Improving Transgene Expression and Cell Viability Using a Biodegradable Polymeric Delivery Platform

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Introduction

Advanced cell therapy research requires the efficient delivery of nucleic acids for use in transgene expression and interference. Novel transgene technologies are transforming and enabling protein production and expanding the fundamental methods used to identify roles of specific enzymes in genetic pathways. Proper end-to-end delivery for these applications requires a stable delivery platform that transports, localizes, and degrades in a non-toxic manner upon release of cargo. Discovery and translational research has witnessed a higher emphasis of gene expression in primary cell types and *in vivo* transfections. Realizing the benefits of these technologies is contingent on developing a reliable method for importing nucleic acids into cells with high efficiency in the most non-invasive/nontoxic manner possible.

Existing Methods Fall Short

Several intrinsically invasive methods have been utilized for the delivery of genetic material into cells. However, they often trigger a cytotoxic response and therefore the chief barrier to using nucleic acids for the development of specific treatments is safe, efficacious delivery of DNA. This is due in large part because the level of disruption to the host cell is directly related to the physical attributes and transmembrane pathway of the nucleic acid. Indeed, high cell viability becomes even more essential when transfection is carried out in sensitive cell types, *in vivo* or in *ex vivo* implanted cells. Traditional means of carrying DNA into cells rely on cell membrane disruption such as electroporation or infection with viruses. Both of these have their own positive attributes and drawbacks, but are generally not conducive to rapid high throughput screening of multiple DNA constructs. Nor are they conducive to large scale protein production because electroporation doesn't scale to larger formats and the presence of a virus complicates protein purification and safety issues. Chemical transfection methods provide a more favorable alternative to electroporation and viruses. The initial choice for nucleic acid delivery was utilizing lipid-based delivery systems, which encapsulate the deliverable gene into a liposomal structure and enter the cell via membrane disruption/diffusion and active uptake. These agents in many cases are very efficacious delivery vehicles, however they exhibit severe drawbacks including extensive gene up-regulation, cell membrane damage, and low transfection efficiency in post-mitosis cells.

Polymeric Delivery

Researchers more recently have turned to cationic polymers to meet their transfection needs, including *poly*-L-lysine (PLL), *poly*-ethyleneimine (PEI), chitosan, and their derivatives. Nucleic acid delivery using these compounds relies on complexation driven by electrostatic interactions between the gene and the polycationic delivery agent. Polymer-DNA complexes condense into particles on the order of 60 - 120 nm in diameter, a size suitable for active endocytosis by mammalian cells. Polymers such as linear PEI and PLL give very high transfection in a variety of primary and cells both *in vitro* and *in vivo*. Toxicity is sometimes associated with these polymers due to the high membrane-disrupting charge they carry.

Glycofect Delivery Platform

Techulon, Inc. (www.techulon.com) has developed a unique polymer-based transfection platform, Glycofect™, which is a carbohydrate-containing *poly*-amidoamine that binds and delivers DNA. The technological advantage to the Glycofect delivery platform is that it is biodegradable which both lowers its toxicity and promotes DNA release in a cell's perinuclear region for maximum gene expression. Studies have indicated that Glycofect is preferentially uptaken by cells via a caveolae-mediated endocytotic route which contributes to the high nuclear delivery. Glycofect has been tested with various reporter plasmids and labeled oligo DNAs in multiple cell lines and primary cell types. Data indicates that similar or better transgene expression levels can be attained using Glycofect Transfection Reagent with lower toxicity in many common cell lines and primary cell types. Currently, therapeutic applications for this powerful delivery platform are being examined.

