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RESEARCH PAPER

Effect of Serum on Transfection by Polyethylenimine/Virus-Like Particle Hybrid Gene Delivery Vectors

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ABSTRACT

Purpose Murine leukemia virus-like particles (M-VLP) complexed with polymers to promote cellular uptake and endosomal escape represent a new class of effective gene delivery vectors. Building upon recent studies of viral-synthetic hybrid vectors, we report the effects of serum on the formation, activity and stability of PEI/M-VLP complexes.

Methods M-VLP were produced by cells grown in serum-supplemented media (M-VLP-S), serum-free media (M-VLP-SF) or serum-free Opti-MEM® I (M-VLP-OM). PEI/M-VLP stoichiometry was varied to investigate complex formation and optimal transfection conditions. The effects of prolonged storage, freeze-thaw cycles, and ultracentrifugation of M-VLP on the stability of vector transduction efficiency were also observed.

Results M-VLP-S required more PEI to form infective complexes than M-VLP-SF and M-VLP-OM. The stoichiometry of PEI/M-VLP-S was dependent on total PEI concentration (7–8 μg/100 μL M-VLP supernatant), while optimal infectivity of PEI/M-VLP-SF and PEI/M-VLP-OM depended on PEI/M-VLP ratios (12–17 μg and 10–14 μg PEI/10^9 M-VLP, respectively). PEI/M-VLP-SF and PEI/M-VLP-OM complexes were significantly more efficient than PEI/M-VLP-S. Stability of the hybrid vectors was not significantly affected by serum.

Conclusions PEI/M-VLP complexes exhibiting increased efficiency were constructed by producing M-VLP in serum-free media. M-VLP could be stored by freezing or refrigeration and concentrated by ultracentrifugation without unacceptable loss of infectivity.

KEY WORDS gene delivery · hybrid vectors · murine leukemia virus · polyethylenimine · virus-like particles

INTRODUCTION

Recombinant viruses have been used as gene delivery vectors in numerous pre-clinical studies and clinical trials to treat conditions including severe combined immunodeficiency (SCID) (1–3), retinal disease (4), HIV infection (5), and several forms of cancer (6–8). Immunogenicity and oncogenicity of viral vectors, along with difficult and expensive production, have hindered clinical implementation, however (9,10). On the other hand, non-viral vectors comprising nucleic acids complexed with cationic lipids and polymers have been employed with great success in the laboratory (11), and cationic lipoplexes have been employed in several clinical trials (12,13). These synthetic vectors hold advantages in safety and versatility, but their relative inefficiency remains a significant challenge.

An alternative approach is to combine viral and synthetic components in a single vector. Several types of such hybrid vectors have been investigated. For example, synthetic vectors have been modified by conjugation to viral proteins such as fusogenic peptides that facilitate endosome escape or nuclear localization signal peptides to improve transgene delivery to the cell nucleus (14–17). In addition, viruses have been modified by attachment of synthetic components such as polymers (18–23). Lee *et al.* and Carlisle *et al.* reported reduction of the immunogenicity of adeno-associated virus by covalent modification of the virus surface with poly(ethylene glycol), which screens recognition

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by antisera (18,19). In each of these studies, however, the viral component of the vector comprised an intact, infectious virus particle.

Ramsey *et al.*, in contrast, recently reported the construction of hybrid vectors comprising inactive, envelope-free murine leukemia virus-like particles (M-VLP) or human immunodeficiency virus-like particles (H-VLP) complexed with polylysine (PLL) or polyethylenimine (PEI) (24,25). The synthetic component provides cell attachment, internalization and endocytic escape, and the virus-like particle is capable of efficient intracellular processing including genomic integration. In particular, PEI/M-VLP vectors promoted high levels of transgene delivery at optimal polymer/M-VLP ratios. The present study expands upon this previous work to more fully characterize the formation and infectivity of PEI/M-VLP vectors. Hybrid vectors containing M-VLP produced in media with and without serum are compared with regard to complex formation stoichiometry, gene delivery efficiency, and M-VLP stability to processing and storage conditions.

MATERIALS AND METHODS

Cell Lines

HEK293 human embryonic kidney cells were purchased from American Type Culture Collection (Manassas, VA). The murine leukemia virus producer cell line, GP293-Luc, was acquired from Clontech (Mountainview, CA). GP293-Luc cells express the MLV viral *gag-pol* genes and the viral insert containing neomycin resistance and luciferase reporter genes. HEK293 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% horse serum and 1% penicillin/streptomycin. GP293-Luc cells were grown in DMEM supplemented with 10% fetal bovine serum. All cells were cultured at 37°C in 5% CO_2.

