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Evaluation of Lentiviral Based Gene Delivery System in Adherent and Suspension *in vitro* Cell Models

(Penilaian Sistem Angkut Serang Gen Berasaskan Lentivirus dalam Model Sel Adheren dan Ampaian secara *in vitro*)

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ABSTRACT

Lentiviruses are a highly robust gene delivery system capable of *in vitro* and *in vivo* gene transfer into multiple cell types. Recent fourth-generation lentiviral systems have been designed for enhanced safety, however, the increased recombination events required to produce infective lentiviral particles may reduce production efficiency. A set protocol for all types of target cells is not recommended and optimization of conditions for gene transfer into different target cells is required. In this study, we aim to evaluate the efficiency and reproducibility of lentiviral production using a fourth-generation lentiviral packaging system and identify optimal parameters for successful transduction in two different cell models, adherent and suspension cells. Lentiviral production, effect of viral volume, sustained gene expression and transduction adjuvants on adherent and suspension gene- cell models were evaluated. Transfection and transduction efficiency of lentiviruses was evaluated by fluorescence microscopy and flow cytometry. This study demonstrates that production of green fluorescent protein (GFP)- lentiviruses using the fourth-generation lentiviral packaging is consistent and reproducible. Optimal transduction of adherent cell types is achieved at lower multiplicity of infection (MOI) compared to suspension cells and produces GFP-expressing cells with higher intensity. Expression of GFP is sustained in all cell types over multiple passages. Polycation DEAE-dextran was determined to improve transduction in suspension cells, however, provides similar transduction efficiency as polybrene in adherent cells. In conclusion, fourth generation lentiviral system reproducibly generates high titre lentiviruses capable of infecting multiple cell types, however transduction protocols for different cell types require further optimization.

Keywords: Gene transfer; *in vitro* model; lentivirus

ABSTRAK

Lentivirus merupakan kaedah pemindahan gen yang sangat teguh yang mampu memindahkan gen secara *in vitro* dan *in vivo* kepada pelbagai jenis sel. Sistem lentivirus generasi keempat yang terbaharu, direka untuk mempertingkatkan keselamatan, walau bagaimanapun, bilangan keadaan rekombinasi yang diperlukan bagi menghasilkan zarah lentivirus berjangkit boleh mengurangkan kecekapan pengeluaran. Protokol yang ditetapkan untuk semua jenis sel tidak digalakkan dan pengoptimuman keadaan bagi pemindahan gen ke dalam sel sasaran yang berbeza diperlukan. Dalam penyelidikan ini, kami berhasrat untuk menilai kecekapan dan kebolehulangan pengeluaran lentivirus menggunakan sistem lentiviral generasi keempat dan mengenal pasti parameter optimum bagi transduksi yang berjaya dalam dua model sel yang

berbeza iaitu sel adheren dan sel ampaian. Penghasilan lentivirus, kesan isi padu virus, pengekspresan gen yang berterusan dan adjuvan transduksi pada model sel adheren atau ampaian telah dinilai. Kecekapan pemindahan dan transduksi lentivirus dinilai oleh mikroskop pendarfluor dan sitometri aliran. Kajian ini menunjukkan bahawa pengeluaran lentivirus *'green fluorescent protein'* (GFP) menggunakan pembungkusan lentiviral generasi keempat adalah tekal dan boleh dihasilkan semula. Transduksi optimum jenis sel adheren dicapai pada kegandaan jangkitan (MOI) yang lebih rendah berbanding sel ampaian dan menghasilkan sel yang mengekspresi GFP dengan keamatan yang tinggi. Ekspresi GFP dikekalkan dalam semua jenis sel yang melalui pemindahan. *Polycation* DEAE-dextran didapati telah meningkatkan transduksi dalam sel ampaian, walau bagaimanapun, ia menghasilkan kecekapan yang sama seperti polibren dalam sel adheren. Kesimpulannya, sistem lentivirus generasi keempat mempunyai kebolehulangan dalam menghasilkan titer lentivirus tinggi yang mampu menjangkiti pelbagai jenis sel, namun protokol bagi transduki bagi jenis sel berbeza memerlukan pengoptimuman selanjutnya.

Kata kunci: *in vitro*; lentivirus; pemindahan gen

INTRODUCTION

Many gene delivery methods have been developed for gene delivery including viral vectors such as lentiviruses, offering several advantages over other gene transfer methods. Lentiviruses can infect quiescent, slow dividing and non-dividing cells which include difficult-to-transfect cells such cardiac-derived cells, hematopoietic stem cells, Schwann cells, neurons, and glial cells (Jang et al. 2020; Kalidasan et al. 2021; Li et al. 2020). Additionally, lentiviral gene transfer allows transfer of large gene constructs and integration into host cell genome, producing long-term transgene expression (Ferreira, Cabral & Coroadinha 2021; Quinonez & Sutton 2002). Lentiviral technology has undergone various modifications in vector design over the last decade. Third-generation lentiviral vector systems are mostly used in pre-clinical and clinical gene transfer applications (Kumar & Woon-Khiong 2011; Milone & O'Doherty 2018). Significant modifications have been made not only to enhance gene transfer ability, but also increase safety during viral production. A major concern in producing lentiviruses is the generation of Replication Competent Lentiviruses (RCL). Biosafety aspect of viral production has improved significantly by removal of non-critical viral accessory proteins, retaining only a fraction of parental HIV genome (Dull et al. 1998). The latest generation of lentiviral systems utilizes a 'split-packaging' approach, separating critical components of viral genome required in viral production into distinct plasmids (Wu et al. 2000). The separation of viral sequences in the packaging plasmids of current fourth generation lentiviral packaging systems offers enhanced safety; however, it may risk reduced efficiency in lentiviral production due to the increased recombination events required to produce infective lentiviral particles.

