1-specific T cells.[35] More recently, this technology was used for finding a route for HIV transmission mechanism.[27] Throughout those studies, this technology proved to be effective in conducting HIV studies for DNA vaccine development. In addition, this can be useful for providing a disposable reaction chamber for HIV samples and delivering the electroporated cells faster.[12] Furthermore, this technology can be potentially beneficial for addressing both issues of safety and efficiency in future HIV research and its vaccine development.

4.2 Stem cell and tissue regeneration studies

Stem cell differentiation is a promising approach for generating a cell source for regenerative medicine. In some cases, it may be desired to deliver RNA or DNA into the cells to direct their behavior. However, stem cells and primary cells are sensitive to environmental changes induced by electroporation such as electrical and physiological factors. (Table 1).

Microcapillary electroporation provides a controlled reaction chamber with enhanced transfection efficiency and viability for stem cells and primary cells. Microcapillary electroporation was used to study bone disease by transfecting bone marrow-derived mesenchymal stem cells (bmMSCs) with small interfering RNA (siRNA) that could then regulate matrix metalloproteinase (MMPs) activity.[28] This technology can also be useful for assessing the differentiation of neuronal stem cells into murine astrocytes by transfecting wild-type astrocytes with plasmid.[29] This study showed that Pax6 is an important transcription factor for neurogenesis and gliogenesis. Similarly, this method was used to transfect siRNAs for studying immune homeostasis and inflammation[50] and liver function.[39]

Induced pluripotent stem (iPS) cells also have an enormous potential for production of patient-specific stem cells.[76] Those cells can be derived by retroviral gene transfection into non-pluripotent cells. However, this technology has a number of major limitations due to the use of potentially harmful genome-integrating viruses.[77] More recently, studies on generation of mouse iPS cells without viral vectors were reported.[77–79] This indicates that retroviral integration in iPS cell generation may not be necessary. Thus, further studies to increase the efficiency of virus-free iPS cells may be of benefit in this area. Therefore, microscale electroporation can become a powerful tool for future applications that involve the derivation and differentiation of iPS cells.

4.3 Cancer studies

Microscale electroporation may also be used to address potential problems in cancer treatments. A successful DNA vaccine candidate for human cancer therapy must have a high efficacy without a large dose. More recently, this effort has led to the use of an adaptive constant-current electroporation (CCE) technique for clinical cancer vaccination.[80] The ability to lower the dose and frequency of vaccinations enables this technique to be both more cost-effective and safer by using much less vaccine for clinical treatment of human patients. Since microscale electroporation can improve transfection efficiency and viability, it may help to further develop the CCE technique for clinical applications. In addition, microscale electroporation uses less toxic materials, generates more uniform electric fields, and leads to less sample contamination for developing future cancer vaccinations.

Microscale electroporation can also be useful for advanced genetic manipulation associated with cancer treatments. For example, it was used to enhance luciferase reporter gene assay by determining the 3D structure and residues of tumor suppressive activity through its interaction with ataxia-telangiectasia mutated (ATM), a serine-specific protein kinase associated with DNA double-strand breaks.[30] This technology can also be used to transfect siRNAs into cancer cells. For example, the induction of hepatocyte growth factor activator (HGFA) gene expression under hypoxia was examined in aggressive invasion of pancreatic cancer.[32] Similarly, microcapillary electroporation can help to study the other cancer cell studies such as human cancer invasion, migration and metastasis,[37] tumor progression,[38] oncogenic regulation,[36] gastric cancer,[33] colorectal cancers,[41] and colon cancer.[49]

Another electroporation approach for cancer treatment is an irreversible electroporation (IRE) technique. Generally, this technique provides an irreversible opening for the cell membrane during electroporation. The IRE technique can be useful for clinical applications such as cancer treatment and surgery in medicine.[81–85] Similarly, the use of microscale electroporation at the single-cell level may be of benefit for developing future IRE applications of cancer cell treatment

without damaging healthy tissues or leaving malignant cells in a controlled manner.

