Supplementary information

Oxidation of MWCNTs

For surface oxidation studies, we used MWCNTs with initial length of 1-5 μm and were designated as long MWCNTs. Briefly, pristine MWCNTs 100mg were dispersed in 20ml of H_2SO_4 and HNO_3 in ratio 3:1. The dispersion was sonicated for 5min. to debundle aggregates and get uniform dispersion. Thereafter , the dispersion was refluxed at 80^0c using hot plate stirrer at 900 rpm [1]. Oxidation was carried out for 8hr. After oxidation, dispersion in acid was diluted up to 5 times of its volume and centrifuged to remove the acid. Ox-MWCNTs then obtained were further dispersed in acetone, centrifuged and supernatant was discarded which formed a dried cake of Ox-MWCNTs after some time.

To analyze the oxidized state of Oxidised MWCNTs, we optimized the oxidation time at different time points (1 to 6 h). Each time point was then characterized by SEM, DLS Sizing and zeta potential analysis to screen out the least toxic, easiest and the maximal cost effective oxidation parameters.

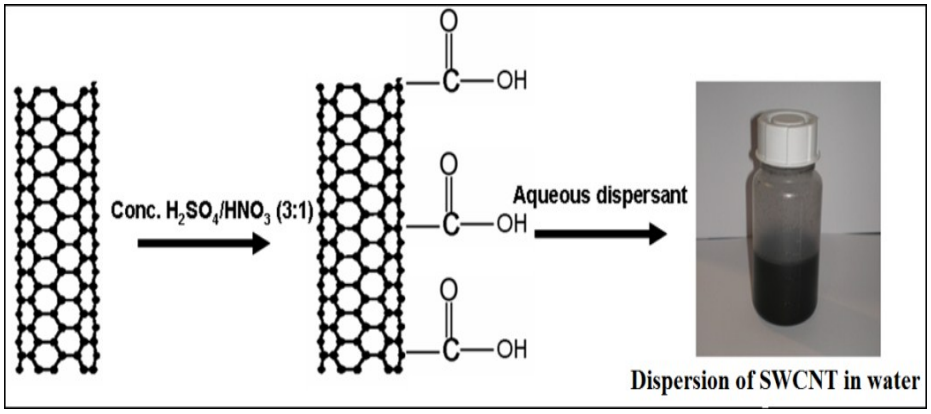

Figure S1: Oxidation of CNT

PEGylationof Ox-MWCNTs using single spacer EDBE

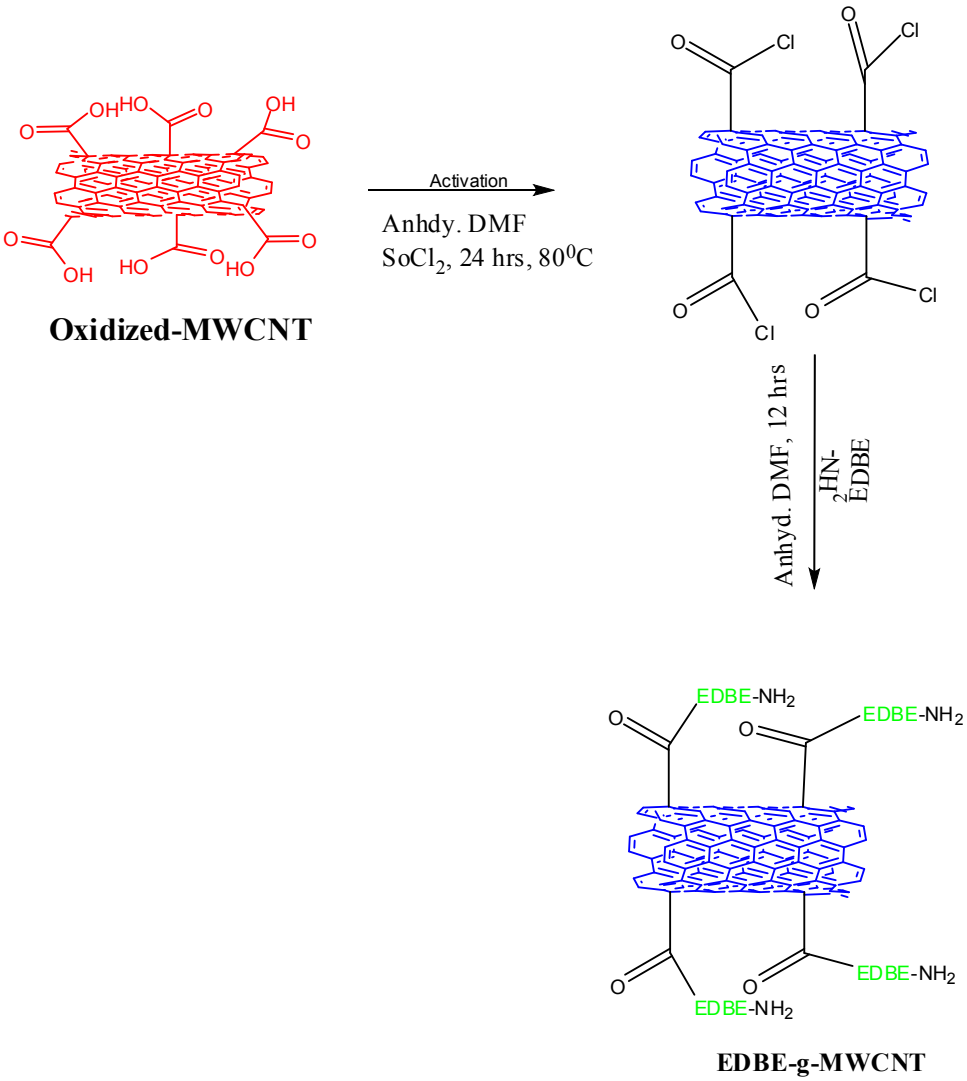

Figure S2: PEGylation of Ox-MWCNTs

