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ARTICLE

Multi-functionalized Graphene Oxide Complex as a Plasmid Delivery for Targeting Hepatocellular Carcinoma Therapy

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A novel efficient graphene-based gene delivery vector was synthesized by continuously covalent functionalization of graphene oxide (GO) with polyethylenimine (PEI), polyethylene glycol (PEG) and folic acid (FA), followed by the loading of the si-Stat3 via electrostatic adsorption (GO-PEI-PEG-FA/si-Stat3). We investigated the effects of the effective diameters and transfection efficiency by changing the content of PEI in complex. It was found that with the content of PEI in complex the increase and the effective diameters reduce in turn until almost unchanged, along with the transfection efficiency of complex became higher. Then the results showed that the transfection efficiency is relatively high with the content of PEI in complex at 61wt% without obvious cytotoxicity. At the same time, the stability of the complex in physiological conditions was improved. The GO-PEI-PEG-FA/si-Stat3 complex exhibited an excellent ability of silencing Stat3 expression for targeting tumor hepatocellular carcinoma *in vitro*.

1. Introduction

Gene therapy is a hot spot research for treating gene related diseases especially cancer in the past two decades. One of the key issues in gene therapy is to develop a suitable gene drug delivery carrier which involved viral and non-viral vectors.[1-3] The non-viral vectors has attracted considerable attention owing to its high safety and efficiency in view of polymers,[4-7] cationic lipids,[8,9] nanoparticles[10-15] and others.[16-18] In recent years, the study of non-viral vectors has obtained great process. However, some problems involving non-specificity to tumor sites, cytotoxicity and biocompatibility in physiological environment have not been well solved. Therefore, the development of powerful nanocarrier is still the present significant challenges for gene therapy.[19]

Recently, graphene, an atom-thick sheets of carbon packed in a two-dimension layered structure with remarkable properties has been widely used in many application fields of electrochemical devices, biomedicine and so on. [20-23] Graphene oxide (GO), graphene's water-soluble derivative has already attracted enormous interests due to its easy manufacture, well-dispersed in water and physiological environment, good colloidal stability and shown potential application in biological fields including biosensors, gene and drug delivery, as well as biological imaging *in vitro* and in vivo.[24-34] Liu et al. first reported that GO was bound with polyehtylenimine (PEI) (1.2 kDa and 10 kDa), then bound with plasmid DNA (pDNA) for intercellular transfection of the EGFP gene in Hela cells.[35] At the same time, Zhang et al. prepared PEI-grafted GO (GO-PEI) via covalent functionalization as an excellent nanocarrier for sequential delivery of siRNA and chemical drugs *in vitro*.[36]

Polyethylene glycol (PEG) as amphiphilic polymers could improve the solubility of nanomaterials in serum, phosphate buffer solution (PBS) and physiological solutions with high aqueous stability.[25,26] Feng et al. synthesized GO-PEI-PEG with excellent solubility in physiological environment and low toxicity for delivering small interfering RNA (siRNA) into cells under the control of NIR light.[37] Meanwhile, Zhang et al. used GO-PEI-PEG for treating Drosophila S2 cells by with the plasmid DNA as transfection agent.[38] Subsequently, Zhang et al. utilized a MMP2-cleavable PLGLAG peptide linkage method to combine GO-PEI-PEG with the anti-tumor drug doxorubicin (DOX) for tumor induced imaging and potential combinational tumor therapy.[39] Recently, Qu et al. demonstrated that the intracellular transportation of nanocarriers (GO-PEG-PEI) was enhanced by using the photo-thermal for light-controllable cytosine-phosphate-guanine CpG delivery for the first time.40 Signal transducer and activator of transcription 3 (Stat3) has been regarded as an oncogene. High expression of Stat3 has been found in Hepatocellular Carcinoma, malignant melanoma, breast carcinoma and prostate cancer.[41,42] Our previous work also reported that plasmid Stat3 siRNA delivered effectively and inhibited Stat3 gene expression in vivo by GO-PEI-PEG, resulting in induction of apoptosis in mouse malignant melanoma and suppressed tumor growth.[43] In order to target deep-seated malignant tumor, we used FA-based complex in our study. FA-based complex as an important plasmid delivery of gene vector can reach directly

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and accurately the tumor site to achieve the effectiveness of tumor treatment.[44,45] Zhang *et al.* successfully functionalized sulfonic GO with FA via conjugation to load two mixed anticancer drugs DOX and CPT for targeting MCF-7 cell.[46] Chen *et al.* reported that GO was conjugated with PEG-co-FA and PyNH2 for targeting intracellular delivery of hTERT siRNA to efficiently knockdown the protein expression level and mRNA level.[47] These results indicated that the multi-functionalized GO-based gene delivery carriers could be applied in gene therapy. However, the efficient and controllable gene delivery nanocarriers should be further researched in the corresponding gene therapy field to establish the structure-property relationship.