Virus and Virus-Like Particle Production

Enveloped MLV with *neo*^{r} antibiotic resistance and *luc* reporter genes were produced from the GP293-Luc cell line transfected with DNA encoding an envelope protein. The cells were seeded in a 10-cm cell culture dish 18–24 h prior to transfection so as to be 80–90% confluent at the time of transfection. The cells were transfected with 24 μg of the Env-encoding plasmid (pVSV-G or pMDM(4070A)) using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) according to standard protocols. The transfection medium was replaced with fresh growth media after 6 h. Viruses were collected 48 h later and filtered through a 0.45-μm surfactant-free cellulose acetate syringe filter. The plasmids pVSV-G and pMDM(4070A) code for VSV-G envelope glycoprotein and amphotropic MLV clone 4070A envelope protein, respectively. pVSV-G was purchased from Clontech, and pMDM(4070A) was a gift from R.C. Mulligan (Children's Hospital, Boston, MA).

Envelope-free M-VLP were also produced from the GP293-Luc cell line. M-VLP-S (M-VLP grown in DMEM supplemented with 10% FBS) were produced in cells cultured for three days before the supernatant was collected. M-VLP-SF (M-VLP grown in serum-free DMEM) and M-VLP-OM (M-VLP grown in Opti-MEM® I) were obtained by replacing the GP293-Luc growth medium with serum-free DMEM or Opti-MEM® I (Gibco/Invitrogen), respectively, after the cells had achieved ~90% confluency. Supernatant was harvested 36 h after medium change and filtered through a 0.45-μm surfactant-free cellulose acetate syringe filter. Filtered M-VLP were used immediately, refrigerated at 4°C for short-term storage (24–48 h), or frozen at −80°C. M-VLP concentration was determined by quantifying the number of viral genome copies using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) as described below.

Polymer Labeling

Branched 750-kDa polyethylenimine (PEI) (Sigma-Aldrich, St. Louis, MO) was labeled with Alexa Fluor® 488 succinimidyl ester (Molecular Probes/Invitrogen, Eugene, OR) (SE) in 0.1 M sodium bicarbonate buffer, pH 8.3. After vortexing for 1 h at room temperature, the conjugated PEI was separated from the unreacted labeling agent using PD-10 gel filtration chromatography columns (GE Healthcare, Chalfont St. Giles, United Kingdom) and eluted with ultra-pure deionized water. Final polymer concentration was determined by measuring primary amine concentration with a ninhydrin assay, and dye content was determined from the Alexa Fluor 488 absorbance at 488 nm. The average degree of labeling was ~2–3 fluorophores per polymer chain. The final product was stored at −20°C.

Cell Transduction and Transgene Expression

HEK293 cells were seeded in 6- or 12-well plates 18–24 h prior to vector exposure. Stock solutions of 750-kDa PEI were prepared in water at a concentration of 5 mg/mL and stored at 4°C. PEI/M-VLP complexes were formed through the drop-wise addition of polymer solution to M-VLP suspension while gently vortexing and incubation at room temperature for 2 h. Immediately before the addition of hybrid vector complexes, the cells were washed with PBS, and the growth medium was replaced with serum-free DMEM. Hybrid vector complexes were then added to each

well, and the cells were returned to the incubator. PEI/M-VLP hybrid vectors were removed after 4 h, and normal growth medium was replaced. For viral infection, MLV supernatant was diluted with growth medium plus 8 μg/mL polybrene prior to cell infection.

Cells transduced with MLV were assayed 24 h after virus administration, while cells transduced with PEI/M-VLP complexes were assayed 48 h after the addition of the vector using the Promega Luciferase Assay System (Madison, WI) and a Lumat LB9507 luminometer (Berthold, Oak Ridge, TN). Protein concentration in the cell lysate was determined by BCA assay (Thermo Scientific, Rockford, IL) and used to normalize luciferase concentration.

M-VLP RNA Extraction and Reverse Transcription-Quantitative PCR

RNA was extracted from M-VLP suspensions using the Qiagen Viral RNA extraction kit following the manufacturer's protocol. Samples were eluted in 60 μL of the provided buffer and stored at −80°C until use. RNA standards (Clontech) and viral RNA samples were prepared for reverse transcription using Taqman Reverse Transcription reagents (Applied Biosystems, Carlsbad, CA). Twenty μL samples were mixed in 200 μL PCR tubes using the reagent concentrations suggested by the kit plus 250 nM sequence-specific primers. The sample tubes were then reverse transcribed using a PTC-100 Thermocycler (MJ Research, Waltham, MA).

Reverse transcription products were diluted in RNase/DNase-free water (10 μL:40 μL). PCR working reagent was created by mixing 20 μL 2× Power SYBR Green PCR reagent (Applied Biosystems), 4 μL each of 2.5 μM forward and reverse primers, and 12 μL of diluted sample. The mixture was vortexed and transferred in triplicate (10 μL/well) to 384-well qPCR-compatible plates. The plate was sealed with a plastic film cover and analyzed with a Taqman 7900 ABI Real-Time PCR Thermocycler (Applied Biosystems). The results were analyzed using SDS software (Applied Biosystems).

