

Cite this: *Lab Chip*, 2011, **11**, 3941

www.rsc.org/loc

## Microfluidic approaches for gene delivery and gene therapy

Jungkyu Kim,<sup>a</sup> Inseong Hwang,<sup>b</sup> Derek Britain,<sup>c</sup> Taek Dong Chung,<sup>b</sup> Yu Sun<sup>\*d</sup> and Deok-Ho Kim<sup>\*c</sup>

Received 16th August 2011, Accepted 10th October 2011

DOI: 10.1039/c1lc20766k

Recent advances in microfluidics have created new and exciting prospects for gene delivery and therapy. The micro-scaled environment within microfluidic systems enables precise control and optimization of multiple processes and techniques used in gene transfection and the production of gene and drug transporters. Traditional non-viral gene transfection methods, such as electroporation, microinjection and optical gene transfection, are improved from the use of innovative microfluidic systems. Additionally, microfluidic systems have also made the production of many viral and non-viral vectors controlled, automated, and reproducible. In summary, the development and application of microfluidic systems are producing increased efficiency in gene delivery and promise improved gene therapy results.

### Introduction

Many diseases are caused by mutated or missing genes, resulting in the production of abnormal proteins that adversely affect cellular function. Gene therapy involves the insertion of genetic materials into cells with genetic defects to correct genetic abnormalities. Gene therapy requires the delivery of foreign genetic materials through the cell membrane without causing cell lysis. The genetic materials must be integrated into the innate genome of the cell and be properly expressed. Successful delivery of exogenous genetic materials across the cell membrane is a major hurdle in gene therapy. Thus, a number of methods have been developed for gene delivery.

Viruses are effective vehicles for delivering genetic materials into cells. The viral genome can be readily edited so that genetic sequences of interest can be coded within the genome without detriment to the viral activity.<sup>1</sup> Because of the diversity of cell types, different types of viral vectors such as adenovirus, retrovirus, lentivirus, and herpes virus have been used for gene delivery.<sup>1–3</sup> Viral vector systems, however, can cause frame-shift mutations by random insertion of target genes into the host genome and inflammatory responses from immune reaction caused by viral components.<sup>4</sup> Other concerns include nonspecific targeting of genes by the infection of multiple cells and complicated procedure for viral vector production.<sup>5</sup> These shortcomings have fueled the development of non-viral vectors that imitate gene delivery functions of viruses and possess larger gene carrying capacity while avoiding adverse immune response and mutagenesis. The two most popular non-viral vectors are

cationic liposomes (lipoplexes) and cationic polymers (polyplexes).<sup>6</sup>

To transport the target gene into cells, physical techniques such as electroporation and sonoporation have been used to create temporary pores in the cell membrane. Electroporation uses electric pulses to enable nucleic acids to pass through the pores. It has become a popular gene transfection method because of its high-throughput and high efficiency.<sup>7,8</sup> Under proper conditions, electroporation was shown to produce transfection rates on par with viral vectors.<sup>7</sup> While electroporation uses electric pulses, sonoporation uses ultrasound to disrupt the cell membrane through acoustic cavitations.<sup>9</sup> The selection of intensity and frequency can be customized for different tissues.<sup>10</sup> The efficacy of the sonoporation technique increases when combined with microbubbles.<sup>11,12</sup> Microbubbles cavitate when they absorb ultrasound waves, thus release local shockwaves and increase pore formation in cell membranes.<sup>9</sup> Since genes can attach to microbubbles, upon cavitation the attached genes are released and absorbed by target cells.<sup>10</sup> Sonoporation is safe and non-invasive, allowing for possible use in internal organs without the need for surgical procedures.<sup>9,10</sup>

Gene transfection in conventional bench-top systems requires complicated operation procedures, lacks accuracy/specificity, and has low cell viability and low transfection efficiency. Rapid advances in microfluidics have created new and exciting prospects for gene delivery and therapy. The microfluidic environment offers increased precision and control for gene delivery and enables controllable and efficient production of vectors and other materials used in gene therapy. In this review, recent progress in microfluidics-based gene delivery and gene carrier fabrication techniques is discussed.

### Microfluidic approaches for gene transfection

Using microfluidics approaches, three gene transfection techniques have been explored, including the use of electric pulse,

<sup>a</sup>Department of Chemistry and Electrical Engineering, University of California, Berkeley, CA, USA

<sup>b</sup>Department of Chemistry, Seoul National University, Seoul, Korea

<sup>c</sup>Department of Bioengineering, University of Washington, Seattle, WA, USA. E-mail: deokho@uw.edu

<sup>d</sup>Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada. E-mail: sun@mie.utoronto.ca

hydrodynamic force, and optical energy. These techniques were conventionally studied with various genes in bulk solution. The microfluidic implementation of these techniques demonstrated higher precision, higher transfection efficiency, and higher cell viability.

### Electrotransfection

Electrotransfection is a method for delivering genes into cells by applying an external electric field. Microfluidics-based electroporation systems employ electric pulses to attract plasmids to the anode and use an electroporation pulse to deliver genes into cells (Fig. 1A–D). Compared to conventional electroporation, microfluidic electroporation requires much lower voltages for gene transfection, resulting in higher cell viability rates. Most of the microfluidic electrotransfection systems consist of metal electrodes on a substrate integrated with microfluidic channels used to localize cells. To enhance transfection capabilities, various chip designs using different materials, electrodes, pulse signal conditions, and microfluidic channel networks have been investigated.