Gene delivery into various cell types has been shown to be achieved by incorporating vesicular stomatitis virus (VSV G) pseudotype envelope protein (Cronin, Zhang & Reiser 2005). Expression rate of the transferred gene could be intensified by the use of cytomegalovirus (CMV) promoters in the 5'LTR (Ailles & Naldini 2002). Despite these modifications, many factors may significantly affect the efficiency of successful transduction in different cell types. The volume of viruses transduced may affect efficiency of gene transfer and expression of the gene in the target cells. Addition of transfection reagents during transduction such as lipofection reagents or polycationic agents diethylaminoethyl (DEAE)-dextran and polybrene work by overcoming the electrostatic repulsion forces between negatively charged cells and viral vectors, although efficacy and toxicity during transfection is dependent on cell type (Swaney et al. 1997; Wang et al. 2018). Ultimately, combinations of several strategies such as combining lipofectamines and viral concentration by spin-column methods accompanied by simple procedural modifications has been shown to increase successful transduction rates (Pirona et al. 2021). However, few studies comparing the effect on efficiency of lentivirus transduction in established cell lines and primary cultures have been carried out.

This study utilizes adherent dermal fibroblast cell lines and suspension peripheral blood monocyte cell lines as models for lentiviral-based gene delivery. Since there is a broad potential for developing a lentiviral based system capable of targeting multiple cell types, there is a need to evaluate protocols for producing highly quality lentiviruses and identify factors that may affect successful gene transfer. This study aims to evaluate the efficiency and reproducibility of fourth-generation lentiviral packaging systems to generate high titre, infective lentiviruses. We determined the effect of multiplicity of infection (MOI) and use of two commonly used adjuvants on optimal transduction in target cells. This study demonstrates the ability of lentiviral transduction in sustaining gene expression over multiple passages.

METHODS AND MATERIALS

CELL LINES

LentiX-293T cells (Takara Bio) were maintained in complete DMEM medium (4 mM Glutamax, 4.5 g/L D-Glucose, 0.1 mM MEM non-essential amino acid, 1 mm sodium pyruvate (Gibco)) containing 10% fetal bovine serum (FBS (Gibco)). BJ (ATCC) human neonatal foreskin fibroblasts were maintained in complete DMEM medium supplemented with 10% FBS. U937 (ATCC) cells were maintained in complete RPMI 1640 medium (2 mM L-glutamine, 2 g/mL D-Glucose) supplemented with 10% FBS.

VIRAL PRODUCTION AND TITRATION

The lentiviral construct pLenti CMV GFP Hygro (656-4) was a gift from Eric Campeau and Paul Kaufman (Addgene plasmid #17446; http://n2t.net/addgene:17446; RRID:Addgene_17446) was packaged into lentiviral particles using Lenti-X Packaging Single Shots Protocol (Takara Bio USA #631275). To produce the fourth-generation lentiviruses, LentiX-293T cells at 80% confluency was co-transfected with the packaging plasmids and 7 μg of vector plasmid in complete DMEM medium. Transfection medium was changed after overnight incubation with fresh complete DMEM medium, and the viral supernatant was collected 36 and 72 h after transfection, respectively.

The supernatant collected at the two time points were combined and centrifuged to remove cell debris. The viral containing supernatant was filtered using a 0.4 μm syringe filter (TPP). Half of the filtered viral containing supernatant was aliquoted and frozen at -80 °C until further use. For concentration, lentiviruses were concentrated using Lenti-X Concentrator (Takara Bio) according to manufacturer's instructions. After removal of supernatant, the remaining pellet was resuspended in serum-free DMEM medium at 1:100 times original volume. Concentrated virus was aliquoted and stored at -80 °C.

To establish viral titres, LentiX-293T cells were plated at 7.5×10^4 cells per well in a 12-well tissue culture plate, allowed to adhere overnight. Adhered cells were incubated with complete DMEM medium, containing serially diluted concentrations of lentiviruses. Next day, equal amounts of fresh medium were added, and cells were incubated for an additional 48 h. Adherent cells were washed with PBS (Gibco) and trypsizned before resuspension in 1% paraformaldehyde (PFA (Sigma)). Fixed cells were analysed by flow cytometry (FACSCanto, BD Bioscience) by measuring expression of Green Fluorescent Protein (GFP). Positively expressing cells were measured based on negative control indicated by non-transduced cells.

Viral titres were calculated according to the following equation: (number of target cells on day 1 × % of GFP positive cells)/volume of viral preparation. Viral titres were calculated only from transductions with dilution factors resulting in between 5% and 20% GFP expressing cells. This was to ensure maximum accuracy and compensate for multiple transduction events per cell. Figures show the results from five independent experiments.

TRANSDUCTION OF CELL LINES

Cell lines were plated in complete medium containing 10% FBS. Adherent cell lines were allowed to attach to the wells overnight. Viruses were added at the indicated multiplicity of infection (MOI) in the presence of adjuvant at indicated concentrations and incubated for 24 h. After 24 h, the viral containing medium was removed and replaced with fresh complete medium and incubated for an additional 48 h. For microscopic analysis, GFP expression of live cells were viewed using CK40 microscope (Olympus) for endogenous GFP expression. Images were processed using ImageJ software.

