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A novel electroporation method using a capillary and wire-type electrode

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Abstract

Electroporation is widely used to achieve gene transfection. A common problem in electroporation is that it has a lower viability than any other transfection method. In this study, we developed a novel electroporation device using a capillary tip and a pipette that was effective on a wide range of mammalian cells, including cell lines, primary cells, and stem cells. The capillary electroporation system considerably reduced cell death during electroporation because of its wire-type electrode, which has a small surface area. The experimental results also indicated that the cell viability was dependent on the change in pH induced by electrolysis during electroporation. Additionally, the use of a long and narrow capillary tube combined with simple pipetting shortened the overall time of the electroporation process by up to 15 min, even under different conditions with 24 samples. These results were supported by comparison with a conventional electroporation system. The transfection rate and the cell viability were enhanced by the use of the capillary system, which had a high transfection rate of more than 80% in general cell lines such as HeLa and COS-7, and more than 50% in hard-to-transfect cells such as stem or primary cells. The viability was approximately 70–80% in all cell types used in this study.

Keywords: Transfection; Electroporation; Gene delivery; Capillary; Electrolysis; pH change

1. Introduction

Introduction of foreign genes into target cells has been an important issue in modern genetic and therapeutic research. Some fields, such as gene therapy and DNA vaccine research, need to deliver genes in order to elucidate the function and regulation of cells (Anderson and Lingrel, 1989; Gale, 1989; Toneguzzo and Keating, 1986). Successful gene delivery requires several important factors, including high transfection efficiency, lack of harmful side effects, and application to a wide variety of cells (Chang et al., 1991). A number of approaches, including virus-mediated, chemical, mechanical, and electrical transfection, have been proposed to improve the delivery and expression of genes in target cells (Luo and Saltzman, 2000). In general, the non-viral transfection method has been known to be less efficient than the viral method, but it has advantages in terms of the larger capacity of DNA insertion and lower risks involved (Jooss and Chirmule, 2003; Oberle et al., 2004).

Since an electrically mediated gene delivery system was proposed in 1982 (Neumann et al., 1982; Zimmermann, 1982), it has become one of the most powerful and effective transfection tools. Electroporation uses electrical energy to create numerous pores in the cell membrane, and then drives genes into the cell to subsequently express the desired genes. However, several problems exist with electroporation: (i) it has a relatively low cell viability and transfection rate, (ii) there is a need for optimization of the cell-specific protocol, and (iii) it involves a sensitive and complicated experimental process (Chang et al., 1991). Additionally, a conventional electroporator has a transfection efficiency of less than 50% in mammalian cell lines, and has far lower transfection efficiency in hard-to-transfect cells such as primary or stem cells. Typically, cell viability is 20–50% (Baum et al., 1994; Kluxen and Lübber, 1993; Patterson, 1979; Yang et al., 1995). Since electroporation is affected by complex factors, the optimal electrical conditions for transfection of untested cells must be empirically determined. Until recently, the electroporation

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mechanism and its subsequent effects on cells have been partially understood, and studies evaluating this mechanism have mainly focused on the relationship between transfection rate and electrical energy (Chang et al., 1991; Čegovnik and Novaković, 2004; Chu et al., 1987; Gabriel and Teissié, 1995; Pliquett et al., 1996; Rols and Teissié, 1998). Although high levels of electrical energy produce more permeable cells, excessive energy might result in cell death (Kim et al., 2007). Additionally, various extra- and intra-cellular factors affect cell viability; for example, stem cells and primary cells are more sensitive to environmental changes induced by electroporation than are general cell lines. All of the factors, including electrical and physicochemical factors, make it rather difficult to determine the optimum conditions for transfection.

Recently, a method using various reactions during electrolysis, referred to as electrochemical treatment (EChT), has been proposed to kill tumor cells (Samuelsson et al., 1980; Nordenström, 1994; Xin, 1994). This procedure utilizes toxic elements induced by electrolysis when direct current (DC) is applied between two electrodes to destroy a tumor adjacent to the electrodes (Euler et al., 2004; Nilsson et al., 1998, 1999; Nilsson and Fonters, 2001; Vijh, 2004; Samaranayake and Sastry, 2005). Although an electrochemical treatment is somewhat different from an electroporation, it allows us to better understand the side effects of electroporation. It is possible to predict considerable changes in near the electrodes, even during electroporation, despite its short running time. Changes in the pH of the background electrolytic buffer due to electrolysis have been reported by Zhu et al. (1994). The initial pH of the electrolytic buffer before the reaction is neutral; however, the electrochemical reaction at the anode decreases the pH, resulting in cell necrosis (Nordenström, 1994). On the other hand, a high pH value at the cathode directly destroys the cell membrane, as well as various enzymes and structures, thus inactivating the cell function. Furthermore, increasing voltage, ionic strength of the buffer, and reaction time contributes to changes in the pH at the electrode.