After oxidation PEGylation of MWCNTs was carried out using 2, 2'– (ethylene dioxy) bis–(ethylene amine) (EDBE/single spacer) MW150 to make them less immunogenic and resistance against RES uptake. Briefly, Ox-MWCNT 100mg was dispersed in 10ml of THF by sonicating them for 1min. To the resultant dispersion, 20ml of $SOCl_2$ was added (addition was done in hood since thionyl chloride is very suffocating). This dispersion was then refluxed at 80^0c for 24hr [2]. Care should be taken to avoid moisture in reaction conditions since moisture hydrolyzes acid chlorides into acid (-COOH)

Solvents were then removed using rotavapour and resultant Ox-MWCNTs were dispersed in anhydrous THF. From TGA analysis, carboxylic density on the surface was determined to be 0.0018 mmoles/mg of CNT. This value was necessary to calculate the exact amount of EDBE. Based on TG results,EDBE (0.270 µg/mg of Ox-MWCNTs) were dissolved in anhydrous DMSO. Pyridine was added under ice-cold conditions to the treated Ox-CNTs and kept for magnetic stirring at 1200 rpm. To the resultant mixture, EDBE was added drop wise and kept for stirring for 18hr (overnight). Precipitation of EDBE functionalized CNTs was perceived after 18hr. The supernatant was discarded and pellet of EDBE-MWCNTs was washed with distilled water, centrifuged to remove reagents and dried by washing with acetone. PEGylation was confirmed by DLS size, zeta potential and FTIR.