In this paper, a novel multi-functionalized GO-based gene vector was prepared for targeting therapy of hepatocellular carcinoma successfully. The GO-PEI-PEG-FA/si-Stat3 gene vector was prepared by covalent modification of GO with PEI, PEG and folic acid (FA) in turn, whereafter loading of si-Stat3 via electrostatic interactions. In this gene vector, PEI was used for controlling the transfection efficiency, PEG for improving the solubility in physiological solutions, FA for targeting tumor therapy and si-Stat3 was used for silencing of Stat3 expression. The controllable structure of complex was studied by adjusting the reaction conditions and the effect of different structure on transfection efficiency of gene vector was investigated. The as-prepared GO-PEI-PEG-FA/si-Stat3 gene vector possessed not only lower toxicity and higher transfection efficiency, as well as better aqueous stability, but also achieved the goal of targeting therapy for tumor mouse hepatocellular carcinoma *in vitro.*

2. Experimental section

2.1 Materials

Natural Graphite powder was supplied from Guangfu Fine Chemical Research Institute (Tianjin, China). Branched polyethylenimine (PEI 25 kDa) was purchased from Sigma (St. Louis, MO, USA). N-hydroxysuccinimide (NHS) was purchased from Aladdin Chemistry Co., Ltd. N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), Triethylamine (TEA) , Folic acid (FA), The molecular mass cutoff dialysis membrane (10 KDa) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China). NH2-PEG-COOH (5 KDa) was purchased from Beijing JenKem Technology Co., Ltd (Beijing, China). Dulbecco's modified eagle's medium (DMEM) were supplied from GIBCO (Carlsbad, CA, USA). SMMC-7721 human hepatoma cell lines were purchased from Bogoo Biological Technology Co., Ltd. (Shanghai, China). Plasmids and strains PGC silencer-si-Stat3 (si-Stat3) preserved as mentioned before. Goat anti rabbit polyclonal IgG-HRP antibody and Beta-actin antibody were purchased from Bioss Inc. (Beijing, China). Stat3 antibody was purchased from Signaling Company American Cell (USA). Enhanced chemiluminescence (ECL) was purchased from Amersham Buckinghamshire Company (UK).

2.2 Preparation of GO-PEI

GO-PEI was synthesized using our previously reported method.[42] The crude GO-PEI was subjected to dialysis in deionized water for 3 days to completely remove unreacted PEI.

2.3 Preparation of GO-PEI-PEG

The as-prepared GO-PEI (1 mg mL^{-1}, 0.5 mL) complex was mixed with NH_2-PEG-COOH (2.5 mg, 0.5 mmol) using EDC/NHS (0.5/0.5 mmol) as coupling agent in the 10 mL flask. The conjugation reaction was stirred at room temperature for 24 h. The resulting GO–PEI-PEG solution was dialyzed in deionized water for 3 days to remove unreacted NH_2-PEG-COOH. The product GO-PEI-PEG was obtained.

2.4 Preparation of GO-PEI-PEG-FA

FA (0.2 mmol), EDC (0.5 mmol) and NHS (0.5 mmol) were put into 1 mL PBS (pH~7.4) solution, then the solution was stirred at room temperature for 6 h. Subsequently, the reaction mixture was added to GO-PEI-PEG suspension (1 mg mL^{-1}, 1 mL) and it was stirred over night at room temperature. The final product GO-PEI-PEG-FA was dialyzed in the deionized water for 3 days.

2.5 GO-PEI-PEG-FA delivering si-Stat3

To observe si-Stat3 plasmid loading onto the GO-PEI- PEG-FA complexes, a gel electrophoresis assay was performed the efficiency of transfection between the complex and the plasmid at different mass ratios (in Fig. 8). We putted 1 μg plasmid to the different nanocomposite mix in total system 20 μl and incubated for 30 min at room temperature. The si-Stat3 plasmid loaded onto GO-PEI-PEG-FA complexes of different weight ratios PEI (1:10,1:20 and 1:40) at various N/P ratios (1:0, 1:2, 1:4, 1:8, 1:12, 1:16, 1:20 and 1:24), as well as the different weight ratios PEI (1:60,1:80 and 1:100) at various N/P ratios (1:0, 1: 0.5, 1: 1, 1: 2, 1: 3, 1: 4, 1: 5 and 1:6). Then we observed the binding rate by 1 % agarose gel electrophoresis with TAE buffer for 20 min at 120 V. Finally, the gel was analysed by atomic force automated imaging system (Tannon 2500 R, Shanghai). SMMC-7721 cells were cultured in 6-well plates at a density of 5×10^5 incubation of plasmid and GO-PEI-PEG-FA at ratios of 2:1 at room temperature after 30 min. We observed the transfection efficiency by fluorescence.