Previously, we developed a microchannel-type electroporation chip based on the presence of a relatively long distance between electrodes (Shin et al., 2004; Kim et al., 2007). This device provides a more symmetrical electric field as the distance between two electrodes increases (Nordenström, 1994). This chip-based device allows high transfection; however, it is difficult to manipulate transfected cells for the following investigations because the cells were fixed in the channel. On the other hand, many biological applications have demonstrated that a capillary system is quite feasible, even though it requires a complicated, highly sensitive liquid handling system.

Therefore, we created a tip-type capillary electroporator for simple handling and high cell transfection by combining a capillary tip with a pipette that was commonly used in the laboratory setting. In this system, a capillary that integrated with the wire-type electrode acted as a substitute for a conventional cuvette as an electroporation reaction chamber. This disposable capillary module with a wire-type electrode enabled rapid, simple, and convenient electroporation. The capillary electroporation minimized the effective surface area of the electrode so that the transfection rate was greatly enhanced compared to that of a conventional cuvette. These experiments demonstrated that the side effects, which occurred as a result of a change in pH during electroporation, were closely related to the geometry of the electroporation chamber. The several pH indicators indicated a more dramatic change in pH in conventional electroporation than in capillary electroporation. Further, we compared the transfection rate and viability of both capillary electroporation and conventional cuvette. We demonstrated that many of the problems of conventional electroporation, not only the change in the pH but also generation of metal ions, were remarkably eliminated in the capillary electroporation system.

2. Materials and methods

2.1. Capillary electroporation system

As shown in Fig. 1A, the capillary electroporation system has a longer distance between electrodes than the conventional cuvette system. The inner diameter, length, and total volume of the capillary are 0.65 mm, 30 mm, and 10 μl, respectively. The electrode was primarily made of copper and nickel (∼1 μm), and was coated with gold (thickness: 80 nm). A single, thin, wire-type electrode was inserted into a hollow capillary. Fig. 1B describes how the electrode handled the sample in a quantitative manner, in a manner similar to that of the piston of a common disposable syringe. This electrode-integrated capillary was useful for achieving both sample injection and ejection of an accurately measured volume rapidly. The moving electrode within the capillary, which was connected to the voltage pulse generator, acted as the anode as shown in Fig. 1C. The cathode was placed inside of the buffer container. Consequently, there was an anode inside the capillary and a cathode outside the capillary; the anode and cathode were separated, but were electrically connected through electrolytes in the buffer. This differs from the cuvette electroporation system, in which both electrodes are present in one electroporation chamber. Fig. 1D illustrates a full schematic of the capillary electroporation system divided into two parts: a high-voltage square wave pulse generator (0–2500 V) and a pipette station.

2.2. Cell preparation

In this study, experiments were conducted using many different cell lines (Jurkat, HL-60, GH3, HiB5, Hs578T, MCF-7, HCT-15, SW480, Hep-3B, SK-Hep-1, BHK-21, COS-7, PC-12, and HeLa), primary cells (human umbilical vein endothelial cell, HUVEC), and stem cells (human mesenchymal stem cell, hMSC) to evaluate the feasibility of the capillary electroporation system. Each cell type was cultured under recommended cell-specific culture conditions. All cells were seeded in a culture flask 2 days prior to the experiments. Before electroporation, cells were detached using trypsin–EDTA (Sigma–Aldrich, USA), and were then washed with DPBS (1×) that did not contain calcium or magnesium. The cells were then centrifuged and resuspended in DPBS-based *Resuspension buffer* (hereafter referred as *R buffer*, NanoEntek, Republic of Korea) at a final concentration of 5×10^6 to 1×10^7 cells ml^{-1}. A reporter gene,

