Optimizing Medium Components for Polyethylenimine Mediated Transient Transfection

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Abstract
In biocomposite scale polyethylenimine (PEI) mediated transient transfection is a cost effective method for producing high quality plasmid DNA for gene therapy trials. A variety of factors affect the transfection efficiency and include the cell lines used, the level of DNA complexes, and the nutrient conditions in the culture medium. At the present time, the most widely used commercial transfection protocols are based on polyethylenimine (PEI) mediated transient transfection, but these are not always adaptable to large scale manufacture. This is particularly true for stem cell lines which require feeder cell to maintain their growth and differentiation. This study evaluated the effect of dextran sulfate, hydrolysate and phosphate concentrations in the transfection formulation on transfection efficiency. We found that the high concentrations of dextran sulfate, hydrolysate and phosphate had detrimental effects on transfection efficiency, with dextran sulfate having the most significant negative effect.

Introduction
Transient transfection is a commonly used method in various biological applications. However, the cost and ease of use (PEI) is a popular transfection agent used at biocomposites scale for gene therapy transfection. Since the early 2000s, transient transfection has been used in many applications, including the production of recombinant cell lines for vaccine production and in vivo gene therapy trials. The purpose of this study was to evaluate the effect of dextran sulfate, hydrolysate and phosphate concentrations in the transfection formulation on transfection efficiency. We found that the high concentrations of dextran sulfate, hydrolysate and phosphate had detrimental effects on transfection efficiency, with dextran sulfate having the most significant negative effect.

Materials and Methods

Cell line and vector
• The HEK 293 EBNA cell line (American Type Culture Collection, ATCC Number CRL-10852) was obtained from the ATCC. The cell line was passaged at least six passages with viability of 95 - 99%.

Culture and transfect cells
- To make the DNA/PEI complex, the cells were seeded at 1 mL/well in a 12-well non-tissue culture treated plate. Two hours after the cells were seeded, the DNA/PEI complex was added to the cell culture.

Quantification of GFP-transfected HEK 293 cells by fluorometry
- Transfection medium Prototype 65237, JRH Item No. 60864, JRH Biosciences, Inc., modified EX-CELL™293 without dextran sulfate and hydrolysate.

Conclusion
- Dextran sulfate has the most significant negative effect on transfection efficiency.

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No other source of funding was disclosed for this study.