2.6 Cell culture

The cells were cultured with Dulbecco's modified eagle medium (DMEM) containing 10 % fetal bovine serum at condition of 37 °C and 5 % CO_2.

2.7 *In vitro* cytotoxicity assay

To detect cytotoxicity effect of GO-PEI-PEG-FA on SMMC-7721 cells, we performed 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) by microplate reader at 490 nm. SMMC-7721 cells were cultured in 96-well plates at a density of 1×10^3 for 24 h. Cells were grown in different

concentrations of gradient GO-PEI-PEG-FA (final concentration of 0, 20, 40, 80 and 160 mg L^{-1}). MTT was added into each well 20 µL solution (5 mg mL^{-1}, *i.e.* 0.5 % MTT) 24 h later. MTT incubated 4 h and added 100 µL DMSO to each well. Finally, we measured the absorbance of each well in the enzyme immunoassay analyzer at OD 490 nm.

2.8 Western Blot assay

The expression of stat3 protein was detected by Western blot through collected tumor tissues from different group. Stat3 nuclear protein was prepared to Western Blot assay. Total protein (30 µg) was separated by 12 % SDS-PAGE and transferred to PVDF membrane, which was incubated with rabbit anti-Stat3 antibody (1:2000) for 1 h. Then the PVDF membrane was incubated by goat anti-rabbit IgG-HRP (1:2000) for 1 h.