To provide high ionic conductivity, Chung *et al.* demonstrated a microfluidic electroporation system incorporated with poly-electrolytic gel electrodes.<sup>13</sup> By applying low DC voltage (5 to 17 V), 60% transfection efficiency and 80% viability of human chronic leukemia cells (K562) were achieved. A similar approach was reported by Lee and colleagues<sup>14</sup> using a serpentine channel made of aluminum on a plastic chip. In this device, the metallic channel acts as an electrode that performs electroporation as the cells flow through it. This method, entitled as semi-continuous flow electroporation, provided better transgene expression (10–15%) and higher cell viability (>50%). Recently, an enhanced system was developed, where DNA was encapsulated in nanometre-sized lipoplexes with targeting ligands and mixed with cells before electroporation. The enhanced system was able to achieve 60% delivery efficiency and 75% cell viability.<sup>15</sup>

Electroporation conditions were studied by varying electrode gap, DNA concentration, pulse voltage, and pulse number.<sup>16</sup> The

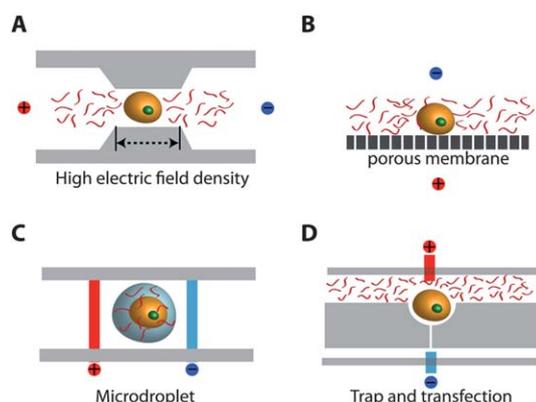
condition of 50  $\mu\text{m}$  electrode gap, 20  $\mu\text{g mL}^{-1}$  plasmid concentration, 6 V and two pulses was shown to be highly effective. In another study, an electric field gradient with a multi-channel microfluidic system was employed for culturing mammalian cell lines and transfecting the cells with enhanced green fluorescence protein (EGFP) plasmids.<sup>17</sup> Transfection rates and cell viability rates were quantified under different electric fields, duration and number of pulses.

Lu and co-workers<sup>18</sup> incorporated a microfluidic valve with an electroporation system to generate electric pulses with fast response. Using a microfluidic valve, ionic buffer flow was switched rapidly in the microchannel, and electric pulses of milliseconds were able to generate actuation pressure and voltage. As an example, electro-permeabilization of suspended and adherent Chinese Hamster Ovary (CHO) cells was demonstrated with 300  $\mu\text{m}$  wide channels, 40 psi pressure, 30 ms pulse, and 209  $\text{V cm}^{-1}$  pulse intensity. Furthermore, they also demonstrated the capability to generate electric pulses either for drug or gene delivery.<sup>19,20</sup> Small molecules and GFP plasmids were transfected into CHO cells with a viability rate up to 75%.<sup>20</sup>

Gene transfection at a single cell level is one of the rapidly emerging topics in neuroscience and stem cell research. The most relevant microfluidic technique employs a hydrodynamic or optical cell trap and a microdroplet to control a single cell. The microfluidic device, reported by van den Berg *et al.*<sup>21</sup> has parallel microfluidic channels with micro-holes for trapping single cells. A vector DNA encoding extracellular signal-regulated kinase (ERK1) protein, known for transducing signals from the cell's environment to the cell nucleus, was transfused into human mesenchymal stem cells (hMSC). To verify gene transfection results, the electroporated cells were activated with fibroblast growth factor (FGF-2), and the expression of ERK1 was quantified after FGF-2 stimulation. Combining an electroporation system with a microfluidic droplet generator was also demonstrated for gene transfection of single cells.<sup>22</sup> The microfluidic device continuously generated micro-droplets containing a single CHO cell and EGFP plasmids. The micro-droplets were then passed through the surface of microelectrodes for electroporation. The electroporated cells were cultured and expressed GFP.

Porous membrane and microwells incorporated with electroporation system have been explored. The design employed a well-defined micro-nozzle array fabricated using femtosecond laser processing. The membrane sandwich electroporation (MSE) device showed more targeted potential distribution and required lower electric voltage. NIH3T3 fibroblast cells were transfected on the device that yielded high and uniform gene transfection rates and excellent cell viability.<sup>23</sup> Jain *et al.* created microwell arrays on indium-tin oxide (ITO) coated glass slides.<sup>24</sup> The microwells confine cells and exogenous molecules (*e.g.*, cDNAs and siRNAs) and achieved highly efficient parallel transfection of human cell lines and primary mouse macrophages. The optical transparency of ITO electrodes also facilitated imaging and phenotypic analysis.

In addition, microfluidic devices were demonstrated for observing neuronal behavior in response to various input, such as chemicals,<sup>25,26</sup> mechanical damages,<sup>25</sup> and electric pulses.<sup>27</sup> In electroporating neuronal cells, electric pulses, if not spatially controlled, can cause unwanted stimulation of nearby neuronal



**Fig. 1** Microfluidic electrotransfection. (A) By controlling microfluidic channel width, electric field is concentrated on designed spots for electroporation. (B) Cells are trapped on the membrane embedded in a microfluidic device. Genes are delivered into cells when an electric field is applied. (C) Microdroplet encapsulating a cell and gene is generated on chip. Electric field is localized on microdroplet to transfer the gene into cell. (D) Hydrodynamic cell trap in a microfluidic device for subsequent electroporation.

cells. Correspondingly, a microfluidic device was reported to stimulate a targeted axon of individual neurons, yielding localized electroporation of plasmids.<sup>27</sup>

