

## **Optimization of ternary complexes as gene delivery systems**

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**Background:** Gene delivery system which is essential for gene therapy needs employing carrier to protect the gene from enzymatic degradation and facilitate membrane entrance. PEI (poly ethylenimine) is a cationic polymer, and it is considered as a standard reference for gene delivery. However, its usage was limited due to its high risk of toxicity. One of the strategies used to reduce the toxicity is using negatively charged polymer to reduce the surface charge. Poly Glutamic acid (PGA), which is a negatively charged, hydrophilic, non-toxic, and biocompatible polymer, could be employed to gene delivery system. Additionally, physicochemical properties of a delivery vehicle have numerous effects on gene delivery. Several studies indicate that nano-sized polyplexes have a higher intracellular uptake. Surface charge of polyplexes is one of the determining factors for cell internalization and polyplexes toxicity. Thus, adjusting the size and zeta potential of the carrier are essential in gene delivery. The size of polyplexes was varied based on the N/P ratio, pH of medium and preparation procedure. Pipetting and syringe pump are methods that could be used for polyplex preparation. In this study, these parameters were examined to find out the optimized condition.

### **Methods:**

Polyplex preparation by pipetting: The polyplexes with different N/P ratios in pH 5,6, and 7 were made by pipetting technique. The HEPES buffer (0.02M) was used, and its pH was adjusted by adding HCl. A stock solution of DNA was diluted with adequate volume of HEPES to reach different desired N/P ratios to obtain polyplexes with different N/P ratios. 500µl of DNA and 500 µl of PEI solution (0.2 mg/ml) was added to the calf DNA and thoroughly mixed by pipetting.

Polyplex preparation by syringe pump method: To obtain the polyplexes, the stock solution of DNA was diluted with adequate volumes of HEPES corresponding to different desired N/P ratios. At first, the 500µl of PEI and DNA were taken. One of the materials, i.e. PEI or DNA, was loaded into the syringe and the other one was transferred to the tube. Then, the syringe pump flow rate was set to the desired value, and the material in the syringe was injected into the tube solution. The mixture gently stirred under magnetic stirring. The stirring speed varies from 100-1000 rpm, and the material adding to each other in 1,3, or 10 min. 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. The 500 DNA µl was loaded to the syringe and in 3 min added to the 500 µl of PEI and the mixture were mixed by 1000 rpm. Then the 500 µl of PGA were added to the prepared polyplex by syringe pump or pipetting with different ratios.

**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 polyplexes formed. 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. Adding PGA reduce the surface charge. The size of the Ternary complexes increased in a comparison of polyplexes.

**Conclusions:** In conclusion ternary complex with N/P/C ratio of 20:1:1 in pH 5 which prepared by a syringe pump in 3 min with the stirring speed of 1000rpm has the small size of 73nm, narrow dispersity (PDI=0.19) and the zeta potential of 30.7mV.