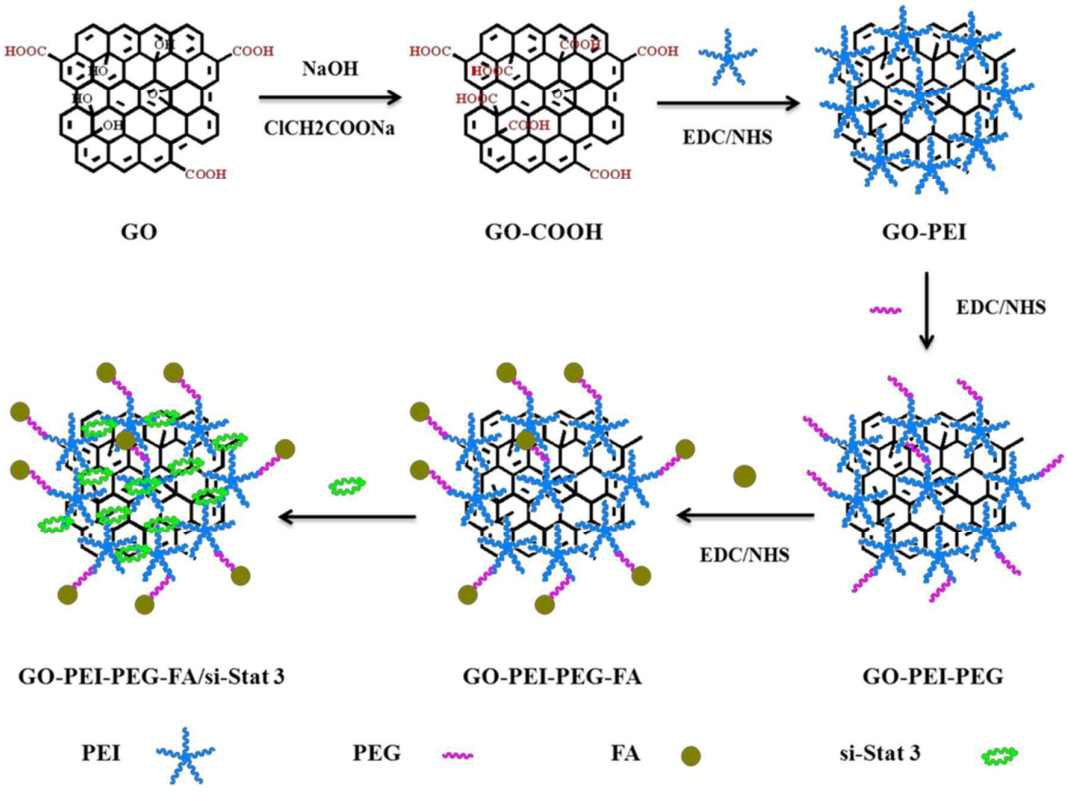
2.9 Instrumentation

Uv-vis spectra was recorded on a Shimadzu UV-2550 spectrophotometer and Fourier transform infrared (FTIR) spectroscopy (VERTEX70 FT-IR spectrophotometer, Bruker Optics) was performed by using powder-pressed KBr pellets for confirming chemical conjugation of all complexes. The effective diameters and zeta potential were measured by using a Zeta sizer 3000 (Malvern, UK). Thermal gravimetric analysis (TGA) was performed on DSC/TGA 1600LF, METTLER TOLEDO instrument with a heating rate of 10 °C min^{-1} under nitrogen flow rate of 60 mL^{-1}. The gel was analyzed by atomic force automated imaging system (Tannon 2500R, Shanghai). MTT assay was performed by microplate reader (BIO-RAD Model 550).

3. Results and discussion

3.1 Preparation and characterization of the GO-PEI-PEG-FA complex

A novel GO-PEI-PEG-FA/si-Stat3 complex gene vector with targeting function was synthesized successfully. The synthesis route consists of five steps (Scheme 1). (1) GO was prepared by modified Hummer's method from natural graphite powder. (2) GO was converted to carboxylate GO (GO-COOH) using NaOH and $ClCH_2COONa$. (3) GO-PEI was synthesized at different weight ratios *i.e.* 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100 by forming an amide linkage using EDC/NHS coupling agent. (4) NH_2-PEG-COOH was covalently linked with GO- PEI to obtain GO-PEI-PEG. (5) The amino ended of GO-PEI-PEG was connected FA to produce the final composite GO-PEI-PEG-FA. (6) The negatively charged si-Stat3 was loaded on the positively charged GO-PEI-PEG-FA via the electrostatic interaction.



Scheme 1 The synthesis processes of GO-PEI-PEG-FA/si-Stat3 complex

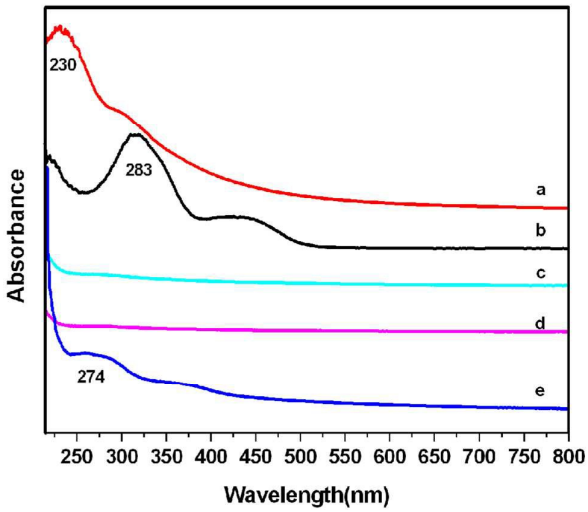


Fig. 1 UV spectra of GO (a), FA (b), GO-PEI (c), GO-PEI-PEG (d) and GO-PEI-PEG-FA (e).

Uv-vis spectroscopy was used to prove that PEI, PEG and FA were continuously covalent grafted on GO (Fig. 1). The spectrum of GO (Fig. 1a) exhibited a peak at about 230 nm, corresponding to the π-π* transitions of aromatic C-C bonds. The peak at 283 nm was attributed to FA (Fig. 1b). The appearance of a new peak at about 274 nm (Fig. 1e) was due to FA on GO-PEI-PEG. These results showed that FA was covalent grafted on GO-PEI-PEG successfully.

The chemical coupling of GO-PEI-PEG-FA was further characterized by FTIR spectroscopy (Fig. 2).The spectrum of GO (Fig. 2a) demonstrates the intense absorption peaks at 1725 cm^{-1}, 1626 cm^{-1} and 1050 cm^{-1}, which was assigned to C=O of carboxylic group, the C=C vibration of aromatic rings and the vibration band of C-O-C respectively. In Fig. 2b, the GO-PEI

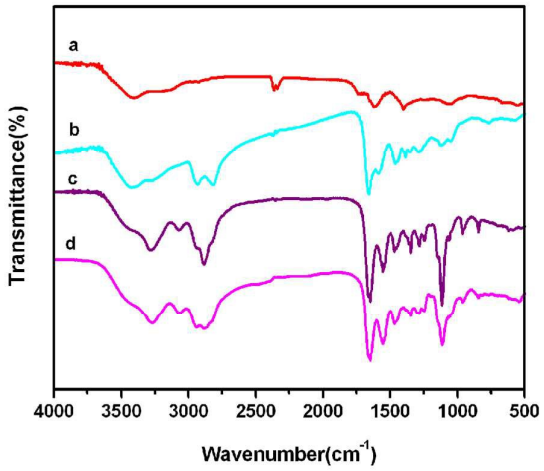


Fig. 2 FTIR spectra of GO (a), GO-PEI (b), GO-PEI-PEG (c) and GO-PEI-PEG-FA (d).