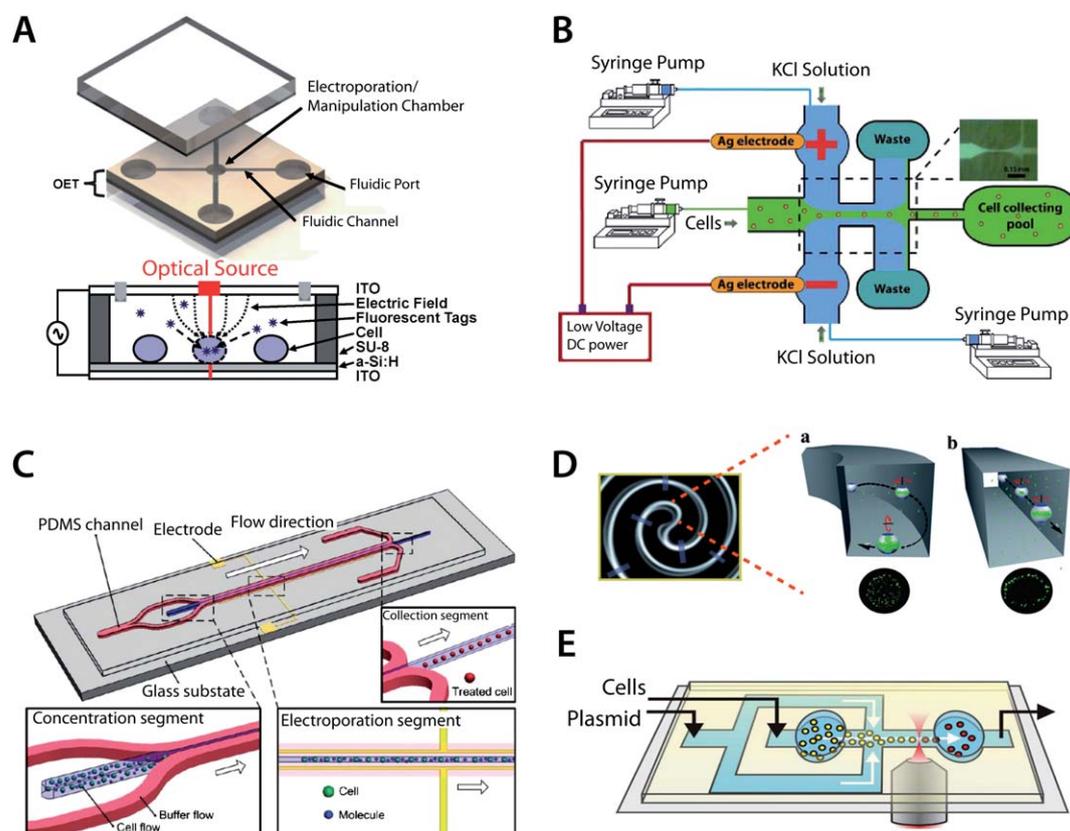
In summary, microelectrodes incorporated with microfluidic systems provide an excellent platform for transfecting exogenous molecules into single cells as well as a controlled number of cells. These devices can also be utilized for other applications, for instance, for releasing small molecules selectively<sup>28</sup> and for eliminating circulating tumor cells from whole blood.<sup>29</sup> Valley *et al.* developed optoelectronic tweezers for parallel manipulation of single cells and electroporation of molecules through the cell membrane.<sup>30</sup> They generated virtual electrodes using a photoconductive surface and patterned light that concentrated the electric field across the cell, resulting in electroporation. An AC bias is applied across the two ITO layers between which a cell is located (Fig. 2A). When a cell is illuminated, the cell experiences a concentrated electrical field for exogenous molecules to permeate into the cell.

### Hydrodynamically enhanced gene transfer

Hydrodynamic focusing, originally developed for flow cytometry to enhance the accuracy of volume calculation and particle

counting, is widely used in microfluidics. Recently, Zhu *et al.* introduced an electroporation system based on hydrodynamic focusing of microfluidic flow with low dc voltage (Fig. 2B).<sup>31</sup> By supplying highly conductive KCl solution as sheath flow, a high electric charge density was generated in the hydrodynamic focusing area. As yeast cells pass through the focused thin layer, they experience a short electric pulse and thereby, electroporation occurs with 70% yield. An advanced approach, exploiting laminar flow electroporation (LFE) *via* hydrodynamic focusing, has been also reported (Fig. 2C).<sup>32</sup> In this system, an additional layer from laminar flow was implemented to isolate electrodes from cell suspension flow in order to minimize inherent damaging effects from electrochemical reactions on electrodes. Using the device, the delivery of DNA and siRNA into several hard-to-transfect cell lines, including Neuro-2A, PC12, and C2C12 cells, was significantly enhanced to produce up to 85% transfection efficiency and 75% viability.

Unlike in conventional macroscale systems, hydrodynamic effects are governed by mechanical and geometrical variations around fluid in microfluidic devices. While hydrodynamic effects can produce desirable outcomes, it is challenging to precisely control the volume and speed of fluid that flows through a microchannel by pressure-driven flow. A PDMS microinjector



**Fig. 2** Hydrodynamic and optical approaches for introducing exogenous molecules into cells. (A) Single cell transfection by integrating optical tweezers and electroporation.<sup>30</sup> (B) Schematic of the electroporation chip with hydrodynamic focusing. Cells passing through the focusing region experience a local electric charge of high density.<sup>31</sup> (C) The LFE system composed of three functional segments of the microfluidic channel; concentration, electroporation, and collection segment. Note the additional sheath flow that isolates electrode from cellular stream line.<sup>32</sup> (D) Microfluidic vortex enables more uniform and larger surface on a cell to contact transfection genes, resulting in enhanced transfection efficiency.<sup>36</sup> (a), whereas non-vortex flow in microfluidic environment does not induce such enhancement (b). (E) Femtosecond laser beam integrated with hydrodynamic focusing microfluidic device.<sup>51</sup> Reproduced by permission of the Royal Society of Chemistry.

was developed using electroosmotic flow (EOF) for precise dosage control of exogenous material.<sup>33</sup> The precision originates from the unique flow profile of EOF. Unlike pressure-driven hydrodynamic lamina flow, which has a parabolic flow profile, EOF has planar flow profile that reduces band broadening. The microinjector combines a mechanical setup for needle movement, two microvalves for single cell trap, and an electrochemical setup for precise EOF control through the needle for gene delivery.<sup>34</sup>

Hydrodynamic effects in microfluidics can generate inertial vortices along curved paths.<sup>35</sup> This secondary flow, also known as Dean flow, is caused by a mismatch of velocity in the downstream direction between fluid in the central region and lateral region, yielding improved mixing of traveling fluid. For improving their previous electroporation technique,<sup>20</sup> Lu *et al.* harnessed the inertial vortices in their electroporator to effectively mix cells and exogenous DNA (Fig. 2D).<sup>36</sup> Cells in such a flow field are exposed to a complex combination of transverse advection and rotation. As a result, a larger cell surface area contacts the exogenous DNA and electric field, resulting in more uniform permeabilization. When compared with a straight microchannel under the same flow conditions without vortices, the vortex-assisted electroporation produced a two-fold increase in transfection efficiency (~30%) while cell viability was similar (~90%).

