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Preparation and evaluation of phospholipid-polyethylene glycoylamine functionalized single walled carbon nanotubes for gene transfer

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Abstract: *In this investigation, single-walled carbon nanotubes (SWCNTs) were non-covalently attached to phospholipid(polyethyleneglycol) amine PL-(PEG)-5000-NH2, to construct efficient carriers for gene delivery. PL- (PEG) amine of 5000 daltons were conjugated to SWCNT to develop vectors possessing effective DNA condensation ability which can interact with cell membrane via both nano-needle mechanism and electrostatic interactions produced by SWCNT and PL- (PEG)amine 5000, respectively. The outcomes demonstrated that SWCNT-PL-PEG-Amine-5000 could pack plasmid DNA p EGFP-C1 to enhance the gene transfer. The efficient vector, which was prepared by attachment of SWCNT-PL-PEGNH2, showed 8.5–10 folds enhancement in transfection activity. We also showed that the selected complex could expeditiously transfer plasmid EGFP-C1 in to prokaryotic and eukaryotic host cells*

Keywords – *gene transfer, nano-needle,, PL-PEG-5000, p EGFPC1, SWCNTS, transfection.*

I. Introduction

The gene transfer systems comprise of viral vectors and non-viral vectors. Viral vectors are the valuable, but their usage is restricted due to their immunogenicity, oncogenicity and the small size of the DNA they can transfer. Non-viral vectors are secure, cheaply available, more consistent and do not offer DNA size limit. The main disadvantage of non-viral vector system is their less transfection efficiency, even though it has been enhanced by various methods and the work is yet unending [1]; The improvement of non-viral transfer systems have a vantage to amplified number of products entering into clinical trials. Nevertheless, viral vector has subjugated the scientific trials in gene therapy for its rather great transfer ability.

Several studies have been carried out to improve the transfection competence of non-viral vectors. Distinctive chemical and physical properties of carbon nanotubes (CNTs) [2] have fascinated immense attention in the field of nanomedicine as potential gene transfer tool. CNTs could simply cross the plasma membrane and precisely pass into the cytoplasm of target cells [3] by nano-needle mechanism with-out any perturbation or interruption in the membrane [4]. In addition, endocytosis-dependent entry of CNTs to cells has been recommended by some researchers. The foremost drawback of CNTs for biological and biomedical applications is their poor solubility [5]. To surmount this problem, water solubility and diffusion of CNTs are improved by functionalization and chemical modifications While oxidation of CNTs increases their solubility in water but they would accumulate in biological environments due to charge screening effects owing to the high salt content, therefore they cannot be absolutely used for biomedical applications. The hydrophilic polymers such as PL-PEGNH2 phospholipid (poly ethylene glycol) (PEG) amine was used to solve this problem.
In this study, we made complex of SWCNT-PL-PEGNH2 for transfer of plasmid p-EGFP1 into prokaryotic and eukaryotic cells. The nanotubes were characterized and their transfection efficiencies were evaluated in-vitro.

II. Physical experiment:

2.1. Materials:

SWCNTs produced by chemical vapour deposition and, ethidium bromide, were purchased from Sigma–Aldrich were used in this investigation. PL-PEG-5000-Amine was obtained from Avanti polar lipids. Plasmid p EGFP-C1, BY-3 Tobacco calluses, Escherichia coli cells, Luria Bertinii medium and U5medium were obtained from GENEI and chick embryos were procured from local vendor.

2.1.1. Production of nanotubes (SWCNTS)

2.1.2. Purification and activation of SWCNTS**:**

SWCNTs were treated with mixture of concentrated H2SO4 (90%) HNO3 (70%) of 3:1 v/v and sonicated in the water bath for two days. The resultant mixture is filtered through polytetraflouroethylene (PTFE) of pore size 450nm and washed thrice with deionized water and methanol respectively. The purified SWCNTs were dried at room temperature [6.7, 8].

