A Thesis for the Degree of Master of Engineering

Development of a Bioelectrospray System Enhanced Gene Transfection into Mammalian Cells

세포 내 유전자 도입 효율증대를 위한 바이오전기분무 시스템 개발

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August 2015

Department of Biosystems & Biomaterials Science and Engineering

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이 논문을 공학석사학위논문으로 제출함

2014년 8월

서울대학교 대학원

바이오시스템○소재학부 바이오시스템공학 전공

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Abstract

To deliver genes into animal cells, viral vectors are generally used because of their high transfection efficiency. However, viral vectors have a risk that it can infect pathogenic genes into the host. Thus, many researchers use non-viral vectors such as synthetic polymers and apply mechanical method. However, their transfection efficiency are lower than that of viral vectors.

In this paper, we tried to combine chemical and mechanical methods for enhancing transfection efficiency. Cationic polymers were selected as chemical method. Then Mechanical method was applied by new way of transfection. The new method is bio-electrospray (BES). The bio-electrospray (BES) is an application of electrospray. This is technique to electrospray living cells on target. We formed a hypothesis that gene transfection efficiency can be increased if the bio-electrospray (BES) method is combined with chemical method (using polymer). After that, as procedures to increase the gene

transfection efficiency in animal cells, we tried to combine polyethylenimine (PEI), a cationic polymer (non-viral carrier) with bio-electrospray (BES) system.

At first, we evaluated cell viability after BES. In order to investigate what solvent elevates transfection level, vector-PEI complex and cells were mixed with several solvents [phosphate buffer saline (PBS), deionized water (DW), Dulbeco's Modified Eagle's Medium (DMEM), and culture media (CM)]. Then in order to investigate what voltage elevate transfection level, the mixtures were electrosprayed with various voltages. Their transfection efficiency is examined by turbo green fluorescent protein (tGFP) expression and luciferase assay.

As a result, when PBS as solvent that mixed cells, DNA and PEI was applied, transfection level was much higher than others. Moreover, when 5kV was applied, transfection level is the highest.

From this study, the new way of transfection was presented and it will be applied at transfection of non-adhesion cells like lymphocyte.

Key words: Transfection, Electrospray, Bio-electrospray(BES), Non-viral vector, Polyethylenimine (PEI)

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List of Terms and Abbreviations

BES	Bio-electrospray
ES	Electrospray
PEI	Polyethylenimine
PBS	phosphate buffer saline
DW	deionized water
DMEM	Dulbeco's Modified Eagle's Medium
FBS	fetal bovine serum
CM	culture media
tGFP	turbo green fluorescent protein
BMSC	bone marrow derived mesenchymal stem cells

1. Introduction

Gene transfection is a method that inserts foreign gene into cells to make genetically modified cells. This is very powerful tool to study various field such as gene regulation, recombinant protein, and gene therapy [1]. Numerous researchers had studied the transfection technique. Thus, many gene transfer methods have been developed [2]. Until now, three typical methods are known by researchers. First method is to use viral vectors as carriers. The virus mediated method have a high efficiency to transfer gene and is easy to use. However, this way has a serious disadvantages. Those are potential risk of virus itself, mutagenesis, and limit of DNA package size.[2, 3] Second method is to use chemicals such as cationic polymers, calcium phosphates, or cationic lipids. The chemical method doesn't need virus vectors. Moreover, it is easy to use. It is also no limit of package size. Although, there are many good points, this method have chemical toxicity problem and transfection efficiency is different depending on the type of cells [2, 4]. Third way is physical methods like direct injection, electroporation, and ultrasound-mediated transfection [5]. Physical method is so simple and less affected by cell types and it doesn't need the vector. However, it needs special device and demands experimental skills [6].

Generally, viral vectors were generally used a lot because of their high efficiency [7]. However, viral vectors have a risk that it can infect pathogenic genes into the host. Thus, many researchers use non-viral vectors like synthetic polymers [8, 9]. Although transfection efficiency of non-viral vectors are lower

than that of viral vectors, it is possible to transfer DNAs with more safety.

To substitute viral method, many researchers also tried to apply mechanical factors [2]. Among physical methods, an electroporation method is a typical technique. The electroporation is technique that introduce DNA or drug into cells by increasing the permeability of cell as using electrical field. From this method, we got a hint to apply transfection using electrospray. That is the bio-electrospray (BES). The bio-electrospray (BES) is application of electrospray methods. It didn't apply to transfer gene before. The bio-electrospray (BES) is technique to electrospray living cells into target [10].

By using this method, we tried to apply transfection into mammalian cell with chemical method. Using cationic polymers were selected as chemical method. As a cationic polymer, we choose polyethylenimine (PEI), a cationic polymer (non-viral carrier), due to its well-known properties, easy availability and high DNA complexation ability.

We formed a hypothesis that gene transfection efficiency can be increased if the bio-electrospray (BES) method is combined with chemical method (PEI).

In this study, in order to increase the gene transfection efficiency in cells, we tried to combine mechanical method and chemical method.

2. Objectives

The objective of this study was to improve efficiency of transfection into cells using bio-electrospray (BES) with cationic polymer. For this, among cationic polymers, polyethylenimine (PEI) was used. The optimized voltage for transfection was examined at bio-electrospray (BES) with PEI vector.

Detailed objectives were as follows:

1) To investigate cell viability after bio-electrospray at several voltage and to find appropriate voltage.

2) To develop a transfection system using bio-electrospray with polyethylenimine (PEI).

3) To confirm and optimize bio-electrospray condition for gene transfection.

3. Literature Review

3. 1. Electrospray

Electrospray, developed long time ago, was a spray system to distribute very small particles by electric force. Electrospray also disperse a liquid sample in a same form [11]. In this technique, high voltage is used to spray a liquid supplied through an emitter.

The electrospray, sometimes called electrohydrodynamic atomization(EHDA), is a modified version of the electrospinning process (electrospun) [12]. Fig.1 shows the effect of several condition on electrospray or electrospinning. Electrospray and electrospun do not have big differences. If concentration of solution is high, fiber shape is made and if it is low, small particles are made.

Electrospray Process is as below. When a potential is applied to a liquid in a nozzle, the liquid is pulled into an oval shape. A surface tension derived force starts to draw the liquid back into the nozzle to minimize surface area. The electrostatic Coulomb attraction draws the liquid to the counter electrode. At appropriate voltage, the oval surface suddenly changes its shape like a sharp cone. From apex of the cone, a spray is emitted [11, 13, 14]. This is called “Taylor cone”[13].

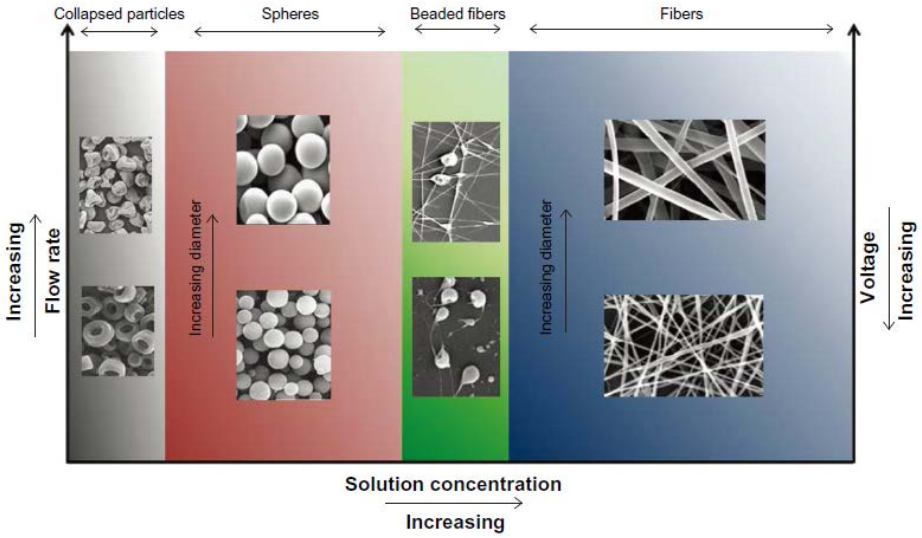

Figure 1. “Schematic illustration of the effect of the concentration, flow rate, and voltage on electrosprayed / electrospun nano / microstructures” [12].

3. 2. Bio-electrospray

Bio-electrospray technique is state of art and application of electrospray method. It is to deposit living cells on various targets with a solution by electric force. At electrospray, materials as polymer with solvent were emitted. However, at bio-electrospray, living cells with solvent were emitted. In other words, as compared to the electrospray, living cells solution as spray solvent were used at bio-electrospray.

Several researchers introduced bio-electrospray as safe technique [10, 15]. Generally, devices of electrospray were set like Fig.2.

S. Sahoo group demonstrated that viability of bone marrow derived mesenchymal progenitor/stem cells (BMSC) after bio-electrospray was fine.