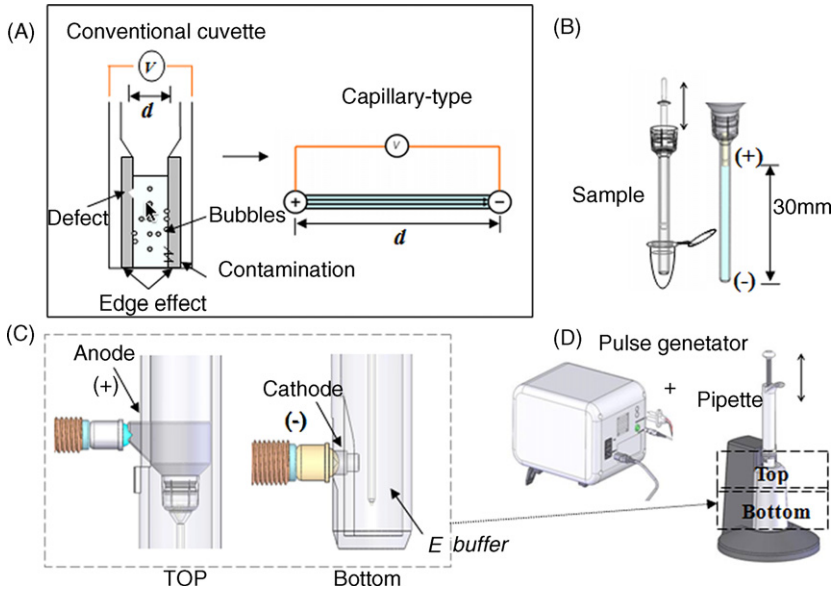

Fig. 1. (A) Comparison of electrochemical side-effects due to the physical geometry of two parallel plate-type electrodes integrated in the cuvette system and a single wire-type electrode used in the capillary system; (B) schematic of the piston-driven capillary tip (diameter: 0.65 mm; length: 30 mm); (C) the position of the anode and cathode in the capillary electroporation system; (D) schematic of the overall capillary electroporation system, which consisted of a high voltage pulse generator and a pipette station.

enhanced green fluorescence protein plasmids (pEGFP-N1), was used to identify transfection and determine the expression levels in cells after electroporation. To accomplish this, pEGFP-N1 was purified using a midi-purification kit (QIAGEN, Germany), and was then added to the resuspended cells at a final concentration of 50 μg ml^{-1}. The cell preparation procedure was the same as that of conventional electroporation experiments.

2.3. Electroporation procedure

For the capillary electroporation system, the capillary was filled with the resuspended cells mixed with pEGFP-N1 using a pipette. Three milliliters of *Electrolytic buffer* (hereafter referred to as *E buffer*, NanoEntek, Republic of Korea) were added to a buffer container. The proposed capillary electroporation system was compared to a conventional electroporator (ECM 830, BTX, USA), which is a typical cuvette-type electroporation device. The cuvette in this device was integrated with two parallel plate-type aluminum electrodes. In general, an electric pulse (5–3000 V, 1 μs–999 ms) was applied between two aluminum electrodes embedded 2 or 4 mm apart.

The mechanical constructions of both systems were so different that the cell-specific electrical conditions for the two electroporation systems should be empirically optimized, respectively. The electrical conditions ranged from 900 to 1500 V and a duration of 1–50 ms, respectively. After electroporation, cells were rapidly dispensed into a 24-well culture plate containing media (5×10^4 to 1×10^5 cells per well) using a pipette, and were then cultured in a CO_2 incubator at 37 °C. After 24 h, the gene expression level was observed using an inverted fluorescence microscope (IX70, Olympus, USA) and analyzed with a conventional flow cytometer (FACSCalibur, Becton Dickinson, USA). Cell viability was measured using propidium iodide (PI) exclusion and analyzed similarly using a flow cytometer. The transfection rate and viability represent the fraction (%) of the GFP-positive cells of the total viable cells and PI-positive cells of the total cell counts, respectively. All experiments were performed in triplicate. All experimental data represent means and standard deviation (±S.D.).

2.4. Detection of pH change during electroporation

In order to estimate the approximate change in pH during electroporation, seven different pH indicators (Sigma–Aldrich, USA) were used (10 ml *R buffer*): pH 0.2–1.8, red to yellow and pH 8.0–9.6, red to purple, cresol red (0.01 g dissolved in 10% ethanol); pH 3.1–4.4, red to orange, methyl orange (0.004 g dissolved in 20% ethanol); pH 3.8–5.4, yellow to blue, bromocresol green (0.01 g dissolved in 5% ethanol); pH 4.8–6.4, yellow to violet, chlorophenol red (0.01 g dissolved in 5% ethanol); pH 6.4–8.0, yellow to red, phenol red (0.01 g dissolved in 5% ethanol); pH 8.0–9.6, colorless to red, phenolphthalein (0.1 g dissolved in 10% ethanol); pH 10.1–12.0, yellow to orange-red, alizarin yellow (0.004 g dissolved in 10% ethanol). Fifty microliters of these pH indicator stock solutions were mixed with 450 μl *R buffer* and then injected into a cuvette. In the capillary electroporation system, the same concentration of the mixture was used. An electric field of 45 kV m^{-1} was induced for a duration of 35 ms. The color change was observed and the photographs were taken with a commercial digital camera, simultaneously. In order to microscopically observe the electroporated cells in the cuvette in real time, a custom-made shock chamber was used. This chamber had a hole in the middle that enabled us to observe the cells following high voltage shock. The cuvette was fixed to the shock chamber on the stage of a microscope, and images of electroporated cells were photographed

simultaneously as soon as a voltage was applied to the shock chamber.

2.5. Metal ion generation during electroporation

In the experiments, we observed additional generation of a metal ions following electroporation. The *R buffer* was used in both the cuvette and the capillary electroporation systems. After electroporation, the buffer was gathered and pretreated with HCl to remove the organic elements or precipitates. To identify the precipitates, a metal complex was separated with a 0.2 μm filter, and dissolved ions in the sample were subsequently analyzed. Metal ions were then detected using an inductively coupled plasma mass spectrometer (ICP-MS, ELAN 6100, PerkinElmer, USA).