For flow cytometry analysis, adherent cell lines were washed with PBS and trypsinized. LentiX-293T, BJ and U937 cells were collected using centrifugation and fixed with 1% PFA. Fixed cells were analysed by flow cytometry, FACS Canto, BD, USA. Positive expressing GFP cells were determined based on negative control consisting of non-transduced cells grown in similar tested conditions. Figures show the results of triplicate wells from one representative experiment.

For analysing the expression of GFP in transduced cells over prolonged time and multiple passages, transduced cells were passaged and plated at 1:4 ratio when reaching confluency at indicated time points. Remaining cells were fixed with 1% PFA prior to flow cytometry analysis.

DATA ANALYSIS

Data significance was assessed using two-way analysis of variance (ANOVA) followed by Sidak's multiple comparison test unless otherwise stated. Student's t-test was used to analyse the significance of viral concentration. A standard level of statistical significance p=0.05 was used. Figures were generated using GraphPad Prism 9.4 program (GraphPad Software Inc).

RESULTS

PRODUCTION OF LENTIVIRUSES AND DETERMINATION OF VIRAL TITRE

To establish an efficient gene delivery system, a fourth-generation lentiviral based vector system which has been

shown to provide high gene transduction efficiency was used (Wu et al. 2000). To assess the transfection efficiency of the tested lentiviral packaging system, fourth-generation lentiviral system and pLenti-CMV-GFP transfer plasmid carrying the green fluorescent protein (GFP) marker were co-transfected into LentiX-293T cells. Visualization under fluorescent microscope showed high expression of GFP in transfected cells (Figure 1(a)). After visualization of the cells, cells were trypsinized and measured for transfection efficiency by flowcytometry analysis of the total cell population. Five separate transfections produced 97.56% cells expressing GFP (min: 95.7, max: 99.1, sem: ±0.625) with an average of mean fluorescence index of 4×10^5 FU (fluorescence units) (Figure 1(b)).

Viral containing supernatant was collected at 48 and 72 h post transfection. Half of the supernatant was concentrated using chemical-based concentration. The resulting viral pellet was resuspended and used for transducing target cells. Functional titer was determined using flow cytometry analysis of LentiX-293T cells transduced with serial dilutions of unconcentrated and concentrated viral particles. 72 h after transduction, cells were analyzed by flowcytometry analysis. The threshold of fluorescence for flow cytometry results was determined according to the negative control represented by non-transduced cells. Viral titer was calculated using transduction of viral dilutions producing between 5-20% GFP expression and measured in transducing units/mL (TU/mL). Mean lentiviral titer following lentiviral transduction of unconcentrated and concentrated lentiviral suspension was 5.65×10^5 TU/mL (n=5, min: 5.14×10^5 TU/mL, max: 5.96×10^5 TU/mL, sem: $\pm 1.6 \times 10^4$) and 1.90×10^7 TU/mL (n=5, min: 1.59×10^7 TU/mL, max: 2.38×10^7 TU/mL, sem: $\pm 1.5 \times 10^6$) respectfully (Figure 1(c)). Viral concentration significantly increased amount of infective viral particles per volume ($p<0.05$). Next, lentiviral transduction on target cells was tested using concentrated lentiviruses produced from a single transfection experiment.

INCREASING MOI INCREASES TRANSDUCTION EFFICIENCY IN TARGET CELLS

The effect of increasing the number of viruses used when transducing cells was tested as to determine the optimal MOI to achieve appropriate levels of transduction. Viral titre obtained by flowcytometry analysis of transduced LentiX-293T cells was to calculate the MOI. Subsequently, LentiX-293T cells, BJ fibroblasts and U937 cells were transduced with lentivirus-GFP at MOI 1, 2, 5, 10 and 15 in standard conditions (serum-containing medium supplemented with polybrene overnight).

In LentiX-293T cells, evaluation under phase contrast microscopy showed that cultures transduced with a higher MOI increased cell debris and reduced cell confluency on day-3 post transduction. Intensity of GFP expression was increased from MOI 1 until MOI 10 and slightly decreased at MOI 15. BJ fibroblasts showed similar trend in transduction efficiency up to MOI 10 and sustained expression at MOI 15. Minimal cell debris was seen with no difference in cell confluency was seen in BJ cells across all tested MOIs. Fluorescent microscopic analysis of U937 cells showed low transduction efficiency until MOI 10 (Figure 2(a)).

After visualization, cells were harvested and measured for transduction efficiency by flowcytometry analysis. All cells showed an increase in GFP-expression with increasing MOI, with BJ cells showing highest transduction efficiency with maximum expression of 93% GFP expressing cells at MOI 10. Similar trend was seen in LentiX-293T cells with maximum expression of 85% GFP expressing cells. U937 cells showed lower transduction efficiency (41%) compared to the other tested cell lines with minimal increase in GFP expression from MOI 1 to MOI 10 (Figure 3(a)).

Intensity of GFP expression was also determined by flowcytometry analysis, measured by the relative mean fluorescent index (MFI), determined according to the negative control represented by non-transduced cells. Relative MFI of BJ and 293T cells were similar, with no significant increase in relative MFI from MOI 5 to 10. U937 cells showed the lowest relative MFI with no significant changes with increasing MOI ($p<0.05$) (Figure 3(b)).