Besides, proliferation and differentiation ability of BMSC was nothing wrong (Fig. 3). Thus, they said that this technique could be applied at tissue engineering like cell patch or scaffold [10]

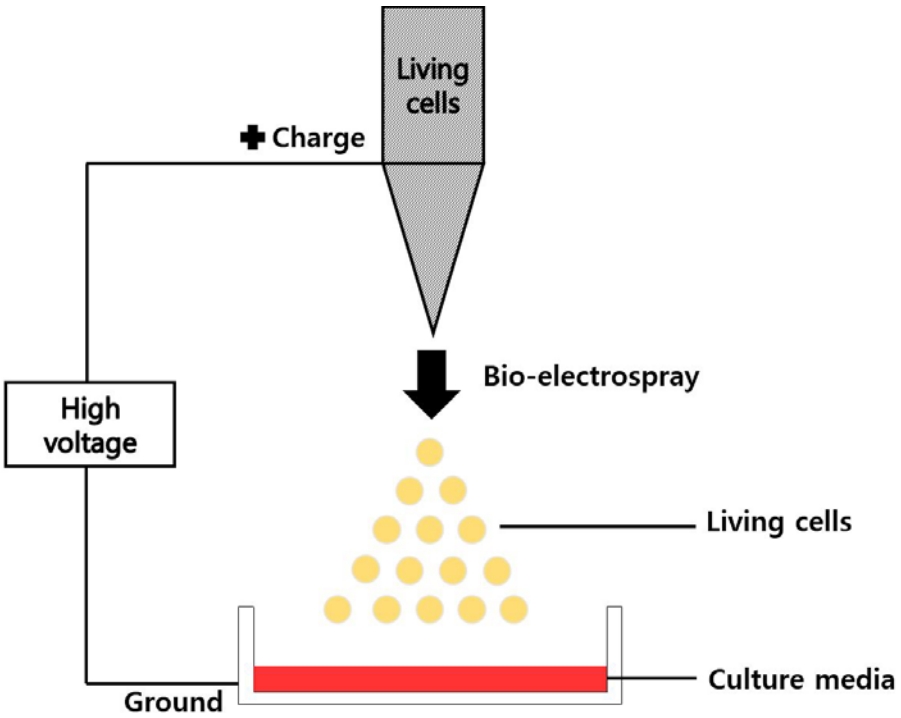

Figure 2. Diagram of Bio-electrospray.

Richard P. Hall group performed to evaluate genetic safety of bio-electrospray using human cells. The bio-electrosprayed cells showed little elevated damage compared to control. However, difference between bio-electrosprayed cells and normal cells was not statistically significant [16].

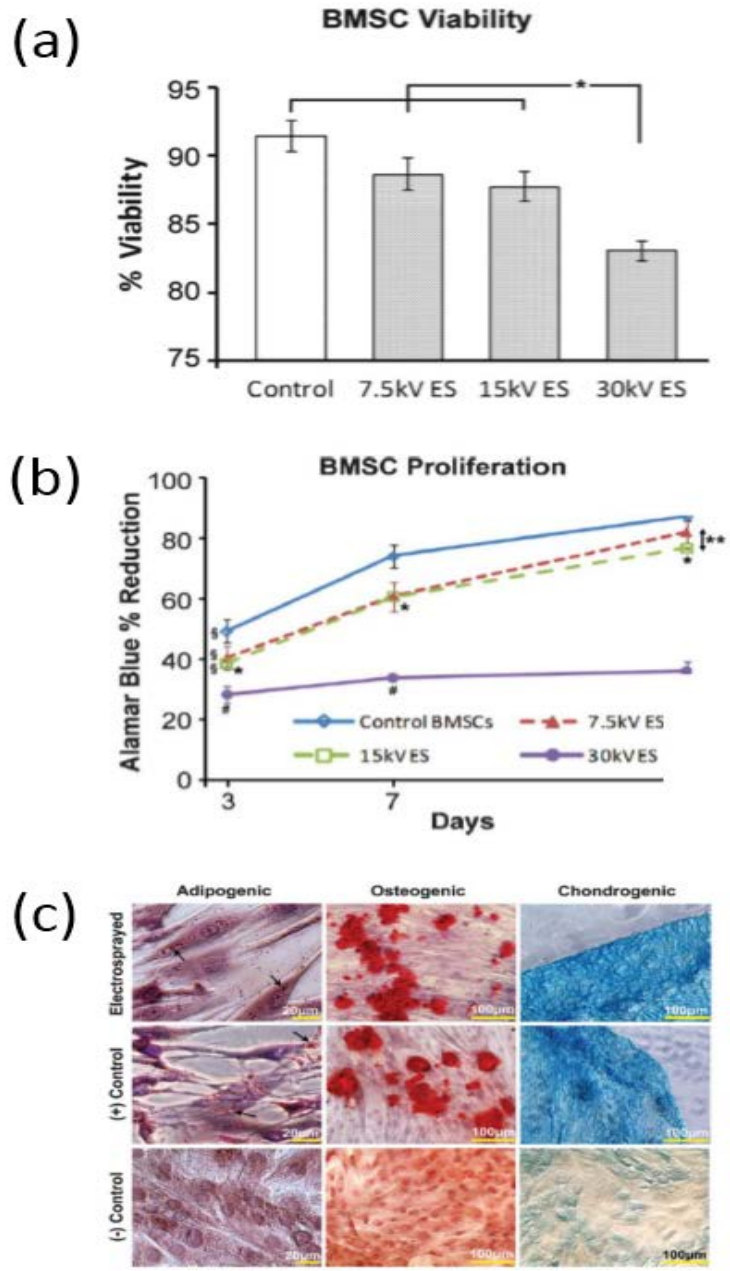

Figure 3. viability, proliferation and differentiation results after bio-electrospray. (a) result of viability, (b) result of proliferation, (c) result of differentiation [10].

3. 3. Transfection

Transfection is technique that transfer foreign gene into cells. Generally, there are three conventional methods for transfection. The most important point of increasing transfection efficiency is selecting the appropriate transfection protocol [17]. According to environment, target, experimenter, etc., each transfection methods was applied differently. Usually, viral vectors were used a lot in transfection [18]. However, viral vectors have a risk that it can infect pathogenic genes into the host. Thus in order to avoid risk of viral vector, chemical and physical method were performed by many researchers.

Class	Method	Advantage	Disadvantage
Biological	using virus	High-efficiency Easy to use Effective on dissociated cells, slices, and in vivo	Potential hazard to laboratory personnel Insertional mutagenesis Immunogenicity DNA package size limit
Chemical	cationic polymer	No viral vector	Chemical toxicity to some cell types
	calcium phosphate	High-efficiency	Variable transfection efficiency by cell type or condition
	cationic lipid	Easy to use Effective on dissociated cells and slices Plenty of commercially available products No package size limit	Hard to target specific cells
Physical	Direct injection	Simple principle and straightforward	Needs special instruments
	biolistic particle delivery	Physical relocation of nucleic acids into cell	Vulnerable nucleic acids
	Electroporation	No need for vector	Demands experimenter skill, laborious procedure

Table 1. “conventional transfection method” [2].

3. 4. Transfection by cationic polymer (PEI)

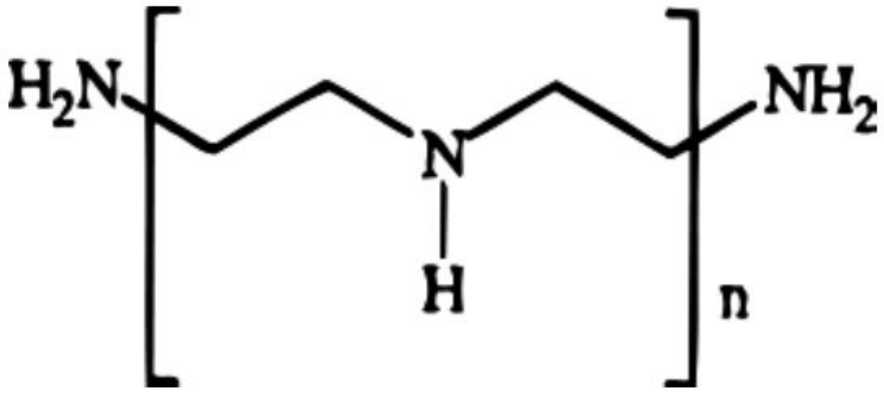

Figure 4. Linear Polyethylenimin (PEI) [19].

Polyethylenimine (PEI, 25k) is commonly used in genetic study field [8]. This is typical cationic polymer and used in transfection. PEI and DNA was bound by charge. Because PEI polymer has plus charge and DNA has minus charge. Also, cell membrane has minus charge. Thus DNA-PEI polyplexes pass through well into cells [9, 19]. PEI vector has lower efficient than viral vector [20]. However, compared to viral, potential risk of using PEI was low. That is not to say PEI polymer didn't have any elements of risk. Polycationic gene-delivery systems can lead to cytotoxicity. The more PEI concentration, the more cytotoxicity is increased in transfected cells [21].