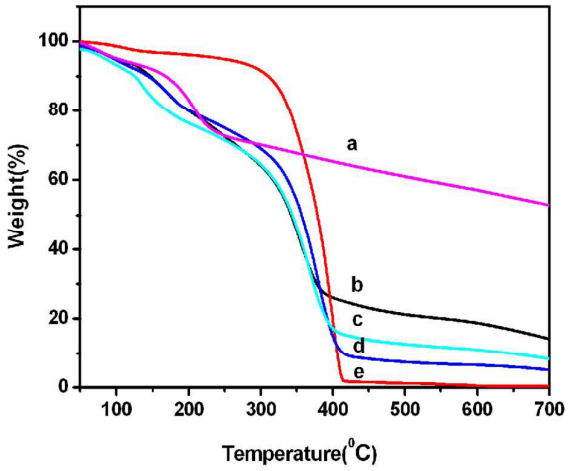


Fig. 3 TGA curves of the GO (a), GO-PEI of different weight ratio 1:10 (b), 1:40 (c), 1:80 (d) and PEI (e).

spectrum appeared the vibration band around 1650 cm^{-1} and 2900 cm^{-1}, that corresponding to the stretching of -NHCO- and N-H of PEI. At the same time, the characteristic absorption band at around 2873 cm^{-1} and 1107 cm^{-1} were corresponded to C-H bond of PEG (Fig. 2c) and the characteristic absorption band at about 1490 cm^{-1} was assigned to the phenyl ring of FA (Fig. 2d). These results suggested that GO-PEI-PEG-FA was successfully covalent coupling.

Thermo gravimetric analysis (TGA) was used to evaluate the content of PEI grafted onto GO as shown in Fig. 3. The TGA curve of GO (Fig. 3a) indicates that GO has 26 wt% weight loss at about 225 °C which belonged to the loss of adsorbed water and oxygen-containing functional groups on GO such as hydroxyl and carboxylic groups. As can be seen from Fig. 3e, the decomposition of pure PEI is between 300 °C and 415 °C.

After functionalization of GO with PEI, the GO-PEI complexes seem to have an obvious decomposition that occurs in two stages, one is the oxygen-containing functional groups on GO before 230 °C, the other arose from the grafted PEI at 300 °C - 415 °C. The content of PEI on GO could be calculated from weight loss at 300°C - 415 °C of Fig. 3b - Fig. 3d.The content of PEI in GO-PEI with weight ratio 1:10, 1:40 and 1:80 are about 35 wt%, 53 wt% and 61 wt% respectively. These results demonstrated that the content of GO-PEI become higher with the increasing weight ratio by controlling reaction conditions strictly.

The effective diameters of GO-PEI, GO-PEI-PEG, GO-PEI-PEG-FA and GO-PEI-PEG-FA/si-Stat3 complexes at different weight ratios of PEI have been demonstrated by DLS in Fig. 4. The effective diameters of GO-PEI complexes at different weight ratios ranged from 1:10 to 1:100 changes from 515 nm to 310 nm, which are larger than the as-prepared GO (~200 nm). Interestingly, we found that the effective diameters were decreased until almost unchanged with an increase of the amount of PEI on GO. This phenomenon suggested that a

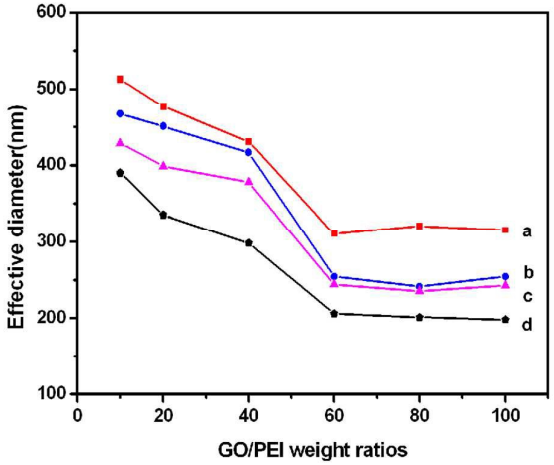


Fig. 4 The effective diameters of GO-PEI (a), GO-PEI-PEG (b), GO-PEI-PEG-FA (c) and GO-PEI-PEG-FA/si-Stat3 (d) at different weight ratios of PEI.