LENTIVIRUS TRANSDUCTION OFFERS SUSTAINED EXPRESSION OF THE TRANSGENE

Most cell lines are immortalized and can proliferate for an undefined time. We tested whether transgene expression would last be following serial passaging over a set period of time. 293T, BJ fibroblasts and U937 cells were transduced with the lentiviruses-GFP at MOI 2 under standard conditions (serum-containing medium supplemented with polybrene overnight or for 8 hours). Transduced 293T, BJ, and U937 cells were passaged and cultured for a period of 21 days, and the samples were subjected to flowcytometry analysis. As shown in Figure 3(c) and 3(d), the expression of GFP was sustained for the entire period of the study in all tested cell lines despite serial passaging over the 3-week period. We also found that the intensity of GFP expression did not change over the time course.

TRANSDUCTION EFFICIENCY IS IMPROVED WITH DEAE-DEXTRAN IN SUSPENSION CELL LINE

Another potential factor that may affect the transduction efficiency of transduced cells is the adjuvant used during transduction. The effect of commonly used adjuvant

polybrene and DEAE-dextran were tested during transduction on BJ fibroblast and U937 cells under standard culture conditions (MOI 2 in serum-containing medium overnight or for 8 h). 72 h following transduction, cells were collected, and transduction efficiency was determined by flowcytometry analysis of the total cell population.

Increasing concentrations of either polybrene or DEAE-Dextran between 2 ug/mL to 12 ug/mL improved transduction efficiency in both BJ cells (Figure 4(a) and U937 (Figure 4(b)). In BJ fibroblasts, transduction with polybrene at lower concentrations significantly improved transduction compared to DEAE-dextran. However, no significant difference was seen in BJ fibroblasts when transduced with either tested adjuvant at higher concentrations.

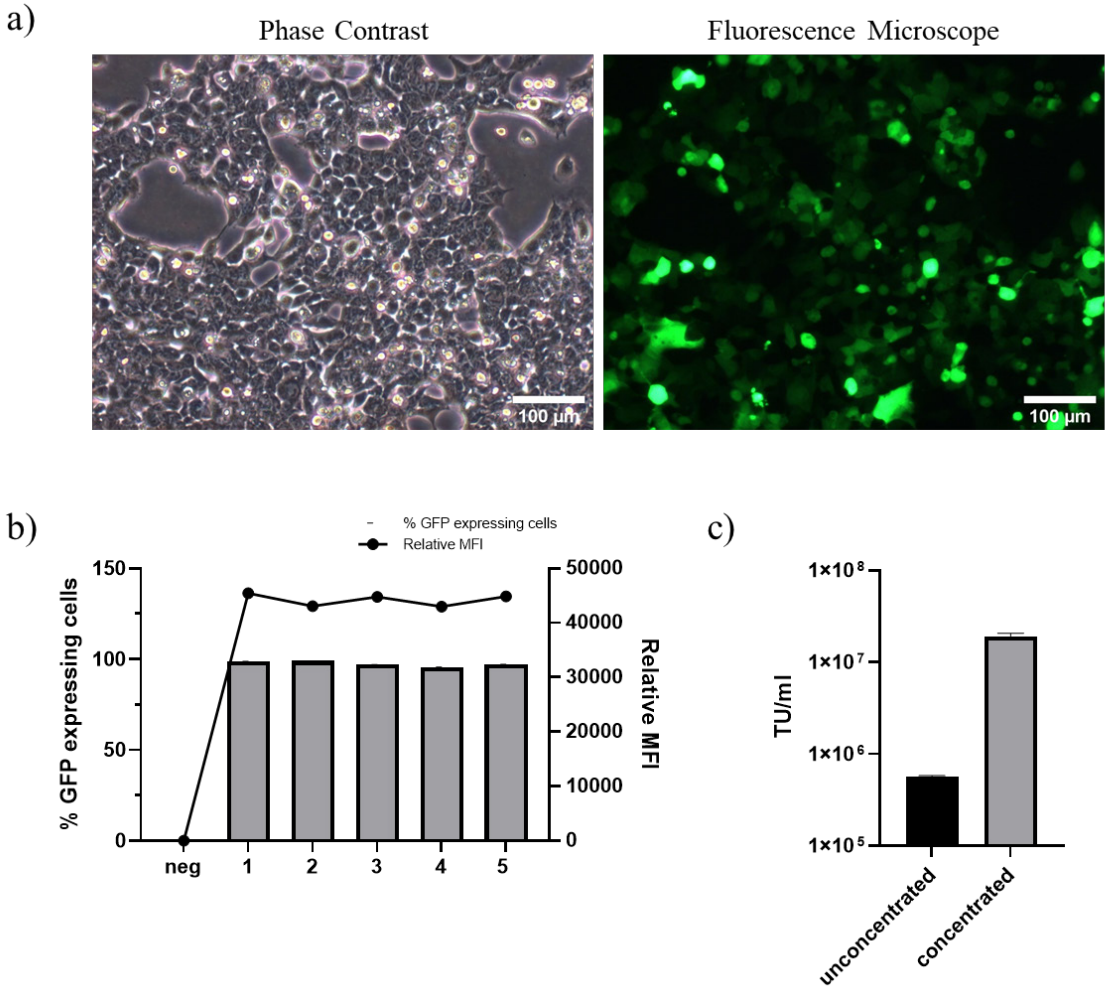

FIGURE 1. Transfection efficiency for fourth-generation lentiviral packaging system. (a) Phase contrast and fluorescence images of LentiX-293T cells co-transfected with fourth-generation lentiviral system and pLenti CMV GFP Hygro vector plasmid at 48 h post transfection. Scale bar = 100 µm. (b) Flow cytometry analysis of GFP expression and intensity in transfected LentiX-293T cells 72 h post-transduction in five separate transfection experiments. (c) Viral titre determined by flow cytometry analysis of LentiX-293T cells transduced with either unconcentrated or concentrated viral preparations (n=5). Positive gated cells and relative mean fluorescence index were determined based on negative control (non-transduced cells) grown and passaged under similar medium conditions. All values are mean ± s.e.m.

