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Optimization Polyplexe Stability in Different Glucose Concentrations

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Abstract: Polyethelenimine (PEI) has been used to synthesis Polyplexesforgene transferring applications, it shows high efficacy in gene expression and formmore stable complex with DNA, different concentration of glucose was used in preparation polyplex with GFP vector in different percentage, retardation assay, dissociation assay and protective activity against DNase was performed to test polyplex stability, the results show that all N/P ratios was high stability except N/P 2 at2%,3%,4%, 5% glucose also it show protective activity against DNase activity.
Key words : polyethylenimine, polyplexe, Glucose.

Introduction

PEI used as a gene delivery system in different cell types It was had buffered property can be protected DNA from degradation by endosomal lysosome so the high density of PEI show high efficacy and low cytotoxicity, this system was synthesis by different methods to decrease its cytotoxicity and increased efficiency. The studies used additive compounds with PEI such as PEI-grafted-PEG in different ratios to reduce cell cytotoxicity and aggregation, although the cytotoxicity of PEI was dependent on its substitution, but don't depend on the molecular weight of this complex[1]. PEI was conjugated with targeting motifs , PIE-grafted-PEG was incorporated in integrin binding of RGD peptide, also it incorporated with monoclonal antibody against human epidermal growth factor receptor-2 (HER-2) in cancer cell, It bond with linear PEI to enhance transfection efficiency in cancer cell, also Folate-polyethylene glycol-folate-grafted-polyethylenimine (FPF-g-PEI) was synthesized by grafting folate-PEG-folate to PEI. It used to transfer small interfering RNA Throw cell surface [2].

Park and co-worker also mention water soluble lipopolymer (WSLP) was synthesized by conjugated cholesterol chloroformate to a low molecular weight of PEI[3], This complex was bound with DNA to form stable colloid partials 70 nm, this complex have buffering effect this useful of endosomal escape and facility transfection complex in side cell , stability of small complex partials of this staircase structure resulted from the hydrophobic cholesterol of WSLP which enhance gene transfer efficiency. Zintchenko *et al*., used it to transfer siRNA to Neuro2ALuc cells , they found that the modified PEI was lower size and more stable than normal PIE, in last recent years large number of studies recorded successful of PEI in gene transfer[4].

Materials and methods:

Polyplex was prepared to form complex between DNA plasmid (Turbu-GFP, Evrogen) and plyethylenimine (branched MW 25000 k, Sigma Aldrich) in a different N/P ratios according to[5] with modification:

1. Polyethylinimine was dissolved in dH2O as a stock solution by 0.0323 g/100 ml and 0.0032g/100ml
2. After its dissolved pH was adjusted at 7.5then solution sterilizeby 0.22 nm Millipore.
3. Solution freezing and thawed 4 times. Then it store at -20 C.
4. PEI solution was added to DNA with mixing according to the equation

PEI (µl) = 3x DNA con µg x(N/P) ratio/ molarity of stock solution (nm/µl)

1. Mixture incubation at room temperature for 30 min.
2. Polyplex assays can be performed.
3. Retardation assay: This assay was performed by electrophoresis complexes in different N/P ratio (2, 4, 6, 8, 10) in 0.7% agarose, 0.5 X TBE buffer for 45 min at 75V.

B- Dissociation complex in different d-glucose solution according to[6]

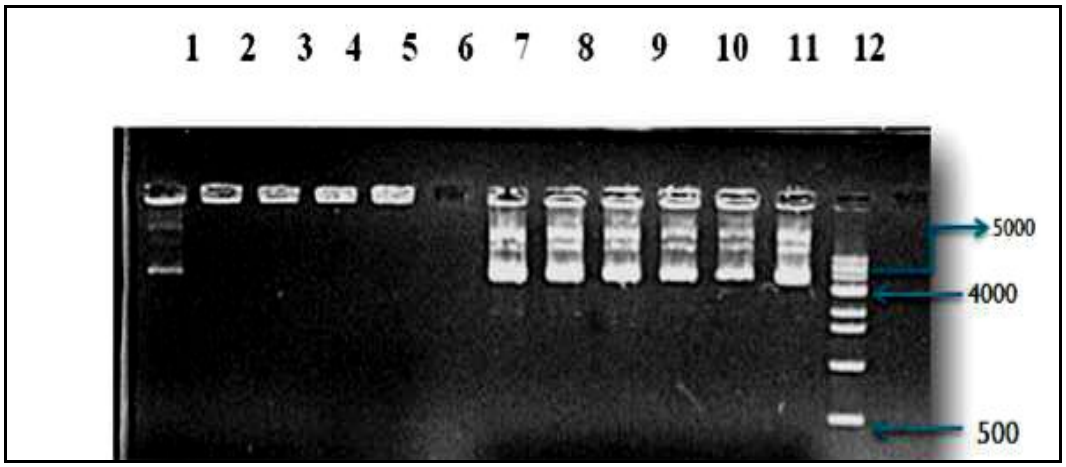
1. Different D-glucose percentage (1%, 2%, 3%, 4%, 5%) was added to the ploy plex solution in N/P ratio 2.
2. Mixture was incubated at 37 Cº overnight,
3. Mixture was centrifugation at 3000 rpm for 20 min.
4. The dissociated DNA was measured at 260 nm in supernant. And pellet was electrophoresis in 0.8% agarose in 0.5X TBE for 40 min in 75 V.