3. 5. Electrospray for transfection

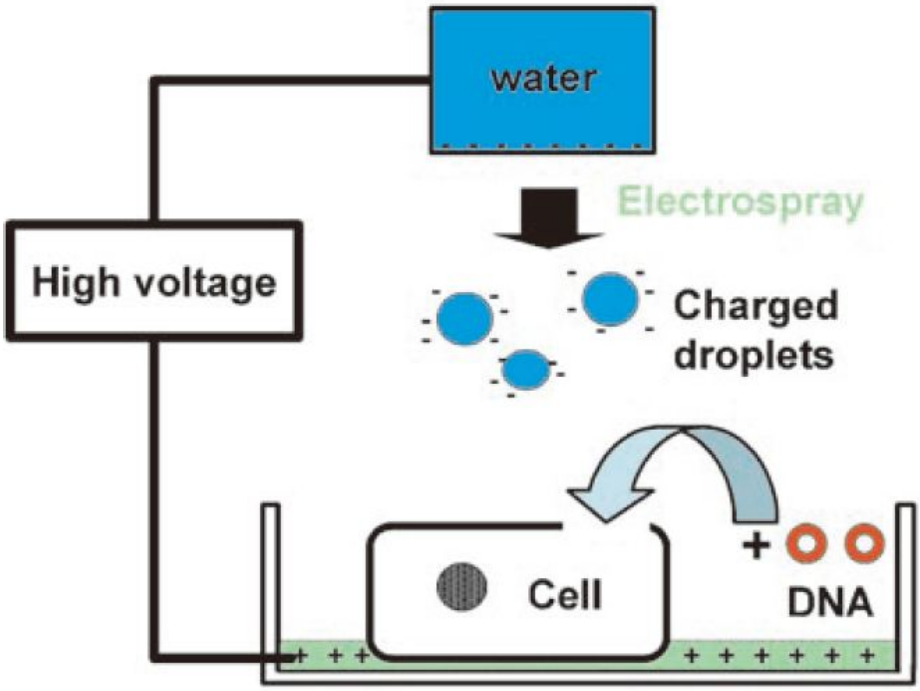

Figure 5. “Diagram of electrospray equipment for gene delivery” [22].

Y. Okubo group reported interesting results of transfection by electrospray [22]. By electrospray, charged droplets were sprayed at dish that cells were attached. Then, foreign gene goes through cells. Consequently, this team had shown that droplet impact by electrospray can be applied to gene transfection. Also, K. Ikemoto group said that size of droplets influences transfection efficiency [23]. However, limit of this technique is that non-adhesion cells or floating cells are not applied.

4. Materials and Methods

4.1. Bio-electrospray(BES)

4.1.1. Preparation of cells for BES

NIH-3T3 (ATCC, USA) cells cultured in a T75 flask (NUNC) for 2 days with culture media (CM) comprised of antibiotics (Welgene Inc., Korea), 10% fetal bovine serum(FBS, Hyclone, USA), Dulbeco's Modified Eagle's Medium(DMEM, Welgene Inc., Korea). NIH-3T3 cells were seeded at a density of $2x10^6$ cells. After 2 days, the cells were harvested and mixed with culture media.

4.1.2. Electrospray with cells

The solution of harvested cell with culture media was put in a 10 ml syringe. Continually, new 35 mm dish(NUNC, USA) was filled with culture media. After then this solution was electrosprayed using a syringe pump and a high power supply at several voltages between a syringe tip and a grounded clip on the 35 mm dish. The cell solution was electrosprayed through a needle equipped to the syringe at a flow rate of 5 mL/h. The electrosprayed cells were dropped on a target 35 mm dish filled culture media. The distance between syringe tip and grounded clip was 3 cm (Fig.6). The electrospray was performed at room temperature.

After electrpspray, treated samples were incubated in CO2 incubator.

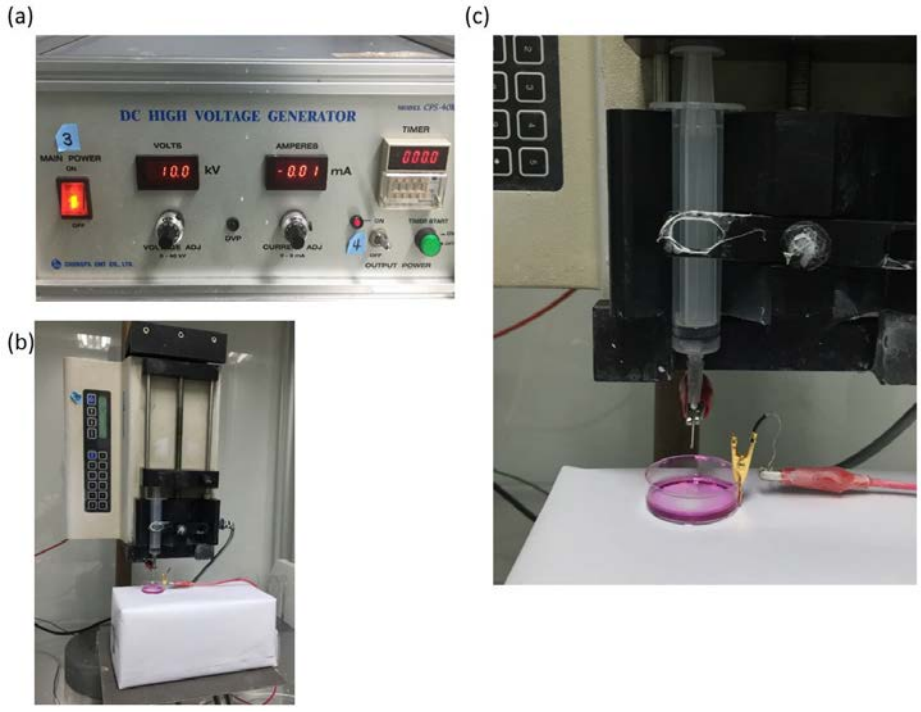

Figure 6. Devices of electrospray. (a) high voltage generator, (b) syringe pump, (c) syringe

4.2. Cell viability after Bio-electrospray (BES)

4.2.1. Observation of cell morphology after BES

After 3 hours of bio-electrospray, morphology of cells was observed by microscope (Ti-E, Nikon, Japan). Sequently, morphology of cells after 1 day of BES was observed again. When BES was run on dish, density of cells was $5x10^5$ and voltage of electrospray was treated at various condition (0kV, 10kV, 15kV, 20kV).

4.2.2. Live / Dead assay

In order to investigate cell viability, Live/Dead Cell Assay kit (abcam ab115347, Mitosciences, USA) was used after 1 day of bio-electrospray. When bio-electrospray was run on dish, density of cells was $5x10^5$ and voltage of electrospray was treated at various condition (0kV, 10kV, 15kV, 20kV).

The concentration of provided Live/Dead Dye was 1000X. When dye is used for assay, it have to be diluted at 5X in PBS. The cells were stained by 5X Live/Dead Dye and incubated 20 min at room temperature. Then the dyed cells were observed by fluorescent microscopy (Ti-E, Nikon, Japan).

4.2.3. WST-1 assay

In order to measure cell viability quantitatively, WST assay was carried out after bio-electrospray. When bio-electrospray was run on dish, density of cells was $5x10^5$ and voltage of electrospray was treated at various condition (0kV, 10kV, 15kV, 20kV). After 1day electrospray, each cell culture dish was added cell viability assay reagent (EZ-cytox, EZ-3000, DOGEN, South Korea) and culture media in dish and reagent were reacted for 3 hours. Then each sample was quantified with the absorbance reader of Sunrise™ (TECAN, Switzerland) at 450nm.

Additionally, cases (0kV, 2.5kV, 5kV, 7.5kV, 10kV) of less than 10kV voltage were run by equal method to check the departmentalized results.

4.3. Transfection study

4.3.1. Transfection by bio-electrospray with PEI polymer

Transfection studies were performed in NIH-3T3 (ATCC, USA) by bio-electrospray using, cationic polymer, bPEI (Mn: 25k). The solution of harvested cell with solvent (500ul) and PEI25k/DNA polyplexes (200ul) were mixed and was put in a 10ml syringe. PEI25k/DNA polyplexes were treated at 15 N/P ratio. This solution was electrosprayed using a syringe pump and a high power supply at several voltages between a syringe tip and a grounded clip on the 35 mm dish filled culture media. The amount of electrosprayed cells were $5x10^5$. As soon as electrospray finished, fresh culture media (1ml) was added at dish.