Polymer Toxicity

The toxicity of PEI was determined using the CellTiter-Blue™ Cell Viability Assay (Promega). HEK293 cells were seeded in a 96-well fluorimeter plate 24 h before polymer was added. Polymer was added to the cells in serum-free medium and incubated for 4 h before the medium was replaced. Twenty-four hours after the medium was replaced, CellTiter-Blue™ reagent was added to each well. The cells were incubated for 4 h at 37°C before fluorescence was measured.

Hybrid Vector Stability

M-VLP samples were collected, filtered, and stored at 4°C as described above. At specified times, samples were transferred from 4°C to the incubation conditions (20°C or 37°C) where they remained until the time of infection or transduction. Stability at 4°C was measured by transferring M-VLP frozen at −80°C to 4°C. M-VLP samples were subsequently analyzed for transfection and M-VLP concentration as described above.

To determine infectivity after freeze-thaw cycles, MLV and M-VLPs were frozen in a −80°C isopropanol bath for 10 min and thawed in a 37°C water bath for 5 min. After the specified number of freeze-thaw cycles, samples were stored at 4°C until assayed. MLV infectivity was determined by expression of the encoded luciferase gene in HEK293 cells. M-VLP samples were used to form PEI/M-VLP hybrid vectors and then analyzed for transfection of HEK293 cells and M-VLP concentration as described above.

The stability of M-VLP to ultracentrifugation was determined by spinning 20 mL samples of filtered virus supernatant at 65,000×*g* for 2 h at 4°C in a 70.1-TI rotor using an L-8 Ultracentrifuge (Beckman Coulter, Fullerton, CA). After ultracentrifugation, samples were gently vortexed to resuspend M-VLP or concentrated by removal of 18 mL of depleted supernatant prior to resuspension. Remaining M-VLP activity was determined by formed PEI/M-VLP hybrid vectors and analyzing transfection of HEK293 cells as described above.

Flow Cytometry

A stock solution of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiD) (Molecular Probes/Invitrogen) was prepared by dissolving DiD in DMSO to give a final concentration of 1 mM. DiD was mixed with M-VLP to give a final supernatant concentration of 2 μM DiD and incubated for 1 h at room temperature. A PD-10 gel filtration desalting column (GE Healthcare) was used to separate the labeled M-VLPs from free dye.

HEK293 cells were transfected with hybrid vectors comprising AF488-PEI and/or M-VLP-DiD as described above. At the specified time after transfection, hybrid vectors were aspirated from the cells, and the cells were rinsed with 0.001% SDS to remove complexes that were not internalized. The cells were then rinsed a second time with PBS and detached from the cell culture dish with 1 mL of trypsin. Cell-associated fluorescence was analyzed on a BD SLR II cytometer (Becton Dickinson, East Rutherford, NJ) using the 488 nm (for Alexa Fluor® 488-labeled PEI) and 633 nm (for DiD-labeled M-VLP) lasers.

Confocal Microscopy

HEK293 cells were seeded at 300,000 cells/well onto sterilized coverslips inserted into 6-well tissue culture dishes and grown overnight. Hybrid vectors comprising AF488-PEI and/or M-VLP-DiD were added to cells and incubated for 1 h, 2 h, or 4 h. The cells were washed once with PBS + 0.001% SDS, washed twice with PBS, and fixed with 3.7% formaldehyde. Cells were washed twice with PBS and mounted on microscope slides. Mounted cells were visualized with a BX60 confocal microscope (Olympus, Center Valley, PA) equipped with a 100× oil-immersion objective and argon-ion and helium-neon lasers.

RESULTS

Effect of Serum on PEI/M-VLP Stoichiometry and Transfection

To study the effect of serum on PEI/M-VLP complexation and to determine optimal PEI/M-VLP ratios for transfection, M-VLP produced in normal serum-supplemented growth medium (M-VLP-S), serum-free DMEM (M-VLP-SF), or Opti-MEM (M-VLP-OM) were complexed with 750-kDa PEI and used to transfect HEK293 cells. (Although this high-molecular-weight polymer has potential for toxicity, previous work (25) demonstrated that lower-molecular-weight PEI resulted in significantly lower gene delivery activity.) The optimal stoichiometry for M-VLP-S complexes increased from 17 μg to 31 μg per 10^9 M-VLP-S as the M-VLP concentration decreased from 3.87×10^6 to 1.54×0^6 M-VLP/μL (Fig. 1a). In contrast, when the same transfection data are plotted against the M-VLP-S supernatant volume, the optimal stoichiometry was 6–8 μg PEI/100 μL regardless of M-VLP concentration (Supplementary Fig. 1). Thus, it appears that serum content, rather than M-VLP-S concentration, controls the complex formation when the hybrid vectors are produced in serum-containing media.

M-VLP-SF and M-VLP-OM exhibited significantly different behavior. The optimal PEI content was relatively constant in the range 12–17 μg PEI/10^9 M-VLP-SF and 10–14 μg PEI/10^9 M-VLP-OM, regardless of M-VLP concentration (Fig. 1b and c). It is also noteworthy that M-VLP-SF and M-VLP-OM required substantially less PEI than M-VLP-S to form infective complexes. At the higher PEI concentrations necessary to form infective complexes with M-VLP-S, complexes with M-VLP-SF were ineffective.

