In: Dextran: Chemical Structure, Applications … ISBN: 978-1-62948-960-5
Editor: Garrett P. Figgs

Chapter 5

APPLICATION OF DEAE-DEXTRAN TO AN EFFICIENT GENE TRANSFER SYSTEM

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ABSTRACT

Up to the present, various materials have been developed and improved for the use of DNA transfection that is required for introduction of plasmid or viral vectors into target cells. Calcium phosphate transfection, liposome-mediated transfection, and electroporation are very frequently applied to efficiently produce cell lines containing stably integrated DNA. However, the transfection efficiency and the reproducibility should be primarily considered for the practical use. DEAE-dextran-based transfection is cost-effective, highly reproducible, and can yield higher transfection efficiency in specific cells compared with other methods. The transfection with DEAE-dextran could contribute to the gene therapy by establishing a gene-transferring system into mammalian cells.

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Keywords: DEAE-dextran; DNA transfection; gene transfer; gene therapy

ABBREVIATIONS

DEAE	diethylaminoethyl
DMSO	dimethylsulfoxide
FCS	fetal calf serum
hGH	human growth hormone
IFN	interferon
iPS cells	induced pluripotent stem cells
LTR	long terminal repeat
Luc	luciferase
MLD	metachromatic leukodystrophy
MMA	methyl methacylate
MMTV	mouse mammary tumor virus
PID	primary immune deficiency disorder
QTL	quantitative trait locus
siRNA	short interference RNA
SV40	simian virus 40
WAS	Wiskott-Aldrich syndrome

INTRODUCTION

DNA transfection is the most fundamental and important method in the laboratory, in which researchers study molecular biology by introducing genes into viable cells [1]. If not for this technique, we could not have examined or even guessed the functions of specific proteins in various cells. Needless to say, it has been necessary to perform DNA transfection to generate transgenic/knockout animals [2], and to establish induced purlipotent stem (iPS) cells [3]. The progress of molecular cell biology has been accomplished by the development of the DNA-introducing technology.

In the laboratory where DNA-recombinant system is required, we usually transform bacteria to amplify DNAs with vectors, including plasmids and bacteriophages. In addition, we are able to carry out the introduction of a variety of expression vectors into cultured cells and animals. Eukaryotes are thought to have evolved from an ancestor by incorporating proteins, DNAs, RNAs, and

organelles, such as mitochondria and chloroplasts [4-6]. Therefore, we could assume that eukaryotic cells can intrinsically acquire any materials, including proteins and nucleotides that are present around or on the outer face of them. Given that the hypothesis is correct, just adding the DNAs to the cells is enough when we want to do transfection of DNAs. However, the efficiency of this method is usually a limiting and often a critical problem for delivery of desired DNAs into eukaryotic cells for biological studies. That is because our evaluating systems have limited sensitivity to detect various outcomes, including analyzing protein amount, functions, and behavior of cells. Even though we have a sensitive method to indicate functions of desired proteins by transfecting expression vectors into cells, it will not work well if the DNA transfection efficiencies were not high enough.

Up to the present, several protocols for DNA-transfection have been established and improved upon. They are mainly classified into four methods as follows [7]:

1. Calcium-phosphate based method
2. Liposome-mediated method
3. DEAE-dextran based method
4. Electroporation

To date, various derivatives have been developed from these methods. Not only reagents but also equipments for each procedure have been improved. In this article, I would focus on the DEAE-dextran based method and discuss about its application to biological sciences at present and in the future.

DNA TRANSFECTION WITH DEAE-DEXTRAN: THE BACKGROUND

DEAE-dextran (diethylaminoethyl-dextran) is a polycationic derivative of dextran, combined with diethylaminoethyl group [8]. Several biological effects have been observed when DEAE-dextran was administrated on animals and cultured cells. For example, DEAE-dextran induces production of interferon (IFN) *in vivo* [9,10], enhances cellular transformation by avian and murine sarcoma virus [11,12], and inhibits tumor growth [13]. The DEAE-dextran was utilized for introducing poliovirus RNA, SV40 and polyomavirus DNAs into cells effectively [14-16]. Negatively charged nucleotides are surrounded by positively

charged DEAE-dextran molecules, and the complex can easily access the negatively charged membrane surface of cells. Then, the DEAE-dextran-nucleotide complex is imported into cells by endocytosis.