3. Results and discussion

3.1. The effect of the design of the capillary electroporation system on transfection

In this study, we attempted to determine the mechanism by which the high electroporation efficiency of the capillary electroporation system was compared to that of the cuvette system, and tried to examine the cause of enhanced transfection efficiency. Scheme S-1 (see Supplementary Material) shows the relationship between the electric field and cell viability. In general, the cell viability and the transfection rate are inversely correlated according to the amount of electrical energy applied (Rols and Teissié, 1998). If high electrical energy is applied, more plasmids are delivered into the cells but more cells also died. The cell death results not only from excessive electric shock, but also from complex intra- and extra-cellular changes such as changes in temperature, pH, and buffer composition during electroporation. It is important to increase the cell viability in order to increase the transfection and expression rates of the cells because only healthy cells survive and function properly.

The electroporated cells in the capillary system maintained a healthier condition than those in the cuvette system. These results indicated that capillary electroporation could enhance not only the transfection rate but also the cell viability, since several harmful factors were eliminated during electroporation. As shown in Scheme S-1 (see Supplementary Material), a remarkable decrease in these side effects resulted in increased cell survival, which allowed an intersection point at which transfection crossed viability to rise and finally extend the range of the optimal condition. The influences of the side effects on cells during electroporation are described in detail in the following sections.

We compared the transfection rate and cell viability of capillary electroporation (Fig. 2D–F) with those of conventional electroporation (Fig. 2A–C) using HeLa, COS-7, and HL-60 cells. Although the experimental ranges were slightly different because of mechanical differences between both systems, the overall viability in capillary electroporation was higher than that of the conventional system in a wide range as shown in Fig. 2. Moreover, when capillary electroporation was used, the optimal conditions of each cell line were so widely applicable that the transfection rate was uniformly high within the range of approximately 100 V and the viability was over 80% within the range although the higher electric field dropped the cell viability. When capillary electroporation was carried out, the overall processing time from setting to dispensing with 24 different cells, exclusive of the cell preparation time, was only 10–15 min. Rapid and effective cell delivery by pipette following electroporation allowed a short processing time and, thus, improved the electroporation efficiency. These results demonstrated that the capillary electroporation system provided high performance and convenience compared to conventional electroporation.

3.2. Transfection rate and cell viability

We performed electroporation using the capillary electroporator on different kinds of cells. Table S-1 (see Table S-1 in Supplementary Material) summarizes the optimal conditions of cells. We tested approximately 70 different cell types (data not shown) and obtained high transfection rates. Fig. 3 shows the results of transfection of 16 representative cells, all of which had a high transfection rate and high cell viability. Successful transfection was observed even in hard-to-transfect cells such as primary cells and stem cells. The transfection rate was greater than 80% in general cell lines, and was greater than 50% in primary cells and stem cells. The cell viability ranged from 70 to 80% for the entire group of cells. These superior percentages have rarely been observed using conventional electroporation and other transfection methods that have been studied.

3.3. Characteristics of capillary electroporation

3.3.1. Physical geometry and electric resistance

In order to find out the reason for the strong performance of capillary electroporation, we examined the differences between capillary- and cuvette-type electroporation in a multilateral manner. The biggest difference between two systems was the effective surface area of the electrode being in contact with the sample. The electrode area of the capillary was much smaller than that of the cuvette, which resulted in the elimination of many of the fundamental problems associated with electroporation.

The capillary system has several unique characteristics. Due to its geometry, the capillary system has a high electrical resistance. The electrical resistance of the electroporation chamber was determined using the following equation. For two parallel plate-type electrodes, $R = rdA^{-1}$, where r is the buffer resistivity ($r = 568\ \Omega$ m, *R buffer*), d is the electrode distance, and A is the electrode area (Nickoloff, 1995). The capillary had a large d (30 mm) and a small A (0.33 mm^2), which resulted in electric resistance being approximately 200-fold higher in the capillary than in the cuvette ($d = 2$ mm, $A = 5$ mm^2; effective surface area in 10 μl). High electrical resistance usually causes a low current, which subsequently induces fewer chemical reactions. Because various harmful effects are induced by these chemical reactions, a small electrode area is necessary in order to minimize damage to the cells. In addition to having high electrical resistance, the capillary system was also unique in that it did not produce vio-