DEAE-dextran mediated transduction in U937 cells showed higher efficiencies compared to standard polybrene at all concentrations. No changes in cell viability, determined by trypan blue exclusion was seen with increasing concentration of either adjuvant in any tested cell line (Figure 4(c) & 4(d)).

DISCUSSION

In this study, the evaluation of a new lentiviral packaging system was carried out along with an assessment of factors that may affect the efficiency of transduction with lentiviral systems in two different cell models, adherent and suspension cells as illustrated in Figure 5. First, the transfection efficiency in LentiX-293T cells that act as producer cells to assemble and generate viral particles was established before transduction was optimized.

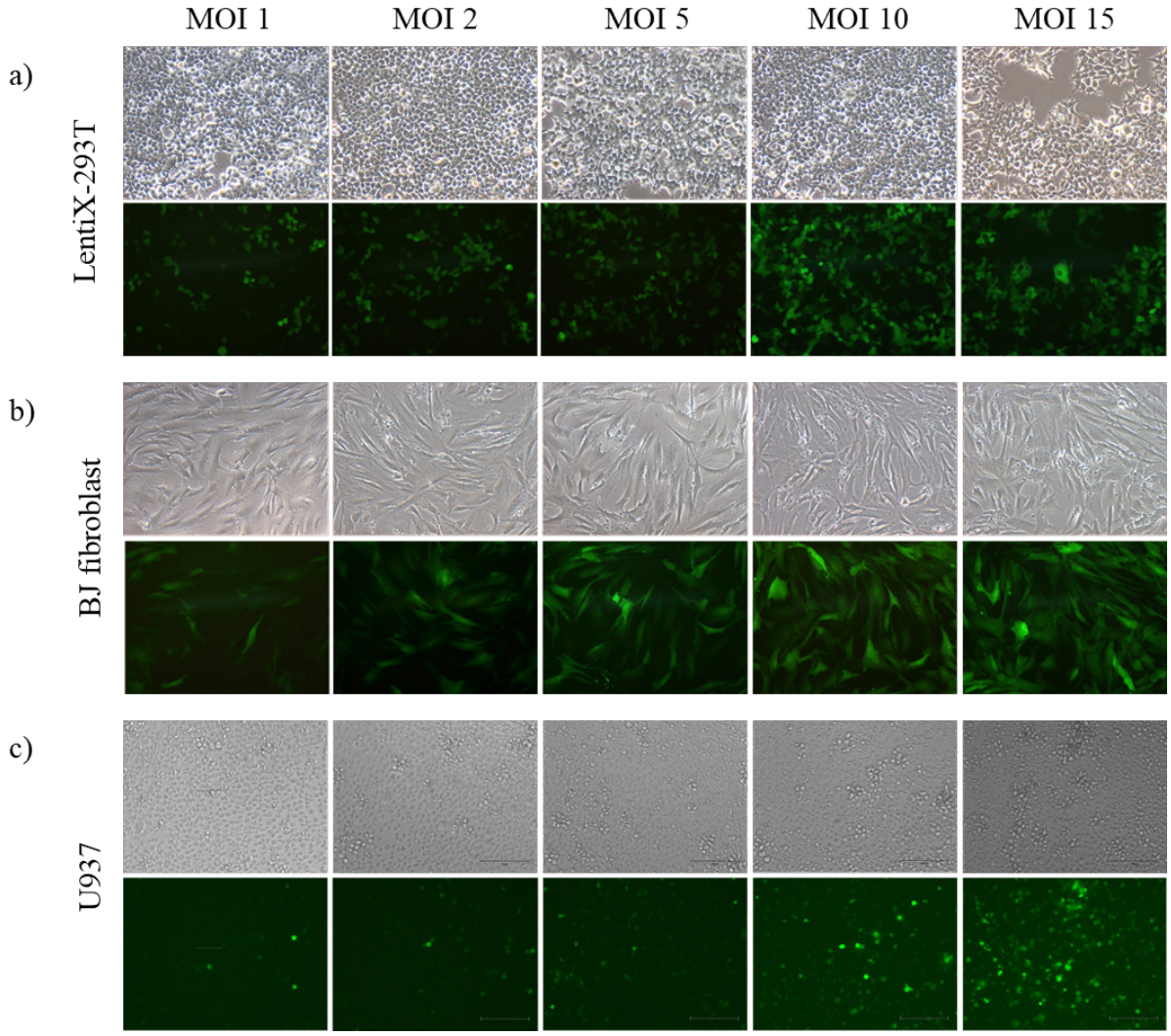

FIGURE 2. Increased GFP expression is seen with increasing viral volume (MOI). Phase contrast and fluorescent images of (a) LentiX-293T, (b) BJ and (c) U937. Cells were transduced with lentivirus carrying GFP gene at indicated MOIs in culture medium supplemented with 10% FBS and polybrene at 8 µg/mL. Images of live cells were viewed for endogenous expression of GFP at 72-h post transduction under 10 X magnification