In order to increase the transfection efficiency, DMSO (dimetylsulfoxide), chloroquine, or glycerol are frequently used as facilitators for DEAE-dextran transfection [7]. In spite of the toxicity, it has been reported to have some advantages in performing DEAE-dextran transfection. For example, double stranded plasmid DNAs that were introduced into mammalian cells by DEAE-dextran method could associate with histone proteins to form minichromosomal structures [17,18]. Thus it is advantageous to analyze biological events in nuclei, including DNA-replication, repair and transcription utilizing DEAE-dextran transfection to deliver and make plasmids assemble as non-integrated minichromosomes. Moreover, its remarkable reproducibility, cost effectiveness, and simplicity are attractive when compared with other transfection methods [19].

DNA TRANSFECTION WITH DEAE-DEXTRAN: PRACTICAL USE IN HIGHLY REPRODUCIBLE REPORTER ASSAY

A recent study, reffered as reverse transfection, demonstrated that plasmid DNAs and siRNAs, if they have been adsorbed on the solid phase prior to transfection, can be delivered to adherent cells [20,21]. Moreover, reverse transfection using RNAi coated multi-well plates with dextran, Lipofectamine, and polyvinyl alcohol, has been reported [22]. Thus, application of these reverse transfection techniques on the reporter assay will enable us to study transcription mechanisms by a high-throughput analysis. We have developed a simple and cost-effective protocol to carry out DEAE-dextran mediated transfection of plasmid vectors into cultured mammalian cells [23]. When necessary to introduce multiple DNA vectors into cultured cells, this new protocol will reduce both expense and time but also perform with high reproducibility. Here, we describe the simple method as follows:

Preparation of Reporter Plasmids

The double-stranded plasmid vectors to be transfected into cultured cells need to be purified before use. This can be easily done using commercially available

column-purification systems. The purity of the plasmids should be checked by electrophoresis in an agarose gel. The concentration needs to be estimated by the absorbance of 260 nm. Plasmid DNAs (0.5-2 μg/μL) are dissolved in distilled water and stored at -20 ^{O}C.

Materials

1. Solution containing reporter plasmid:
   Plasmid DNAs that are to be adsorbed on the multi-well plates are diluted (50 ng/μL) before use.
2. DEAE-dextran:
   DEAE-dextran (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) is dissolved in distilled water and sterilized by filtration. The solution (5 μg/μL) can be stored at 4 ^{O}C at least two years.
3. TBS (Mg^{2+},Ca^{2+}):
   25 mM Tris-HCl (pH 7.4), 137 mM NaCl, 5 mM KCl, 0.6 mM Na_2HPO_4, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$
4. Multi-well cell culture plates:
   Sterile, disposable plastic-made 96-well cell culture plates with flat bottoms are used.
5. Cell culture medium (DME or RPMI 1640) containing 10% FCS

Simple Protocol to Introduce DNAs into Cultured Cells

Simple protocol to perform multiple DEAE-dextran based transfection is schematically shown in Figure 1.

1. Luc-reporter plasmid (100 ng) and DEAE-dextran (10 μg) is spotted onto each well and dried completely under vaccum (Figure 2). The DEAE-dextran/DNA adhered plates can be stored at 4 ^{O}C for at least ten months in a desiccated condition.