In addition, transgene expression in cells transfected with PEI/M-VLP-SF or PEI/M-VLP-OM complexes was significantly greater than with PEI/M-VLP-S complexes at equivalent M-VLP concentrations. RT-qPCR assays revealed lower M-VLP concentration in serum-free media ($4.5\pm1.0\times10^6$ M-VLP-S/μL; $1.6\pm0.3\times10^6$ M-VLP-SF/μL; $3.2\pm0.7\times10^6$ M-VLP-OM/μL), suggesting that the greater transfection is not related to the total number of M-VLP applied, but may be attributed to the formation of more stable or effective PEI/M-VLP-SF and PEI/M-VLP-OM complexes.

Cytotoxicity of Hybrid Vectors

Cytotoxicity of the hybrid vectors was determined as the relative metabolic activity of HEK293 cells transfected with PEI/M-VLP at varying stoichiometry within the range used for transfections. Metabolic activity decreased with PEI concentration at 1–8 μg PEI/100 μL M-VLP-S and 1–4 μg PEI/100 μL M-VLP-SF or M-VLP-OM. Viability of cells transfected with PEI/M-VLP-S at 8–12 μg PEI/100 μL M-VLP was approximately 50% (Fig. 2). PEI/M-VLP-OM appeared to be slightly less toxic than PEI/M-VLP-S and PEI/M-VLP-SF complexes at the same PEI concentrations.

Hybrid Vector Uptake and Intracellular Localization

M-VLP were labeled with the lipophilic dye DiD to allow analysis by flow cytometry and visualization by microscopy. (Because of interactions between serum proteins and DiD, only M-VLP-SF and M-VLP-OM could be used for these experiments.) To determine the amount and rate of hybrid vector uptake, HEK293 cells transfected with labeled PEI/M-VLP were analyzed by flow cytometry 1 h, 2 h, and 4 h after transfection (Table I). The percentage of cells containing hybrid vectors increased from 20–30% at 1 h to 80% at 4 h post-transfection. Significant increases in mean fluorescence were also observed over the same time period. No correlation between uptake and PEI/M-VLP ratio was observed (data not shown). Laser scanning confocal microscopy was also employed to visualize internalization and subcellular localization of the hybrid vectors 4 h post-transfection (Fig. 3). In general, M-VLP and PEI appeared to be co-localized and likely were still complexed within the cells. A significant amount of free PEI was present, but free M-VLP was much less commonly observed (with the exception of a small percentage of cells that appeared red throughout, apparently as a result of DiD staining of the cell membrane).

Stability of M-VLP and Hybrid Vectors

To assess the stability of the viral particles, in comparison to the known instability of intact MLV, M-VLP were incubated at 37°C, 20°C or 4°C and analyzed for M-VLP concentration by RT-qPCR and gene delivery activity