Fourth generation lentiviral packaging systems used in this study are said to provide more safety for the user during the production of lentiviruses as it utilizes a split genome approach (Gurumoorthy et al. 2022; Kumar & Woon-Khiong 2011; Wu et al. 2000). The removal of further unnecessary viral sequences and the separation of viral packaging sequences over five plasmids compared to three plasmids requires increase recombination events to produce infective viral particles (Elegheert et al. 2018; Gándara, Affleck & Stoll 2018; Kappes, Wu & Wakefield 2003). This causes a lower chance for creating RCLs in theory, making it safer compared to previous generations as a gene transfer strategy. However, increasing the recombination events may result in less efficient lentiviral production. This study has demonstrated that the fourth-generation lentiviral system is highly efficient and resulted in high transfection of 293T cells. Concentration of viruses allows for lower transduction volumes, which has been shown to improve transduction efficiency (Lin et al. 2012; Pirona et al. 2021). In addition, this study demonstrated that the previously developed transfer vector plasmid was compatible with the tested lentiviral production protocol.

Having established a lentiviral production protocol using the fourth-generation lentiviral system, the next phase of the study assessed factors that could influence lentiviral transduction. A baseline for comparison with

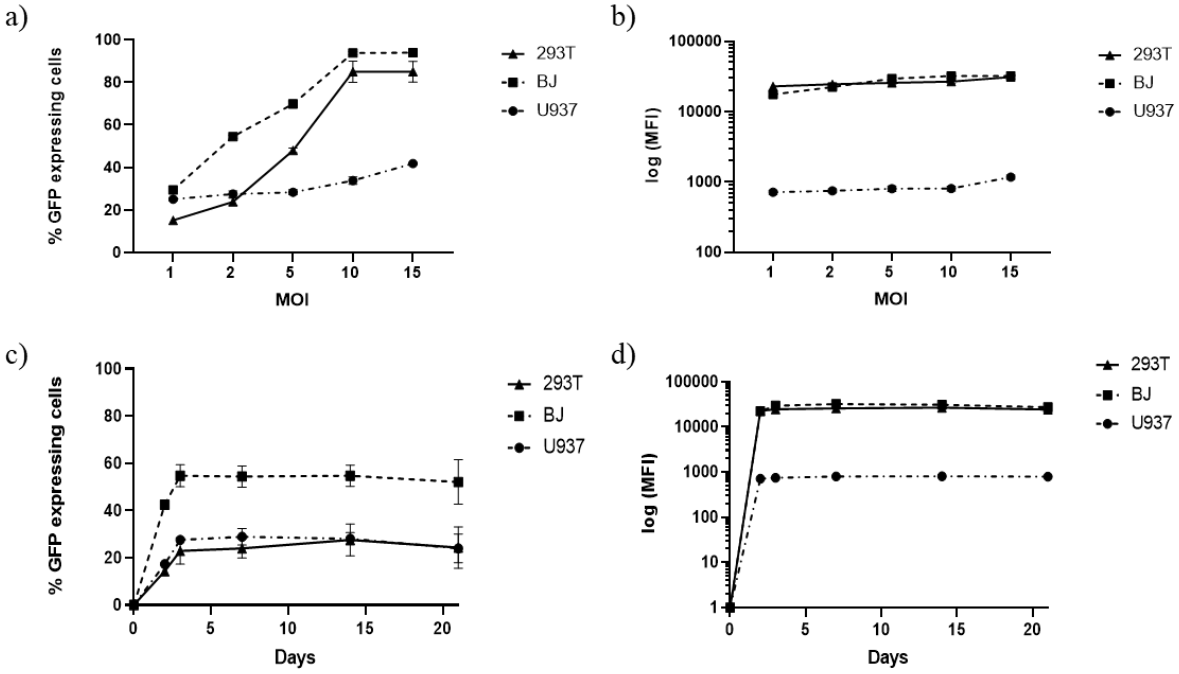

FIGURE 3. Transgene expression is affected by viral volume and is sustained over time and multiple passages. LentiX-293T, BJ and U937 cells were transduced with lentivirus carrying GFP gene at indicated MOIs in culture medium supplemented with 10% FBS and polybrene at 8 µg/mL. (a) Percentage of GFP expressing cells were determined at 72-h by flow cytometry. (b) Relative intensity of GFP expression in transduced cells at the indicated MOIs measured by flow cytometry. (c) Percentage of GFP expressing cells following serial passaging on days 3, 7, 14 and 21 post-transduction. (d) Relative intensity of GFP expression in transduced cells following serial passaging on days 3, 7, 14 and 21 post-transduction. Positive gated cells and relative mean fluorescence index were determined based on negative control (non-transduced cells) grown and passaged under similar medium conditions. Representative results of transduction were carried out in triplicate wells from one representative experiment. All values are mean ± s.e.m.

overnight transduction in 500 µL serum containing medium, with the addition of 8 µg/mL polybrene, using an MOI of 2 was used. This study next investigated variables affecting transduction, including type of target cells, MOI, the ability of gene transfer to produce sustained expression and use of transduction adjuvant to improve transduction efficiency.