C-Protective effect against DNase activity, the different N/P ratios of polyplex was incubated with DNase (eurx) as fallowing:

1. One Microliter of RQ1RNase-free DNase buffer 10X was added to polyplex solution.
2. RQ1 RNase-free DNase was added tothe mixture.
3. Mixture was completed to 10 µl by DH2O.
4. Mixture was incubated at 37 Cº for 30 min.
5. Microliter of RQ1 DNase stop solution was added to the mixture.
6. Mixture was incubated at 65 Cº for 10 min.
7. Mixture was electrophoresis in 0.8% agarose in 0.5X TBE for 40 min at 75 V.

Results

The results of the present study show that the polyplex was formed when used 7.5 mM of polyethylinimineexcept N/P 2 it show disassociation from complex at N/P 2 , while 0.75 mM don't formed any complex as shown in figure (1).



Figure(1) Retardation assay of polyplex complex in 7.5 mM and 0.75mM of polyethylinimine at different N\P ratio lane 1,2,3,4,5 polyplex at 2,4,6,8,10 N\P of 7.5 mM; lane 6,7,8,9,10 refer to 2,4,6,8,10 N\P ration at 0.75mM respectively .

Protective effect against DNase activity show high efficiency in all N/P ratios as shown in figure(2)

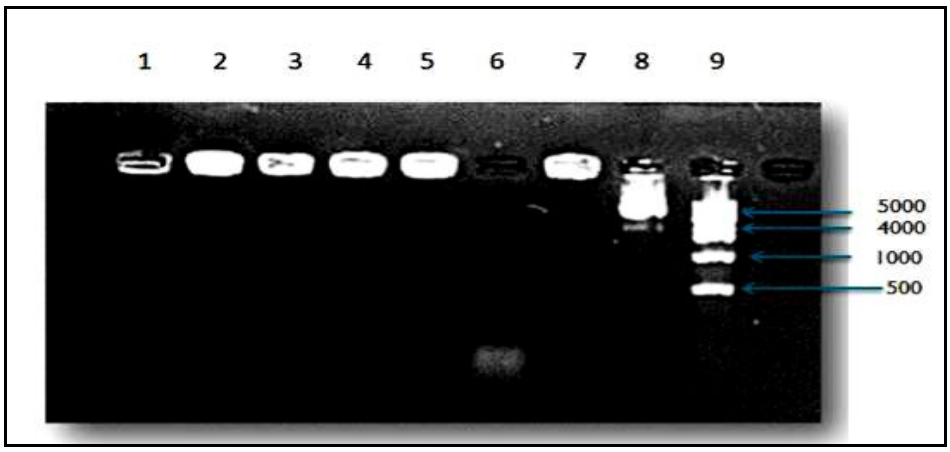


Figure (2) Electrophoresis patterns of DNase activity against polyplex in (2,4,6,8,10 N\P ratio, lane 1,2,3,4,5, polyplex in 2,4,6,8,10 N\P ratio respectively ; lane 6 digestion D1 vector by DNase; lane 7 polyplex without any additive ; lane 8 D1 vector without any additive ; lane 9 DNA marker.