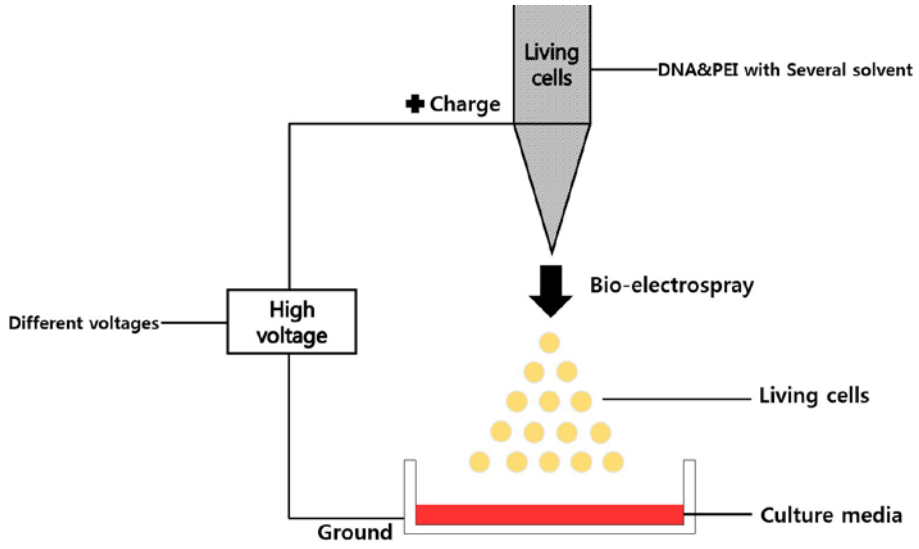

Figure 7. Diagram of Bio-electrospray transfection

4.3.2. The study of transfection by bio-electrospray using several solvents

NIH-3T3 cultured in a T75 flask (NUNC) for 2 days with culture media (CM) comprised of antibiotics (Welgene Inc., Korea), 10% fetal bovine serum(FBS, Hyclone, USA), Dulbeco's Modified Eagle's Medium(DMEM, Welgene Inc., Korea). NIH-3T3 cells were seeded at a density of $2x10^6$ cells.

After 2 days, when the cells were harvested and electrosprayed, sevral solvents were used at electrospray to find solvent which increases transfection efficiency. As solvent, phosphate buffer saline (PBS, Welgene Inc., Korea), deionized water (DW), Dulbeco's Modified Eagle's Medium (DMEM, Welgene Inc., Korea), and culture media (CM) were used. The solution of harvested cell with solvent (500ul) and PEI25k/DNA polyplexes (200ul) were mixed and was put in a 10ml syringe. The solution was electrosprayed using a syringe pump and a high power supply at 10kV between a syringe tip and a grounded clip on the 35 mm dish filled culture media. The amount of electrosprayed cells were $5x10^5$. As soon as electrospray finished, fresh culture media (1ml) was added at dish.

4.3.3. The study of transfection at different voltages of bio-electrospray

NIH-3T3 cultured in a T75 flask (NUNC) for 2 days with culture media (CM) comprised of antibiotics (Welgene Inc., Korea), 10% fetal bovine serum(FBS, Hyclone, USA), Dulbeco's Modified Eagle's Medium(DMEM, Welgene Inc., Korea). NIH-3T3 cells were seeded at a density of $2x10^6$ cells.

After 2 days, various voltages were used at electrospray to investigate voltage which increases transfection efficiency. As solvent, phosphate buffer saline (PBS, Welgene Inc., Korea), was used. The solution of harvested cell with PBS (500ul) and PEI25k/DNA polyplexes (200ul) were mixed and was put in a 10ml syringe. The solution was electrosprayed using a syringe pump and a high power supply at several voltages (0kV, 5kV, 7.5kV, 10kV) between a syringe tip and a grounded clip on the 35 mm dish filled culture media. The amount of electrosprayed cells were $5x10^5$. As soon as electrospray finished, fresh culture media (1ml) was added at dish.

Additionally, case of less than 5kV voltage were run equal method to confirm transfection efficiency at low voltages (0kV, 2.5kV, 5kV, 7.5kV, 10kV).

4.3.4. The study of transfection for various animal cells using bio-electrospray

Three types cell line were applied at bio-electrospray. As cell lines, NIH-3T3 (ATCC, USA), CHO (ATCC, USA), HeLa (ATCC, USA) were used. Each cells cultured in a T75 flask (NUNC) for 2 days. In order to culture NIH-3T3 cell, Dulbeco's Modified Eagle's Medium (DMEM, Welgene Inc., Korea) including antibiotics (Welgene Inc., Korea), 10% fetal bovine serum(FBS, Hyclone, USA) were used. Also, in order to culture CHO and HeLa cells, Roswell Park Memorial Institute Media (RPMI1640, Welgene Inc., Korea)

including antibiotics (Welgene Inc., Korea), 10% fetal bovine serum(FBS, Hyclone, USA) were used.

After 2 days, each cell was harvested and applied at electrospray. As solvent, phosphate buffer saline (PBS, Welgene Inc., Korea), was used. The solution of harvested cell with PBS (500ul) and PEI25k/DNA polyplexes (200ul) were mixed and was put in a 10ml syringe. The solution was electrosprayed using a syringe pump and a high power supply at several voltages (0kV, 5kV, 7.5kV, 10kV) between a syringe tip and a grounded clip on the 35 mm dish filled culture media. As soon as electrospray finished, fresh culture media (1ml) was added at dish. The amount of electrosprayed NIH3T3 cells were $4x10^5$ and The amount of electrosprayed CHO and HeLa cells were $8x10^5$.

4.4 Evaluation of Transfection

4.4.1. turbo Geen Fuorescent Potein (tGFP) assay

The green fluorescent protein (GFP) gene was obtained from Clontech (Palo Alto, CA, USA). PEI25K/GFP (4ug) polyplexes were treated at 15 N/P ratio. After transfection using PEI/GFP by bio-electrospray, cells were cultured for 48 hours in CO2 incubator. Then transfected cells were observed by fluorescent microscopy (Ti-E, Nikon, Japan).

4.4.2. Luciferase Assay

Luciferase reporter, pGL3-vector with SV-40 promoter and enhancer encoding firefly (Photonus pyralis) luciferase was obtained from Promega (Madison, WI, USA). PEI25K/pGL3 (4ug) polyplexes were treated at 15 N/P ratio. After transfection using PEI25K/ pGL3 by bio-electrospray, cells were cultured for 48 hours in CO2 incubator. Then luciferase assay was performed according to the manufacturer's protocol. A Multiple Plate Reader (victor3, Perkin Elmer, USA) was used to measure relative light units (RLUs) normalized with protein concentration in the cell extract estimated using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

4.5 Statistical Data Analysis

The statistic analysis was performed by statistical analysis program as R v3.2.1 software (The R Foundation, http://www.r-project.org). Statistic significance between comparison groups and control groups was compared with one-way ANOVA at *$p<0.05$, **$p<0.01$, ***$p<0.001$. The data were reported as the mean ± standard deviation, n=3.

5. Results and Discussion

5.1. Cell viability

5.1.1. Cell morphology after bio-electrospray

After 3 hours to bio-electrospray cells, morphology of cells was observed by microscope (Fig.7). Density of cells at 0kV and 10kV didn't decrease and cells was attached on the dish well. However, at voltage more than 15kV, Density of cells was significantly decreased. Especially, electrosprayed cells at 20kV were hardly attached on the dish.

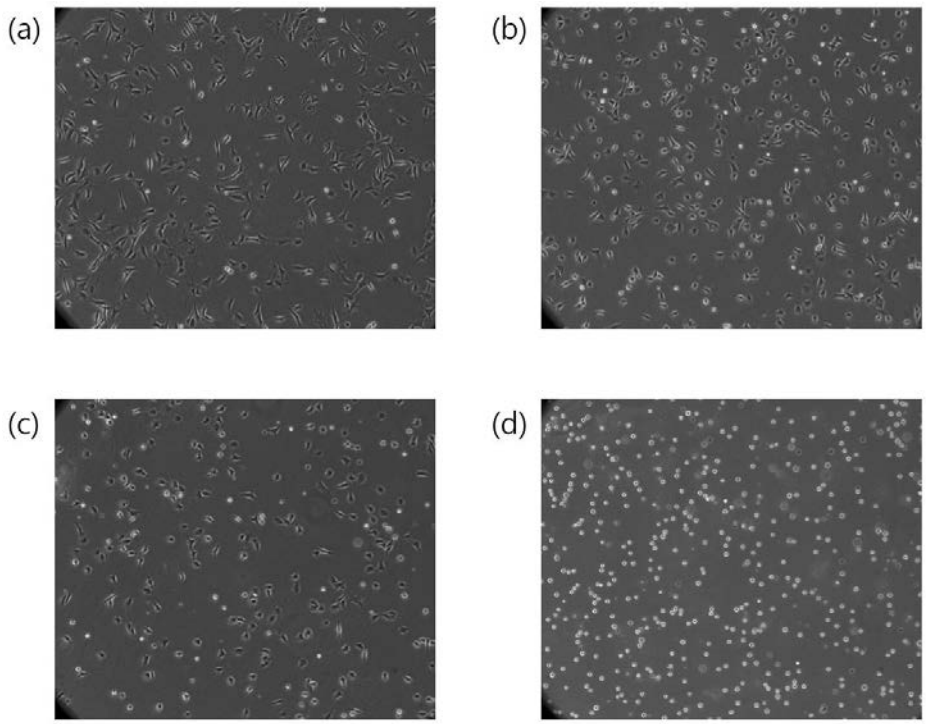

Figure 8. Cell morphology after 3 hours to bio-electrospray cells. (a) 0kV, (b) 10kV, (c) 15kV, (d) 20kV. Case of 0kV is negative control (no treat bio-

electrospray). The pictures were taken at 10x magnification.

After 1 day to bio-electrospray cells, morphology was observed by microscope (Fig.8). Like the morphology after 3hours, density of cells from 0kV to 10kV was high. Density of cells at 15kV was little lower than 10kV. However, electrosprayed cells at 20kV were still small population.