Firstly, the efficiency of lentivirus-GFP transduction on adherent and suspension cells were compared. Transduction was found to be more efficient in adherent cell line BJ fibroblasts and comparable to 293T cells used as control. 293T cells are transformed human embryonic kidney cells that contain simion virus 40 (SV40) large T-antigen are highly transfectable and produces high levels of protein expression (Kumar & Woon-Khiong 2011; Merten, Hebben & Bovolenta 2016). The vector plasmid carrying GFP gene used in this study contains cytomegalovirus (CMV) promoter region, which is often silenced in hematopoietic and stem cell lines (Ailles & Naldini 2002), possibly causing the under expression of GFP in suspension cell line U937. As U937 cells are grown in suspension, surface contact between virus and cells during transduction may be reduced compared to adherent cells. Alternative methods to improve transduction such as centrifugation or also known as spinoculation have been shown to increase transduction efficiency in both adherent human mesenchymal stem cells (hMSC) (Lin et al. 2012), and suspension T-cells (O'Doherty, Swiggard & Malim 2000) by increasing virus-cell surface contact.

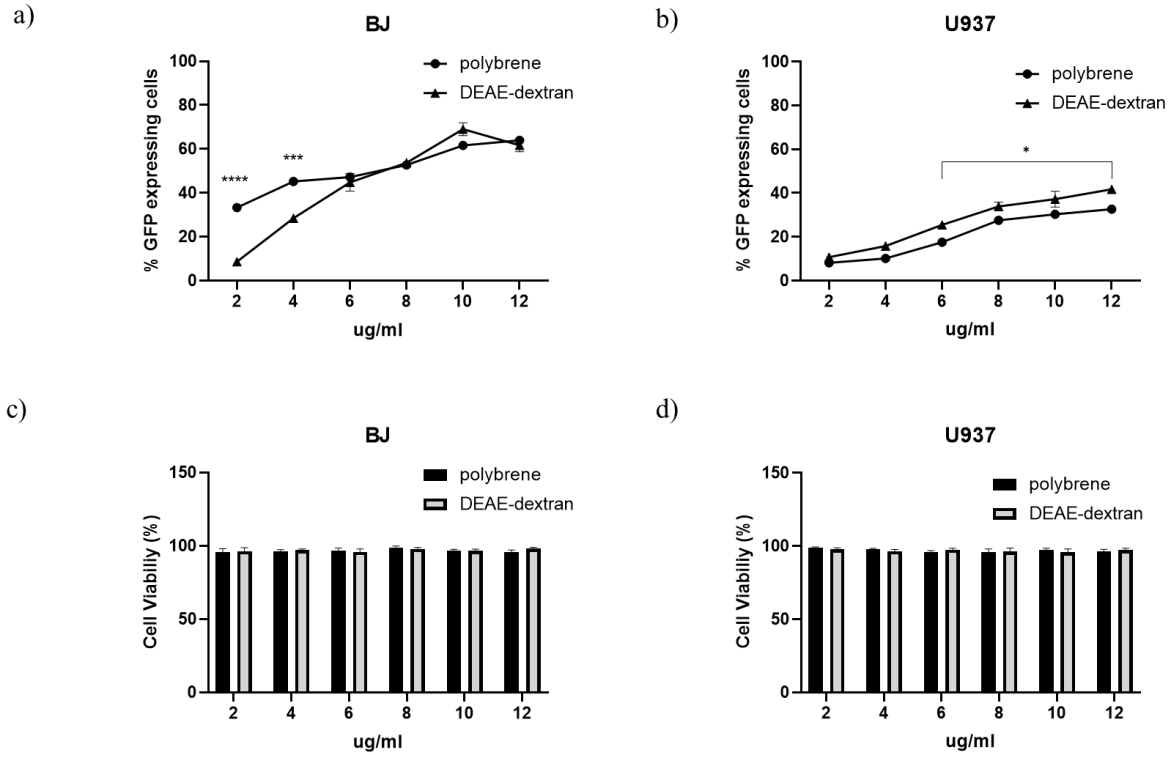

FIGURE 4. DEAE-Dextran improves lentiviral transduction efficiency compared to polybrene of lentiviruses only in suspension cells without affecting cell viability. BJ (a) and U937 (b) cells were transduced with lentivirus carrying GFP gene at MOI 2 in culture medium containing serum supplemented with either polybrene or DEAE-Dextran at the indicated concentrations. The percentage of GFP expressing cells were determined at 72 h post transduction by flow cytometry. Cell viability was determined in both BJ (c) and U937 (d) cells by trypan blue exclusion. Representative results of transduction were carried out in triplicate wells from one representative experiment. All values are mean ± s.e.m.

Increasing the MOI of lentiviruses also effects the number of transduced cells in all tested cell lines. One previous study demonstrated that increasing the MOI did not increase transduction efficiency in a primary pancreatic exocrine cell line (Balak et al. 2019). Another study showed hMSC incubated with increasing MOI produced a sharp initial rise in transduction efficiency followed by smaller increments with further amounts of lentiviruses (Lin et al. 2012). Transduction in our tested adherent cell lines was similar to that reported in hMSCs but reached a plateau at a lower MOI. In our adherent cell lines, transduction reached a maximum of approximately 80-90%. This indicates that the efficiency of gene transfer is MOI dependent, and the determination of optimal MOI is cell line specific. Higher viral volumes may also be toxic to the target cells.