4.4 Other disease studies

To obtain higher transfection efficiency and viability, microcapillary electroporation can be useful for other disease studies. In particular, more stable and efficient gene delivery into living organisms can be used to understand complex pathways of delivery to the nucleus. For example, this method was used for efficient electroporation of primary sensory neurons to study the role of up-regulation of intracellular GTP in persistent sodium currents.[34] This approach was also used to study insulin-dependent gene transduction into 3T3-L1 adipocytes to study the chromatin remodeling complex for mediating insulin-dependent regulation of gene expression.[40]

Furthermore, microscale electroporation was used to study efficient intracellular gene delivery and its interaction mechanisms such as PERK-dependent compartmentalization of endoplasmic reticulum (ER) associated degradation during ER stress[42] and statin-mediated cytoprotection of human vascular endothelial cells.[43] Similarly, it can also help to examine complex genetic mechanism by its higher transfection efficiency and cell viability. For example, it was used to perform other disease studies such as glial cell death,[44] infected human fibroblasts,[45] infected human T cells,[46] aspirin hypersensitivity,[47] and autoimmune disease.[48]

6. Conclusions

Integrating microscale engineering with biology is potentially beneficial for practical applications in life sciences and biotechnology. In the past few years, various approaches have been developed to advance this field in a synergistic manner. In this review, we focus on recent advances of microscale electroporation and its biological applications. Specifically, we also discuss current challenges and perspectives of microscale electroporation for clinical applications. This technology is divided into two main categories: microchannel and microcapillary electroporation. Specifically, this approach has strong advantages in comparison to its macroscale counterpart which include: (i) *in situ* visualization of molecular uptake, (ii) real-time monitoring of intracellular responses, (iii) formation of uniform or symmetrical electric fields, (iv) generation of a controlled reaction chamber, and (v) rapid optimization of transfection protocols. In addition, it can also be combined with the other techniques such as electrophoresis, dielectrophoresis, and electrochemical impedance spectrometry, especially within physically modified geometries. This can be useful for conducting single cell electroporation as well as a number of cells electroporation in a controlled and high-throughput manner. These efforts have successfully led to widespread applications for studying hard-to-cure diseases (HIV and cancer), regenerative medicine (stem cells regeneration), and other diseases-related cells. To promote a wider use of microscale electroporation in clinical fields, however, it may be required to enhance the efficiency as well as increase the ease of use. Furthermore, this approach will help researchers to promote a comprehensively integrative approach to advanced genetic and cellular research in life sciences.

Acknowledgements

This work was supported by the National Institute of Health (NIH EB007249, DE019024 and HL092836) and the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-357-D00035).