series of GO-PEI complexes were prepared successfully. It was found that the effective diameters become larger than GO at low weight ratios of PEI, which may be due to the situ grafting of PEI between GO sheets to form the intercalated layer structure, like GO-PEI-GO/PEI-GO-PEI sandwiched structure. The effective diameters of GO-PEI become smaller with the increasing of the weight ratio. It may be attributed to the increased amount of PEI on GO sheets which strengthen the electrostatic interaction between GO-PEI sheets. The electric repulsion made GO-PEI complex form exfoliated structure. Therefore, the GO-PEI sheets were unfolded at relatively low weight ratio with large effective diameters, on the contrary, the conformation of complexes sheets display the state of fold which led the effective diameters become smaller until unchanged. When the weight ratio reached to 1:60-1:100, GO-PEI complexes may form a coil as PEI chains wrapped with GO sheets. After conjugating GO-PEI with PEG, FA and plasmid si-Stat3, the trends of size changes of complexes are nearly as

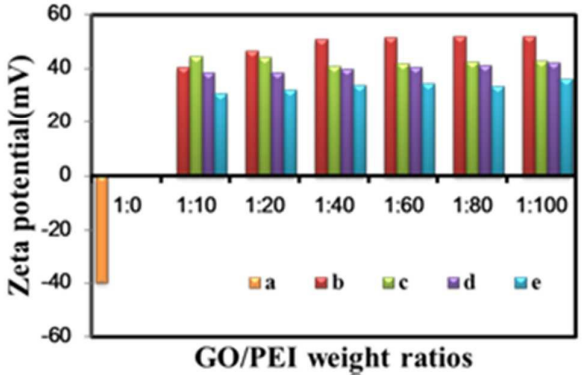


Fig. 5 Zeta potential of GO (a), GO-PEI (b), GO-PEI-PEG (c), GO-PEI-PEG-FA (d) and GO-PEI-PEG-FA/si-Stat3 (e) at different weight ratios of PEI.

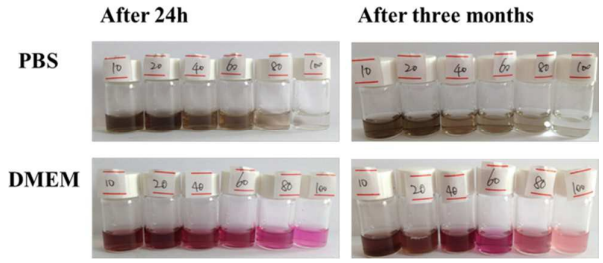


Fig. 6 Colloidal stability of GO-PEI-PEG-FA in PBS and DMEM (0.4 mg mL^{-1}) at different weight ratios of PEI.

same as GO-PEI. Therefore, we could control the size of GO-PEI-PEG-FA/si-Stat3 at about 400 nm - 200 nm by adjusting the graft ratio of PEI on GO with positive charge, which are beneficial for intracellular trafficking and cellular uptake.

The surface charges of GO-COOH, GO-PEI, GO-PEI-PEG, GO-PEI-PEG-FA and GO-PEI-PEG-FA/si-Stat3 complexes were measured by zeta potential (Fig. 5). The GO suspension exhibits highly negative charge about -39.8 mV, caused by the presence of hydrophilic carboxyl groups on the GO-COOH. The surface charges change to +52.1 mV after PEI covalent grafted onto GO. After functionalization of GO-PEI with PEG and FA, the surface zeta potential had maintained at above +40 mV. And the zeta potential of GO-PEI-PEG-FA/si-Stat3 complex at different weight ratios of PEI is above +30 mV. The positive zeta potential of GO-PEI-PEG-FA/si-Stat3 complex can promote the attachment to the negatively-charged cellular membranes and achieve the cellular uptake of si-Stat3 complexes.

