Optimizing Medium Components for Polyethylenimine Mediated Transient Transfection

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Abstract

At bioreactor scale polyethylenimine (PEI) mediated transient transfection is a well established method for recombinant protein expression in the biopharmaceutical industry. However, transient transfection efficiency and protein production levels can vary widely. Furthermore serum-free (SF) and animal-component free (ACF) media are highly desired for target validation and downstream drug development processes in support of regulatory compliance. During the development of a transient transfection platform using HEK 293 EBNA cells, it was observed that several commonly used components such as dextran sulfate, hydrolysate and phosphate could promote cell growth thus improving protein production. Conversely, these components were only able to support high transfection efficiency and protein production when added at certain steps of the transfection process. Transfection of HEK 293 EBNA cells could be inhibited or completely diminished if such critical components were present at other steps of the process. In conclusion these data demonstrated that a transfection protocol can be as critical to efficient PEI mediated transient transfection of HEK 293 EBNA cells as the transfection medium.

Introduction

Transient transfection is a commonly used method for various industrial applications. Because of its low cost and ease of use, PEI is a popular transfection reagent used at bioreactor scale for transient transfection. Serum-free media that support cell growth, transfection and protein expression for PEI mediated transient transfection can better meet regulatory compliance and simplify downstream product purification. Because components required for cell growth and protein production can inhibit or diminish transfection efficiency when present in transfection medium, development of one medium for the entire transient transfection process can be extremely difficult. A protocol that utilizes different media at different stages of the transfection process not only improves the transfection efficiency but also the viable cell density and total protein production. Our data demonstrate that optimization of the transfection protocol for increased recombinant protein production is as important as medium optimization.

Materials and Methods

Cell line and vector

- Transformed primary human embryonic kidney cells 293 expressing Epstein-Barr virus nuclear protein (HEK 293 EBNA), American Type Culture Collection, ATCC Number CRL-10852
- The vector used for transfection was gWIZ™ Green Fluorescent Protein (GFP) Expression Vector under control of an optimized human cytomegalovirus (CMV) promoter, Gene Therapy Systems Inc., San Diego, California USA. The vector concentration was 1 mg/mL.

Transfection reagent and media

- Polyethylenimine, Linear MW 25,000 (PEI), PolySciences, Inc. (Warrington, Pennsylvania USA), Catalog No. 23966, sterile solution at 1 mg/mL in WFI water supplied by R&D department, JRH Biosciences, Inc. (Lenexa, Kansas USA)
- Complete transfection medium EX-CELL™ 293 Serum-Free Medium for HEK 293 Cells, JRH Catalog No. 14570*, JRH Biosciences, Inc., containing dextran sulfate, hydrolysate and phosphate
- Transfection medium Prototype 60864, JRH Item No. 60864, JRH Biosciences, Inc., modified EX-CELL™ 293 without dextran sulfate and hydrolysate
- Transfection medium Prototype 65237, JRH Item No. 65237, JRH Biosciences, Inc., modified EX-CELL™ 293 without dextran sulfate, hydrolysate and minimal phosphate
- Dextran sulfate, hydrolysate and phosphate to titrate into deficient medium were made in the JRH Biosciences, Inc. R&D department

Culture and transfect cells

- HEK 293 EBNA cells were cultivated in serum-free, chemically defined (CD) and ACF medium, Prototype 60864, supplemented with 4 mM L-glutamine solution, JRH Catalog No. 59202, JRH Biosciences, Inc.
- HEK 293 EBNA cells were inoculated at approximately 3 x 10^5cells/mL. The cell culture was maintained in 125 mL shaker flasks with 25 mL of cell suspension in a 37 C, 5% CO_2 incubator.
- To make the DNA/PEI complex for 1 mL of cell suspension, 3.6 µg of PEI was added to 200 µL of transfection medium and the mixture vortexed for 30 seconds. 2.4 µg of DNA was then added in the mixture. After 30 minutes, the DNA/PEI complex was added to the cell suspensions.
- Cells in log phase growth were diluted with variations of transfection media at a 1:4 ratio to reach a cell density of (4-6)e^5/mL. 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.
- At 16 hours post-transfection, feeds were added into the cell culture if needed. At 72 hours after transfection the cells were monitored by fluorescence microscopy and then collected into 96-well plate. The intensity of fluorescence was quantified.