References

1 A. Khademhosseini, R. Langer, J. Borenstein and J. P. Vacanti, Microscale technologies for tissue engineering and biology, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 2480–2487.
2 J. West, M. Becker, S. Tombrink and A. Manz, Micro total analysis systems: latest achievements, *Anal. Chem.*, 2008, **80**, 4403–4419.
3 A. L. Paguirigan and D. J. Beebe, Microfluidics meet cell biology: bridging the gap by validation and application of microscale techniques for cell biological assays, *Bioessays*, 2008, **30**, 811–821.
4 E. Berthier, J. Warrick, H. Yu and D. J. Beebe, Managing evaporation for more robust microscale assays. Part 1. Volume loss in high throughput assays, *Lab Chip*, 2008, **8**, 852–859.
5 U. Demirci and G. Montesano, Single cell epitaxy by acoustic picolitre droplets, *Lab Chip*, 2007, **7**, 1139–1145.
6 Y. Du, E. Lo, S. Ali and A. Khademhosseini, Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 9522–9527.
7 S. M. Kim, S. H. Lee and K. Y. Suh, Cell research with physically modified microfluidic channels: a review, *Lab Chip*, 2008, **8**, 1015–1023.
8 W. G. Lee, H. Bang, H. Yun, J. Lee, J. Park, J. K. Kim, S. Chung, K. Cho, C. Chung, D. C. Han and J. K. Chang, On-chip erythrocyte deformability test under optical pressure, *Lab Chip*, 2007, **7**, 516–519.
9 W. G. Lee, H. Bang, H. Yun, J. Min, C. Chung, J. K. Chang and D. C. Han, An impulsive: electropulsation-driven backflow in microchannels during electroporation, *Lab Chip*, 2008, **8**, 224–226.
10 J. A. Kim, K. Cho, Y. S. Shin, N. Jung, C. Chung and J. K. Chang, A multi-channel electroporation microchip for gene transfection in mammalian cells, *Biosens. Bioelectron.*, 2007, **22**, 3273–3277.
11 M. Khine, C. Ionescu-Zanetti, A. Blatz, L. P. Wang and L. P. Lee, Single-cell electroporation arrays with real-time monitoring and feedback control, *Lab Chip*, 2007, **7**, 457–462.
12 J. A. Kim, K. Cho, M. S. Shin, W. G. Lee, N. Jung, C. Chung and J. K. Chang, A novel electroporation method using a capillary and wire-type electrode, *Biosens. Bioelectron.*, 2008, **23**, 1353–1360.
13 X. Cheng, Y. S. Liu, D. Irimia, U. Demirci, L. Yang, L. Zamir, W. R. Rodriguez, M. Toner and R. Bashir, Cell detection and counting through cell lysate impedance spectroscopy in microfluidic devices, *Lab Chip*, 2007, **7**, 746–755.
14 N. Odani, K. Ito and H. Nakamura, Electroporation as an efficient method of gene transfer, *Dev., Growth Differ.*, 2008, **50**, 443–448.
15 T. Iimura and O. Pourquie, Manipulation and electroporation of the avian segmental plate and somites *in vitro*, *Methods Cell Biol.*, 2008, **87**, 257–270.
16 V. V. Fedorov, V. P. Nikolski and I. R. Efimov, Effect of electroporation on cardiac electrophysiology, *Methods Mol. Biol.*, 2008, **423**, 433–448.
17 M. P. Rols, Mechanism by which electroporation mediates DNA migration and entry into cells and targeted tissues, *Methods Mol. Biol.*, 2008, **423**, 19–33.
18 K. Anwer, Formulations for DNA delivery *via* electroporation *in vivo*, *Methods Mol. Biol.*, 2008, **423**, 77–89.
19 M. Takahashi, T. Nomura and N. Osumi, Transferringgenes into cultured mammalian embryos by electroporation, *Dev., Growth Differ.*, 2008, **50**, 485–497.
20 J. Gehl, Electroporation for drug and gene delivery in the clinic: doctors go electric, *Methods Mol. Biol.*, 2008, **423**, 351–359.
21 A. Luxembourg, C. F. Evans and D. Hannaman, Electroporation-based DNA immunization: translation to the clinic, *Expert Opin. Biol. Ther.*, 2007, **7**, 1647–1664.
22 L. M. Mir, Application of electroporation gene therapy: past, current, and future, *Methods Mol. Biol.*, 2008, **423**, 3–17.

