Self Assembled Nanostructures of Triblock Co-polymer and Plasmid DNA for Gene Delivery

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ABSTRACT

The formation of the self-assembled nanostructures of tri-block copolymer (PEO-PPO-PEO or TBCP) and plasmid DNA (pDNA) and their function as a non-viral vector to deliver pDNA is shown in the present work. Green fluorescence protein (GFP) pDNA was used as a model DNA. Addition of pDNA to the aqueous solution of TBCP, led to the formation of self assembled nanostructures of pDNA/TBCP. Encapsulation of DNA within the polymer micelles was proved by the gel electrophoresis experiments wherein pDNA/polymer composite didn't show any mobility under the electric field. Mixtures of different weight ratios of pDNA and TBCP have been made to screen the optimum ratio to achieve high transformation. We observed upto 6 folds higher transformation with the use of TBCP at the weight ratio of 1:10. Further, we demonstrated the integrity of the pDNA in the complex of pDNA/TBCP and the function of the pDNA in the cellular environment using *E. coli* DH 5α as a model microorganism.

Keywords: tri-block copolymer, non-viral vector, plasmid DNA, self assembled complex, transformation

1 INTRODUCTION

Numerous human disease stem form defective genes [1] and gene therapy or gene silencing are common approaches used to treat such diseases. Gene therapy has been regarded as one of the most promising and ultimate cure for many life threatening and debilitating diseases such as cancer [2]. Viral vectors have been conventionally used as DNA carriers, which have potential side effects. Hence it is important to develop efficient non-viral gene delivery vectors in order to realize the full potential of gene therapy in curing the afore-mentioned diseases. Among the non-viral DNA delivery vehicles, functionalized gold nanoparticles, mesoporous silica materials [3-5] and cationic polymers [1, 5-7] have attracted significant attention.

The major disadvantage of using cationic polymers as DNA delivery vehicles is the DNA condensation within the polymer vesicle, which result in low transfection efficiency. [8]. Thus we have used a neutral triblock copolymers poly (ethylene oxide) - poly (propylene oxide) – poly (ethylene oxide) (PEO-PPO-PEO), which have been widely used in the chemical and pharmaceutical industries as detergents, colloidal dispersion stabilizers and in cosmetic products [9]. These triblock systems show thermoreversible gelation around body temperature (37ºC) and therefore, are particularly appropriate for biomedical applications such as drug delivery and tissue engineering [6].

We have used these TBCP as DNA delivery vehicles after their formation of self-assembled superstructures with pDNA [10]. Very little is known about the physicochemical aspects that govern the association of the negatively charged polyelectrolyte that is DNA, with these self-organizing non-ionic amphiphilic block copolymers. We have studied their interaction using different analytical techniques to establish a correlation between physicochemical properties of PEO-PPO-PEO (TBCP)/DNA formulations and their efficiency in DNA delivery. In curent work, we demonstrate the use of TBCP as a non-viral vector to deliver pDNA. We have screened the optimum ratio of pDNA/TBCP complexes to achieve high transformation and showed integrity of the pDNA in the complex of pDNA/TBCP using *E. coli* DH 5α by the expression of green fluorescence protein (GFP) gene .

2 EXPERIMENTAL SECTION

2.1 Reagents and Materials

Nutrient agar (microbiology grade), Luria-Bertani (molecular biology grade) and triblock copolymer (PEO-PPO-PEO) were obtained from oxoid, US Biological and Sigma-Aldrich, respectively and used as received. LB broth and nutrient agar medium were used to grow and maintain the bacterial culture as per the standard protocol. All the solutions were prepared using deionised MilliQ water (18.2 MΩ-cm).

2.2 Preparation of pDNA/TBCP complex

pDNA containing green fluorescence protein (GFP) gene and ampicillin resistance gene was isolated

according to the Shambrook *et al.* with slight modifications [11].

Stock solution of TBCP was prepared by dissolving 10 gm of TBCP in 100 ml of deionised miliQ water at 50°C.

To screen the optimum ratio of pDNA/TBCP to achieve high transformation, 10 µg pDNA (1µg/µl) was incubated with different concentraions of TBCP (10 µg, 50 µg, 100 µg, 150 µg, 200 µg, 500 µg and 1000 µg) for 2 hours at 37°C for complex formation.