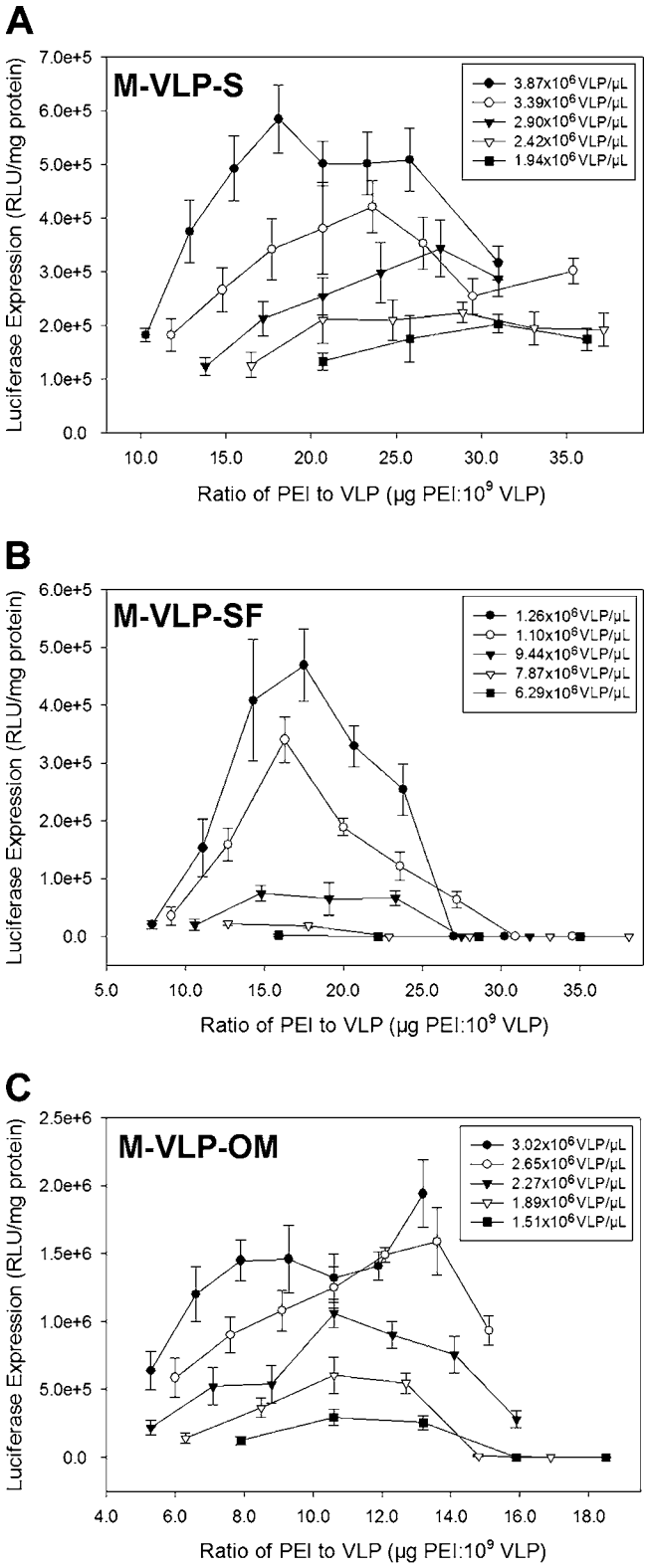

Fig. 1 Transfection with HEK293 cells with (**A**) PEI/M-VLP-S, (**B**) PEI/M-VLP-SF, and (**C**) PEI/M-VLP-OM hybrid vectors at varying PEI/M-VLP ratio. ($n = 6$; *error bars* represent standard deviation).

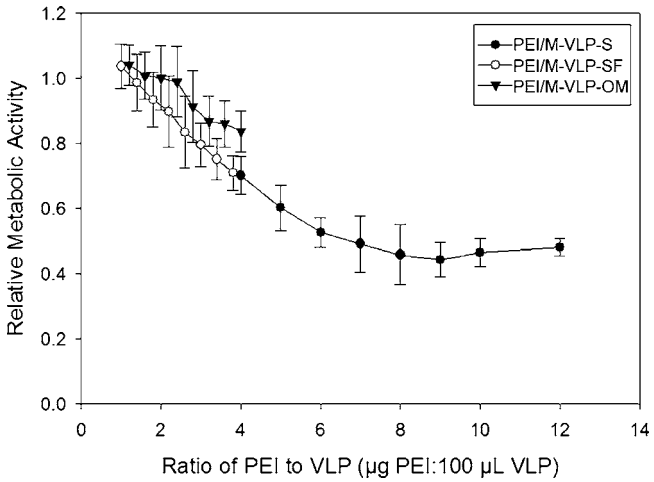

Fig. 2 Cytotoxicity of PEI/M-VLP complexes. Relative metabolic activity of HEK293 cells transfected with PEI/M-VLP complexes was determined using the CellTiter-Blue™ assay. Relative metabolic activity = $(A_{sample}-A0)/(A_{100}-A_0)$; A_{sample} = relative absorbance of sample at 570/600 nm, A_0 = relative absorbance in wells without cells, A_{100} = relative absorbance in samples without added complexes. ($n = 8$, *error bars* represent standard deviation).

(Fig. 4). Changes in M-VLP concentration were statistically insignificant in all cases, indicating that the viral genomic RNA remains protected, presumably within intact M-VLP particles. M-VLP incubated at 4°C retained nearly 100% transfection activity for the full 16-day observation period, while M-VLP incubated at 37°C lost 70–85% activity after 48 h, regardless of the presence or absence of serum. M-VLP incubated at 20°C retained 50–75% infectivity after 4 days but lost nearly all activity after 16 days. M-VLP-OM displayed slightly slower loss of activity than M-VLP-S or M-VLP-S-SF at 20°C.

Transfection efficiencies of M-VLP samples were determined after a series of freeze-thaw cycles (Fig. 5a). After one freeze-thaw cycle, approximately 30–40% of transfection activity was lost, regardless of the presence or absence of serum. Repeated freezing and thawing resulted in 10–30% decrease in transfection efficiency with each cycle. As expected, M-VLP concentration was not significantly affected by freezing and thawing (data not shown), as encapsidated viral RNA was not damaged by the freeze-thaw process.

To evaluate ultracentrifugation as a method of concentration, M-VLP were centrifuged at 65,000×*g* at 4°C for 2 h (Fig. 5b). M-VLP were either resuspended in their original supernatant medium or concentrated 10-fold by removing 90% of the supernatant medium prior to resuspension. M-VLP-S infectivity was relatively stable to ultracentrifugation, maintaining more than 80% transfection activity. M-VLP-SF and M-VLP-OM, in contrast, were significantly less stable, losing 50–60% of

