Optimization of ternary complexes as gene delivery systems

Nazgol Karimi Dastgerdi [1,2], Cameron Alexander[1], Pratik Gurnani[1], Rasoul Dinarvand[2], Keith Spriggs[1]

[1] Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, NG7 2RD, UK; [2]School of Pharmacy, Tehran University of Medical Science, Iran

Background: Gene delivery systems are essential for gene therapy to protect the gene from enzymatic degradation and facilitate membrane translocation. PEI (polyethyleneimine) is a widely used cationic polymer in gene delivery, however, its usage has been limited due to high cytotoxicity. One strategy to reduce this toxicity is to use a negatively charged polymer coating to reduce the surface charge and thus membrane interaction. Poly (glutamic acid) (PGA), is a polyanionic, hydrophilic, biocompatible and biodegradable polymer which fits these criteria. Furthermore, polyplex surface charge is one of the determining factors for cell internalization. However, due to the increased complexity of these formulations containing a third component, we sought to systematically evaluate the effects of particle size and surface charge of PEI/DNA/PGA (N/P/C) polyplexes under different preparation conditions.

Methods: Polyplexes were prepared via two different methods, direct pipetting and slow addition using a syringe pump. In both cases binary polyplexes (i.e. DNA and PEI only) were prepared with branched PEI (20 M_w = 20 kg mol^{-1}) and model calf thymus DNA at N/P = 20:1 in HEPES (20 mM). For the pipetting method, 500µl of DNA and 500 µl of PEI solution (0.2 mg/ml) were thoroughly mixed by pipetting. Additional experiments were performed to study polyplex formulation under different pH buffers. For the syringe pump method one solution (either DNA or PEI) was fed into a stirred vial of the other solution (either DNA or PEI) at a variety of flow rate. The effects of adding either PEI to DNA or vice versa, stirring rate (100-1000 rpm) and rate of addition (over 1,3 or 10 min) were systematically evaluated. Preparation of ternary complexes: DNA/PEI/PGA ternary complexes were constructed with different N/P/C charge ratios by adding PGA to pre-prepared polyplexes prepared above. Then the different concentrations of PGA were added to the prepared polyplex by syringe pump or pipetting with different ratios, and the resulting stability of these complexes were monitored by DLS and zeta potential.

Results: These experiments confirmed that the pH and ratio of each material (i.e. PEI, PGA and DNA) and the fabrication procedure (pipetting or syringe pump) influence polyplexes. At N/P ratio of 20, PEI condensed and entrapped the DNA and the 78 nm polyplexes formed with zeta potential of 41.6mV. The pH value of 5 formed a larger polyplex because of the more DNA binding ability of PEI at the low pH. A small, monodispersed and reproducible polyplexes were gained by syringe pump synthesizing method. Slowly DNA addition reduced the polyplexes size, however, this change was statistically insignificant. Additionally, high-speed mixing of DNA and PEI mixture prevented polyplexes aggregation and consequently, we could have the smaller polyplexes. Adding PGA to polyplexes reduced the surface charge up to 30.7mV. The method of adding PGA (syringe or pipetting) didn't affect the particle size. By adding the PGA up to a value of 2.5 the size didn't change dramatically and after that, the size increased rapidly. Because the more amount of PGA couldn't interact with PEI to form ternary complexes.

Conclusions: In conclusion, to achieve small ternary polyplexes the syringe pump method is the most viable route to add DNA to the polymer slowly (in 3 min) and mix gently with high speed (1000rpm). Low pH (pH=5) and optimal N/C/P (20:1:1) are important in polyplex preparation. Additionally, coating the polyplex with PGA can reduce the surface charge.