23 Y. Isaka and E. Imai, Electroporation-mediated gene therapy, *Expert Opin. Drug Delivery*, 2007, **4**, 561–571.
24 T. F. Yuan, Vaccine submission with muscle electroporation, *Vaccine*, 2008, **26**, 1805–1806.
25 D. Luo and W. M. Saltzman, Synthetic DNA delivery systems, *Nat. Biotechnol.*, 2000, **18**, 33–37.
26 C. Ionescu-Zanetti, A. Blatz and M. Khine, Electrophoresis-assisted single-cell electroporation for efficient intracellular delivery, *Biomed. Microdevices*, 2008, **10**, 113–116.
27 S. Sowinski, C. Jolly, O. Berninghausen, M. A. Purbhoo, A. Chauveau, K. Kohler, S. Oddos, P. Eissmann, F. M. Brodsky, C. Hopkins, B. Önfelt, Q. Sattentau and D. M. Davis, Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission, *Nat. Cell Biol.*, 2008, **10**, 211–219.
28 J. L. Lin, M. J. Wang, D. Lee, C. C. Liang and S. Lin, Hypoxia-inducible factor-1alpha regulates matrix metalloproteinase-1 activity in human bone marrow-derived mesenchymal stem cells, *FEBS Lett.*, 2008, **582**, 2615–2619.
29 K. Sakurai and N. Osumi, The neurogenesis-controlling factor, Pax6, inhibits proliferation and promotes maturation in murine astrocytes, *J. Neurosci.*, 2008, **28**, 4604–4612.
30 K. J. Kim, M. C. Park, S. J. Choi, Y. S. Oh, E. C. Choi, H. J. Cho, M. H. Kim, S. H. Kim, D. W. Kim, S. Kim and B. S. Kang, Determination of three-dimensional structure and residues of the novel tumor suppressor AIMP3/p18 required for the interaction with ATM, *J. Biol. Chem.*, 2008, **283**, 14032–14040.
31 X. Y. Tang, Y. F. Li and S. M. Tan, Intercellular adhesion molecule-3 binding of integrin alpha L beta requires both extension and opening of the integrin headpiece, *J. Immunol.*, 2008, **180**, 4793–4804.
32 Y. Kitajima, T. Ide, T. Ohtsuka and K. Miyazaki, Induction of hepatocyte growth factor activator gene expression under hypoxia activates the hepatocyte growth factor/c-Met system *via* hypoxia inducible factor-1 in pancreatic cancer, *Cancer Sci.*, 2008, **99**, 1341–1347.
33 T. Otsubo, Y. Akiyama, K. Yanagihara and Y. Yuasa, SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis, *Br. J. Cancer*, 2008, **98**, 824–831.
34 J. A. Ostman, M. A. Nassar, J. N. Wood and M. D. Baker, GTP up-regulated persistent Na+ current and enhanced nociceptor excitability require NaV1.9, *J. Physiol.*, 2008, **586**, 1077–1087.
35 S. K. Wang, P. H. Liang, R. D. Astronomo, T. L. Hsu, S. L. Hsieh, D. R. Burton and C. H. Wong, Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3690–3695.
36 M. C. Kwon, B. K. Koo, J. S. Moon, Y. Y. Kim, K. C. Park, N. S. Kim, M. Y. Kwon, M. P. Kong, K. J. Yoon, S. K. Im, J. Ghim, Y.-M. Han, S. K. Jang, M. Shong and Y.-Y. Kong, Crif1 is a novel transcriptional coactivator of STAT3, *EMBO J.*, 2008, **27**, 642–653.