Quantification of GFP-transfected HEK 293 cells by fluorometry

- The level of GFP was quantitated after 72 hours post-transfection by fluorescence microscopy using a Gemini XPS plate reader (λex=480 nm λem=510 nm), Molecular Devices Corporation (Sunnyvale, California, USA).
- Readings were performed on each well and the relative background was subtracted from the readings.

Results and Discussion

To determine what components in the transfection formulation inhibit transfection, a transfection assay was performed for formulations with different component combinations based on literature searches. The data from this experiment (Figure 1) showed that the formulations containing either dextran sulfate, a high concentration of hydrolysate or a high concentration of phosphate, yielded zero or significantly reduced transfection efficiencies.

GFP fluorescence intensities were measured from cells transfected in five media with the same transfection protocol. Fluorescence intensity for cells transfected in dextran sulfate containing medium was equivalent to the background level. With either hydrolysate or phosphate concentrations at 100% of those in complete medium, GFP fluorescence intensity was reduced by about 20% as compared to medium lacking these components. Without these components in the medium viable cell density was decreased (Figure 2A and 2B).

Feeding the cells with complete medium containing dextran sulfate, hydrolysate and phosphate 16 hours after transfection improved total protein productivity (Figure 3). Photos for transfected HEK 293 EBNA cells were taken 72 hours after transfection under the fluorescent microscope as shown in Figure 4A, 4B, 4C and 4D.

Conclusion

- Dextran sulfate has the most significant negative effect on transfection efficiency;
- at the concentration in the medium, it completely inhibited transfection;
- at 100% concentration hydrolysate and phosphate also demonstrated significant negative effects on transfection efficiency by causing 20% reduction in efficiencies;
- however, by performing the transfection process in a deficient medium, followed by feeding cells with a complete medium 16 hours after transfection, 50% transfection efficiency was achieved in combination with good viable cell densities, viabilities and protein production.

*Since this research was conducted, this product is no longer a catalog product. JRH Catalog No. 14570 has been replaced by JRH Catalog No. 14571

Figure 1: GFP Fluorescence Intensity Comparison for Different Transfection Media

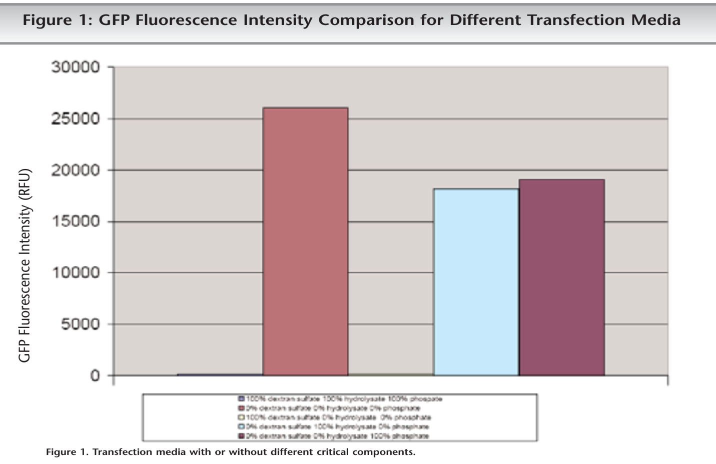

Figure 1. Transfection media with or without different critical components.

Figure 2A: Cell Growth in Complete Medium

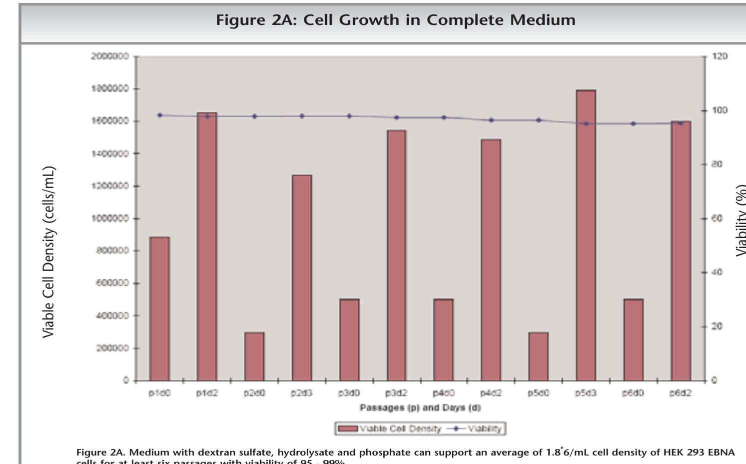

Figure 2A. Medium with dextran sulfate, hydrolysate and phosphate can support an average of 1.8^{e}6/mL cell density of HEK 293 EBNA cells for at least six passages with viability of 95 - 99%.