Table I Uptake of Hybrid PEI:VLP Complexes by HEK293 Cells

Time post-transfection	% Fluorescence-positive[a]		Mean fluorescence[b]	
	VLP-SF	VLP-OM	VLP-SF	VLP-OM
1 h	30.3 ± 4.2	19.3 ± 1.6	2.36 ± 0.28	1.59 ± 0.07
2 h	37.7 ± 2.8	43.2 ± 3.1	3.35 ± 0.23	3.87 ± 0.22
4 h	80.7 ± 1.0	83.2 ± 2.4	23.9 ± 1.5	31.8 ± 0.9

[a] Percentage of cells gated as fluorescence-positive for VLP-DiD uptake. Control cell population (no DiD) was defined as 2.5% fluorescence-positive

[b] Mean fluorescence of cell population normalized to control cells ± standard deviation ($n = 3$)

transfection activity after centrifugation. The activity upon 10-fold concentration of M-VLP-S, M-VLP-SF, and M-VLP-OM increased by factors of 12-, 2-, and 7-fold, respectively.

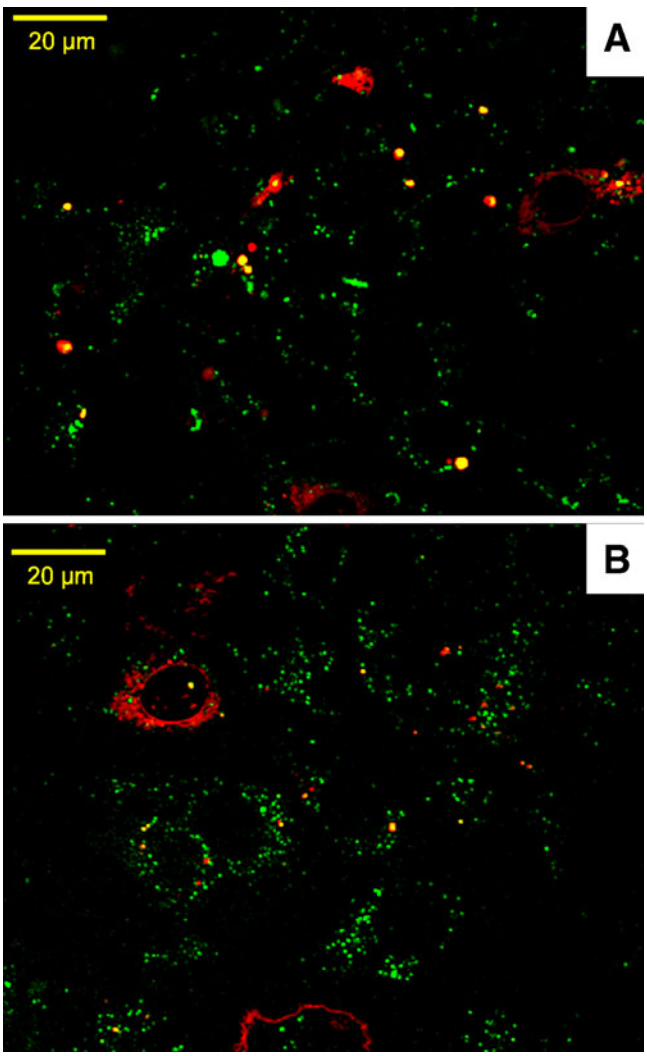

Fig. 3 Confocal fluorescence microscopy of HEK293 cells transfected with PEI/M-VLP complexes. Cells were imaged 4 h following transfection with PEI/M-VLP-SF (**A**) or PEI/M-VLP-OM (**B**). *Green*: AF488-labeled PEI; *Red*: DiD-labeled M-VLP; *Yellow*: colocalization.