As geometrical dimensions are reduced, fluids flowing through microfluidic channels experiences high pressure. This in turn gives rise to shear stress to the residing cells within the channel. Since cells can uptake exogenous materials at a different rate under stresses, it was shown that the transfection yield on primary neurons exposed to lipoplex of DNA and liposome was enhanced by hydrodynamic shear stress.<sup>37</sup> The shear stress effect, however, should be applied with considerations of cell types and exogenous molecules, as in some cases the transfection efficiency was shown to decrease at certain stress levels.<sup>38,39</sup>

### Optical gene delivery

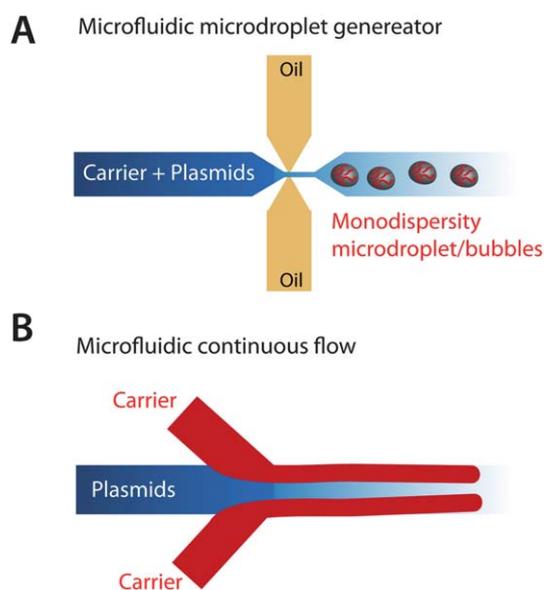
Light is an electromagnetic wave that can be synchronized, focused, and amplified to give rise to lasers whose level of energy can be manipulated. Thus, stress applied to cells by optical energy can be harnessed for delivering genetic materials into living cells, which is called 'optical transfection'.<sup>40</sup> Optical transfection has been conducted using various laser sources, such as continuous wave lasers and pulsed lasers. Continuous wave lasers and nanosecond pulsed lasers rely on localized heating to induce the transient formation of cavities in cell's plasma membrane. These lasers also generate bubbles and thermoelastic stresses. The generation of bubbles can be utilized to disrupt the cellular membrane for subsequent microinjection of cargo without needing a micropipette to penetrate the cell.<sup>41</sup> However, bubbles and thermoelastic stresses are generally undesirable, often resulting in low transfection efficiency.<sup>42,43</sup> In contrast, near-infrared femtosecond pulsed lasers that exert multi-photon effects and high repetition rates produce less stress to open a single pore in cell membrane through which genetic substances pass, allowing high efficiency and cell viability.<sup>44-47</sup> Additionally, not only could the optical energy be used as a source of stress that disturbs cell membranes, it could also act as tweezers that

transfer macromolecules,<sup>48</sup> nanoparticles,<sup>49</sup> and microspheres<sup>50</sup> into living cells. In these cases, the particles are loaded with genetic materials that can be released within cells since single-cell transfection using lasers is a serial process. Dholakia *et al.* demonstrated a microfluidic system<sup>51</sup> that used hydrodynamic focusing to align single cells with a focused laser beam (Fig. 2E). The microfluidic system successfully delivered propidium iodide into HEK293 with an efficiency of ~28% and demonstrated the possibility for continuous, high-throughput operation.