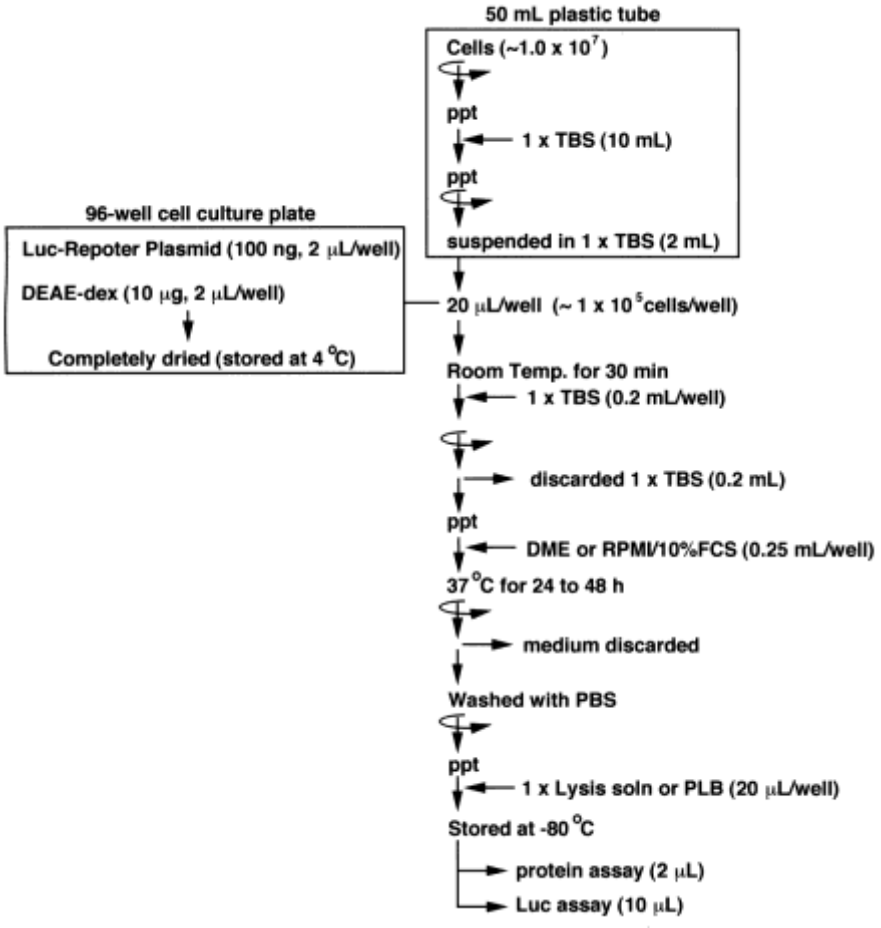

Figure 1. Multiple DEAE-dextran based transfection of Luc reporter plasmids.
Prior to the transfection, Luc-reporter plasmid/DEAE-dextran adhered multi-well plate should be prepared. Usually, solution (2 μL) containing Luc-reporter plasmid and DEAE-dextran is separately spotted on the bottom of each well. Each well needs to be completely dried.

2. Cells (1 x 10^7) are washed with 10 mL of TBS and then suspended in 2 mL of TBS (containing 1 μg of pGL4.74[hRluc/TK] vector [Promega, Madison, WI, USA], for dual assay). An equal number of cells (1 to 2 x 10^5) is dispensed into aliquot each DNA/DEAE-dextran treated well and left for 30 min at room temperature.
3. TBS (200 μL) is added and centrifuged for 5 min at room temperature.

4. The supernatant is discarded and the cells are cultured for 24 h in complete medium (DME or RPMI 1640) containing 10% FCS (250 μL).
5. Luc and dual-luc assays are performed according to the manufacturer's instruction manual (Promega). Luc activities can be normalized by the protein amount or renilla-luc activities.

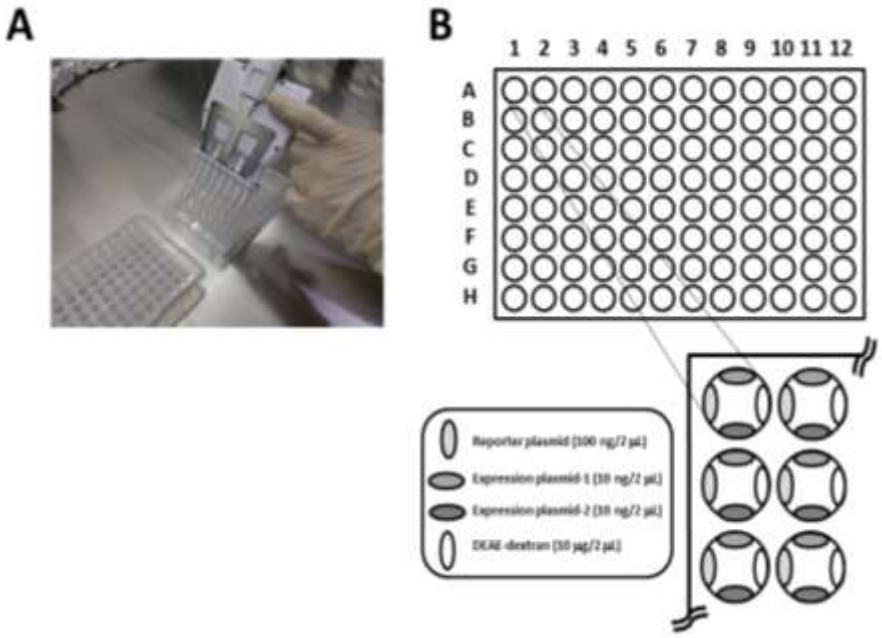

Figure 2. Preparation of DEAE-dextran/DNA-adhered 96-well plate.
(A)Each Luc reporter plasmid (100 ng/2 μL) is spotted onto the left side bottom of each well.
(B)A 96-well plate is schematically shown. DEAE-dextran (10 μg/2 μL) is spotted onto the right side bottom of each well. The front and rear sides can be used for spotting specific gene-expression plasmids.

Luc activity was detectable in the sample extract from HeLa S3 and Jurkat cells that were transfected with pGL3-Promoter vector (Promega) [23]. We have reported simplicity, reproducibility and cost-effectiveness of the multiple DEAE-dextran transfection method [23-25].

