Development of a Polycationic Reagent for Transient Transfection of Mammalian Cells

Veiczi, M.[1,#], Puengel, S.[2,#], Welsink, T.[2], Faust, D.[2], Vater, V.[2], Weglöhner, W.[2], Levison, D.[1], Moeller, U.[1]

[1] *emp Biotech GmbH, Berlin, Germany* [2] *InVivo Biotech Services GmbH, Hennigsdorf, Germany* [#] these authors contributed equally

To whom correspondence should be addressed: dlevison@empbiotech.com

Synthesis and Characterization of INVect

Non-viral transfection reagents (TR) facilitate uptake of DNA into cells by one of two mechanisms, polyplex or lipoplex formation. Negatively charged DNA binds to polycations and the complex is taken up by the cell via endocytosis. After entering the cell, the DNA is transcribed and translated into target protein of interest.

Polyethylenimine (PEI) is a polycation which is commonly utilized as a gene carrier in transient transfection. However, the high charge density of PEI is believed to be associated with high cell toxicity, limiting its general application. To improve gene transfection efficiency while maintaining low toxicity, a set of various polycations of different lengths and chemistries has been synthesized and investigated.

Optimal performance has been observed from a low molecular weight polycation crosslinked with an enzyme-degradable linking reagent. The novel transfection reagent, named INVect, delivers high transfection efficiency of up to 90%. Furthermore, INVect displays extremely low toxicity for mammalian cells such as CAP-T, HEK293-F or CHO-S. INVect demonstrates chemically stability between pH 2 through 11 and at temperatures up to 50°C in our studies. In light of its superior transfection efficiency and low toxicity, INVect provides a new and powerful tool for the transfection of mammalian cells.

In ***Fig.1***, the ability of INVect to both complex with plasmids and to protect the plasmid from DNAse degradation is demonstrated with green fluorescent protein (GFP) plasmid and secreted alkaline phosphatase (SEAP) plasmid. PEI (25 kDa) is used as a control.

An optimal equivalent ratio of INVect:plasmid was determined after combining TR with varying plasmid amounts (0.5 to 10 µg, using either GFP-p or SEAP-p) in water at RT for 30 minutes and determining the amount of TR required for migration in gel electrophoresis to no longer be observed. Again, PEI (25 kDa) was used as a control. A ratio of 6:1 TR:plasmid was selected (***Fig. 1A***) for subsequent evaluation of resistance to DNAse degradation.

The TR/plasmid complexes were next incubated with DNAse at three time intervals (t = 30, 60, and 120 minutes) at a temperature of 37°C, followed first by deactivation with 250 mM EDTA (pH = 8,0) and then with complex denaturation using 1% SDS (see ***Fig.1B***). Plasmid DNA (SEAP-p) was then analyzed using gel electrophoresis (0,8%-agarose, EtBr staining, U = 100V, marked by yellow circle and arrow) and compared with PEI (25 kDa).

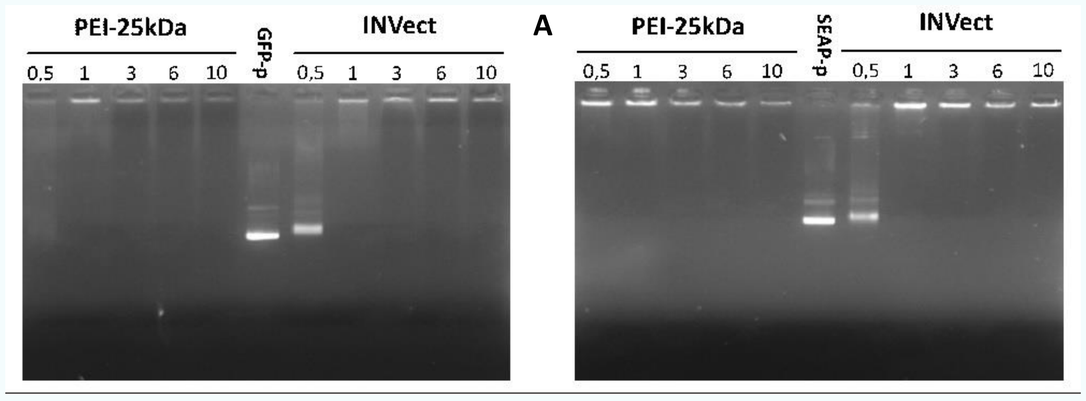

Fig.1 : **Illustration of plasmid complex formation (A) and plasmid protection (B) ability of INVect in comparison to PEI-25kDa using SEAP plasmid (p) over a variey of TR/Plasmid ratios (µg/µg), Control = SEAP-p/TR-polyplex (TR/plasmid = 6) in buffer without DNAse after treatment with 1% SDS.**

Use of INVect

Over 20 different candidates were evaluated for cell toxicity by using 0.4 to 40 pg transfection reagent per cell. Only once did a TR candidate demonstrate observable toxic characteristics, while morphological abnormities in cells were observed in two further candidates but at concentrations far out of the normal application range. Low toxicity candidates were checked for transfection efficiency over a wide range of DNA to transfection reagent ratios.