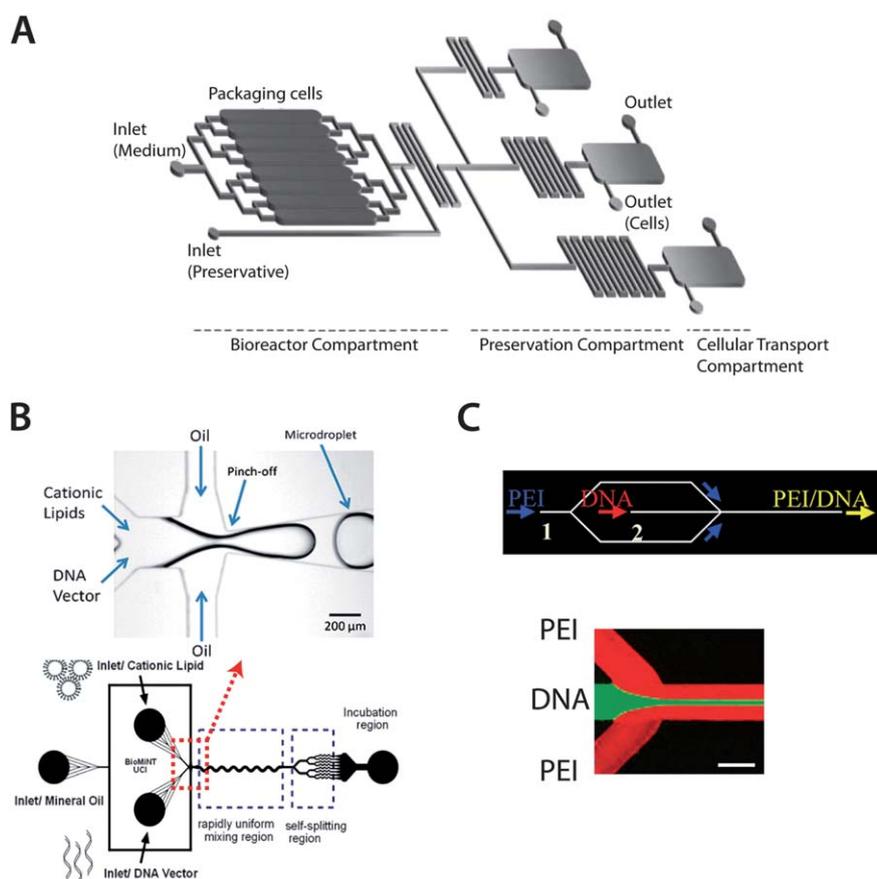
### Production of gene carriers

Conventional approaches to producing viral and non-viral gene carriers are labor intensive and time consuming. Besides the amenability to automated operation, microfluidics provides excellent platforms that are also able to control the shape, size and composition of non-viral carriers (Fig. 3) and the culture condition of viral carriers. A microfluidic system was developed to improve the production of retroviral vectors.<sup>52</sup> Viral vectors using PT67-GFP packaging cells were produced in microfluidic devices (Fig. 4A). PDMS channel walls were coated with bovine serum albumin (BSA) to increase the virus production rate.

As a non-viral carrier, a multi-functional envelope-type nano-device consisting of a DNA, RNA, or protein core condensed by polycation and lipid bilayer shell was developed.<sup>53</sup> Because of the complex fabrication process and the amount of waste produced, it was not suitable for customized gene carrier fabrication. These limitations were improved by implementing the microscale biosynthesis reactor using a microfluidic system. The DNA-polycation complex (DPC) was synthesized from plasmid DNA, water, and poly-L-lysine (PLL) in a microfluidic system that consisted of three inlets, a mixing chamber, and one outlet. DPC and lipid solutions reacted within the mixing and reaction zone in the microfluidic system. Through optimizing flow rate and initial concentration of each solution, the polyplex size and uniformity



**Fig. 3** (A) Microfluidic microdroplet/bubble generator. (B) Microfluidic continuous flow used to make well-controlled gene carrier for gene transfection.



**Fig. 4** Microfluidic gene carrier production. (A) Multiple compartment and fluidic channel for viral gene carrier production.<sup>52</sup> Reproduced by permission of the Royal Society of Chemistry. (B) Generation of lipoplex with a microdroplet system.<sup>54</sup> Reproduced by permission of the Royal Society of Chemistry. (C) PEI/DNA complex generation using microfluidic continuous-flow regime.<sup>55</sup> Reprinted with permission from ref. 55. Copyright 2009 American Chemical Society.

**Table 1** Summary of microfluidic gene transfection techniques

Gene transfection technique		Cell type	Delivered gene	Efficiency (%)	Viability (%)	Selectivity	Ref
Electroporation	pDADMAC Salt bridge	K562 (human leukemia cell)	EGFP plasmid	60	80	Low	13
	Single cell trap	Mouse myoblastic C2C12 cells and hMSC	Encoding ERK1 protein	75	100	High	21
	Microdroplet mediated Enhanced semi-continuous flow	CHO cell	EGFP plasmid	11	68	High	22
		K562	Liposome nanoparticles conjugated with G3139 (anti-sense ODN)	60	75	Low	15
	Membrane	NIH 3T3 fibroblasts cell	EGFP and secreted alkaline phosphatase plasmid	40	90	Low	23
Geometry modification	CHO cell	EGFP plasmid	40–75	60–90	Low	20	
Hydrodynamic force	Hydrodynamic focusing	Yeast	Fluorescein <sup>a</sup>	40–95	70	Low	31
	Hydrodynamic focusing	HEK293, HeLa, Neuro-2A, C2C12, PC12	EGFP plasmid siRNA	70–90	55–75	Low	32
	Microinjection	Zebrafish embryo	Methylene blue <sup>a</sup>	100	100	High	33
		HeLa Cell	Fluorescein <sup>a</sup>	N/A	98	High	34
	Hydrodynamic vortex	CHO cell	EGFP plasmid	30	90	Low	36
Hydrodynamic shear stress	Neuroal cell line (NIE-115)	GFP plasmid with lipoplex carrier	45	N/A	Low	37	
Optical energy	Femtosecond laser	CHO cell	EGFP plasmid	~100	~100	High	47
	Femtosecond laser under hydrodynamically focused channel	HEK293	Propidium iodide <sup>a</sup>	28	67	High	51

<sup>a</sup> Included as virtual genetic materials.

was characterized, and the non-viral carrier proved to increase the efficiency of gene delivery.

A microfluidic droplet generator was used for the production of lipoplex that is a well known non-viral gene delivery tool.<sup>54</sup> The size of lipoplex is a critical parameter for delivering genes consistently into target cells. Variables, such as the component of cationic lipids, the order and rate of mixing of vectors, and the incubation time of the mixture, must be well controlled for achieving uniformity in size. The microfluidic droplet generation system precisely controlled these variables and efficiently created uniform lipoplexes (Fig. 4B). As a demonstration, lipoplex containing EGFP plasmids was produced. The carriers were transfected into U2OS cells, and EGFP was expressed with high reproducibility.

Polyethylenimine (PEI) and plasmid DNA complexes are commonly used for non-viral gene delivery. Well controlled N/P ratio (the molar ratio of nitrogen in PEI to phosphate in DNA) is a key factor for effective gene transfection. A microfluidic device was developed to precisely control the N/P ratio.<sup>55</sup> PEI/DNA complexes were created by both bulk mixing methods and the microfluidic setup (Fig. 4C). These complexes were transfected into mouse embryonic stem cells and NIH3T3 cells, and the gene transfection efficiency and cell viability were compared. The complexes created by the microfluidic device had ~10% improvement in cell viability with the N/P ratio 3.3 and 6.7. Gene expression was also ~2 fold at 2 and 4 days post transfection. Using a similar approach, lipoplex nanoparticles containing antisense oligonucleotide (ODN) were used to down-regulate anti-apoptotic protein encoded by a Bcl-2 gene in K562 leukemia cell. The oligonucleotide carriers, fabricated using bulk mixing method and the microfluidic method, were transfected into cells. The carriers produced from both methods showed a decrease in the anti-apoptotic protein expression level; however, the microfluidic system showed a lower expression level by ~20%.<sup>56</sup> Microfluidic devices permit high uniformity and controllability of size and ratio, leading to lower toxicity and higher exogenous gene expression in transfected cells.