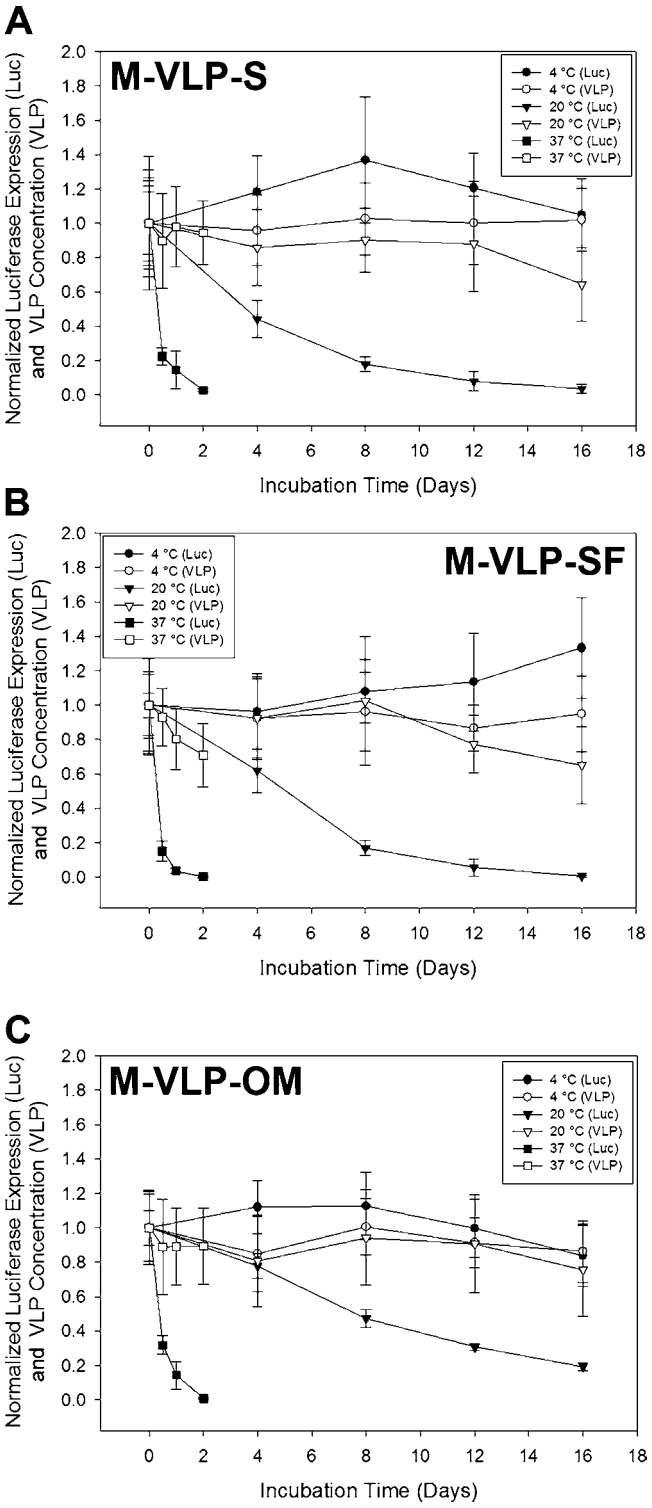

Fig. 4 Thermal stability of (**A**) M-VLP-S, (**B**) M-VLP-SF and (**C**) M-VLP-OM. Stability at 4°C, 20°C, and 37°C was determined using M-VLP concentration as determined by RT-qPCR (M-VLP) and transfection efficiency of PEI/M-VLP complexes (Luc), normalized by M-VLP concentration and transfection efficiency at $t = 0$, respectively. ($n \geq 6$, error bars represent standard deviation).

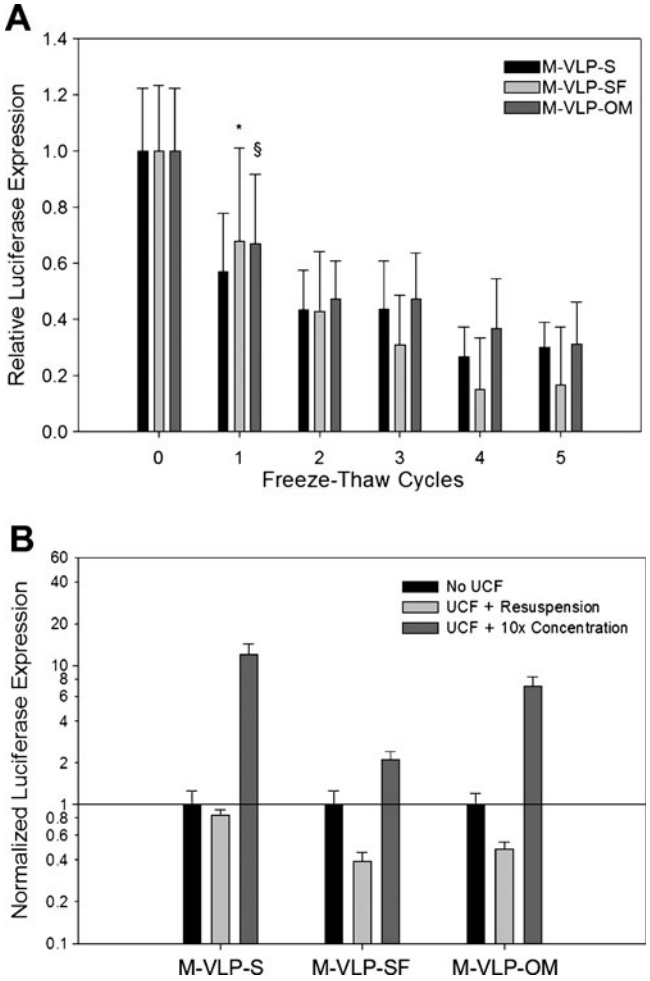

Fig. 5 Stability of M-VLP to freezing and concentration by ultracentrifugation. (**A**) M-VLP were frozen and thawed (at −80°C and 37°C, respectively) 0 to 5 times prior to complexing with PEI and assaying transfection efficiency relative to unfrozen M-VLP. (**B**) M-VLP suspensions were pelleted by ultracentrifugation, and M-VLP were subsequently resuspended in the full supernatant volume (UCF + resuspension) or following removal of 90% of supernatant medium (UCF + 10× concentration). Stability was determined by transfection efficiency of PEI/M-VLP vectors relative to uncentrifuged M-VLP. ($n = 6$, *error bars* represent standard deviation).