2.3 Instrumentation and Characterization

The pDNA/TBCP complexes were characterised by Transmission Electron Microscopy (TEM) and Agarose gel electrophoresis. TEM imaging of these samples were carried out using a JEOL 100 KV instrument. BioRad gel documentation imaging system was used to visualize gel under UV light. Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Photoelectron Spectroscopy (XPS) were also used to characterize the physiochemical interaction between pDNA and TBCP (data not included for brevity).

2.4 Mobility gel assay to study pDNA/TBCP complex through agarose gel electrophoresis

Agarose gel electrophoresis was used to study the mobility of pDNA/TBCP complexes under electric field through mobility gel assay. pDNA/TBCP complexes were prepared at varying pDNA:TBCP ratios (1:0, 1:1, 1:5, 1:10, 1:15, 1:20, 1:50 and 1:100). Ampicillin resistance and green fluorescence protein pDNA was selected as model DNA. 10 µL of the formed complex solution was mixed with 1 µL of loading dye and loaded onto a 0.8% agarose gel containing 0.5 µg/ml EtBr. Electrophoresis was carried out at 90 Volt for 1 hour in TAE buffer solution. The gel was visualized on BioRad gel documentation imaging system (Figure 2).

2.5 Transformation studies

E. coli DH 5α was used as model microorganism for transformation studies. Competent cells were prepared with $CaCl_2$.$MgCl_2$ mediated methods in the presence of tetracyclin. More specifically, single colony of *E. coli* was inoculated in 10 ml of LB broth with tetracyclin (10 µg/ml) and incubated overnight at 37ºC, 200 rpm on orbital shaker. 1 ml of freshly grown bacterial culture was used to inoculate 50 ml LB (10µg/ml tetracyclin) and incubated for 3 hours at 37ºC on orbital shaker at 200 rpm till we arrive the optial density 0.5-0.6 at 600 nm. The broth was kept on ice for 1 hour and transferred into the ice cooled sterilized centrifuge tubes. The content was centrifuged at 4000 rpm for 10 min at 4ºC. The pellet was resuspended in 20 ml freshly prepared ice cold $CaCl_2$.$MgCl_2$ solution (20 mM $CaCl_2$ 80 mM $MgCl_2$). The bacterial cells were recovered by centrifugation at 4000 rpm for 10 minutes at 4ºC and the cells were suspended in 2 ml of 100 mM $CaCl_2$ and used for transformation experiments.

To perform transformation, according to table 1 different weight ratio of pDNA and TBCP were incubated for 2 hours at 37ºC and 200 rpm on orbital mixer incubator. After incubation of pDNA:TBCP, 200 µl of freshly prepared competent cells were added and further incubation on ice for 30 minutes, followed by heat shock at 42ºC for 90 seconds and immediately transferred in ice for next 2 minutes. Then 800 µl of LB media was added in this, while 200 µl of competent cell and 800 µl of LB media were used as negative control. These were kept for further incubation at 37ºC at 200 rpm on orbital mixer incubator for 1 hour. After incubation 100 µl aliquots were spread on ampicillin containing plates (10 µg/ml in each plate). All the plates were incubated at 37ºC and colonies grown after overnight incubation were counted.

S.No.	pDNA	TBCP	pDNA:TBCP
1	10 µg	-	1:0
2	10 µg	10 µg	1:1
3	10 µg	50 µg	1:5
4	10 µg	100 µg	1:10
5	10 µg	150 µg	1:15
6	10 µg	200 µg	1:20
7	10 µg	500 µg	1:50
8	10 µg	1000 µg	1:100

Table 1: Weight ratios of pDNA and TBCP used to screen optimum ratio of pDNA/TBCP complex for transformation.

3. RESULTS AND DISCUSSION

In aqueous solution, TBCP have a tendency to self assemble into spherical micelles and their size is concentration dependent and in higher concentrations, they form different shape micells. Since it has the hydrophilic PEO segment, it weakly coordinates to the DNA by electrostatic interactions and form spherical vesicles wherein DNA entrapped inside [8].