## Conclusion and prospects

In this review, we discussed several microfluidic techniques for gene transfection. Conventional methods produce ~10% transfection efficiency and ~50% cell viability.<sup>57,58</sup> In contrast, microfluidic approaches have achieved higher transfection efficiency and cell viability. The microfluidic environment allows for increased spatiotemporal control of the target cell, the exogenous genetic material, the transfection stimulus (E-field, photons, *etc.*), and the environment in which all three of these factors interact. Microfluidics eliminates the randomness involved in traditional transfection methods. One can be more certain of the extent to which a cell is exposed to the exogenous genetic material, and have better control over the strength and duration of the transfection stimulus experienced by a particular cell. These abilities could permit the creation of optimal or close-to optimal transfection conditions, leading to increased transfection efficiencies. Such conditions can also lead to higher cell viability, as no cell is exposed to detrimental transfection stimuli beyond the minimum necessary to achieve transfection.

Table 1 summarizes microfluidics-mediated gene transfection methods. Several microfluidic devices performing electroporation and optical transfection produced transfection efficiency over 50% and cell viability above 90%. Additionally, incorporating an electrotransfection system with an automated microfluidic network<sup>59,60</sup> would make it feasible to inject multiple plasmids simultaneously into target cells. The hydrodynamic technique's transfection efficiency (30%) was lower compared to electroporation; however, it has a high cell viability rate (90%) and has a higher throughput due to its high flow rate. Optical gene transfection has shown almost 100% transfection efficiency and viability;<sup>47</sup> however, throughput demands improvement. Microfluidic electroporation devices were used to transfect almost any type of cells, including adherent cells, non-adherent cells, primary cells, and cell lines. Differently, the hydrodynamic approach may not be suitable for transfecting primary cells due to the long double time of primary cells. This technique is only able to carry plasmids into the cytoplasm, not into the nucleus. Only during cell division when the nuclear membrane is transiently disintegrated can the plasmids enter the nucleus. Compatibility of optical transfection with microfluidic set-ups is a clear advantage.<sup>61</sup> However, optical transfection requires expensive laser and peripheral optical equipment to perform gene transfection task, which can be mitigated by integrating inexpensive laser components on microfluidic chips. In terms of selectivity, hydrodynamic single cell trap using electroporation, microinjection, and optical methods present high selectivity, and these methods demonstrated both high cell viability and high efficiency. The microdroplet mediated method showed high selectivity and multiplex transfection capability, although viability and efficiency require improvement.

The integration of optical systems with microfluidics has been evolving quickly and is highly relevant to gene delivery and cell transfection. Optofluidic techniques enable increased precision control, and microfluidic channels can be used as optical waveguides to localize strong optical energy on specific spots.<sup>62</sup> To take advantage of the ease and accuracy of optical methods for cell transfection, researchers have found light-sensitive ion-channel proteins and used them to control neuronal activities.<sup>63-65</sup> Furthermore, the emerging technology *optogenetics* relies on efficient viral transfection using specific promoters for targeted expression.<sup>66</sup> Once the gene transfers into cells and is expressed, the gene product can be activated by light of a specific wavelength.<sup>63,64</sup> Consequently, among millions of neuronal cells, only transfected ones respond. Such techniques can be transferred to microfluidic platforms to benefit studies of single neurons or patterned neurons.<sup>67,68</sup> Neuronal cell patterning on microfluidic chips is an active research topic in neurophysiology where high-resolution control over gene expression is of importance.<sup>25,26,69</sup> One would envision a microfluidic system on which neuronal cells are cultured and patterned, and optical transfection would perform precise targeting and spatial control<sup>40,70</sup> while optical activation would activate targeted neurons with high specificity.

Besides gene transfection, microfluidic systems have also demonstrated significant value for non-viral gene carrier fabrication by flexibly controlling the size and compositions of polymer-DNA complexes. For instance, changing the flow rate alone, the ratio and composition of polymer-DNA complex can

be readily and precisely altered. Thus, the near future would witness the invention of automated microfluidic systems for gene carrier production, which will significantly reduce time and cost compared to conventional fabrication approaches.

Microfluidic techniques have demonstrated various utilities for gene transfection and the production of gene carriers. On-chip microelectrodes and the capability for producing homogeneous microbubble carriers and lipoplexes enable high efficiency gene transfection at the single cell level. Moreover, it becomes feasible to measure single-cell response from gene transfection accurately. Improvement in precision control of microfluidic conduits, throughput, cell survival and stability, and homogeneity of transfection will further enhance practical adoption of microfluidic transfection technologies.

Finally, the generation of a stably transfected cell line is significant for gene therapy. Establishing a stable cell line, however, typically takes longer than three months *via* conventional gene transfection. Conventional gene transfection<sup>71</sup> involves selecting proper antibiotics, collecting homogeneous colonies, and culturing and passaging cells for several generations. Microfluidics-assisted transfection systems have thus far demonstrated high efficiency and cell viability. Further progress would result in practical microfluidic systems for homogenous transfection, long-term single cell culturing, and single cell sorting to replace the laborious processes and reduce time involved in conventional gene transfection processes.

## Acknowledgements

D. H. Kim thanks the Department of Bioengineering at the University of Washington for the new faculty start-up fund. D. H. Kim is also supported by a Perkins Coie Award for Discovery. Y. Sun acknowledges financial support from the Canada Research Chairs program. This research was also supported by the Nano/Bio Science and Technology Program (M10536090001-05N3609-00110) and the Public Welfare & Safety Research Program (No. 2011-0021117) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology. The authors thank Dr. Sungphil Cho of the University of Utah for scientific discussions.