2.1.3. Characterization of pristine and oxidised swcnts

The name "carbon nanotube" indicates an entire class of materials, which vary in their numbers of walls, diameter distribution, length distribution, chirality, purity, catalyst material, impurity types and defects [9, 10, 11]. Hence, characterization of the functional matter was essential to guarantee quality and reproducibility. The SWCNT in this effort were characterized by scanning electron microscopy (SEM) Fig-1, 2 and Raman imaging Fig-3, 4

III. Peglyation of SWCNT WITH PL-PEG-5000-Amine

The conjugation of SWCNT with PL-PEG-5000-Amine was achieved by noncovalent interaction between SWCNT and –NH2 of PL-PEG5000. The functionalization of CNTs can also be accomplished by noncovalent surface finishing by low-molecular-weight surfactants, surface packaging with polymers, or through π-π stacking interaction between the aromatic rings of the loaded materials and π-electrons of graphite layer on the surface of CNTs. [12, 13] Concisely, the swcnts were added to PL-PEG-5000-Amine suspended in double distilled water in a glass beaker. The mixture was sonicated in probe sonicator. The sonication was done for 4cycles of 10seconds on and 4cycles of 10seconds off for 40seconds . This helps in proper dispersion and mixing of the surfactant (PL-PEG-5000-Amine) and the SWCNT. Further the suspension was sonicated in the sonicator for two hours carefully at room temperature. After completion of sonication this suspension was centrifuged at 13200 rpm for 10 minutes. The supernatant was pipette out of the centrifuge tube and filtered using centrifugal devices with cellulose filter of 100KD by centrifuging at 13200 rpm for five minutes. The filtrate was washed for three times with 50µl of double distilled water. The supernatant was resuspended in double distilled water.

3.1. Characterization of SWCNT-PL-PEG-5000-Amine

The evaluation of SWCNT-PL-PEG-5000-Amine was made by Raman imaging Fig-5. The evaluation was done to interpret the complex formed between SWCNT-PL-PEG-5000-Amine.

IV. Preparation of SWCNT-PL-PEG-5000-Amine plasmid DNA (p EGFP-C1)

The pegylated SWCNT was bonded by mixing the plasmid (p EGFP-C1) with the afore mentioned suspension. The mixture was allowed to stand at room temperature for half an hour. The complex of SWCNT-PL-PEG-5000-Amine functionalized p EGFP-C1 was formed. [14, 15, 16,]

4.1. Characterization of SWCNT-PL-PEG-5000-Amine functionalized p EGFP-C1

The evaluation of SWCNT-PL-PEG-5000-Amine functionalized p EGFP-C1 was made by scanning electron microscopy Fig-6 and agarose gel electrophoresis Fig-7. The evaluation was done to interpret the amount of bound plasmid DNA and free plasmid DNA. [17, 18, 19]

V. Bioassay of SWCNT-PL-PEG-5000-Amine functionalized p EGFP-C1

The bioassay of SWCNT-PL-PEG-5000-Amine functionalized p EGFP-C1 was done using E.coli cells, BY3 tobacco cells and chick embryo [20,21 ,22 ,23]. The assay method showed the transfer of plasmid p EGFP-C1 into the host cells by SWCNT-PL-PEG-5000-Amine**.**