Figure 1 shows TEM images of TBCP and pDNA-TBCP nanohybrid complex of different weight ratios. TBCP was spherical in shape and ranging from 0.1 µm to 10 µm in size when concentration of polymer increased from 0.1 mg/ml to 10 mg/ml. It can be clearly seen that after complex formation (interaction of pDNA-TBCP) TBCP vesicles encapsulate pDNA. As we increase the concentration of TBCP compare to pDNA, it enhanced the distance between individual TBCP vesicles. At a sufficient charge ratio of nitrogen to phosphate (N:P), the polymer can condense DNA to sizes compatible with celluar uptake and provides steric protection from nuclear degradation [12].

Self assembled complexs of pDNA/TBCP were prepared at varying ratios such as 1:1, 1:5, 1:10, 1:15, 1:20, 1:50 and 1:100. After complex formation, 10 µL of the pDNA/TBCP solution was mixed with 1 µL of loading dye and loaded onto agarose gel and the gel was

visualized on BioRad gel documentation imaging system. Agarose gel electrophoresis demonstrated the formation of pDNA/TBCP complexes.

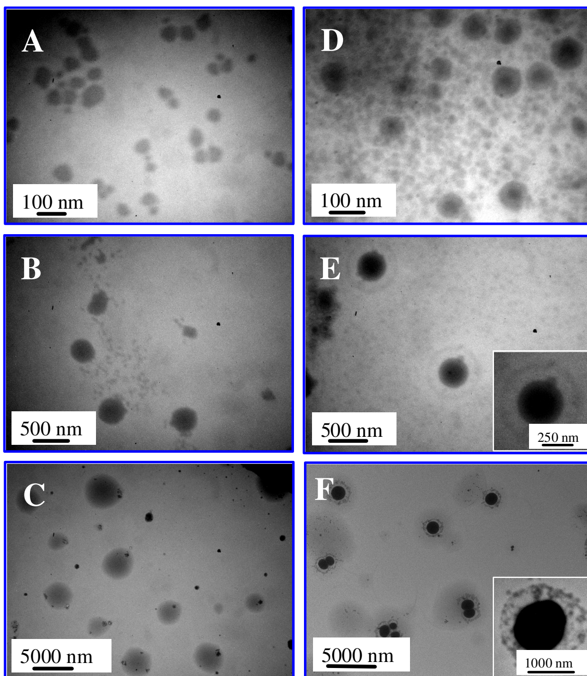

Figure 1: TEM images of as-formed TBCP micelles when their concentration is 0.1 mg/ml (A), 0.5 mg/ml (B), and 1 mg/ml (C). TEM images of pDNA:TBCP complex after incubation of different ratios of pDNA and TBCP such as 1:1 (D), 1:5 (E), and 1:10 (F).

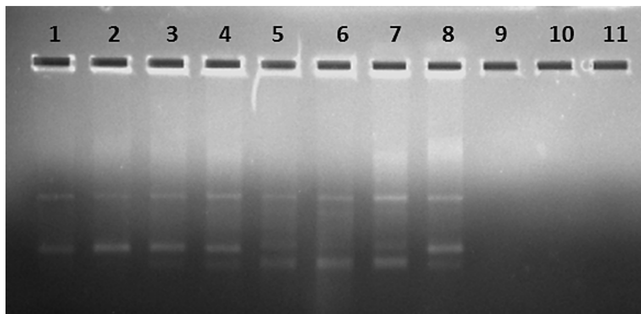

Figure 2: Agarose gel electrophoresis of plasmid DNA and pDNA/TBCP complexes :- **Lane 1**- pDNA, **lane 2-8**- pDNA:TBCP 1:1, 1:5, 1:10, 1:15, 1:20, 1:50 and 1:100, respectively and **lane 9-11** TBCP in the concentration of 0.1, 0.5 and 1.0 mg/ml, respectively.