Good biocompatibility of nanomaterials is necessary for its biological application. Accordingly, we had investigated the colloidal stability of a series of GO-PEI-PEG-FA complexes with different weight ratio in PBS and DMEM (Fig. 6). Obviously, the complexes with weight ratio 1:10 appeared as the darkest colored solution. The color of GO-PEI-PEG-FA complexes dispersion became light in turn with higher loadings of PEI which was consistent with the results of TGA (Fig. 3). After several months, noticeable aggregation of weight ratio of PEI 1:10 and 1:20 were observed in DEME, and the remaining solution displayed long-term stability. The occurrence of this phenomenon is resulting that formed reaction of the charge repulsion among GO-PEI-PEG-FA complex and its effective diameters. According to the Stokes-Einstein Equation, the classical theory of colloids $D=KT/6\pi\eta Rh$, (D: diffusion coefficient, Rh: hydrodynamic radius or effective diameters, T: temperature, η: solvent viscosity and K: Boltzmann constant), the particle velocity is inverse proportion to the hydrodynamic radius and solvent viscosity and it is proportion to temperature.

In our experiment, the aggregation of GO-PEI-PEG-FA complex with weight ratio at 1:10 and 1:20 in DMEM was due to the weak electrostatic interaction and the larger hydrodynamic radius which accelerated the particle sedimentation rate under the same experiment conditions with others. Therefore, the solution stability of complex was control by the particle size and electrostatic interaction among

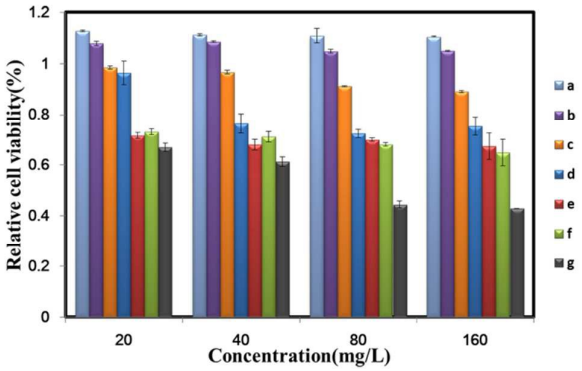


Fig. 7 Relative cell viability showed the cytotoxicity of different concentration GO-PEI-PEG-FA (at different weight ratios of PEI) to SMMC-7721 cells. a, b, c, d, e, and f was representative of the different weight ratios of PEI 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100 respectively.

sample-sample and sample-solvent interaction. We could obtain the long-term stable colloidal solution even under physiological conditions by adjusting the different weight ratio of PEI.

3.2 Biological properties

Cytotoxicity of different concentrations of GO-PEI-PEG-FA complexes was evaluated with SMMC-7721 cells by cell viability assay *in vitro* (Fig. 7). MTT showed that relative cell viability was softly reduced 10 %, when the different ratios of GO-PEI were 1:10 and 1:20. In contrast, the cell viability with GO: PEI at 1:100 was reduced to 65 %. Meanwhile, the relative cell viability of 1:100 reduced to 44 % at the concentrate of 80 and 160 mg L^{-1}. In cytotoxicity view, the results showed that the ratio of GO: PEI was too higher cytotoxicity to SMMC-7721 cells at 1:100.

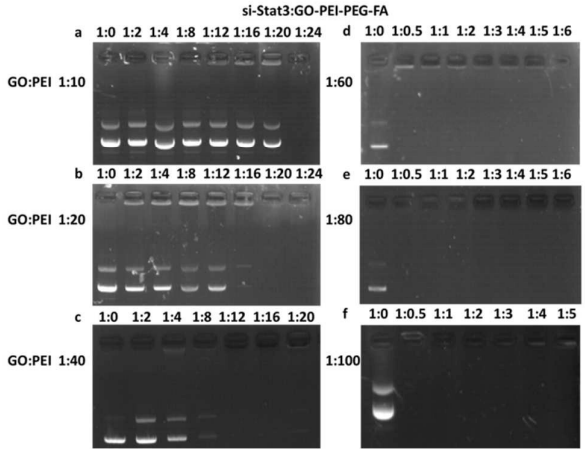


Fig. 8 Gel retardation analysis showed combining efficiency of complexes at various N/P ratios of GO-PEI-PEG-FA and Si-stat3. a, b, c, d, e, and f was representative of the different weight ratios of PEI 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100 respectively.