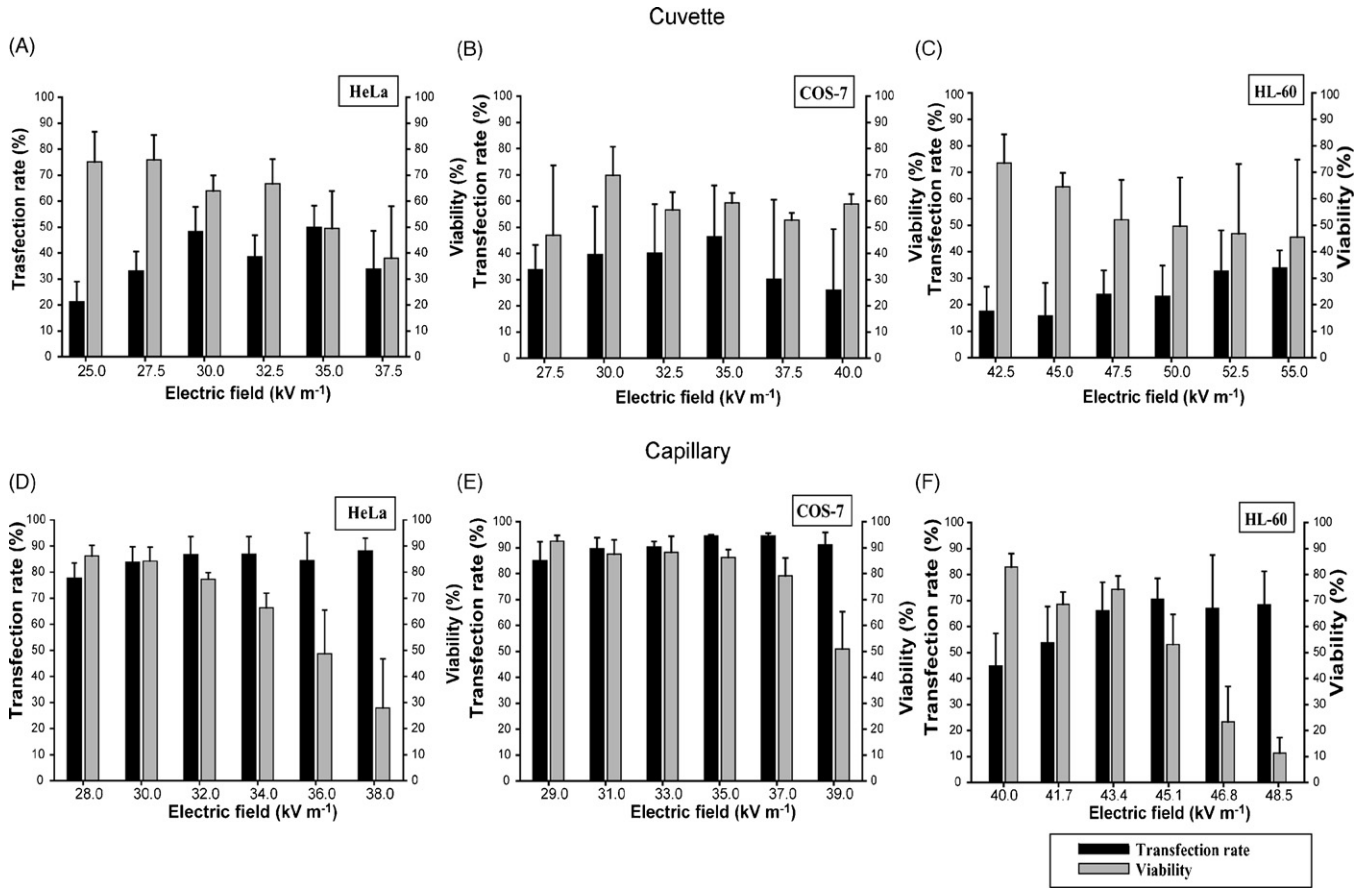

Fig. 2. Comparison of the transfection efficiency of HeLa, COS-7, and HL-60 cells by conventional (A–C) and the capillary electroporation (D–F). The three cell lines were transfected in a broad range of electric fields. Other electrical conditions such as pulse width, pulse number, and pulse repetition frequency were fixed when the two types of electroporation were evaluated. The pulse width and repetition was 35 ms and twice, in HeLa cells, 30 ms and twice, in COS-7 cells, and 35 ms and once, in HL-60 cells, respectively. The transfection rate and viability were measured using a flow cytometer 24 h after transfection. The transfection rate and cell viability of the HeLa, COS-7 and HL-60 cells when the capillary electroporation system was used were superior to those obtained using conventional electroporation, in terms of the efficiency and the stability in a wide range of electric fields. Data shown represent the mean ± S.D. of results obtained from three independent transfection experiments.