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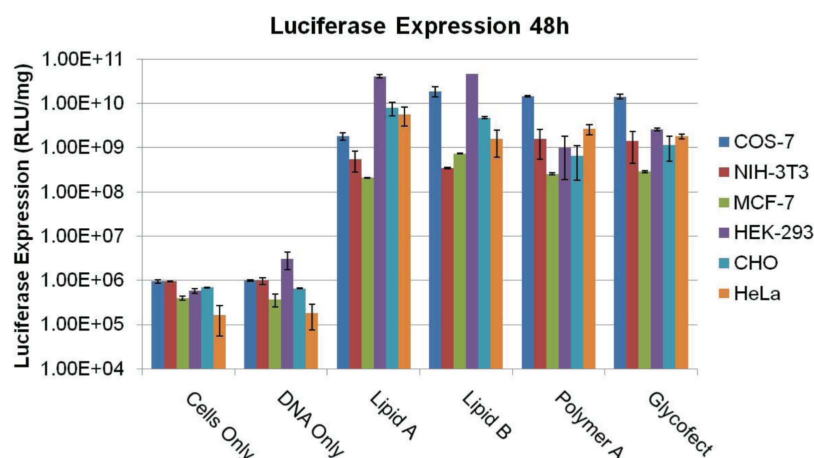
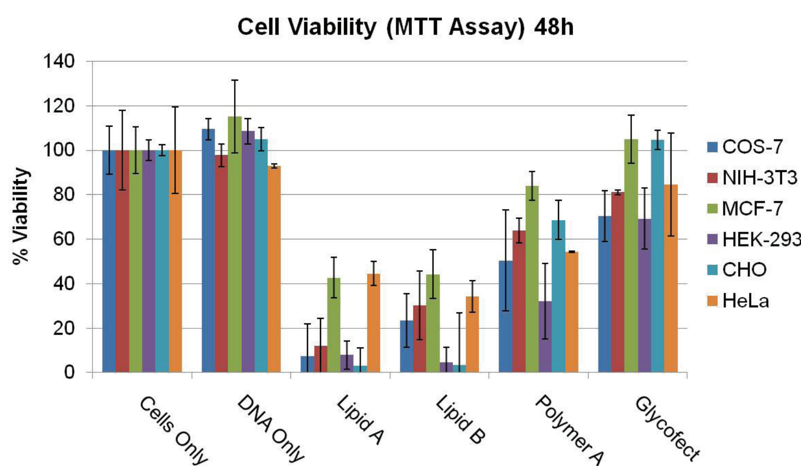
FIGURE 1



Expression of pCMv-Luc transgene (relative light units per milligram of lysate protein (RLU/mg)) using different commercially available transfection reagents assayed after 24h.

FIGURE 2



Cell viability via MTT assay.
Cells were treated with MTT containing media after 47 h and lysed at 48 h.

Experimental Methods

Luciferase Gene Expression Assay. Prior to transfection, 5 commonly utilize cell lines were seeded on 24-well plates at a density of 50,000 - 100,000 cells per well depending on cell size, approximately 70% confluence. Cells were incubated in supplier recommended media (varies by cell type, but generally DMEM or EMEM with 10% FBS), for 24 h at 37C in a 5% CO2 environment. Cells were starved of serum 30 minutes prior to transfection. Transfection reagents (two leading lipid reagents, one leading polymer-based reagent and Glycofect) were formulated with pDNA based upon their recommended protocols. Transfection efficiency was optimized in accordance with instructions of each of the respective protocols. Solutions of transfection reagent-pDNA (gWiz-Luciferase, Aldevron, Fargo, ND) complexes for each transfection reagent were added in triplicate to corresponding wells (1 µg pDNA per well). Plates were briefly swirled and incubated for four hours, after which more media supplemented with 10% FBS (500 µl) was added to each well. Cells were incubated for an additional 20 h, followed by a media change with serum containing media and 24h of additional incubation time. Media was evacuated from wells and cells were lysed in 100 µl Cell Lysis Buffer (Promega, Madison, WI). Cell lysates were deposited on 96-well plates and analyzed for luciferase activity on a luminometer plate reader (Tecan GENios Pro). Conditions for each reagent yielding best gene expression are reported. Total protein concentration per well was measured using a BCA assay as to quantify RLU of luciferase/mg of protein.

MTT Assay. Cells were prepared and transfected using the same methodology as reported above under the luciferase assay. However, at the 47 h time point media was evacuated from each well and replaced with media containing 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, [MTT] = 0.5 mg/ml). Cells were incubated for an additional 1h then washed with PBS and lysed in 250 µl DMSO. Sample cell lysates were analyzed via absorbance vs. cells only lysate's absorbance to determine cell viability.

Results

It is clear from **Figures 1 and 2** that total transgene expression and cell viability vary substantially from cell line to cell line. Both Lipid Reagent A and Lipid Reagent B show very high levels (1000x to 10,000x of control) of luciferase expression in all cell lines, however at these high expression levels (all within manufacturer recommended dosages) cell viability suffers substantially in all cell lines tested. Transgene expression was also very high, giving RLU/mg responses similar to the lipids, in all cell lines tested with the polymeric transfection reagent. Cell viability improved substantially compared with the lipid-based reagents, however viability generally was between 50% and 70%, with over 60% cell death in HEK-293 cells. Glycofect exhibited similar high transgene expression as with the lipid and polymer reagents, however cell viability was higher than 70% viability all the way to greater than control viability. Maintaining a high recovery rate is especially important for researchers studying sensitive cells and stable cell production.

Conclusion

Delivery method choice is an important factor that contributes to total transgene expression, level of throughput, and cell viability. These factors are critical in sensitive cells, protein production, and the generation of stable cell lines. The Glycofect delivery platform has demonstrated improved DNA transfection in several commonly used cell lines by showing high transgene luciferase expression in with lower toxicity at higher expression conditions. This data indicates that polymeric transfection agents generally show lower toxicity compared with lipid reagents at highest gene expression conditions and are an excellent reagent choice for DNA transfections.

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