## References

- Z. Debyser, *Curr. Gene Ther.*, 2003, **3**, 495–499.
- S. Gogev, F. Schynts, F. Meurens, I. Bourgot and E. Thiry, *Curr. Gene Ther.*, 2003, **3**, 597–611.
- M. K. Chuah, D. Collen and T. VandenDriessche, *Curr. Gene Ther.*, 2003, **3**, 527–543.
- N. B. Woods, V. Bottero, M. Schmidt, C. von Kalle and I. M. Verma, *Nature*, 2006, **440**, 1123.
- C. E. Thomas, A. Ehrhardt and M. A. Kay, *Nat. Rev. Genet.*, 2003, **4**, 346–358.
- C. Tros de Ilarduya, Y. Sun and N. Düzgüneş, *Eur. J. Pharm. Sci.*, 2010, **40**, 159–170.
- F. André and L. M. Mir, *Gene Ther.*, 2004, **11**(Suppl. 1), S33–42.
- S. Mehier-Humbert and R. H. Guy, *Adv. Drug Delivery Rev.*, 2005, **57**, 733–753.
- M. S. Al-Dosari and X. Gao, *AAPS J.*, 2009, **11**, 671–681.
- H. D. Liang, J. Tang and M. Halliwell, *Proc. Inst. Mech. Eng.: H*, 2010, **224**, 343–361.
- R. Suzuki, E. Namai, Y. Oda, N. Nishiie, S. Otake, R. Koshima, K. Hirata, Y. Taira, N. Utoguchi, Y. Negishi, S. Nakagawa and K. Maruyama, *J. Controlled Release*, 2010, **142**, 245–250.
- N. G. Lee, J. L. Berry, T. C. Lee, A. T. Wang, S. Honowitz, A. L. Murphree, N. Varshney, D. R. Hinton and A. A. Fawzi, *Invest. Ophthalmol. Vis. Sci.*, 2011.
- S. K. Kim, J. H. Kim, K. P. Kim and T. D. Chung, *Anal. Chem.*, 2007, **79**, 7761–7766.
- S. N. Wang, X. L. Zhang, W. X. Wang and L. J. Lee, *Anal. Chem.*, 2009, **81**, 4414–4421.
- S. N. Wang, X. L. Zhang, B. Yu, R. J. Lee and L. J. Lee, *Biosens. Bioelectron.*, 2010, **26**, 778–783.
- S.-C. Yang, K.-S. Huang, H.-Y. Chen and Y.-C. Lin, *Sens. Actuators, B*, 2008, **132**, 551–557.
- J. A. Kim, K. Cho, Y. S. Shin, N. Jung, C. Chung and J. K. Chang, *Biosens. Bioelectron.*, 2007, **22**, 3273–3277.
- J. Wang, M. J. Stine and C. Lu, *Anal. Chem.*, 2007, **79**, 9584–9587.
- H. Y. Wang and C. Lu, *Biotechnol. Bioeng.*, 2008, **100**, 579–586.
- T. Geng, Y. H. Zhan, H. Y. Wang, S. R. Witting, K. G. Cornetta and C. Lu, *J. Controlled Release*, 2010, **144**, 91–100.
- A. Valero, J. N. Post, J. W. van Nieuwkastele, P. M. Ter Braak, W. Kruijer and A. van den Berg, *Lab Chip*, 2008, **8**, 62–67.
- Y. Zhan, J. Wang, N. Bao and C. Lu, *Anal. Chem.*, 2009, **81**, 2027–2031.
- Z. Fei, S. Wang, Y. Xie, B. E. Henslee, C. G. Koh and L. J. Lee, *Anal. Chem.*, 2007, **79**, 5719–5722.
- T. Jain, R. McBride, S. Head and E. Saez, *Lab Chip*, 2009, **9**, 3557–3566.
- A. M. Taylor, M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman and N. L. Jeon, *Nat. Methods*, 2005, **2**, 599–605.
- U. Hengst, A. Deglincerti, H. J. Kim, N. L. Jeon and S. R. Jaffrey, *Nat. Cell Biol.*, 2009, **11**, 1024–U1263.
- W. C. Chang and D. W. Sretavan, *Biosens. Bioelectron.*, 2009, **24**, 3600–3607.
- N. Bao, J. Wang and C. Lu, *Electrophoresis*, 2008, **29**, 2939–2944.
- N. Bao, T. T. Le, J. X. Cheng and C. Lu, *Integr. Biol.*, 2010, **2**, 113–120.
- J. K. Valley, S. Neale, H. Y. Hsu, A. T. Ohta, A. Jamshidi and M. C. Wu, *Lab Chip*, 2009, **9**, 1714–1720.
- C. Y. Xiong, T. Zhu, C. X. Luo, J. Y. Huang, Q. Ouyang and J. Fang, *Biomed. Microdevices*, 2010, **12**, 35–40.
- Q. Du, Z. W. Wei, D. Y. Zhao, X. M. Li, M. X. Wu, W. Wang, H. Huang, X. X. Wang, Z. C. Liang and Z. H. Li, *Anal. Chem.*, 2011, **83**, 5881–5887.
- A. Noori, P. R. Selvaganapathy and J. Wilson, *Lab Chip*, 2009, **9**, 3202–3211.
- A. Adamo and K. F. Jensen, *Lab Chip*, 2008, **8**, 1258–1261.
- D. Di Carlo, *Lab Chip*, 2009, **9**, 3038–3046.
- J. Wang, Y. H. Zhan, V. M. Ugaz and C. Lu, *Lab Chip*, 2010, **10**, 2057–2061.
- N. L. Jeon, H. S. Shin, H. J. Kim and S. J. Sim, *J. Nanosci. Nanotechnol.*, 2009, **9**, 7330–7335.
- N. Li and P. K. Wong, *Bioanalysis*, 2010, **2**, 1689–1699.
- A. van der Meer, M. Kamphuis, A. Poot, J. Feijen and I. Vermes, *J. Controlled Release*, 2008, **132**, e42–e44.
- D. J. Stevenson, F. J. Gunn-Moore, P. Campbell and K. Dholakia, *J. R. Soc. Interface*, 2010, **7**, 863–871.
- T. H. Wu, T. Teslaa, S. Kalim, C. T. French, S. Moghadam, R. Wall, J. F. Miller, O. N. Witte, M. A. Teitell and P. Y. Chiou, *Anal. Chem.*, 2011, **83**, 1321–1327.
- H. Schneckenburger, A. Hendinger, R. Sailer, W. S. L. Strauss and M. Schmitt, *J. Biomed. Opt.*, 2002, **7**, 410–416.
- A. V. Nikolskaya, V. P. Nikolski and I. R. Efimov, *Cell Commun. Adhes.*, 2006, **13**, 217–222.
- J. Baumgart, W. Bintig, A. Ngezahayo, S. Willenbrock, H. M. Escobar, W. Ertmer, H. Lubatschowski and A. Heisterkamp, *J. Laser Micro/Nanoeng.*, 2009, **4**, 111–117.
- C. T. A. Brown, D. J. Stevenson, X. Tsampoula, C. McDougall, A. A. Lagatsky, W. Sibbett, F. J. Gunn-Moore and K. Dholakia, *J. Biophotonics*, 2008, **1**, 183–199.
- M. Lei, H. P. Xu, H. Yang and B. L. Yao, *J. Neurosci. Methods*, 2008, **174**, 215–218.
- U. K. Tirlapur and K. König, *Nature*, 2002, **418**, 290–291.
- F. Stracke, I. Rieman and K. König, *J. Photochem. Photobiol., B*, 2005, **81**, 136–142.
- Y. Arita, M. L. Torres-Mapa, W. M. Lee, T. Cizmar, P. Campbell, F. J. Gunn-Moore and K. Dholakia, *Appl. Phys. Letters*, 2011, **98**.
- M. Terakawa and Y. Tanaka, *Opt. Lett.*, 2011, **36**, 2877–2879.
- R. F. Marchington, Y. Arita, X. Tsampoula, F. J. Gunn-Moore and K. Dholakia, *Biomed. Opt. Express*, 2010, **1**, 527–536.

