Development of a Cationic Reagent for Transient Transfection of Mammalian Cells

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Introduction:
For rapid recombinant protein production in small to medium size volumes, transient transfection of mammalian cells is still the method of choice in biotechnology. However, the available transfection reagents represent a bottleneck due to the high costs associated with the commercial use of lipofectamins and other polycationic transfection reagents such as the widely used polyethylenimine (PEI). While these reagents produce seemingly high transient transfection rates, there is still a strong desire for transfection reagents which allow more secure and easier handling and higher recombinant protein production. To maintain competitiveness, Invivo Biotech Services initiated a joint venture with emp Biotech to develop novel reagents (NB) for transient transfection and recombinant protein production in mammalian cells.

Biological Analysis:
Toxicity:
More than 20 different candidates were checked on toxicity from 0.4 to 40 pg potential transfection reagent per cell. Only one of those candidates showed toxic characteristics. When using two other candidates, cells showed morphological abnormalities, but at concentrations far out of application range.

Transfection Efficiency:
Non toxic candidates were checked for transfection efficiency in a far-ranging DNA to transfection reagent ratio. Selected experiments are shown in Fig.1. Candidates NB 33, NB 97 and NB 98 turned out to work best. Most other candidates showed no or very low transfection efficiency. The optimum in DNA to transfection reagent ratio was tested for most convenient candidates.

Productivity:
Cell culture supernatant of transient transfected cells using new synthesized reagents being most efficient in transfection efficiency named NB 33 and NB 97 were checked on production of secreted alkaline phosphatase (SEAP). We compared those transfections where jetPEI™ (PolyPlus) or PEI were used.

Synthesis:
Required properties:
The task at hand for emp Biotech was the design and synthesis of various functionalized cationic and hydrophilic co-polymers that can potentially mediate between polyanionic plasmid DNA and the negatively charged cell surface, thereby facilitating uptake of DNA into the cells. Two improvements for transfection reagents were of primary interest, namely that of increased solubility and lower cytotoxicity.

Synthesis strategy:
We focused on the introduction of hydrophilic functional groups into the polymer structure to enhance solubility as well as on potential structures which allow rapid intracellular degradation and decrease overall cytotoxicity.

Results:
Several different cationic polymer candidates were chemically synthesized and provided to Invivo Biotech. All products are chemically defined and animal component free and consequently highly suitable for use in cell culture systems.

Increased yields of recombinant proteins using NB33:
We wanted to verify the profitability using NB33 as transfection reagent, shown at SEAP expression levels, under manufacturing conditions. We chose to express transiently thrombomodulin, a highly glycosylated protein, with an molecular weight of around 60 kDa.

Application of NB33 in different cell lines:
Transient transfection NB33 was checked for applicability on transient transfections of different cell lines. Relative SEAP expression increased in each cell line when we used NB33 instead of PEI as transfection reagent.

Improvement of Synthesis:
Experiments have been performed to reproduce synthesis of NB33 and prove long term storage of the product, which have been proceeded successfully. Critical in-process parameters like temperature and duration of the significant reaction step were tested. These variations did not implicate any improvement of transfection efficiency.

Future works to be done:
We want to optimize synthesis parameter to yield a transfection reagent with even better capabilities than NB33. Furthermore there is a need to develop optimized protocols for transient transfection of different mammalian cell lines with NB33.

Conclusion:
We synthesized several candidates as novel cationic reagents for transient transfection of mammalian cells. When most promising candidate NB33 was used in productivity experiments, yield of recombinant proteins was increased up to 3 times, when compared to conventional transfection reagent PEI. NB33 is also suitable to be routinely used for cell lines HEK293F, CHO-S, and CAP-T cells as well.

Take home message:
• New developed transfection reagent
• Chemically defined and animal component free
• More productivity than using PEI or jetPEI™
• Up to 3 times increased productivity as compared to conventional PEI
• Tested for transfection of CHO, HEK and CAP-T cells