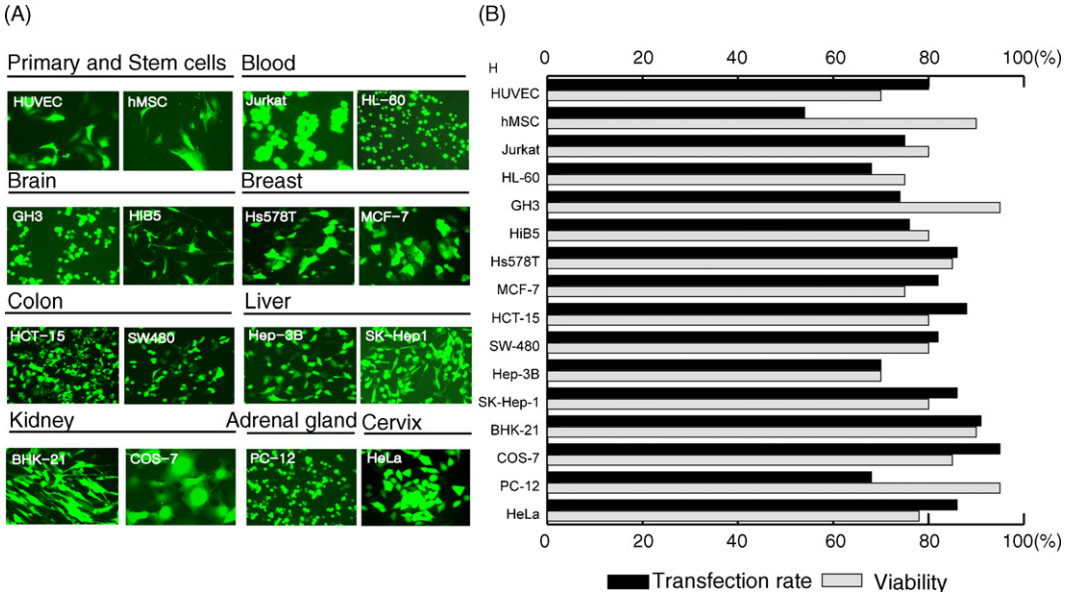

Fig. 3. Electroporation of 16 different cell types with pEGFP-N1 (50 μg ml^{-1}) using the capillary electroporation system. (A) Fluorescence images and (B) average transfection rate and cell viability at 24 h after capillary electroporation.

lent bubble generation and turbulence within the capillary. In the cuvette system, however, bubble generation and turbulent flow occurred in the buffer during electroporation, which promoted the spread of the hydroxyl and hydrogen ions from the area near the electrode to the cells. As a result, cells were more likely to be damaged. In contrast, the buffer solution throughout the capillary could be assumed to be neutral, with the exception of a very thin layer adjacent to the anode. The geometry of the capillary also limits the diffusion and the mixing of the hydroxyl and hydrogen ions during electroporation. These results are experimentally supported in the following sections.

Finally, the simple liquid handling procedure of the capillary system reduced unnecessary cell death. In the capillary system, it took only a few seconds to transfer electroporated cells into fresh media for culture following electroporation by using a pipette, whereas the transfer took a few minutes in the conventional electroporation system. Consequently, the capillary and pipette minimized the time for which the cells were exposed to the harmful environment, which might have increased cell viability. Even slightly damaged cells could be safely transferred for the next step on account of their delicate handling.

3.3.2. *Local pH change during electroporation*

Several recent studies have been conducted regarding changes in pH and buffer composition due to electrolysis that occurs during electroporation (Nilsson et al., 1998; Fredrich et al., 1998; Murthy et al., 2003; Saulis et al., 2005). Kinetic expression of the electrochemical reactions at each electrode was introduced as follows (Fredrich et al., 1998; Murthy et al., 2003; Saulis et al., 2005). In the cuvette system, the main reactions at the anode consist of the dissolution of aluminum in Eq. (1) and the evolution of chlorine in Eq. (2) because the anode material is soluble when aluminum is used as an electrode in an aqueous sodium chloride solution.

$$\mathrm{M} \rightarrow \mathrm{M}^{n+} + n\mathrm{e}^{-} \quad (1)$$

$$2\mathrm{Cl}^{-} \leftrightarrow \mathrm{Cl}_2 + 2\mathrm{e}^{-} \quad (2)$$

$$2\mathrm{H}_2\mathrm{O} \leftrightarrow \mathrm{O}_2 + 4\mathrm{H}^{+} + 4\mathrm{e}^{-} \quad (3)$$

Conversely, in the capillary system, the electrode is coated with insoluble gold so that the dissolution of gold is negligible. The anode reaction mainly produces chlorine in Eq. (2) and oxygen gases in Eq. (3). At the cathode, the main reaction that occurs in both the capillary and cuvette consists of the evolution of hydrogen.

$$2\mathrm{H}_2\mathrm{O} + 2\mathrm{e}^{-} \leftrightarrow \mathrm{H}_2 + 2\mathrm{OH}^{-} \quad (4)$$

The change in pH that occurred near the electrodes was influenced by the concentrations of hydroxyl and hydrogen ions generated during this electrolysis. In the cuvette, bubbles and turbulent flow were generated violently, and promoted the spreading of these ions throughout whole solution. It can also potentially hinder the uniform electric field (Pliquett et al., 1996). We investigated the changes in the pH during electroporation using the pH indicators described in the Section 2.4. Fig. 4 shows the approximate changes in the pH of the cuvette and capillary systems after electroporation. We approximated the pH values based on the changes in the color of buffer solution. In the cuvette system, after electroporation, the pH changed to approximately 11 at the cathode and 3 at the anode, respectively (Fig. 4A). Conversely, in the capillary system, only a very thin layer adjacent to the end of the electrode (i.e., anode) changed color, as shown in Fig. 4B. Additionally, there was almost no color change observed within the capillary, which was due to the fact that the cathode was not present inside the capillary.