Figure 2B: Cell Growth in Deficient Medium

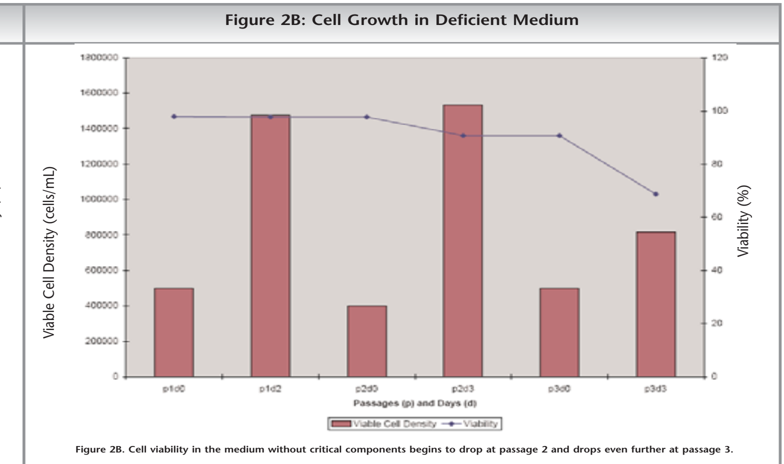

Figure 2B. Cell viability in the medium without critical components begins to drop at passage 2 and drops even further at passage 3.

Figure 3: GFP Fluorescence Intensity for Transfected GPF Cells with Different Feed

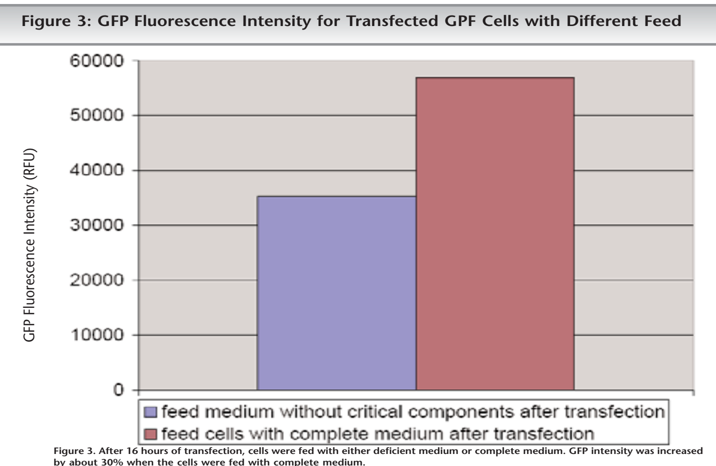

Figure 3. After 16 hours of transfection, cells were fed with either deficient medium or complete medium. GFP intensity was increased by about 30% when the cells were fed with complete medium.

Figure 4: Cells Transfected in Complete Medium

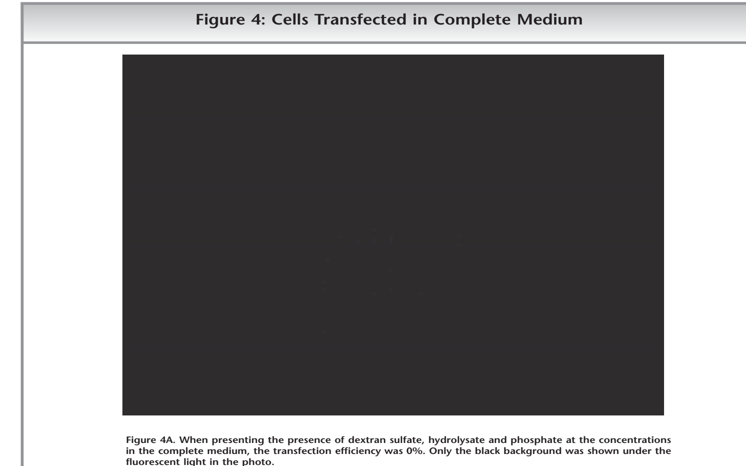

Figure 4A. When presenting the presence of dextran sulfate, hydrolysate and phosphate at the concentrations in the complete medium, the transfection efficiency was 0%. Only the black background was shown under the fluorescent light in the photo.