After identifying an optimal MOI, we tested the ability of transduce cells to sustain gene expression over a specific time frame. The transcriptional activity of the widely used CMV promoter is highly robust, but prone to inactivation over time (Yew et al. 2001). We tested whether GFP expression would be diminished in transduced cells over serial passaging for 21 days. Expression of GFP was maintained in all tested cell lines despite passaging, indicating that the parent cells were able to transfer the transduced gene to daughter cells upon cell division without compromising gene expression.

Finally, the use of adjuvants to improve transduction efficiency was investigated. Polybrene is a widely used adjuvant in improving transduction in multiple cell lines (Denning et al. 2013; Jensen, Chen & Miller 2003; Le Doux et al. 2001), however, may cause toxicity to certain

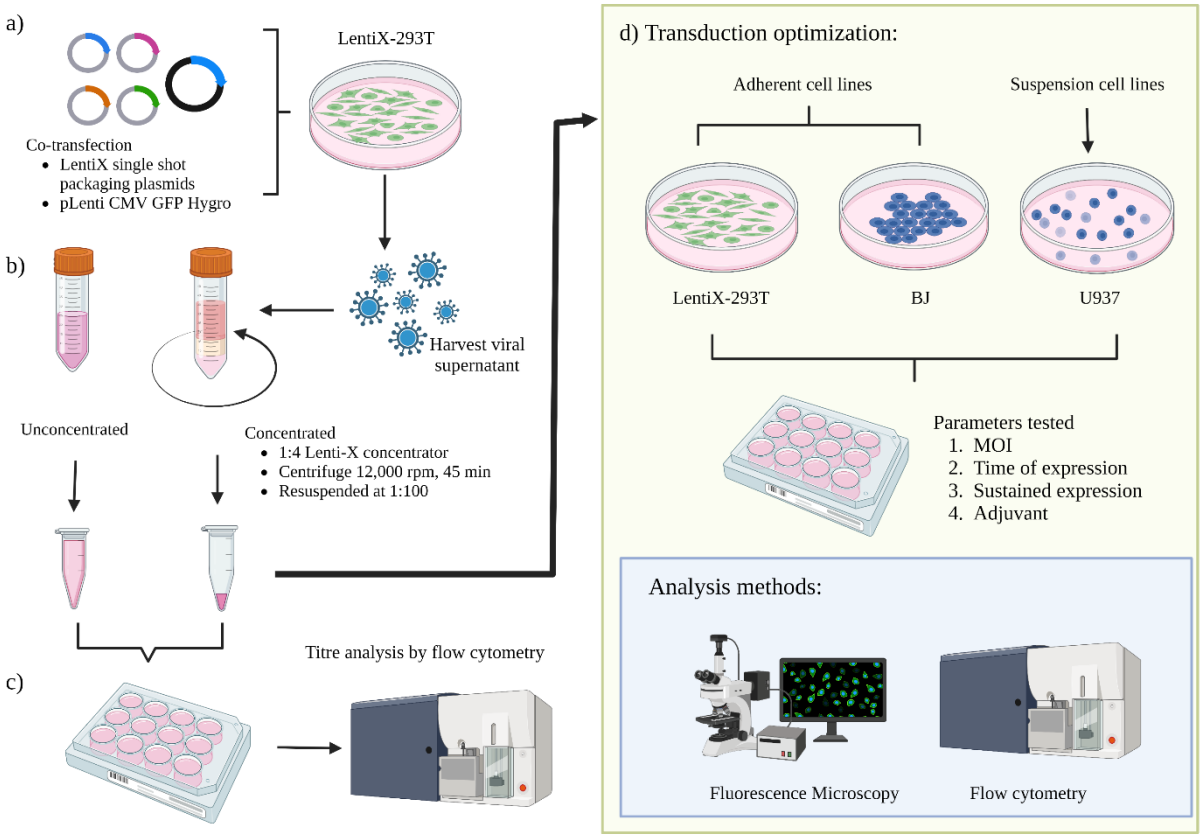

FIGURE 5. Diagrammatic workflow of the experiment. Workflow of (a) lentivirus production, (b) concentration, and (c) determination of viral titre. (d) Optimization of transduction on adherent and suspension cell lines was carried out testing for the effect of MOI, time required to achieve maximum expression, ability of transduced cells to sustain gene expression and the effect of adjuvant type and concentration on transduction

cell lines. It has also been reported that DEAE-Dextran has improved transduction efficiency compared to polybrene (Denning et al. 2013; Lizée, Gonzales & Topalian 2004). Our results indicate that in BJ fibroblasts, commonly used concentrations of either polybrene or DEAE-dextran do not confer any improvement in transduction efficiency and increasing the concentrations does not cause any toxicity. Transduction of U937 cell lines on the other hand improves with the addition of higher concentrations of DEAE-Dextran. This further indicates that the choice of adjuvant must be optimized for every different cell type.

Collectively, the presented results show that the use of the fourth-generation lentiviral system is efficient in producing infective lentiviral particles for gene transfer. Additionally, we have presented optimized transduction parameters for efficient gene transfer in adherent and suspension cell models. Our results suggest that efficient gene transfer can be achieved in vitro using different cell models, however no single protocol is applicable to achieve optimal transduction efficiency and each cell type requires thorough optimization of individual parameters. These findings can be applied to the clinical translation of potential lentiviral based gene therapies, where human cells, whether adherent or suspension cells, can be specifically targeted to achieve optimal gene transfer.

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