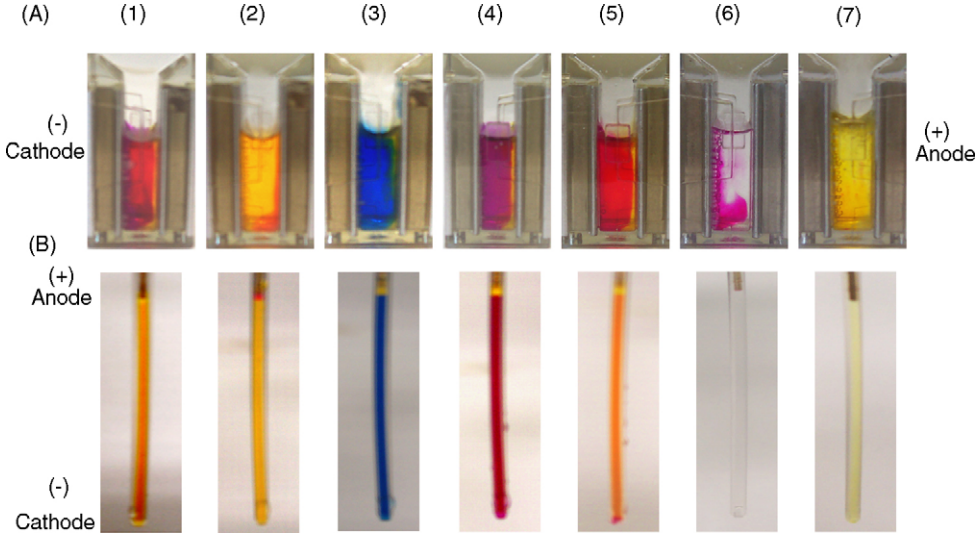

Fig. 4. Detection of the pH change that occurred due to electrolysis during electroporation in the cuvette (A) and in the capillary (B) systems, which was obtained using 7 different pH indicators. (1) pH 0.2–1.8, red to yellow and pH 8.0–9.6, red to purple, cresol red; (2) pH 3.1–4.4, red to orange, methyl orange; (3) pH 3.8–5.4, yellow to blue, bromocresol green; (4) pH 4.8–6.4, yellow to violet, chlorophenol red; (5) pH 6.4–8.0, yellow to red, phenol red; (6) pH 8.0–9.6, colorless to red, phenolphthalein; (7) pH 10.1–12.0, yellow to orange-red, alizarin yellow. The images were immediately captured after applying electric voltage. The distance between electrodes within the cuvette was 4 mm. The *R buffer* used as the electroporation buffer had a resistivity of 568 Ω m. The electric field was 45 kV m^{-1} and the pulse duration was 35 ms.