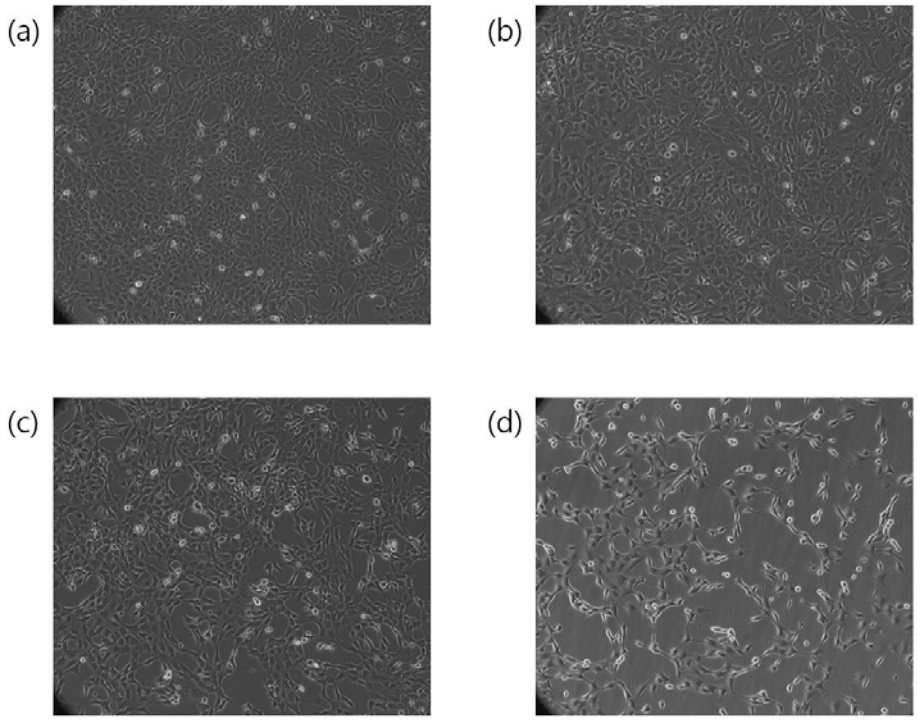

Figure 9. Cell morphology after 1day to bio-electrospray cells. (a) 0kV, (b) 10kV, (c) 15kV, (d) 20kV. Case of 0kV is negative control (no treat bio-electrospray). The pictures were taken at 10x magnification.

By these data, morphology of cells and density were fine up to 10kV at

electrospray. At voltage higher than 10kV, density of cells was decreased. Applied at electrospray using excessively high voltages, cells were damaged and lost adhesion ability.

However, only morphology was not enough to confirm cell viability. In order to investigate cell viability precisely, Live/Dead assay and WST-1 assay were performed at same condition.

5.1.2. Live/Dead assay after bio-electrospray

In order to examine cell viability visually, Live/Dead assay was performed after 1 day to bio-electrospray cells (Fig. 9). A green color said that cells lived. A red color said that cells were damaged or died.

Electroeprayed cells at 0kV and 10kV almost lived. Density of 0electrosprayed cells at 15kV seems to be a lot. However many dead cells were also showed. At 20kV, density of cells seems to be very low.

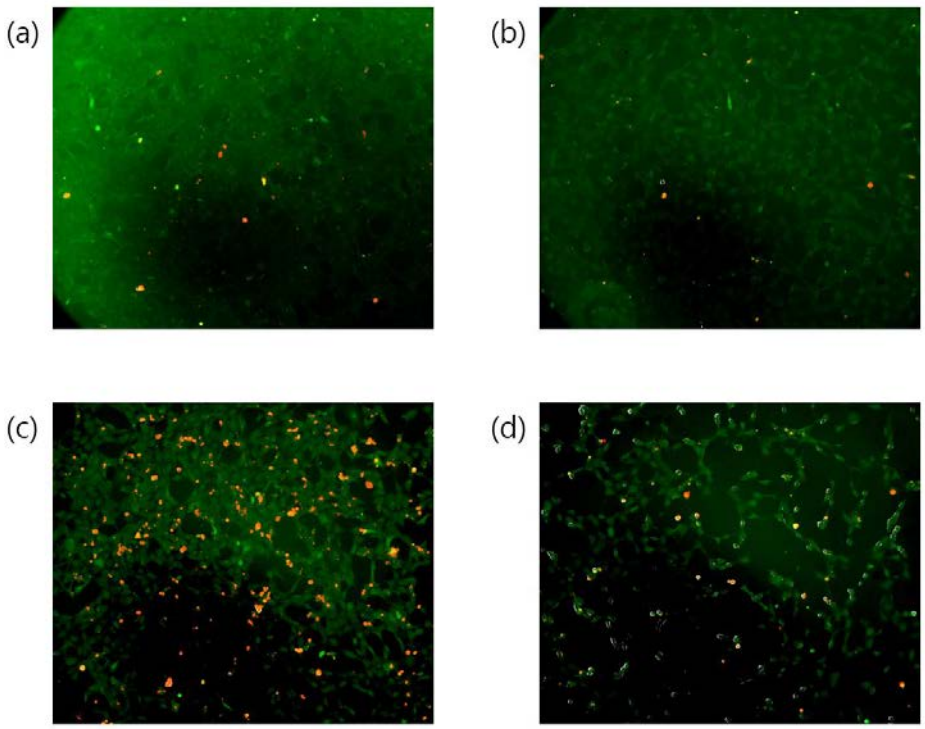

Figure 10. Live/Dead assay after bio-electrospray. (a) 0kV, (b) 10kV, (c) 15kV, (d) 20kV. Case of 0kV is negative control (no treat bio-electrospray). Live/Dead assay pictures were taken at 10x magnification.

In this assay, an intact plasma membrane and intracellular enzymatic activity form the basis of intracellular esterase activity to generate green fluorescence and exclusion of red dye. Dead cells are identified by lack of esterase activity and non-intact plasma membrane which allows red dye staining.

From these data, viability of electroprayed cells at extremely high voltages (15kV, 20kV) was remarkably decreased and cell viability by electrospray was fine up to 10kV.

Consequently, Live/Dead assay showed visual information of cell viability. However, WST assay was also performed to get more numerical data of cells viability.

5.1.3. WST-1 assay after bio-electrospray

WST assay was carried out after 1 day to bio-electrospray (Fig. 10). The results of this experiment was similar to Live/Dead assay. In voltage over 15kV, The cell viability was decreased sharply.

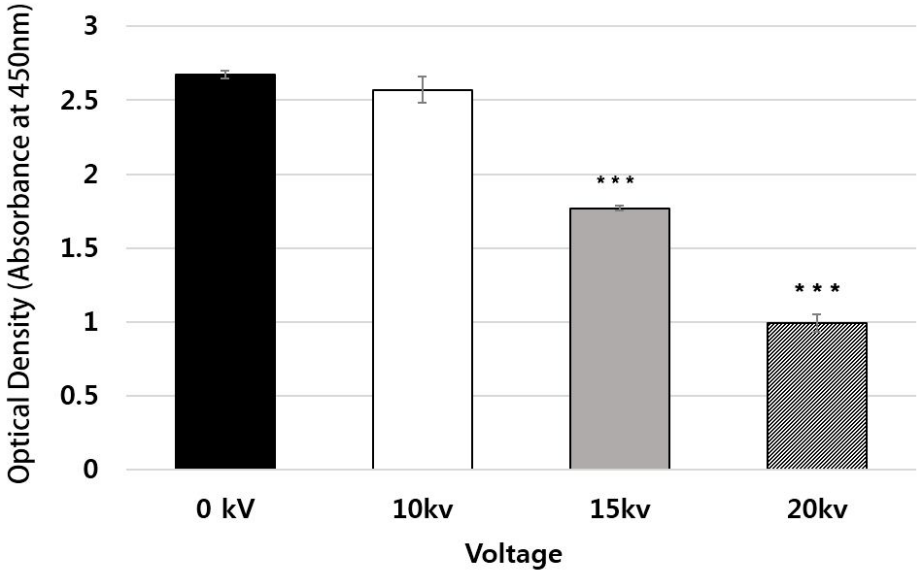

Figure 11. WST assay after bio-electrospray from 0kV to 20kV. Case of 0kV is negative control (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

By WST assay, cell viability was measured quantitatively. Additionally, WST assay was performed from 0kV to 10kV for more detailed analysis (Fig. 11).

In conclusion, The more voltage of electrospray elevates, the more viability of cells down. However, cell viability was good under 10kV. According to

statistic analysis, viability by bio-electrospray was no relevant difference at voltage of from 0kV to 10kV.