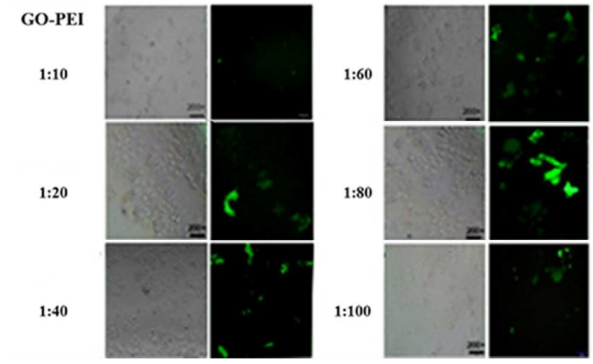


Fig. 9 Inverted fluorescence microscope image of transfection efficiency of different ratios of GO-PEI-PEG-FA /si-Stat3 complexes carried the same quality si-Stat3 in SMMC-7721 after 72 h at different weight ratios of PEI.

Combining efficiency is an important index of reaction material synthesis. The combining efficiency of nanoparticles loaded with si-Stat3 reflected the advantage of nanocomposite, in another word the higher rate of combining efficiency, the more low cost nanocomposite carrier. To obtain higher efficiency among our complex substance, we further tested the combining efficiency by agarose gel electrophoresis (Fig. 8). 1.0 % agarose gel electrophoresis showed Lane 1 was naked si-Stat3 vector as control and Lane 2-6 was GO-PEI-PEG-FA/si-Stat3 complexes with different N/P ratio. a, b and c showed si-Stat3 was loaded onto GO-PEI-PEG-FA completely at ratios of 1:20, 1:20 and 1:12 respectively. e, f and g showed si-Stat3 was loaded onto GO-PEI-PEG-FA completely at ratios of 1:0.5 all. In summary, the different weight ratios of PEI with higher combining efficiency of GO-PEI-PEG-FA were 1:60, 1:80 and 1:100.

Furthermore, to detect proper proportion of nanocomplex is more efficient to deliver DNA into cells, transfection efficiency was observed by inverted fluorescence microscope (Fig. 9). Carrying the same quality of si-Stat3, different ratios GO-PEI-PEG-FA complexes obtained different transfection efficiency in SMMC-7721 cells as follows: the transfection efficiency of 1:10 and 1:100 of GO: PEI was 10 %, the transfection efficiency of 1:20, 1:40 and 1:80 of GO: PEI was 35 %, 50 % and 70 % respectively. The results indicated that 1:80 of GO: PEI has the highest transfection efficiency. We found that the transfection

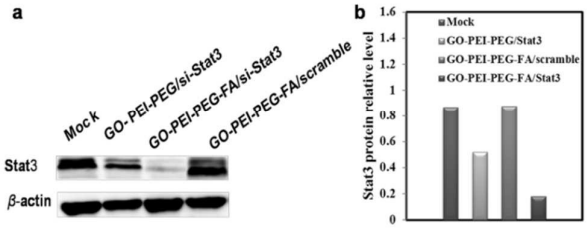


Fig. 10 Western blot to investigate si-Stat3 related protein expression (a) and calculation of relative Stat3 protein expression in each group (b).

efficiency was improved with the increasing of weight ratios of PEI as our expectation. According to the results of cytotoxicity of cells, combining efficiency and transfection efficiency, it is the most appropriate proportion of GO-PEI at 1:80.

In order to verify the effect of complex carrier delivering si-Stat3 *in vitro*, Western blot results (Fig. 10) proved that expression of Stat3 in the GO-PEI-PEG-FA/si-Stat3 was significantly reduced compared with other group. The results indicated the folate targeted better delivery of si-Stat3 into SMMC-7721 cells reduced more significant protein expression.

4. Conclusions

In summary, we have synthesized a novel, effective and safe multi-functionalized GO-PEI-PEG-FA/ si-Stat3 gene delivery vector for treating hepatocellular carcinoma successfully. The effective diameters of the as-prepared GO-PEI-PEG-FA/si-Stat3 complex could be control by the content of PEI. And the structure of complexes was changed from an intercalated layer structure to a fully exfoliated structure with the increasing content of PEI. The long-term stability of the complex in physiological conditions was realized, allowing for applying in gene delivery. It was found that the complex possess favorable properties of lower cytotoxicity and higher controllable transfection efficiency. Consequently, the as-prepared GO-PEI-PEG-FA/si-Stat3 realizes the silence of si-Stat3 expression by targeting therapy. Further investigation will involve the exact release mechanisms and apoptosis of si-Stat3 from the complexes *in vitro* and *in vitro.*

Acknowledgements

This work was funded by the National Natural Science Foundation of China (No. 81201188 and No.81472344).

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Multi-functionalized Graphene Oxide Complex as a Plasmid Delivery for Targeting Hepatocellular Carcinoma Therapy

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A novel efficient graphene-based gene delivery vector was prepared, the effects of the size, the stability and transfection efficiency by changing the content of PEI were investigated.