37 H. Jung, K. P. Lee, S. J. Park, J. H. Park, Y. S. Jang, S. Y. Choi, J. G. Jung, K. Jo, D. Y. Park, J. H. Yoon, J.-H. Park, D.-S. Lim, G.-R. Hong, C. Choi, Y.-K. Park, J. W. Lee, H. J. Hong, S. Kim and Y. W. Park, TMPRSS4 promotes invasion, migration and metastasis of human tumor cells by facilitating an epithelial-mesenchymal transition, *Oncogene*, 2008, **27**, 2635–2647.
38 M. Tomita, G. L. Semenza, C. Michiels, T. Matsuda, J. N. Uchihara, T. Okudaira, Y. Tanaka, N. Taira, K. Ohshiro and N. Mori, Activation of hypoxia-inducible factor 1 in human T-cell leukaemia virus type 1-infected cell lines and primary adult T-cell leukaemia cells, *Biochem. J.*, 2007, **406**, 317–323.
39 S. Sawada, T. Kinjo, S. Makishi, M. Tomita, A. Arasaki, K. Iseki, H. Watanabe, K. Kobayashi, H. Sunakawa, T. Iwamasa and N. Mori, Downregulation of citrin, a mitochondrial AGC, is associated with apoptosis of hepatocytes, *Biochem. Biophys. Res. Commun.*, 2007, **364**, 937–944.
40 Y. S. Lee, D. H. Sohn, D. Han, H. W. Lee, R. H. Seong and J. B. Kim, Chromatin remodeling complex interacts with ADD1/SREBP1c to mediate insulin-dependent regulation of gene expression, *Mol. Cell. Biol.*, 2007, **27**, 438–452.
41 S.-K. Chung, M.-G. Lee, B.-K. Ryu, J.-H. Lee, J. Han, D.-S. Byun, K.-S. Chae, K.-Y. Lee, J.-Y. Jang, H.-J. Kim and S.-G. Chi, Frequent alteration of XAF1 in human colorectal cancers: implication for tumor cell resistance to apoptotic stresses, *Gastroenterology*, 2007, **132**, 2459–2477.
42 M. Kondratyev, E. Avezov, M. Shenkman, B. Groisman and G. Z. Lederkremer, PERK-dependent compartmentalization of ERAD and unfolded protein response machineries during ER stress, *Exp. Cell Res.*, 2007, **313**, 3395–3407.
43 F. Ali, S. S. Hamdulay, A. R. Kinderlerer, J. J. Boyle, E. A. Lidington, T. Yamaguchi, M. P. Soares, D. O. Haskard, A. M. Randi and J. C. Mason, Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1, *J. Thromb. Haemostasis*, 2007, **5**, 2537–2546.
44 Y. J. Byun, S.-B. Lee, D. J. Kim, H. O. Lee, M. J. Son, C. W. Yang, K.-W. Sung, H.-S. Kim, O.-J. Kwon, I.-K. Kim and S.-W. Jeong, Protective effects of vacuolar H+-ATPase c on hydrogen peroxide-induced cell death in C6 glioma cells, *Neurosci. Lett.*, 2007, **425**, 183–187.
45 J. J. Park, Y. E. Kim, H. T. Pham, E. T. Kim, Y. H. Chung and J. H. Ahn, Functional interaction of the human cytomegalovirus IE2 protein with histone deacetylase 2 in infected human fibroblasts, *J. Gen. Virol.*, 2007, **88**, 3214–3223.
46 G. Mlechkovich and N. Frenkel, Human herpesvirus 6A (HHV-6A) and HHV-6B alter E2F1/Rb pathways and E2F1 localization and cause cell cycle arrest in infected T cells, *J. Virol.*, 2007, **81**, 13499–13508.