DISCUSSION

M-VLP can be produced by cells growing in serum-free as well as serum-supplemented media. Complexes of M-VLP produced in serum-free DMEM and OptiMEM exhibited increased gene delivery activity relative to M-VLP produced in the presence of serum. As M-VLP-S samples were diluted, the optimal complex stoichiometry varied, suggesting that serum proteins play an important role in complex formation such that PEI/M-VLP-S may be best described as complexes of all three components. M-VLP-SF and M-VLP-OM, in contrast, appear to form binary complexes with PEI, as the gene delivery activity was optimal at constant PEI to M-VLP number ratio, regardless of M-VLP concentration.

The well-known disadvantage of PEI as a component of gene delivery vectors is toxicity. Although low-molecular-weight PEI (800–2,000 Da) is minimally toxic (26), 25-kDa PEI complexed with plasmid DNA has been shown to result in a 30–50% decrease in relative cell metabolic activity at 5 μg/mL (26,27). For the 750-kDa PEI/M-VLP complexes used here, cytotoxicity was found to be a function of PEI concentration and was unrelated to complex stoichiometry. PEI/M-VLP-S were significantly more toxic than PEI/M-VLP-SF or PEI/M-VLP-OM due to the higher PEI content required to form vectors with high transfection efficiency. In future studies, modified polymers (such as biodegradable cross-linked PEI (26)) may be used to generate less toxic hybrid vectors.

Uptake of M-VLP complexes increased substantially during the 4 h following transfection. The relatively slow uptake of these hybrid vectors is believed to be a consequence of the large size of PEI/M-VLP complexes (1–1.5 μm), which are most likely taken up by macropinocytosis (25). Observations from confocal micrographs are consistent with this hypothesis. Four hours after transfection, a large fraction of internalized M-VLP appeared as punctate fluorescence and appeared to be coincident with or adjacent to PEI, suggesting that a majority of complexes were still intact, possibly inside an endosomal environment. This suggests inefficiencies in the endosomal release of PEI/M-VLP complexes. Further study of uptake and endosome escape may be useful for development of improved hybrid vectors.

The MLV upon which the hybrid vectors are based are notoriously unstable (28,29). The half-life for wild-type MLV is approximately 5–7 h and 17 days at 37°C and 4°C, respectively (25,30,31). Thus, the stability of M-VLP is also important. In these studies, hybrid vectors were observed to be infective after medium-term (16 days) storage of M-VLP at 4°C and retained significant infectivity for several days when stored at ambient temperature (20°C). M-VLP concentration was not affected significantly compared to the effects on particle infectivity. In addition, we found that M-VLP infectivity was quickly lost at 37°C, similar to MLV.

While refrigerated M-VLP remained infective for several weeks, storage for longer duration may require freezing. The infectivity of MLV has been shown to decrease significantly upon freezing and thawing (32). Similarly, gene delivery by hybrid vectors was reduced by half with one freeze-thaw cycle, and progressive cycles resulted in additional degradation. M-VLP can be frozen for storage if necessary, but repeated freezing and thawing should be avoided. Under most circumstances, however, refrigeration of M-VLP is preferable.

Concentration of M-VLP by ultracentrifugation was found to be a useful method for increasing PEI/M-VLP transfection efficiency. More concentrated M-VLP samples can be used to apply a greater number of viral particles per target cell. Some viral vectors have been observed to lose infectivity due to the physical stress associated with ultracentrifugation (33). M-VLP also lost infectivity during ultracentrifugation, but these effects were more than compensated for by enhanced transfection with concentrated samples. Further sample concentration may allow even better transfection efficiency and would be an appropriate subject for future study.

CONCLUSIONS

We found that M-VLP can be produced in serum-free medium, resulting in hybrid PEI/M-VLP with improved infectivity. Serum proteins have a significant effect on complex binding, dominating the PEI/M-VLP-S interaction and possibly limiting the number of M-VLP contained in those complexes. M-VLP-SF and M-VLP-OM formed optimally infective complexes within a consistent PEI-to-M-VLP window. Though transfection efficiency of PEI/M-VLP complexes remains lower than the efficiencies mediated by intact MLV, PEI/M-VLP-SF and PEI/M-VLP-OM were capable of efficiencies within an order of magnitude of MLV. Slow uptake and intracellular processing of PEI/M-VLP are likely causes for the observed inefficiencies and are subjects for further study. Modification of PEI may yield vectors with lower toxicity and improved trafficking.

M-VLP remained infective following storage at 4°C, but lost infectivity gradually when stored at 20°C, and quickly at 37°C. Frozen M-VLP was roughly half as infective as unfrozen M-VLP, indicating that samples may be stored in this fashion if long-term storage is necessary, but refrigeration is preferable in most cases. Some infectivity was lost during ultracentrifugation of M-VLP samples, but not enough to preclude concentration by this method. Indeed, greater than 10-fold increases in transfection efficiency were observed following concentration of M-VLP samples by ultracentrifugation.

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