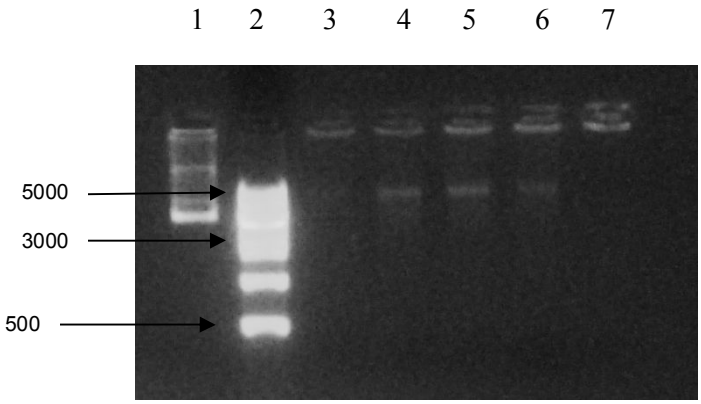


Figure (3) Electrophoresis pattern of stability assay of glycatedpolyplex in 1%, 2%, 3%, 4%, 5%,Lane 1 DNA plasmid without any additive; Lane 2 1kb DNA Ladder.Lane 3, 4, 5, 6, 7 polyplex in 1%, 2%, 3%, 4%, 5% glucose respectively at N/P 2.

The result shown that 2%,3% and 4% of glucose causes leakage of DNA from complex when it incubate with glucose, only 1% and 5% don't cause DNA leakage as in figure (3).

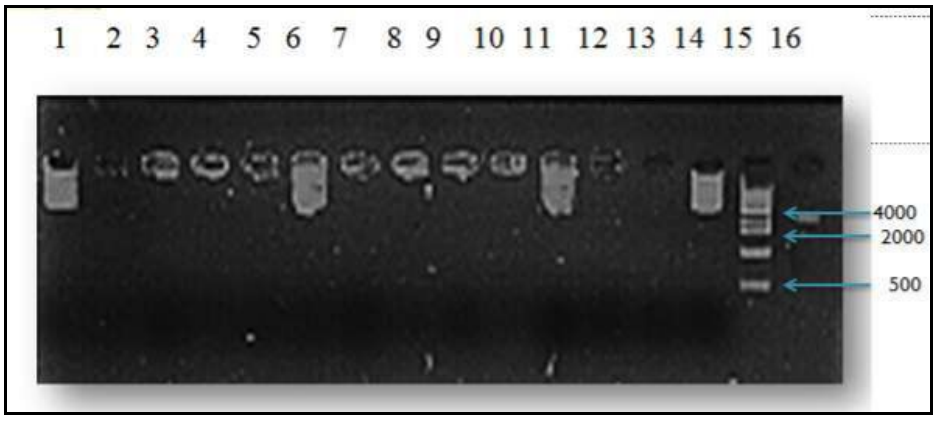


Figure (3) Electrophoresis pattern of stability test of glycatedpolyplex in 5% and 10% glucose, lane 1,2,3,4,5 polyplx in 2,4,6,8,10 N\P ratio at 5% glucose; lane 6,7,8,9,10 polyplex in 2,4,6,8,10 N\P ratio in 10% glucose ; lane 12 plolyplex without any additive ; lane 13 DNA marker; lane 14 vector without any additive.

Discussion

As show in the review of literatures different methods were used to prepare polyplex from PEI and plasmid, these different methods show different results in pre-tests which used to confirm PEI efficacy in protecting DNA and complex stability.

In association assay different N\P ratios were used with two concentrations of PEI, these ratios and 7.5 mM shown a good association except N\P 2 it show dissociation from the complex , the complex was formed by branched PEI that have more than two-NH_2 in its structure which can be condense DNA if the amount of N was enough to form linking reaction between P and N[7].

Ikonen *et al*., used PEI –DNA at N\P ratio 2.4,the choosing this ratio was because it did not have net charge[17], this according to Choosakoonk ring and his worker[8] which reported that the changes of negative to positive zeta potential occur between N/P 2 and 4.

The retardation assay used to determinate efficiency of complication between DNA and PEI because only charged molecules can be migration in electricity field thus all DNA molecules which bind with PEI cannot migrate in the gel.

Yue and his scientific team clarified that the polyplex formation depending on the hydrodynamic radius distribution they found that polyplex were formed completely at N/P 3 when no free DNA was observed in PBS buffer after complication[9].They also used unique methods for enhancing transfection efficiency by using complex at N/P 3 and added free PEI to become N\P 10 they found that this excessive PEI was increased transfection efficacy, this because the role of PEI in navigate intracellular trafficking barriers like endolysosomal formation and escape from these pathways[9].