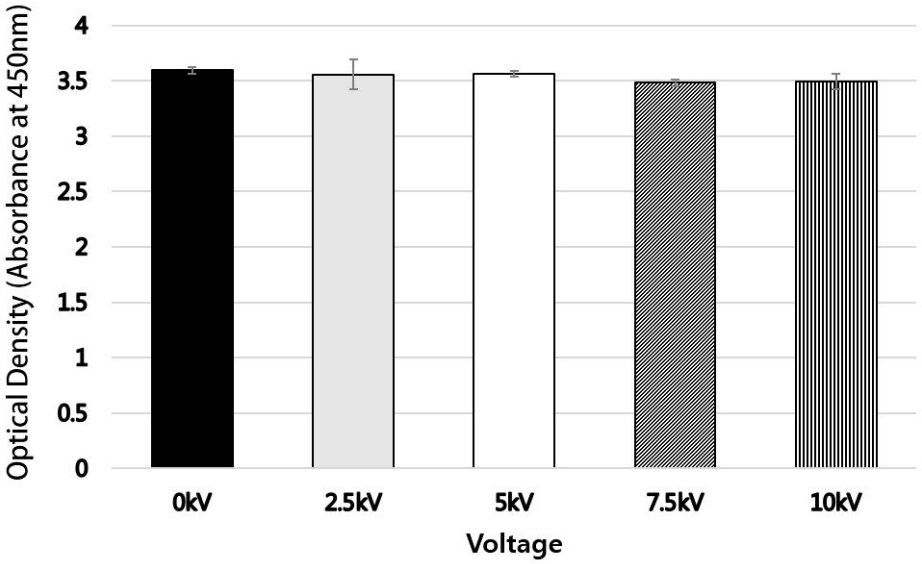

Figure 12. WST assay after bio-electrospray from 0kV to 10kV. Case of 0kV is negative control (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

5.2. The evaluation of transfection by bio-electrospray with PEI polymer

5.2.1 Transfection by bio-electrospray using several solvents

For investigating transfection level, luciferase assay and GFP expression assay were performed using several solvents (Fig. 12, Fig. 13).

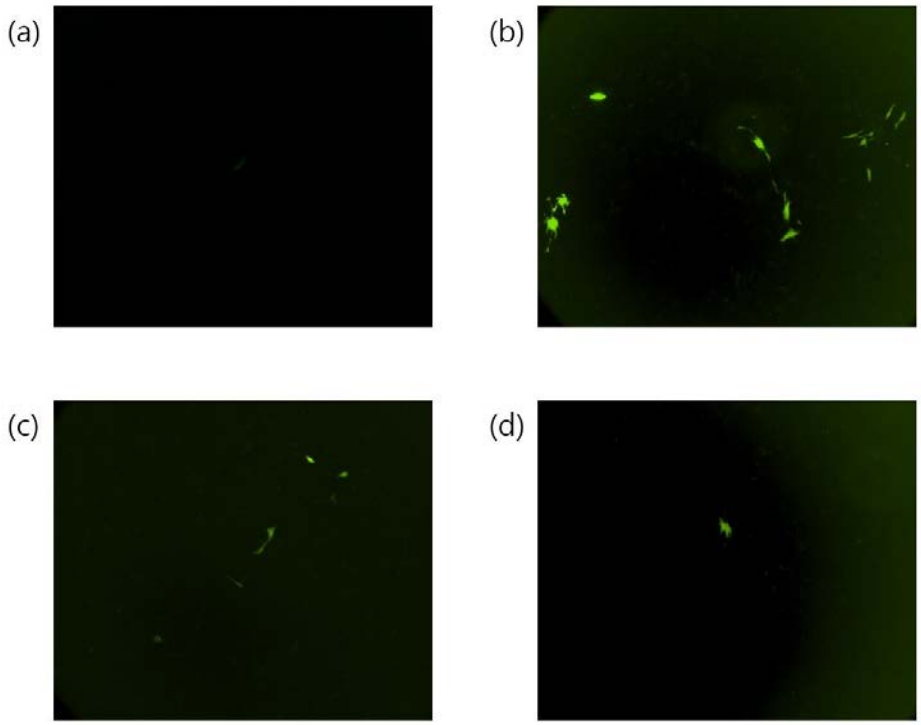

Figure 13. GFP expression using bio-electrospray with several solvents. (a) BES+DW, (b) BES+PBS, (c) BES+DMEM(serum free media), (d) BES+CM(culture media). GFP expression pictures were taken at 10x magnification.

As solvents, 1x phosphate buffer saline (PBS, Welgene Inc., Korea), deionized water (DW), Dulbeco's Modified Eagle's Medium (DMEM, Welgene Inc., Korea), and culture media (CM) were used.

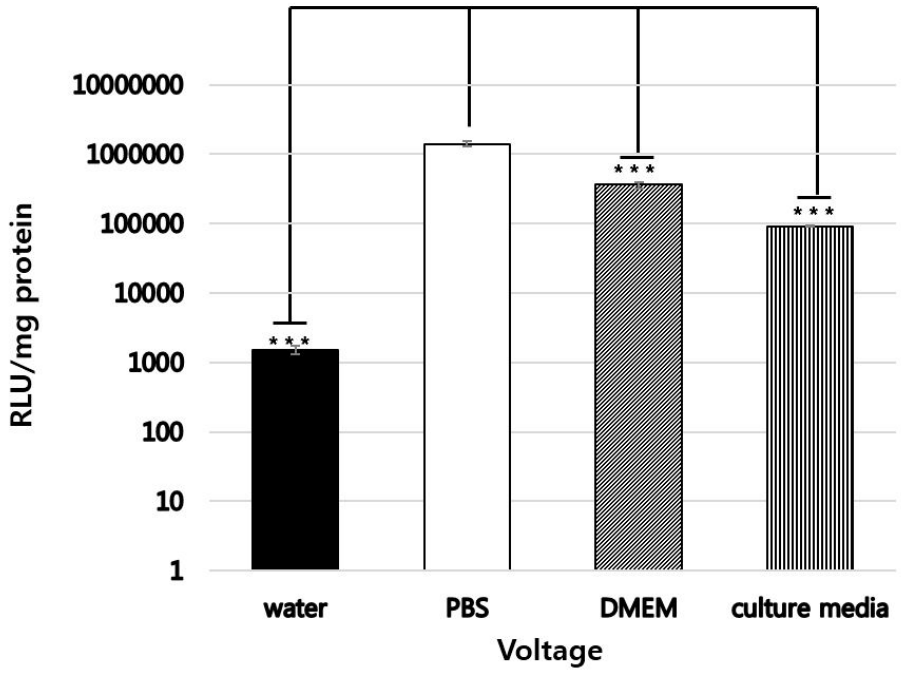

Figure 14. Luciferase activity using bio-electrospray with several solvents. A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

In GFP expression assay, case of BES+PBS showed a lot of green fluorescence than other cases overall. Moreover, luciferase assay also indicated that case of ES+PBS was better than others.

From results of GFP expression and luciferase assay, it showed that the best solvent is PBS. By statistic analysis, there is significant difference between

using PBS and using others.

We expected that case of DMEM is the highest of all cases. CM contained serum. Generally, serum interfere to combine DNA and PEI. Mixed cells and DNA-PEI complex, we thought that DMEM contribute to survive cell. However, case of PBS as solvent was highest of all cases unexpectedly. Thus, PBS as solvent was continuously used after this.

Moreover, we had a hypothesis that appropriate high voltage is good for transfection. Thus, the electrospray was performed at 10kV. Because 10kV didn’t damage cell viability. However, in order to find optimal voltage, electrospray carried out at several voltage.

5.2.2 Transfection at different voltages of bio-electrospray

At first, the transfection using electrospray was performed at 0kV, 5kV, 7.5kV, and 10kV (Fig. 14).

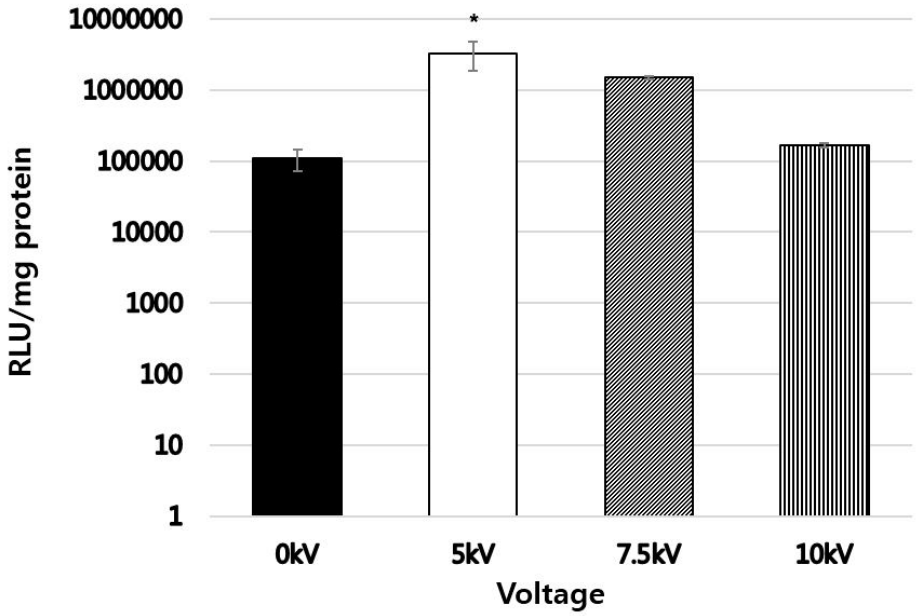

Figure 15. Luciferase assay results using bio-electrospray at from 0kV to 10kV. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

Comparing to the results of luciferase assay, transfection level at 5kV was better than other conditions. The transfection level of 5kV was about 30 times higher than control group (0kV). The case of 7.5kV was also relatively higher than control group (0kV).