One transfection reagent, “INVect”, provided the best transfection efficiency of about 45% and was further investigated. Subsequent stepwise optimizations of INVect, including synthetic parameters and transfection procedures, ultimately led to a transfection efficiency of about 90% as shown in ***Fig. 2*** (a). Further verification was obtained by determination of post-transfection cell productivity. Cells were transfected with a SEAP-gene harboring plasmid, with product concentration being measured on day 3 and 6 post-transfection. The optimized transfection system using INVect provided a minimum 2-fold increase in SEAP productivity over PEI (25 kDa) based transfection.

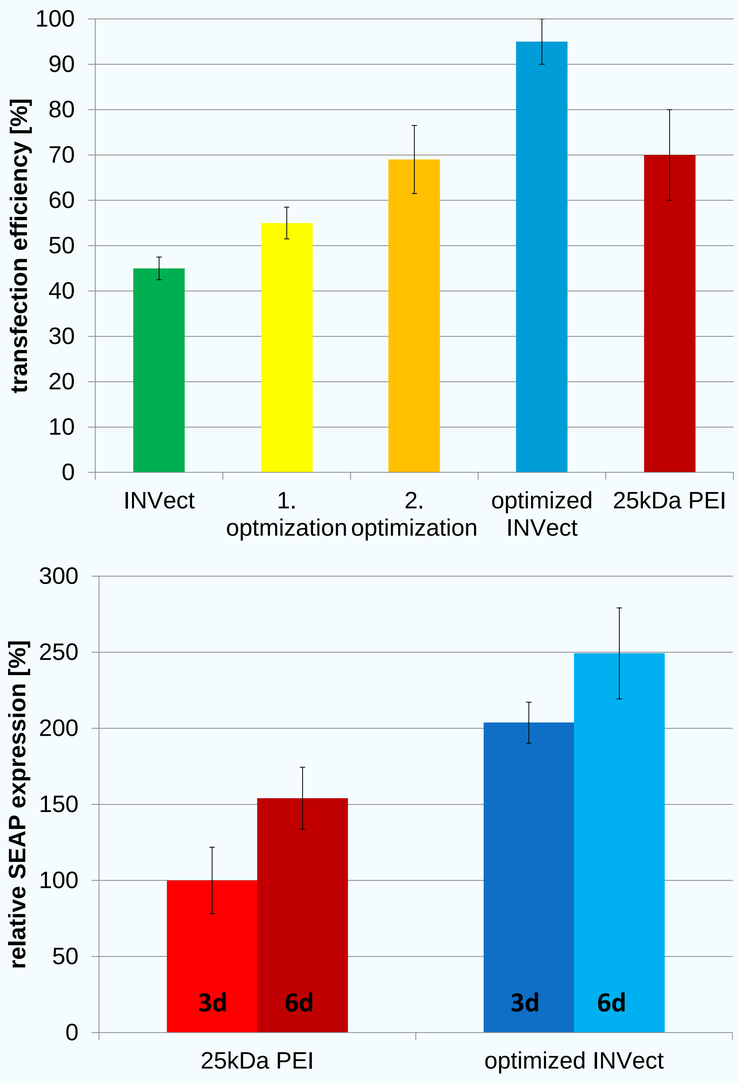

Fig.2: **(a) Optimization of synthetic parameters and transfection procedure. In the optimized protocol cells were transfected with 10 µg per mL of a GFP-gene harboring plasmid at a cell density of 5 x 10^6 cells per mL in 4 mL FreeStyle™ 293 Expression Medium (Life Technologies) with transfection reagent to DNA ratio of 6:1 (w/w). Cultures were supplemented 2 hour post transfection with same volume Protein Expression Medium (Life Technologies). Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells using a FACSCalibur (Becton, Dickinson and Company).**
(b) Relative SEAP expression following transient transfection using INVect. Cells were transfected with optimized INVect according to the protocol above. SEAP expression was determined in cell culture supernatant of day 3 and day 6 post transfection by a photometric pNPP turn-over assay. SEAP expression was normalized to sample “PEI – day 3 post transfection”.