As shown in Figure 2 lane 1, control plasmid DNA moved under the influence of electric field, while lanes 2-8 showed movement of pDNA present in pDNA/TBCP complexes. Land 2-8 showed that in pDNA/TBCP complexes only small fraction of pDNA moved in electric field and most of the pDNA remained in the wells (lane 2-8) as the fluorescence intensity is quite high near wells compare to lane 1. This suggested the binding between pDNA and polymer. It also suggest that when we apply electric field on these pDNA/TBCP complexes, fraction of pDNA dissociated from the complexes due to the electic field, while most of the pDNA stay in complex system which contributed in the fluorescence intensity near the wells (lane 2-8). Control experiments were also performed where pure TBCP in the concentration of 0.1, 0.5 and 1.0 mg/ml was used in gel electrophoresis (lane 9-12) to show that fluorescence near the wells was originated from pDNA. As in the case of pure polymer, fluorescence was not observed.

Further, to prove the integrity of the pDNA in the complexes of pDNA/TBCP, we demonstrated the function of the pDNA in the cellular environment using *E. coli* DH 5α as a model microorganism. As it is reported that after transformation, if GFP pDNA is delivered into cells and stay functional, GFP will be synthesized in the cell through the expression of GFP gene and it will be fluorescent. Figure 3 showes expression of green fluorescence protein (GFP) plasmid DNA in transformed colonies.

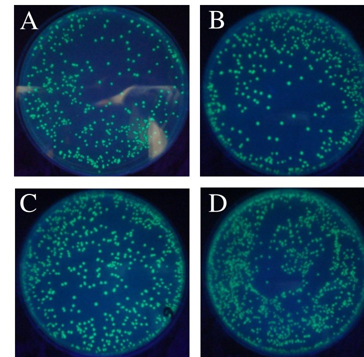

Figure 3: Expression of GFP plasmid DNA in transformed colonies grown on antibiotic plates. pDNA (A), pDNA/TBCP complexes (B-D) as 1:1 (B), 1:5 (C), and 1:10 (D) respectively.

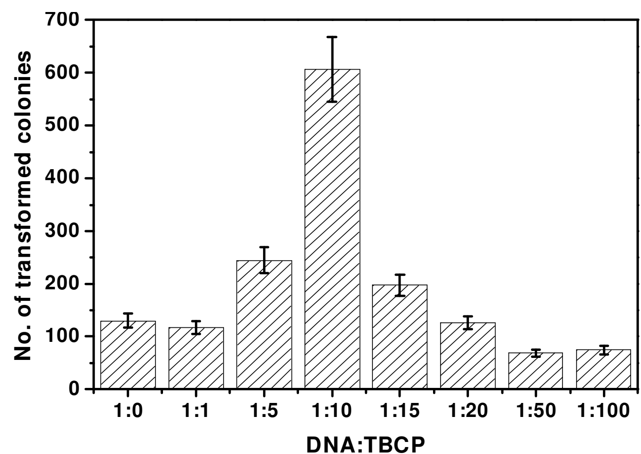

Figure 4: Number of transformed colonies grown on ampicillin plates.

According to table 1 different weight ratios of pDNA and TBCP were incubated and their transformation studies were performed on *E. coli* competent cells. Competent cells were prepared by calcium chloride / magnesium chloride ($CaCl_2.MgCl_2$) mediated metho. Figure 4 shows number of transformed colonies grown on ampicillin plates (10 µg/ml) after transformation. All the plates were incubated at 37ºC and colonies grown after overnight incubation were counted. Our results demonstrated that with the increasing ratio of TBCP, transformation increased upto certain amount and then it stared to decline. We found that pDNA:TBCP at 1:10 weight ratio, transformation was maximum and compare to only pDNA, it showed 6 folds higher transformation.

4 CONCLUSIONS

In conclusion, we have formed the self-assembled nanostructures of triblock copolymer (TBCP) and pDNA and investigated their potential as a non-viral DNA delivery vector. The copolymer is composed of PEO-PPO-PEO and it was capable to self assemble with plasmid DNA forming a compact TBCP-DNA nanoconjuagte system. These nanostructures were found to have spherical shape and the size of this complex depends on the concentration of TBCP. TBCP showed greatly enhanced transformation efficiency. Further, we have shown integrity of the pDNA in the complex of pDNA/TBCP and the function of the pDNA in the cellular environment. These results revealed that TBCP has a potential to become one of the biocompatible and efficient DNA delivery carriers.

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