47 S. H. Kim, E. M. Yang, H. J. Park, Y. M. Ye, H. Y. Lee and H. S. Park, Differential contribution of the CysLTR1 gene in patients with aspirin hypersensitivity, *J. Clin. Immunol.*, 2007, **27**, 613–619.
48 M. Yamauchi, M. Hashimoto, K. Ichiyama, R. Yoshida, T. Hanada, T. Muta, S. Komune, T. Kobayashi and A. Yoshimura, Ifi202, an IFN-inducible candidate gene for lupus susceptibility in NZB/W F1 mice, is a positive regulator for NF-kappaB activation in dendritic cells, *Int. Immunol.*, 2007, **19**, 935–942.
49 T. Fujimoto, M. Koyanagi, I. Baba, K. Nakabayashi, N. Kato, T. Sasazuki and S. Shirasawa, Analysis of KRAP expression and localization, and genes regulated by KRAP in a human colon cancer cell line, *J. Hum. Genet.*, 2007, **52**, 978–984.
50 N. Mizutani, T. Sakurai, T. Shibata, K. Uchida, J. Fujita, R. Kawashima, Y. I. Kawamura, N. Toyama-Sorimachi, T. Imai and T. Dohi, Dose-dependent differential regulation of cytokine secretion from macrophages by fractalkine, *J. Immunol.*, 2007, **179**, 7478–7487.
51 Y. S. Shin, K. Cho, J. K. Kim, S. H. Lim, C. H. Park, K. B. Lee, Y. Park, C. Chung, D. C. Han and J. K. Chang, Electrotransfection of mammalian cells using microchannel-type electroporation chip, *Anal. Chem.*, 2004, **76**, 7045–7052.
52 M. Khine, A. Lau, C. Ionescu-Zanetti, J. Seo and L. P. Lee, A single cell electroporation chip, *Lab Chip*, 2005, **5**, 38–43.
53 M. Golzio, J. Teissie and M. P. Rols, Direct visualization at the single-cell level of electrically mediated gene delivery, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 1292–1297.
54 T. Jain and J. Muthuswamy, Bio-chip for spatially controlled transfection of nucleic acid payloads into cells in a culture, *Lab Chip*, 2007, **7**, 1004–1011.
55 M. B. Fox, D. C. Esveld, A. Valero, R. Luttge, H. C. Mastwijk, P. V. Bartels, A. van den Berg and R. M. Boom, Electroporation of cells in microfluidic devices: a review, *Anal. Bioanal. Chem.*, 2006, **385**, 474–485.
56 A. Valero, J. N. Post, J. W. van Nieuwkasteele, P. M. Ter Braak, W. Kruijer and A. van den Berg, Gene transfer and protein dynamics in stem cells using single cell electroporation in a microfluidic device, *Lab Chip*, 2008, **8**, 62–67.
57 H. Y. Wang and C. Lu, Microfluidic electroporation for delivery of small molecules and genes into cells using a common DC power supply, *Biotechnol. Bioeng.*, 2008, **100**, 579–586.
58 J. Wang, N. Bao, L. L. Paris, H. Y. Wang, R. L. Geahlen and C. Lu, Detection of kinase translocation using microfluidic electroporative flow cytometry, *Anal. Chem.*, 2008, **80**, 1087–1093.
59 C. de la Rosa, R. Prakash, P. A. Tilley, J. D. Fox and K. V. Kaler, Integrated microfluidic systems for sample preparation and detection of respiratory pathogen Bordetella pertussis, *Conf. Proc. IEEE Eng. Med. Biol. Soc.*, 2007, **2007**, 6303–6306.

