Efficient Gene Delivery Nanovectors Based on Functionalization Of Single wall Carbon Nanotubes (SWNT) with Polyethylenimine (PEI)

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Abstract: Carbon nanotubes (CNTs) have found their uses in the biological sciences at molecular and cellular levels. Studies interfacing CNTs with biological molecules indicate that such interfaces can be established while the functions of biological molecules are preserved. We used functionalization procedures to improve the solubility and biocompatibility of SWNT. PEI (polyethyleneimine) have been extensively searched for its binding ability to plasmid due to positive surface charge of its porotonated amine groups in biologic pH and different vectors are synthesized by modifying PEI molecules to improve its viability . By attaching PEI to the surface of SWNT, synthesized vectors will be able to bind plasmid efficiently and internalize the cell without causing allergic reactions and can be used as delivery vehicles for gene delivery purpose.

Keywords: Single wall Carbon Nanotube, Functionalization, Polyethyleneimine, Gene Delivery

Introduction

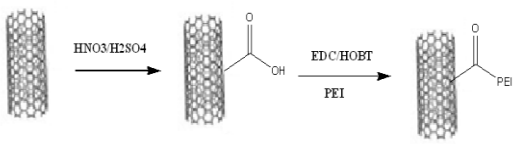
Since the discovery of SWNT in 1993 as a new allotrope of carbon, lot of attention has been focused on this magic one dimensional (1D) carbon structure [1]. They have been subjected to wide researches, due to their potential application, ranging from gas storage [2],field effect transistors, biosensors and catalyst support. Their poor solubility limits their application for biological purposes which can be overcamed by functionalization. Attachment of PEI, polymer known for its excellent in vivo and in vitro gene delivery [4], via different linkers onto the surface of CNT not only leads to aqueous solubility but improves the transfection efficiency with respect to polymer.

Experimental

Three different approaches were used for attachment of PEI to the surface of SWNT, as follows:

1- Direct Attachment

PEI is attached directly via amide bond to the Carboxyl groups introduced in first step. Three nanovectors are synthesized using PEI with different molecular weights: 1800, 10000, 25000 Dalton , named V1.8,V10, V25.

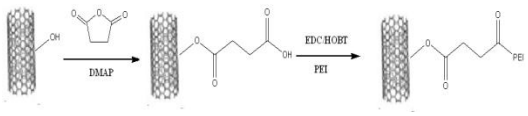


2-Attachment using SUCCINC ACID linker

Two different reactions are used to attach the succinct linker to the surface of SWNT:

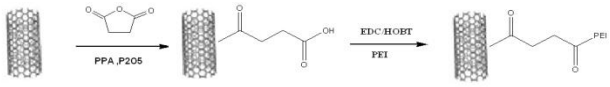
A- Attachment via Esterification Reactions

After Hydroxylation of SWNT, esterification with succinc anhydride provided us with succinic spacer. Then PEI attachment carried out by amidation reaction. Three other vectors are synthesized and named S1.8,S10, S25 repectively.



B- Attachment via Friedel- Craft Acylation:

Succinct anhydride is attached to SWNT through a carbonyl group via Friedel- carft acylation and attachement of PEI is carried out by amide bond formation. Three other vectors are synthesized and named Ac1.8, Ac10, Ac25 respectively.



Results and Discussion

Characterization of structures were carried out by FT-IR technique. Size and zeta potential of vector were determined using Malvern Zeta sizer.

Condensation Assay

Vector were investigated for their binding ability to plasmid using Ethidium Bromide method.

Transfection

Gene delivery and transfection efficiency of vectors were evaluated using pRL-CMV in N2A cells by luciferase assay(Promega). results are summarized in Figure1, 2, 3.

MTT ASSAY

Cell viabilities were by evaluated MTT assay(Fig 4,5,6).

Conclusions

Data from zeta potential shows that all structures bear positive charges, due to the cationic polymer on the surface. Size of nanovectors lay in the range of 90-130 nm (Fig 7).

In each set best results were obtained in the case of vector synthesized based on PEI 1800 and comparing the attachment mode of PEI onto the surface, vectors with succcinc linker were much more efficient in transfection. In the case of Ac1.8, at C/P=6 transfection is increased 65 times and for S1.8 at C/P=8, 8 fold increasement in transfection is observed. Vectors showed no cytotoxicity, especially for the vector with succinic linker which viability exceeds 100 percent.

It seems that the lower the size of polymer , the more polymer molecules are attached to a specific area of SWNT surface and transfection increases compare to polymer.

References

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[2] EoghanP.Dillon,ChristopherA. Crouse, and Andrew R.Barron,s ,*Acs Nano*,2 (2008), 156-164.

[3] KeWua,CraigA.Meyers, *Brain Research* 1008 (2004) 284–287.