- 52 H. N. Vu, Y. Li, M. Casali, D. Irimia, Z. Megeed and M. L. Yarmush, *Lab Chip*, 2008, **8**, 75–80.
- 53 H. Kuramoto, Y. S. Park, N. Kaji, M. Tokeshi, K. Kogure, Y. Shinohara, H. Harashima and Y. Baba, *Anal. Bioanal. Chem.*, 2008, **391**, 2729–2733.
- 54 A. T. Hsieh, N. Hori, R. Massoudi, P. J. Pan, H. Sasaki, Y. A. Lin and A. P. Lee, *Lab Chip*, 2009, **9**, 2638–2643.
- 55 C. G. Koh, X. Kang, Y. Xie, Z. Fei, J. Guan, B. Yu, X. Zhang and L. J. Lee, *Mol. Pharmaceutics*, 2009, **6**, 1333–1342.
- 56 C. G. Koh, X. Zhang, S. Liu, S. Golan, B. Yu, X. Yang, J. Guan, Y. Jin, Y. Talmon, N. Muthusamy, K. K. Chan, J. C. Byrd, R. J. Lee, G. Marcucci and L. J. Lee, *J. Controlled Release*, 2010, **141**, 62–69.
- 57 U. Lakshmipathy, B. Pelacho, K. Sudo, J. L. Linehan, E. Coucouvanis, D. S. Kaufman and C. M. Verfaillie, *Stem Cells*, 2004, **22**, 531–543.
- 58 F. Cao, X. Xie, T. Gollan, L. Zhao, K. Narsinh, R. Lee and J. Wu, *Mol. Imaging Biol.*, 2009, **12**, 15–24.
- 59 E. C. Jensen, B. P. Bhat and R. A. Mathies, *Lab Chip*, 2010, **10**, 685–691.
- 60 E. C. Jensen, Y. Zeng, J. Kim and R. A. Mathies, *JALA*, 2010, **15**, 455–463.
- 61 M. Tsukakoshi, S. Kurata, Y. Nomiya, Y. Ikawa and T. Kasuya, *Appl. Phys. B: Photophys. Laser Chem.*, 1984, **35**, 135–140.
- 62 D. Psaltis, S. R. Quake and C. Yang, *Nature*, 2006, **442**, 381–386.
- 63 F. Zhang, A. M. Aravanis, A. Adamantidis, L. de Lecea and K. Deisseroth, *Nat. Rev. Neurosci.*, 2007, **8**, 577–581.
- 64 M. Scanziani and M. Hausser, *Nature*, 2009, **461**, 930–939.
- 65 S. Kleinlogel, K. Feldbauer, R. E. Dempksi, H. Fotis, P. G. Wood, C. Bamann and E. Bamberg, *Nat. Neurosci.*, 2011, **14**, 513–518.
- 66 V. Gradinaru, F. Zhang, C. Ramakrishnan, J. Mattis, R. Prakash, I. Diester, I. Goshen, K. R. Thompson and K. Deisseroth, *Cell*, 2010, **141**, 154–165.
- 67 A. Vishwanathan, G. Q. Bi and H. C. Zeringue, *Lab Chip*, 2011, **11**, 1081–1088.
- 68 L. J. Millet, M. E. Stewart, R. G. Nuzzo and M. U. Gillette, *Lab Chip*, 2010, **10**, 1525–1535.
- 69 J. Wang, L. Ren, L. Li, W. Liu, J. Zhou, W. Yu, D. Tong and S. Chen, *Lab Chip*, 2009, **9**, 644–652.
- 70 L. E. Barrett, J. Y. Sul, H. Takano, E. J. Van Bockstaele, P. G. Haydon and J. H. Eberwine, *Nat. Methods*, 2006, **3**, 455–460.
- 71 J. Mairhofer, M. Cserjan-Puschmann, G. Striedner, K. Nöbauer, E. Razzazi-Fazelib and R. Grabher, *J. Biotechnol.*, 2010, **146**, 130–137.