VI. Figures

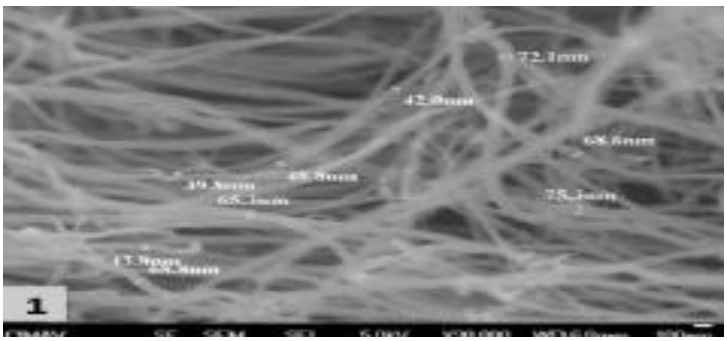


Figure-1 SEM image of pristine SWCNTS

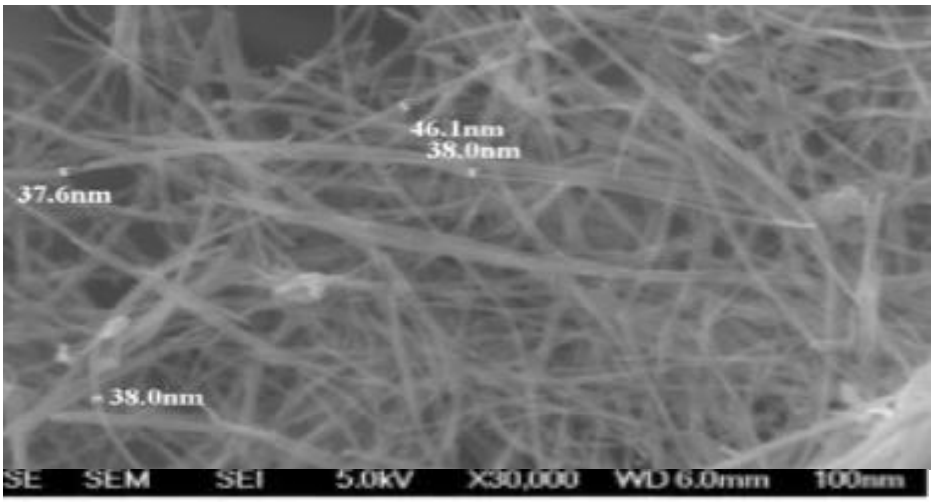


Figure-2 SEM image of activated SWCNTS

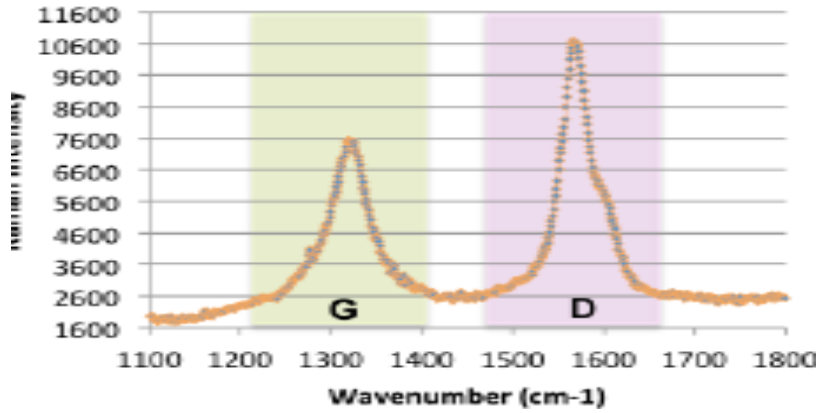


Figure-3 Raman imaging of pristine SWCNTS

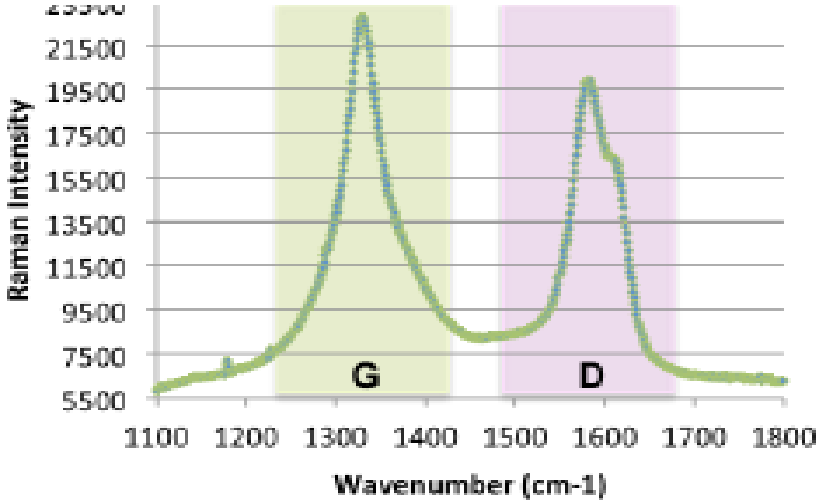


Figure-4 Raman imaging of activated SWCNTS

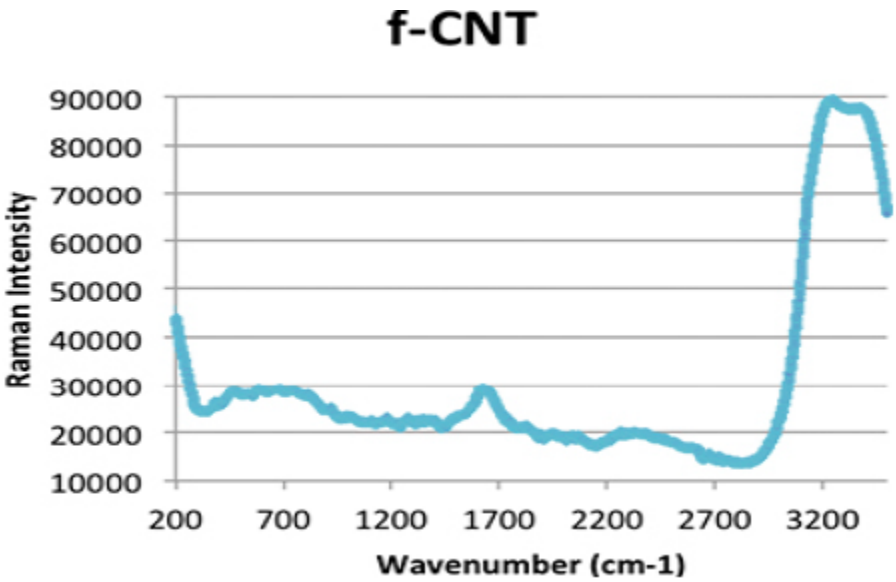


Figure-5 Raman imaging of activated SWCNTS

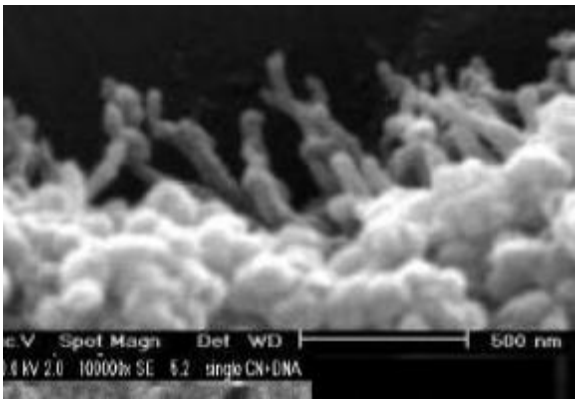
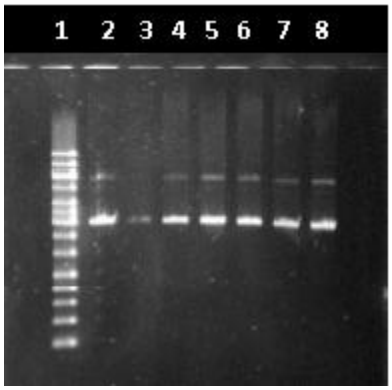


Figure-6 SEM image of PL-PEG-5000-NH2- SWCNT p-EGFP-C1



Lane 1: ladder,
lane 2: p EGFP-c1 plasmid alone 9.3 ng.µl-1
lanes 3-8: f-SWCNT:plasmid DNA complexes with plasmid 9.3 ng. µl-1

Figure-7 Agarose gel electrophoresis of PL-PEF-NH2-5000-SWCNT p-EGFP-C1

VII. Conclusion

The activation SWCNTS resulted in the formation of debundled and tangled SWCNTS which was observed in scanning electron microscopy images. The structure of the SWCNTS was changed which was

interpreted by Raman imaging as there was shift in the intensity G-band. The SWCNTS were further complexed with PL-PEG-5000-NH2 to increase the efficiency of gene transfer. The characterization of pegylated SWCNTS further showed that binding of PL-PEG-5000-NH2 with SWCNTS noncovalently by raman imaging. The suspension of SWCNT- PL-PEG-5000-NH2 was functionalized with the plasmid p EGFP-C1.The non covalent binding of plasmid with the complex SWCNT- PL-PEG-5000-NH2 was characterized by scanning electron microscopy and agarose gel electrophoresis. The plasmid p EGFP-C1 transfer efficiency of SWCNT- PL-PEG-5000-NH2 was further bioassayed using E.coli cells, BY3 tobacco cells and chick embryos. The SWCNT- PL-PEG-5000-NH2 functionalized p EGFP-C1 transfer into host cells was proved to be more efficient than viral vectors. Hence SWCNT- PL-PEG-5000-NH2 was considered as one the best alternative non viral vector for gene transfer.