Temperature and pH stability of INVect

To determine the chemical and temperature stability of INVect, long-term studies were implemented, demonstrating the overall functional stability of this novel transfection reagent. The function of INVect was not observed to be negatively affected by incubation in buffers within the pH range of 2-11 or by temperatures ranging from 37 °C to -80 °C, as INVect consistently provided transfection efficiencies of greater than 80% after incubation (***Fig.3***).

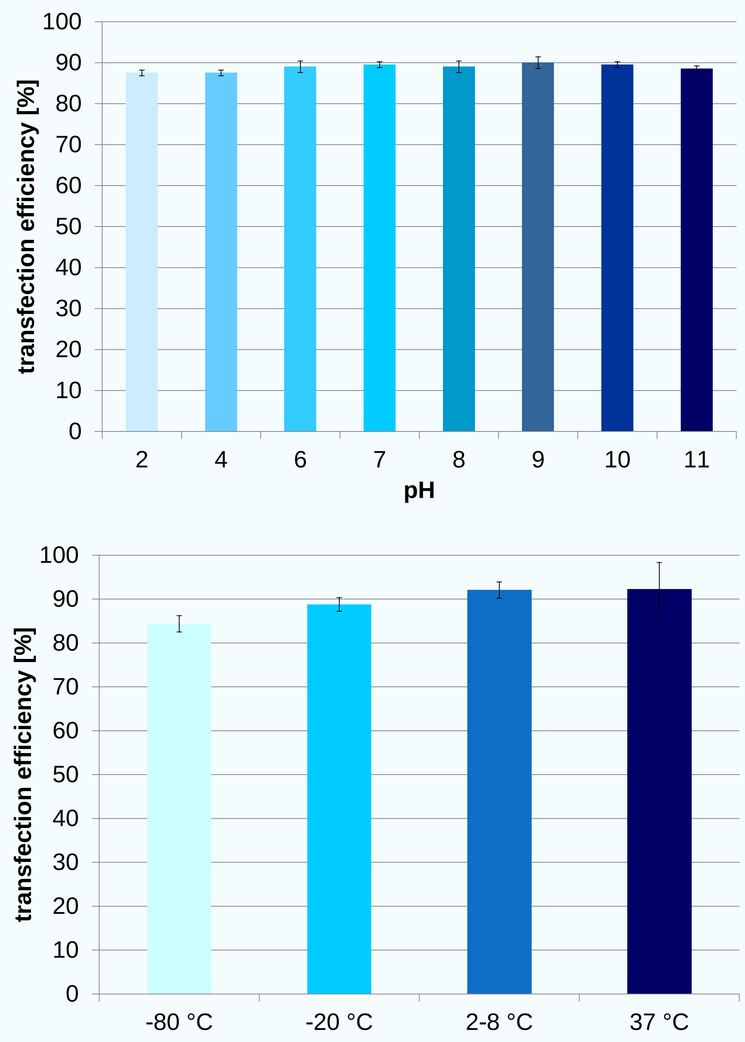

Fig.3: ***(a)*** **pH stability of INVect. INVect was dissolved in water at a concentration of 1 mg/mL and pH was adjusted in a range from 2 to 11 two weeks before use. Cells were transfected with 10 µg per mL of a GFP-gene harboring plasmid at a cell density of 5 x 10^6 cells per mL in 4 mL FreeStyle™ 293 Expression Medium (Life Technologies) with the novel transfection reagent INVect and DNA to transfection reagent ratio 1 to 6. Cultures were supplemented 2 hour post transfection with same volume Protein Expression Medium (Life Technologies). Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells using a FACSCalibur (Becton, Dickinson and Company).**
(b) Temperature stability of INVect. INVect was dissolved in water at a concentration of 1 mg/mL and stored at different temperatures for several months. Cells were transfected with optimized INVect according to the protocol above.

Conclusion:

INVect is a novel polycationic transfection reagent which demonstrates low cell toxicity for transient transfection of mammalian cells while delivering extremely high transfection efficiencies of up to **90% 24 hours post transfection.** The stability of INVect at elevated temperatures and across a wide range of pH has been investigated. INVect can be used effectively with all common cell lines, including HEK293-F, CHO-S, and CAP-T cells.