Sun and his team concluded from their worker that the binding between DNA and PEI depending on the protonation ratios, they concluded that PEI was bound to DNA bone by hydrogen bond with oxygen, 46% protonated PI bind directly with DNA and 23% bind by indirect interaction mediated by water molecules also the binding was stable at low pH because it high protonated molecules[10]. Tang and Szokadescribed the interaction between DNA and PEI was electrostatic interactive , a 90%of these molecules were charged which neutralized by condense DNA[11].

The polyplex complex volume was smaller than naked DNA it was 10^4-10^6 times smaller than naked DNA. Increasing in amount of PEI which lead to an increased N/P ratio to 20 resulted to decreased partials size from 1000-(100-200) nm[12].

The condensation DNA inpolyplex complex depending on PEI feature and ionic strength of the buffer, another study used PBS and NaCl in preparation polyplex they found that used sodium phosphate was beneficial to use in injection than other buffer glucose was used in the present study because glucose reduced polyplex size (30-60) nm than other buffer[1].

Also PEI have buffering capacity resulting from of present the secondary Amin groups in PEI, Mady*et al*[13] used different PEI molecular mass in polyplex formation , they found that the buffering capacity reduced with increased molecular mass in it; thus 25 k has lowest this facilitate gene transfer and enhancing gene expression according to Forrest et al. [14]. The coagulation of polyplex depending on factors molecular mass, N\P ratio and Type of buffers, when it prepares in water at N\P 1 the positive surface charge preventing complex to aggregation, This was improved by Mady *et al*[13] when it used with NaCl.

PEI has been used in gene transfer as a gene carrier because the polyplex ranging in size from 50 to 1000 nm, it depends on the types of PEI linear or branch and types of buffer glucose , PBS, TE and sodium chloride.

Used sodium chloride in polyplex preparation causes agitation of the complex which causes enhanced transfection *in vitro* while it lowest *in vivo*, thus in present study used glucose to ploylexprepare. Studies showed that the protonation ability of PEI is important for gene transfer, it gives a cationic polymer buffering capacity against change in pH also it induced facilitate endosome escape due to the uptake protons by the basic

amino group when pH decreased in endosome thus buffering effect causes an increase in osmatic pressure in endosome lading to disruption endosome membrane to facilitate polyplx transport into the cytoplasm[15-19].

Nakamura and his workers[20] used jet injection kit*in vivo*-jet PEI (Polyplus-Transfection, Illkirch, France) that consist of PEI for transgene mouse liver they found that used this martials as a gene carrier they found high gene expression of GFP because the size of the complex was 80-100 nm this sizes enable the complex partials to cross cell membranes because it is smaller than hepatocytes pours.

The pH used in this polyplex formation was 7.5, according to the Reed *et al* clarified that pH of polyplex was ranging from 7-8 after modulation Ph using HCl this because the physiological pH is 7 it the better for cells functions also this pH was suitable for DNA[21].