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References

[1]. Yafei Zhang, Lingang Zei, Covalent sidewall functionalization of single-walled carbon nanotubes via one-electron reduction of benzophenone by potassium Chemical Physics Letters 446(1) · September 2007.
[2]. Amir Khashayar Varkouhi, Stéphanie Foillard, Twan Lammers, Gert Storm, SiRNA delivery with functionalized carbon nanotubes nternational Journal of Pharmaceutics 416(2):419-25 · February
[3]. Liu Z, Tabakman SM, Chen Z, Dai H. Preparation of carbon nanotube bioconjugates for biomedical applications. Nature protocols. 2009;4(9):1372-1382. doi:10.1038/nprot.2009.146.
[4]. Naghmeh Hadidi,1 Farzad Kobarfard,2 Nastaran Nafissi-Varcheh,3 and Reza Aboofazeli PEGylated Single-Walled Carbon Nanotubes as Nanocarriers for Cyclosporin A Delivery.
[5]. Jayanth Panyama, Vinod Labhasetwara,b , Biodegradable nanoparticles for drug and gene delivery to cells and tissue Advanced Drug Delivery Reviews 55 (2003) 329–347
[6]. MuFei Tang 1鄢 , Lei Lei 1鄢 , ShengRong Guo 1 , WenLin Huang 2,3Recent progress in nanotechnology for cancer therapy Chinese Journal of Cancer 2010 Sep;29(9):775-80
[7]. Auti Snehal D.1*, and Gadhave Manoj v2carbon nanotube: a promising drug carrier in cancer therapy world Journal of Pharmaceutical Research Volume 4, Issue 2, 906-926
[8]. Victor Ramos-Perez , Anna Cifuentes , Núria Coronas , Ana de Pablo , and Salvador Borrós Paolo Bergese and Kimberly Hamad-Schifferli (eds.), Modifi cation of Carbon Nanotubes for Gene Delivery Vectors. Nanomaterial Interfaces in Biology: Methods and Protocols, Methods in Molecular Biology, vol. 1025, DOI 10.1007/978-1-62703-462-3_20, © Springer Science+Business Media New York 2013
[9]. Rasti Krajcik a, Adrian Jung b, Andreas Hirsch b, Winfried Neuhuber c, Oliver Zolk a,*R. Krajcik et al. /Functionalization of carbon nanotubes enables non-covalent binding and intracellular delivery of small interfering RNA for efficient knock-down of genes Biochemical and Biophysical Research Communications 369 (2008) 595–602
[10]. Cheng Q, Blais MO, Harris G, Jabbarzadeh E (2013) PLGA-carbon nanotube conjugates for intercellular delivery of caspase-3 into osteosarcoma cells. PLoS.One. 8:e81947
[11]. Liu Z, Tabakman S, Welsher K, et al. Carbon nanotubes in biology and medicine: in vitro and in vivo detection, imaging and drug delivery. Nano Res. 2009;2:85–120.
[12]. Richard C, Balavoine F, Schultz P, et al. Supramolecular self-assembly of lipid derivatives on carbon nanotubes. Science. 2003;300:775–778.
[13]. Wang H, Zhou W, Ho DL, et al. Dispersing single-walled carbon nanotubes with surfactants: a small angle neutron scattering study. Nano Lett. 2004;4:1789–1793.
[14]. Pantarotto D, Briand JP, Prato M, Bianco A (2004) Translocation of bioactive peptides across cell membranes by carbon nanotubes. Chem Commun (Camb)16–17
[15]. Pantarotto D, Singh R, McCarthy D, Erhardt M, Briand JP, Prato M, Kostarelos K, Bianco A (2004) Functionalized carbon nanotubes for plasmid DNA gene delivery. Angew Chem Int Ed Engl 43:5242–5246
[16]. Construction, DNA wrapping and cleavage of a carbon nanotube– polypseudorotaxaneconjugate w Yong Chen,a Lu Yu,a Xi-Zeng Feng,b Sen Houb and Yu Liu*a Chem. Commun., 2009, 4106–4108.
[17]. B.F. Pan, D.X. Cui*, P. Xu, T. Huang, Q. Li, R. He, F. Gao Cellular Uptake Enhancement of Polyamidoamine Dendrimer Modified Single Walled Carbon Nanotubes Journal of Biomedical & Pharmaceutical Engineering 1:1 (2007) 13-16 ISSN: 1793-4532
[18]. Pu Chun Ke1,3 and Rui Qiao2 Carbon nanomaterials in biological systems J. Phys.: Condens. Matter 19 (2007) 373101 (25pp)
[19]. C.Y. Li1, C.K. Hsu1, F.H. Lin2, L. Stobinski3 Diamine biomolecules surface functionalization single-walled carbon nanotube as gene-delivery vector European Cells and Materials Vol. 13. Suppl. 3, 2007 (page 24)
[20]. Jin H, Heller DA, Strano MS (2008) Single-particle tracking of endocytosis and exocytosis of single-walled carbon nanotubes in NIH-3T3 cells. Nano Lett 8:1577–1585
[21]. Ben-Yair R, Kalcheim C. Single Cell Transfection in Chick Embryos. Journal of Visualized Experiments : JoVE. 2010;(43):2133. doi:10.3791/2133.
[22]. Tan H, Fu L, Seno M. Optimization of Bacterial Plasmid Transformation Using Nanomaterials Based on the Yoshida Effect. International Journal of Molecular Sciences. 2010;11(12):4961-4972. doi:10.3390/ijms11124962.
[23]. N. Lalitha Jyotsna 1*, Dr. N. Saritha identification of genetic transformation by single walled carbon nanotube for plasmid dna delivery. Journal of scientific research in pharmacy. Vol 6, supply -1, (2017)