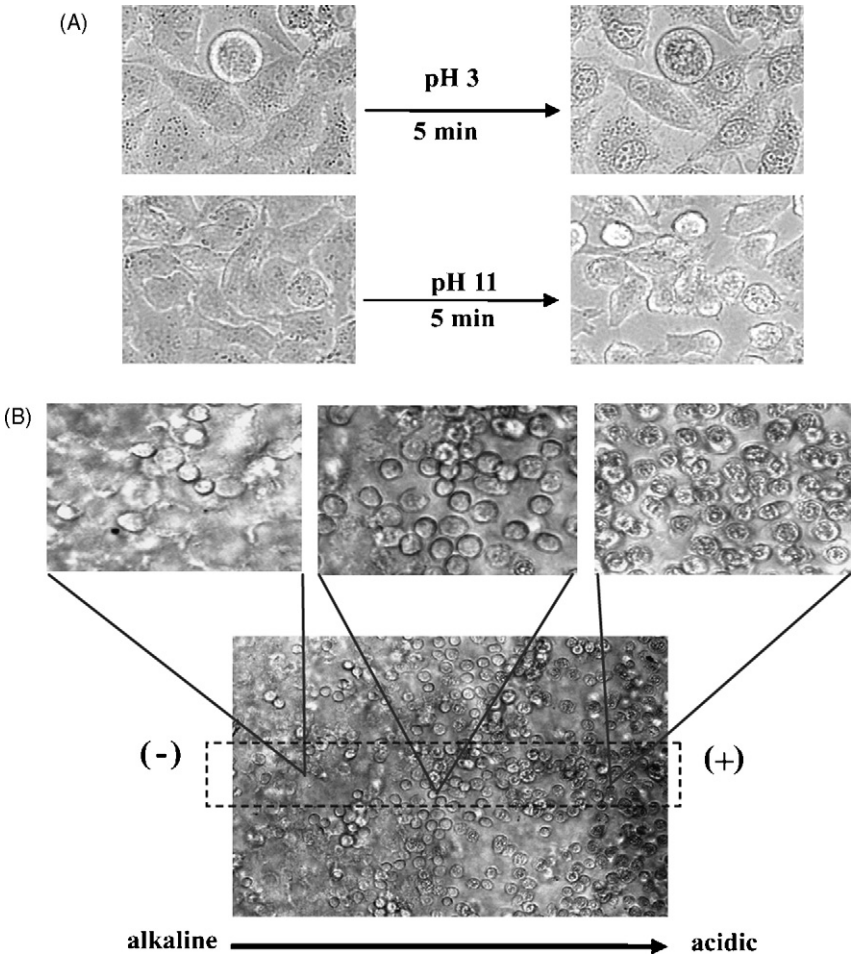

Fig. 5. The effect of the pH change on cell death. (A) HeLa cells were treated with the acidic (pH 3) and alkaline *R buffer* (pH 11) for 5 min (as a control). These cells were severely damaged by the pH change. (B) The damage of HL-60 cells near the electrodes of the cuvette (4 mm distance) during electroporation. The image was captured within 5 min. The electric field was 45 kV m^{-1} and the pulse duration was 35 ms. At the anode, cells were ruptured by osmotic lysis, whereas cells were dehydrated at the cathode.

A change in pH resulted in a subsequent destructive effect on cells near the electrodes because of the significant production of hydrogen and hydroxyl ions, which in turn created strongly acidic or basic conditions around the cells. The microscopic images of damaged cells as a result of the change in pH in the cuvette system are displayed in Fig. 5. In healthy cells (e.g., HeLa), the progression of damage to the cells caused by rapid changes in pH occurred relatively slowly (Fig. 5A). Meanwhile, as soon as the electric pulse (45 kV m^{-1} and 35 ms) was applied to the HL-60 cells, electroporated cells near the electrode were dramatically damaged, swollen, and eventually ruptured (Fig. 5B). The sudden increase in temperature of the buffer solution as a result of the high level of electric shock might locally accelerate this phenomenon. These results supported the idea that changes in pH that occur during electroporation might be a strong factor in cell death.

3.3.3. Metal ion generation during electroporation

When aluminum electrodes are used in conventional electroporation, many secondary chemical reactions with ions can occur (Nickoloff, 1995). One of the reactions can be expressed by the following reaction form:

$$Al^{3+} + 3OH^- \leftrightarrow Al(OH)_3 \quad (5)$$

A considerable amount of $Al(OH)_3$ was produced after the electroporation process was completed. Because a precipitate was observed in the cuvette using the naked eye, we attempted to analyze the spent *R buffer* containing the precipitate, as well as, filtered *R buffer* using ICP-MS, as shown in Table 1. As a result, the precipitate that was produced after electroporation revealed an aluminum complex. It was possible for ionized aluminum produced at the anode to be transported to the porous membrane of the electroporated cells by diffusion or migration, which may have toxic effects (Pliquett et al., 1996). Although it remains somewhat debatable that aluminum ions are toxic, aluminum complex can conglomerate with cell debris or other elements in media, which may hinder cell attachment and growth on culture plates. In the capillary electroporation system, the electrodes were coated with passive material (gold) and the surface area of each electrode was very small. Therefore the metal dissolution

Table 1
Detection of metal ion after electroporation in the cuvette (aluminum electrode) and the capillary electroporation system (gold electrode) (ppb = μg/l) using ICP-MS

Sample	Al	Au
1	12,231.88	ND[a]
2	10.8	ND
3	ND	ND
4	ND	ND

Sample 1: after electroporation by BTX electroporator, the *R buffer* was analyzed. Sample 2: sample 1 was filtered and then analyzed. Sample 3: deionized water (control). Sample 4: after electroporation by the capillary electroporation, the *R buffer* was analyzed.

[a] ND = non-detected. Al < 5 ppb, Au < 0.5 ppb.

in this system was negligible. A small amount of metal ion was observed in the capillary electroporation system, as indicated in Table 1.

4. Conclusions

In this study, we demonstrated that our capillary electroporation system considerably reduced many of the harmful side effects that occur when conventional electroporation methods are used. We also achieved an excellent transfection rate in a wide range of mammalian cells, including hard-to-transfect cells and high and reproducible viability of 70–80%. The capillary electroporation system provided a uniform electric field and diminished the various side effects of electrolysis due to the small surface area of the electrode. Additionally, the simplicity of the integrative capillary and pipette station produced a very high efficacy compared to that of conventional electroporation. The capillary method was also rapid, reproducible, and free of cell loss, and therefore, was applicable to high-throughput experiments. Furthermore, the unique design of the capillary electroporation system can be expanded to other biological application tools in which the reaction and handling process need to be unified.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2007.12.009.

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