Figures

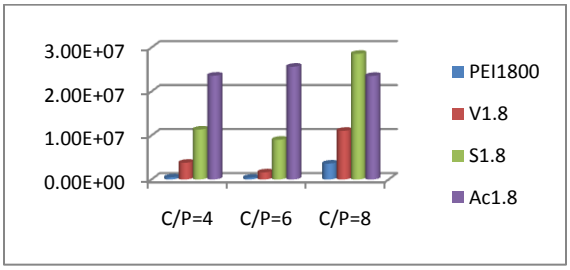

Fig1:Transfection results for vector based on PEI1800 D.

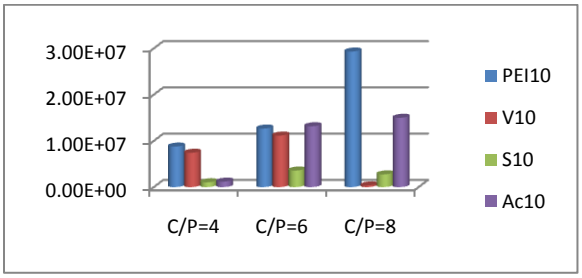

Fig2:Transfection results for vector based on PEI 10 kD.

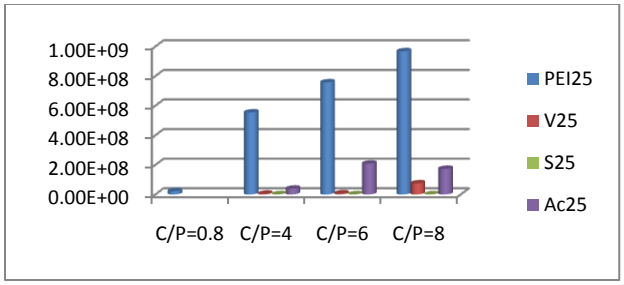

Fig3: Transfection results for vector based on PEI 25 kD.

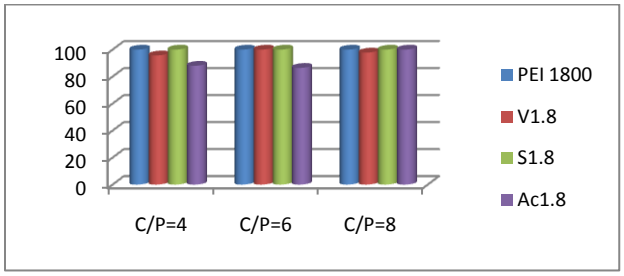

Fig 4: Viability of vectors based on PEI 1800D

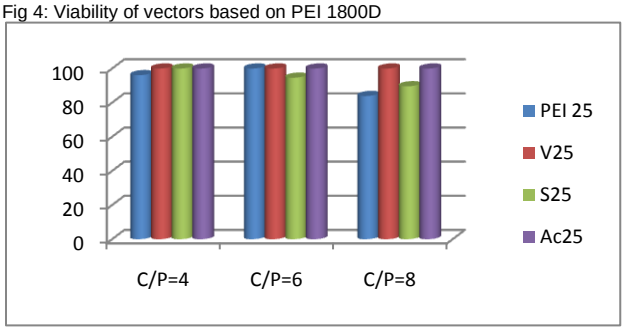

Fig 5: Viability of vectors based on PEI 10KD

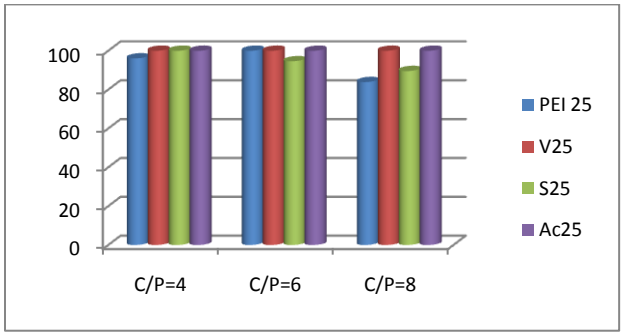

Fig6: Viability of vectors based on PEI 25 kD

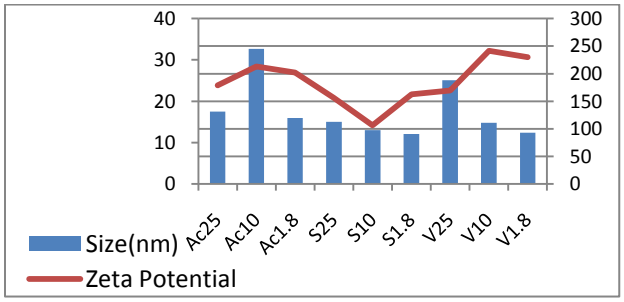

Fig7:Size(nm) and Zeta potential