To confirm optimal voltage of electrospray for transfection, additional experiment carried out. The more detailed comparison groups were set. The luciferase assay and GFP expression were used to check transfection level using electrospray (Fig. 15, Fig. 16)

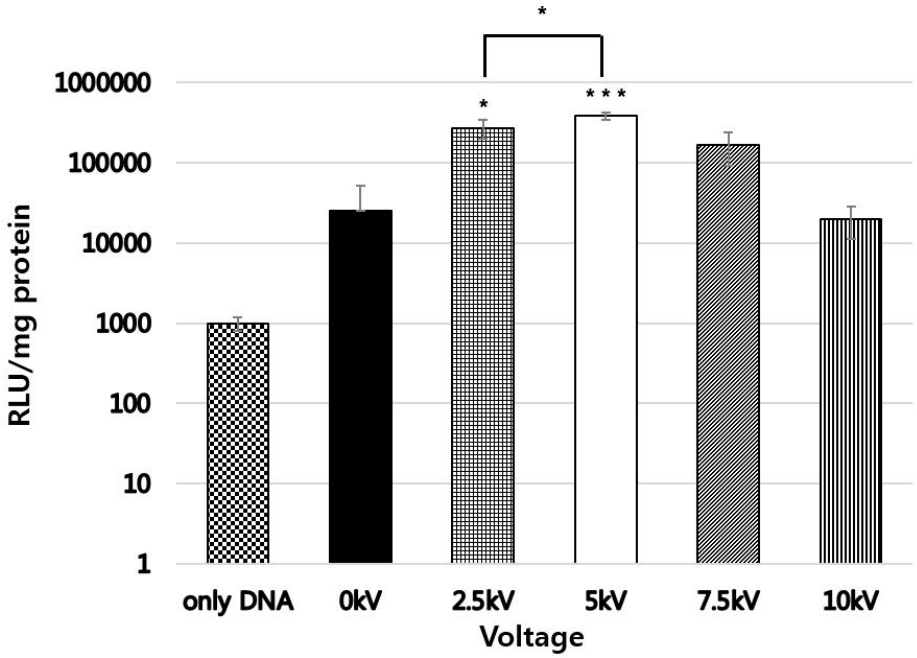

Figure 16. Luciferase assay results using bio-electrospray at more detailed voltages. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

As shown above, the gene expression from 2.5kV to 7.5kV were relatively high. Among them, case of 5kV was the highest.

From results of GFP expression and luciferase assay, it showed that the

optimal voltage of electrospray for transfection was 5kV.

By these data, hypothesis that high voltage is good for transfection was broken. The transfection level at 5kV was higher than 10kV and it was the best of all cases.

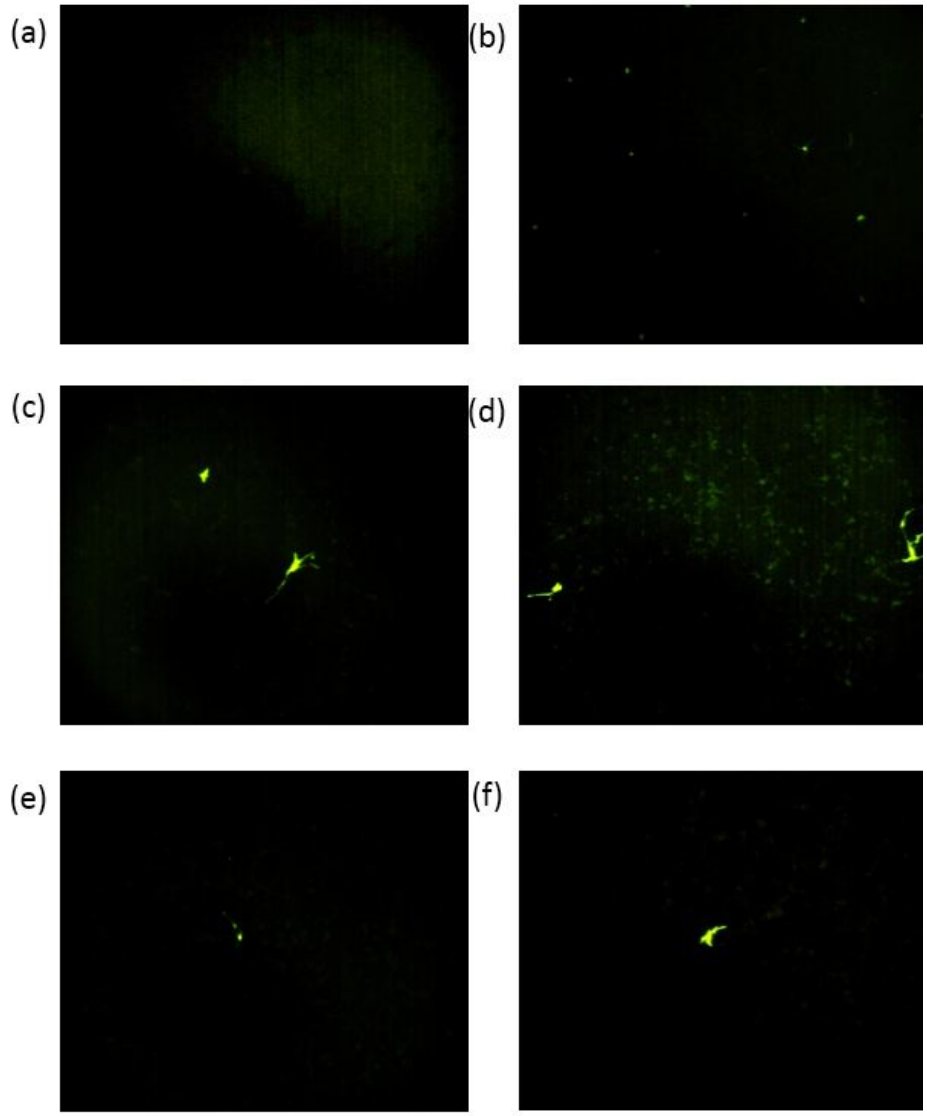

Figure 17. GFP expression results using bio-electrospray at more detailed voltages. (a) only DNA, (b) 0kV, (c) 2.5kV (d) 5kV, (e) 7.5kV, (f) 10kV. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). GFP expression pictures were taken at 10x magnification.

5.2.3. Transfection into various animal cells using bio-electrospray

In previous experiments of electrospray in this paper, NIH-3T3 cell line was used. We tried to apply bio-electrospray into various cells. In this experiment, NIH 3T3, CHO, and HeLa cells were used to bio-electrospray for transfection. In order to investigate transfection level, luciferase assay carried out. (Fig. 17, Fig. 18, Fig. 19)

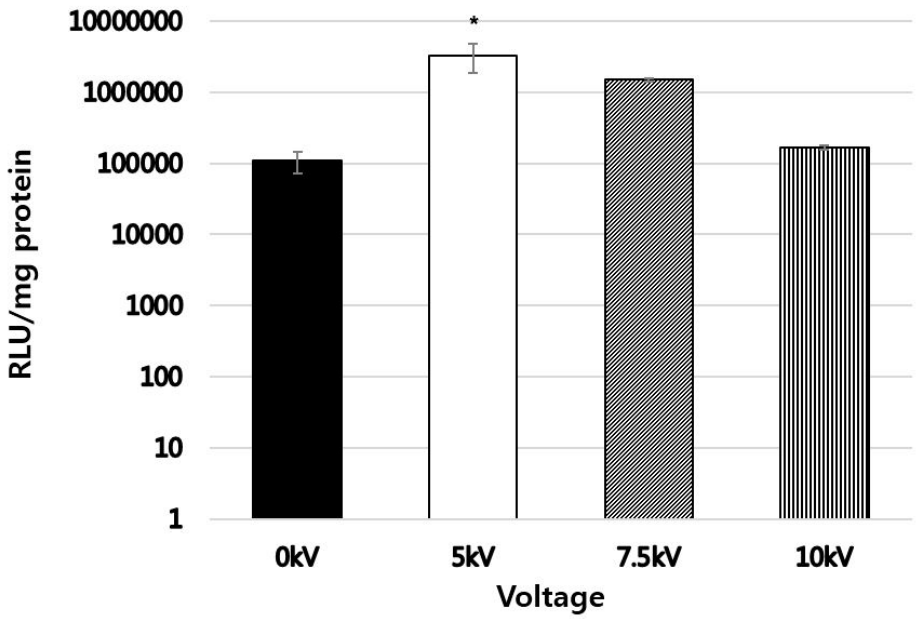

Figure 18. Luciferase assay results of bio-electrospray using NIH-3T3 cell. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