References

1. Ilarduyaa, C. D.; Sunb, Y. and Düzgünes,N. (2010)Gene delivery by lipoplexes and polyplexes,European Journal of Pharmaceutical Sciences 40 159–170.
2. Grosse , S. , Thévenot , G. , Aron, Y., Duverger E. , Abdelkarim , M. , Roche ,A.C. , Monsigny , M. Fajac ,I. (2008) In vivo gene delivery in the mouse lung with lactosylated polyethylenimine, questioning the relevance of in vitro experiments. Journal of Controlled Release 132 (2008) 105–112.
3. Park, T.G.; Jeong, J.H. and Kim, S.W. (2006).Current status of polymeric gene delivery systems. Advanced Drug Delivery Reviews, *58*, 467-486.
4. Zintchenko, A.; Philipp, A.; Dehshahri, A. and Wagner, E. (2008) Simple Modifications of Branched PEI Lead to Highly Efficient siRNA Carriers with Low Toxicity, BioconjugateChem, 19, 1448–1455.
5. Reed, S.E., Staley, E.M., Mayginnes, J.P., Pintel, D.J., Tullis, G.E., (2006). Transfection of mammalian cells using linear polyethylenimine is a simple and effective means of producing recombinant adeno-associated virus vectors. J. Virol. Methods 138,85–98.
6. Mahato, R., D. Fungeson, A. Maheshwari, S. Han, S.W. Kim (2000) Polymeric genedelivery for cancer treatment, In: Biomaterials and Drug Delivery Towards New Millennium,K.D. Park, I. Kwon, N. Yui, S. Jeong, K. Park eds, Han Rim Wonn Publishing: Seoul, Korea,249–280.
7. Deng, R.; Yue, U; Jin, F; Chen, Y.;. Kung, H.; Lin, M. ; Wu, C. (2009) . Revisit the complexation of PEI and DNA − How to make low cytotoxic and highly efficient PEI gene transfection non-viral vectors with a controllable chain length and strucuture? J. Control. Release. 140 (1) 40–46.
8. Choosakoonkriang, S., B. A. Lobo, G. S. Koe, J. G. Koe, and C. R. Middaugh. 2003a. Biophysical characterization of PEI/DNA complexes. J. Pharm. Sci. 92:1710–1722.
9. Yue,A. , Jin , F., Deng R, Cai J, Chen Y, Marie C.M. Lin; Kung H, Wu C.(2010).Revisit complexation between DNA and polyethylenimine— Effect of uncomplexed chains free in the solution mixture on gene transfection,Journal of Controlled Release xxx (2010) xxx–xxx.
10. Sun,C. ;Tang,T.; Uluda_H.andCuervo, J (2011) Molecular Dynamics Simulations of DNA/PEI Complexes: Effect of PEI Branching and Protonation State, Biophysical Journal Volume 100, 2754–2763.
11. Tang, M. X., and F. C. Szoka. (1997). The influence of polymer structureon the interactions of cationic polymers with DNA and morphology ofthe resulting complexes. Gene Ther. 4:823–832.
12. Erbacher, P., Bettinger, T., Belguise-Valladier, P., Zou, S., Coll, J.L., Behr, J.P., Remy, J.S., (1999). Transfection and physical properties of various saccharide, poly(ethyleneglycol) and antibody-derivatizedpolyethylenimines (PEI). J. Gene Med. 1,210–222.
13. Mady M,; Mohammed M.W. ; El-Guendy, N. and EL-Sayed, (2011).Effect of polymer molecular weight on the DNA\PEI polyplexes properties Roman J. biophys.21, 2, 151–165.
14. Forrest, M.L.; Gabrialson, N.; Pack, D.W (2004) Reduction of polyethylenimine buffering capacity enhances in vitro gene delivery activity, MolTher., 9, S138.
15. Boussif, O., Lezoualc'h, F., Zanta, M. A., Mergny, M. D., Scherman, D., Demeneix, B., and Behr, J. P. (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc. Natl. Acad. Sci. U.S.A. 92*, 7297–7301.
16. Aljebori, A. M.; Alshirifi, A. N., 2012. Effect of Different Parameters on the Adsorption of Textile Dye Maxilon Blue GRL from Aqueous Solution by Using White Marble, Asian journal of chemistry, 24: 5813-5816.

17. Aljeboree A. M., Alshirifi A. N., and Alkaim A. F., 2014. Kinetics and equilibrium study for the adsorption of textile dyes on coconut shell activated carbon, Arabian J. Chem., 10.1016/j.arabjc.2014.01.020.
18. Al-Saadi, A.H., Zaidan, K.I., Mohammed, T., Zaidan, H.K., Al-Terehi, M. 2015. Dental sex determination by multiplex PCR in iraqi samples. Source of the Document Research Journal of Pharmaceutical, Biological and Chemical Sciences 6 (6), pp. 1572-1577.
19. Salman, J.M., AL-Mamoori, A.M.J., Al-Saadi, A.H., al Terehi, M.N. 2012. Dna damage in common carp fish (cyprenus carpio l.) induced by acute exposure to copper & cadmium. World Applied Sciences Journal 20 (5), pp. 679-682
20. Nakamura, S Maehara, T. Watanabe, S. Ishihara,M; and Masahiro Sato, (2013) Improvement of Hydrodynamics-Based Gene Transfer of NonviralDNA Targeted to Murine Hepatocytes. BioMed Research International Volume, Article ID 928790, 9 pages.
21. Ikonen, M,;Murtom, L.; Ky,A. ; Kontturi, O. (2008). Controlled complexation of plasmid DNA with cationic polymers: Effect of surfactant on the complexation and stability of the complexes, Colloids and Surfaces B: Biointerfaces66, 77–83.

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