60 L. A. MacQueen, M. D. Buschmann and M. R. Wertheimer, Gene delivery by electroporation after dielectrophoretic positioning of cells in a non-uniform electric field, *Bioelectrochemistry*, 2008, **72**, 141–148.
61 H. Sedgwick, F. Caron, P. B. Monaghan, W. Kolch and J. M. Cooper, Lab-on-a-chip technologies for proteomic analysis from isolated cells, *J. R. Soc. Interface*, 2008, **5**, S123–S130.
62 N. Bao, J. Wang and C. Lu, Microfluidic electroporation for selective release of intracellular molecules at the single-cell level, *Electrophoresis*, 2008, **29**, 2939–2944.
63 H. He, D. C. Chang and Y. K. Lee, Nonlinear current response of micro electroporation and resealing dynamics for human cancer cells, *Bioelectrochemistry*, 2008, **72**, 161–168.
64 J. Wang, M. J. Stine and C. Lu, Microfluidic cell electroporation using a mechanical valve, *Anal. Chem.*, 2007, **79**, 9584–9587.
65 S. K. Kim, J. H. Kim, K. P. Kim and T. D. Chung, Continuous low-voltage dc electroporation on a microfluidic chip with polyelectrolytic salt bridges, *Anal. Chem.*, 2007, **79**, 7761–7766.
66 N. Miyano, Y. Inoue, Y. Teramura, K. Fujii, F. Tsumori, H. Iwata and H. Kotera, Gene transfer device utilizing micron-spiked electrodes produced by the self-organization phenomenon of Fe-alloy, *Lab Chip*, 2008, **8**, 1104–1109.
67 T. Jain and J. Muthuswamy, Microsystem for transfection of exogenous molecules with spatio-temporal control into adherent cells, *Biosens. Bioelectron.*, 2007, **22**, 863–870.
68 H. He, D. C. Chang and Y. K. Lee, Using a micro electroporation chip to determine the optimal physical parameters in the uptake of biomolecules in HeLa cells, *Bioelectrochemistry*, 2007, **70**, 363–368.
69 K. S. Huang, Y. C. Lin, K. C. Su and H. Y. Chen, An electroporation microchip system for the transfection of zebrafish embryos using quantum dots and GFP genes for evaluation, *Biomed. Microdevices*, 2007, **9**, 761–768.
70 I. Zudans, A. Agarwal, O. Orwar and S. G. Weber, Numerical calculations of single-cell electroporation with an electrolyte-filled capillary, *Biophys. J.*, 2007, **92**, 3696–3705.
71 A. Agarwal, I. Zudans, E. A. Weber, J. Olofsson, O. Orwar and S. G. Weber, Effect of cell size and shape on single-cell electroporation, *Anal. Chem.*, 2007, **79**, 3589–3596.
72 J. Olofsson, M. Levin, A. Stromberg, S. G. Weber, F. Ryttsen and O. Orwar, Scanning electroporation of selected areas of adherent cell cultures, *Anal. Chem.*, 2007, **79**, 4410–4418.
73 Report on the global HIV/AIDS epidemic 2008: http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp.
74 E. R. A. Van Gulck, P. Ponsaerts, L. Heyndrickx, K. Vereecken, F. Moerman, A. De Roo, R. Colebunders, G. Van den Bosch, D. R. Van Bockstaele, V. F. I. Van Tendeloo, S. Allard, B. Verrier, C. Marañón, G. Hoeffel, A. Hosmalin, Z. N. Berneman and G. Vanham, Efficient stimulation of HIV-1-specific T cells using dendritic cells electroporated with mRNA encoding autologous HIV-1 Gag and Env proteins, *Blood*, 2006, **107**, 1818–1827.
75 A. D. Cristillo, L. Galmin, S. Restrepo, L. Hudacik, J. Suschak, B. Lewis, R. Draghia-Akli, N. Aziz, D. Weiss, P. Markham and R. Pal, HIV-1 Env vaccine comprised of electroporated DNA and protein co-administered with Talabostat, *Biochem. Biophys. Res. Commun.*, 2008, **370**, 22–26.
76 G. Q. Daley, Towards the generation of patient-specific pluripotent stem cells for combined gene and cell therapy of hematologic disorders, *Hematology Am. Soc. Hematol. Educ. Program*, 2007, **2007**, 17–22.
77 M. Stadtfeld, M. Nagaya, J. Utikal, G. Weir and K. Hochedlinger, Induced Pluripotent Stem Cells Generated Without Viral Integration, *Science*, 2008, **322**, 945–949.
78 T. Aoi, K. Yae, M. Nakagawa, T. Ichisaka, K. Okita, K. Takahashi, T. Chiba and S. Yamanaka, Generation of pluripotent stem cells from adult mouse liver and stomach cells, *Science*, 2008, **321**, 699–702.
79 K. Okita, M. Nakagawa, H. Hyenjong, T. Ichisaka and S. Yamanaka, Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors, *Science*, 2008, **322**, 949–953.
80 C. Curcio, A. S. Khan, A. Amici, M. Spadaro, E. Quaglino, F. Cavallo, G. Forni and R. Draghia-Akli, DNA immunization using constant-current electroporation affords long-term protection from autochthonous mammary carcinomas in cancer-prone transgenic mice, *Cancer Gene. Ther.*, 2008, **15**, 108–114.
81 B. Rubinsky, Irreversible electroporation in medicine, *Technol. Cancer. Res. Treat.*, 2007, **6**, 255–260.
82 B. Rubinsky, G. Onik and P. Mikus, Irreversible electroporation: a new ablation modality-clinical implications, *Technol. Cancer Res. Treat.*, 2007, **6**, 37–48.
83 C. Bertacchini, P. M. Margotti, E. Bergamini, A. Lodi, M. Ronchetti and R. Cadossi, Design of an irreversible electroporation system for clinical use, *Technol. Cancer Res. Treat.*, 2007, **6**, 313–320.
84 J. Lavee, G. Onik, P. Mikus and B. Rubinsky, A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation, *Heart Surg. Forum*, 2007, **10**, E162–167.
85 E. Maor, A. Ivorra, J. Leor and B. Rubinsky, Irreversible electroporation attenuates neointimal formation after angioplasty, *IEEE Trans. Biomed. Eng.*, 2008, **55**, 2268–2274.
86 J. T. Nevill, R. Cooper, M. Dueck, D. N. Breslauer and L. P. Lee, Integrated microfluidic cell culture and lysis on a chip, *Lab Chip*, 2007, **7**, 1689–1695.