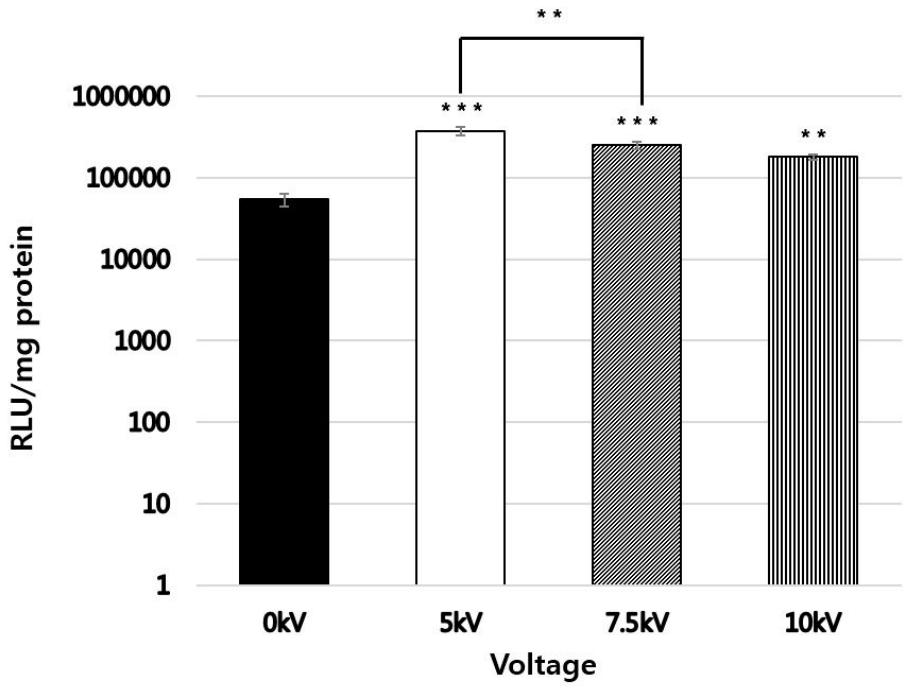

Figure 19. Luciferase assay results of bio-electrospray using HeLa cell. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

In case of HeLa cells, it was similar to the result of NIH-3T3. Transfection level by electrospray at 5kV was the highest of other cases. However, in case of CHO cells, Transfection level by electrospray at 7.5kV was higher than other cases.

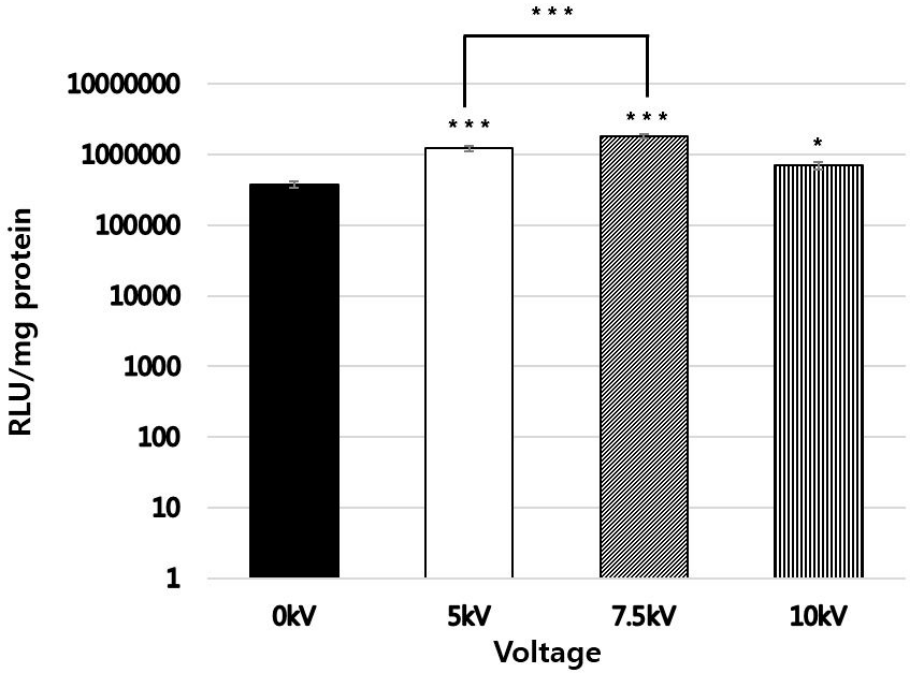

Figure 20. Luciferase assay results of bio-electrospray using CHO cell. Case of 0kV was treated by only PEI+DNA (no treat bio-electrospray). A P-value <0.05 was considered to be statically significant, n=3 (*P<0.05, **P<0.01, ***P<0.001).

6. Conclusions

The objectives of this thesis were to improve efficiency of transfection into cells using bio-electrospray (BES) with cationic polymer (PEI). In this study, we had three key questions. First is “Which solvent is used at bio-electrospray for transfection ?”. Second is “Which voltage is applied at bio-electrospray for transfection?”. Third is “Is this method can be applied another mammalian cells?”.. Followings are the main results :

1) The bio-electrospray over 10kV was fatal for cells. The viability of electrosprayed cells from 10kV to 20kV was significantly decreased. Thus, in order to apply bio-electrospray, voltage under 10kV was appropriate.

2) The electrosprayed solvent that was used to mix cell pellet and PEI solution was very important when mixture of cell and PEI was made. Because result of transfection changed according to use some solvents. As a solvent, 1x phosphate buffer saline (PBS) was the best of all.

3) Efficiency of transfection by bio-electrospray at 5kV was significantly high at NIH-3T3. Transfection level of all cases from 2.5kV to 7.5kV was higher than control group. However, case of 5kV was relatively higher than others.

Consequently, Bio-electrospray (BES) increases efficiency of transfection into cells using cationic polymer (PEI).

4) The transfection level by bio-electrospray technique could be different by type of cells. According to type of cells, It is better to adjust voltage of bio-electrospray.

5) Bio-electrospray technique for transfection could be applied to transfer gene into non adhesion cells as blood cells. Because this technique was to transfer gene into cell of float state. By transfection of bio-electrospray, we expect to make patch sprayed transformed cells and to study transfection into non adhesion cell.

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동물세포 내 유전자 도입 효율증대를 위한 바이오전기분무 시스템 개발

서울대학교 대학원

바이오시스템·소재학부 바이오시스템공학전공

이 명 철

초 록

세포 내로 유전자를 전달하기 위해 일반적으로 바이러스 벡터가 많이 이용된다. 그 이유는 바이러스 벡터를 이용할 때 유전자 전달의 효율이 매우 높기 때문이다. 그렇지만, 바이러스

벡터는 그것을 이용할 때 숙주세포가 잠재적인 병원성 유전자에 감염될 수 있는 위험성을 가지고 있다. 그래서 많은 연구자들은 합성 고분자와 같은 비 바이러스 성 벡터를 이용하거나 물리적인 방법을 이용하여 유전자 전달을 한다. 그러나 이러한 방법들의 유전자전달 효율은 바이러스 성 벡터에 비해 낮다. 이러한 낮은 효율 극복하기 위해 새로운 합성고분자를 이용하는 방법이나 물리적으로 유전자를 전달시키는 여러 방법들이 개발되어왔다.

위 논문에서는 세포 내 유전자전달 방법으로써 화학적인 방법과 물리적인 방법을 합치는 것을 시도하였다. 화학적인 방법으로써 양이온 고분자를 이용하였고, 물리적인 방법으로서는 새로운 방법을 적용하였다. 그 새로운 방법은 바이오-전기 분무법이다. 바이오-전기 분무법은 살아있는 세포를 원하는 곳에 전기 분무하는 방법이다. 우리는 만약 바이오 전기분무법과 고분자를 이용한 화학적인 방법을 함께 적용하면 세포 내로 유전자 전달효율이 증가 될 것이라고 가설을 세웠다. 그 후 세포 내 유전자도입 효율을 증가시키기 위한 방법으로써 양이온 고

분자인 폴리에틸렌이민 (PEI)을 이용한 유전자 도입 방법을 바이오-전기분무시스템과 접목시켰다.

먼저 바이오-전기분무 후 세포의 생존도를 실험하였다. 다음으로 바이오 전기분무를 할 때 세포와 DNA-PEI 복합체를 섞어 분무용액을 만들게 되는데, 이 때 어떤 용매를 이용할 때 유전자도입의 효율이 높아지는지 다양한 용매 (PBS, DW, DMEM, CM) 들을 이용하여 실험하였다. 그리고 얼마의 전압으로 전기분무를 할 때 유전자 도입효율이 높아지는 지 보기 위해 다양한 전압으로 바이오-전기분무를 하였다. 유전자 도입의 효율을 알아보기 위한 방법으로 GFP 발현확인과 Luciferase assay를 이용하였다

그 결과 바이오 전기분무 용매로써 PBS를이용했을 때 유전자 도입 효율이 다른 용매를 이용했을 때보다 높았고, 바이오 전기분무를 할 때 5kV의 전압을 가하여 주었을 때 가장 유전자 도입의 효율이 높은 것으로 나타났다.

본 연구를 통해 유전자 도입 방법의 새로운 방법을 제시하였다. 특히 혈구세포와 같은 비 부착의존성을 갖는 세포 내 유

전자 도입에 적용할 수 있을 것이다.

핵심어 유전자도입, 전기분무, 바이오-전기분무, 비 바이러스 벡터, 폴리에틸렌이민