Figure 4B: Cells Transfection in the Medium Containing 100% Hydrolysate and Phosphate

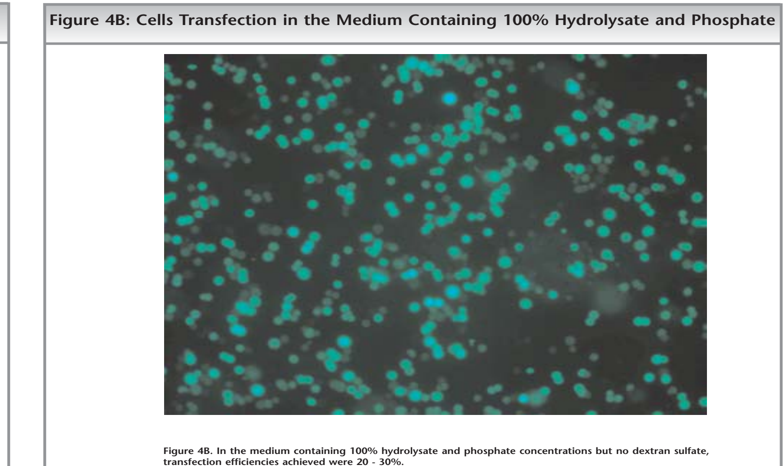

Figure 4B. In the medium containing 100% hydrolysate and phosphate concentrations but no dextran sulfate, transfection efficiencies achieved were 20 - 30%.

Figure 4C: Cells Transfected in Deficient Medium (no dextran sulfate, hydrolysate and minimal phosphate)

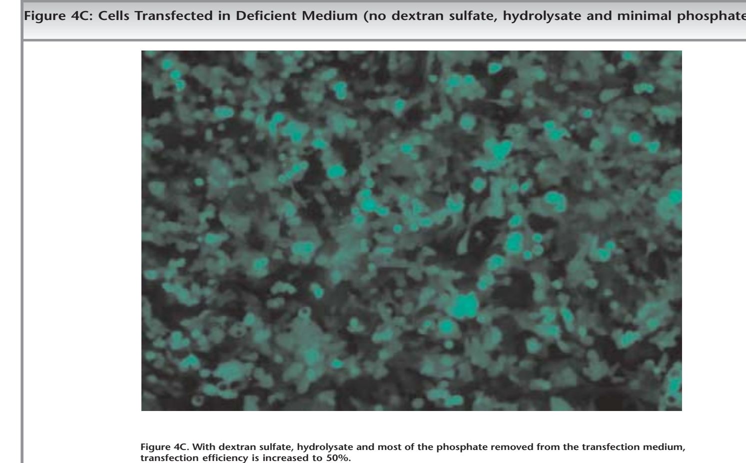

Figure 4C. With dextran sulfate, hydrolysate and most of the phosphate removed from the transfection medium, transfection efficiency is increased to 50%.

Figure 4D: Cells Transfected in Deficient Medium and Fed with Complete Medium 16 Hours After Transfection

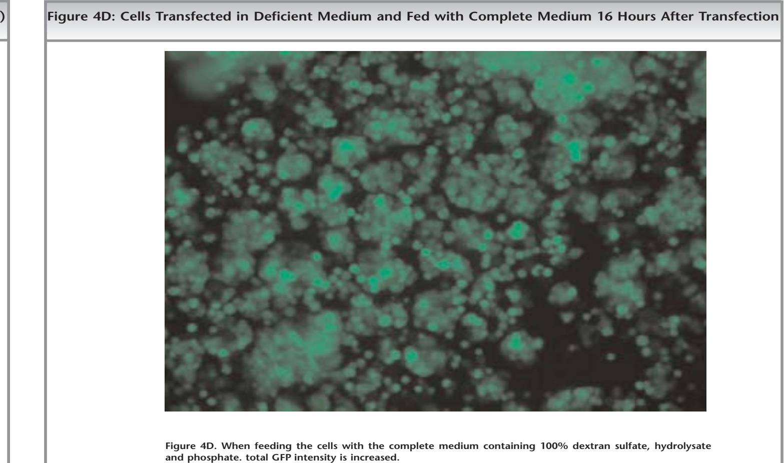

Figure 4D. When feeding the cells with the complete medium containing 100% dextran sulfate, hydrolysate and phosphate. total GFP intensity is increased.

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