DNA TRANSFECTION WITH DEAE-DEXTRAN: APPLICATION TO MEDICINE

Presently, most gene therapies are carried out with viral vector systems to express genes that are deficient in the patients [26]. The procedure is simply started with isolation of cells from a patient, and then viral infection *ex vivo* is executed [27]. The last step is to return the cells to the patient. In this way, gene therapy by introducing lentiviral vectors to hematopoietic stem cells from Wiskott-Aldrich syndrome (WAS) and metachromatic leukodystrophy (MLD) patients was recently reported to have clinical benefit [28,29]. Introduction of viral vectors *in vivo* or *ex vivo* will successfully integrate the desired genes into chromosomes and their expression will be expected to be high enough to achieve therapeutic benefit for primary immunodeficiency disorders (PIDs) [30]. This therapeutic concept will soon be applied to a regenerative medicine transferring a gene(s) into iPS cells. Despite these advantages, it is necessary to take into account for the integration sites of viral vectors on the chromosomes. For example, recent studies showed that mouse mammary tumor virus (MMTV) is the causative agent of human breast cancer [31]. Furthermore, it is well known that integration of retroviruses into chromosomes eventually causes leukemia or tumors [32-34]. The long terminal repeat (LTR), which acts as a strong promoter, could be a causative agent for leukemia or cancer when it is integrated close to functional cellular genes that are associated with cellular growth or cell cycle progress. Hence, careful monitoring the viral vector-integrated sites by genomic analysis should be done when retroviral vector systems are utilized for gene therapy.

Although DEAE-dextran has a toxic effect on cell viabilities [7], it is expected to be applied on a gene therapeutic use. Human growth hormone (hGH) gene-expression plasmid has been successfully introduced to a guinea pig mammary gland by the DEAE-dextran method [35]. The procedure enabled delivery of plasmid DNA into vascular smooth muscle cells that were derived from the thoracic aorta of rat [36]. These results suggest that DEAE-dextran method could be applied to *ex vivo* gene transfer therapy. Methyl methacylate (MMA) modification has been introduced to DEAE-dextran to generate DEAE-dextran-MMA and its utility in non-viral gene transfer was examined [37,38]. In addition, 5-azacytidine enhances effect of the DEAE-dextran to deliver plasmid DNA into macrophages [39]. These lines of evidences suggest that DEAE-dextran or its derivatives may contribute to establishing an advantageous method to transfer non-viral vectors into cells.

At present, however, the multiple throughput DEAE-dextran based transfection method would be possibly applicable for evaluating the transcriptional ability in the isolated cells from animals, including human iPS cells. The protocol consists of only three steps as follows:

1. Isolation of cells from a patient and a healthy control.
2. DEAE-dextran based DNA transfection is carried out to introduce various promoter-driven reporter plasmids.
3. Reporter assay is carried out and results are evaluated to estimate transcription ability profile.

The results will show which *cis*-element is active or inactive in the (iPS) cells from a patient. For example, if the (Luc) reporter activity is extremely high in the patient cells that were transfected with GC-box containing reporter vector, Sp1 or GC-box binding transcription factors are activated. Multiple analysis *cis*-element or their combinations will show the transcriptional state in the cells from patient. This analysis may contribute to establish a diagnosis of a specific disease(s) if it was already known that a specific *cis*-element is either activated or suppressed.

CONCLUSION

In this article, we have reviewed past, present and the future of the DEAE-dextran based DNA transfection technique. This method has been developed to introduce DNAs into cultured mammalian cells, including COS cells [40]. Despite its toxicity on viability of cells, this non-viral vector transferring system is preferred if the transfection efficiency is higher than other method. The toxicity could be reduced by the modification or improvement of the material reagent. Although, recent gene therapy has been progressed with the development of viral vectors, careful attention is needed if the integration of the LTR in the chromosomes activates cellular genes that drive cellular functions, including proliferation, cell cycle control, apoptosis, or signal transduction systems. Therefore, the non-viral and minichromosomal vector introducing system will be an alternative gene therapy when the effect of the LTR should not be ignored.

Recently, diagnosis of cancer and diseases that are thought to be occurred from genomic alterations is carried out with the second-generation sequencing [41]. Moreover, recent studies on genomics showed alterations in gene expression in many human diseases [42,43]. Very recently, it was reported that the transcriptome indicating *cis*- quantitative trait loci (QTLs) is valuable in revealing

gene expression and transcript state in cells from patients of specific disease [44]. The DEAE-dextran based DNA transfection/reporter assay, which is principally different from the transcriptome and genome sequencing analysis, could monitor transcriptional profile in nuclei. The method could be also applied to the reporter assay to examine a specific *cis*-element(s) is activated or suppressed in iPS cells. The results will in turn show which gene expression should be modulated by a gene therapy.

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