Synthesis of Es-PEG-MWCNT-conjugate

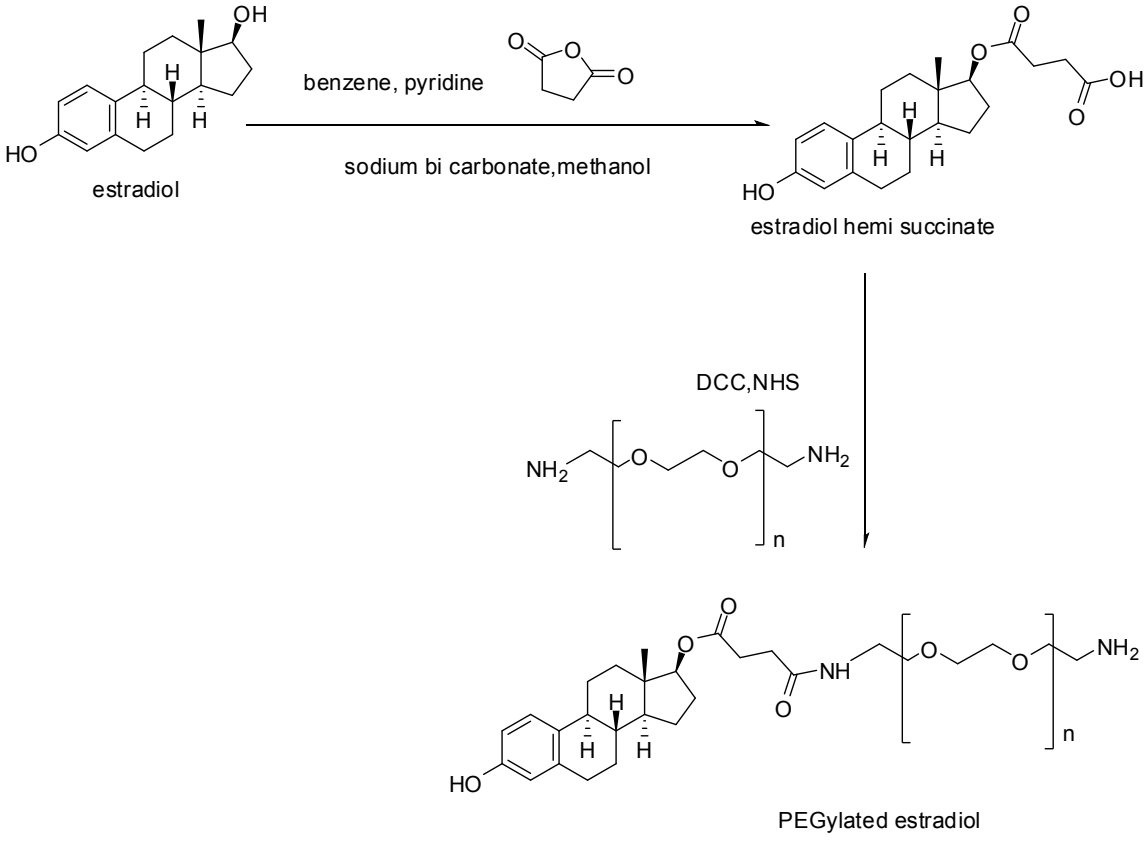

Figure S3: Schematic representation of preparation of PEGylated estradiol

Synthesis of Estradiol 17 β- hemisuccinate

It was prepared by a simple, rapid, high yield and relatively inexpensive procedure. Briefly, 17β-Estradiol (300 mg, 1.1mmol) was dissolved in anhydrous benzene (10 ml) and refluxed with approximately 5 fold molar excess of succinic anhydride (550 mg, 5.5 mmol) in presence of pyridine (2 ml). The reflux was continued for approximately 24h, until no Es was detectable by thin layer chromatography (TLC). On cooling at room temperature excess succinic anhydride precipitated out from the solution, which was filtered off. The filtrate was concentrated under reduced pressure by rotary evaporation, following which, the residue (E2-3, 17 disuccinate) was dissolved in methanol (10 ml) and stirred overnight with an excess of sodium bicarbonate (1g suspended in 10 ml of water) to complete selective hydrolysis of the phenolic ester. Completeness of the reaction was ensured by thin layer chromatography (TLC) using a 1:1 (v/v) mixture of dichloromethane and methanol as the eluent. The reaction mixture was subjected to filtration to remove unreacted NaHCO3. After filtration of NaHCO3, water (10 ml) was added to the reaction mixture. The alkaline solution was extracted 3 times with diethyl ether (10ml ×3). The aqueous phase was brought to pH 7 with 1N HCl and then poured into a mixture of 0.1N HCl and crushed ice. The white crystalline product was removed by vacuum filtration, washed repeatedly with water and air-dried. The crude Es-hemisuccinate was recrystallized from boiling benzene[3].

PEGylation of E2-hemisuccinate: Synthesis of E2-PEG derivative

E2-hemisuccinate (42 mg, 0.1 mmol) in dichlormethane (DCM, 2 ml), pyridine (2 ml), DCC (25 mg, 0.12 mmol), NHS (15 mg, 1.3 mmol) were sequentially added. The resultant mixture was stirred for 1h, following which PEG-bisamine (350 mg/0.1mmol dissolved in 1 ml of DCM) or EDBE (single spacer) was added. After 24h, finely suspended, white precipitate of dicyclohyxyl urea (DCU) was envisaged in the reaction mixture, ensuring successful transformation of E2-hemisuccinate to the corresponding NHS ester. The precipitate was removed by filtration, following which the filtrate was added dropwise to ice cold ether (30-40 ml) to precipitate the PEGylated E2 derivative as a white semi-solid. The crude product was air-dried, washed repeatedly with cold methanol to remove unreacted PEG-bisamine and finally dried under vacuum[3].

Functionalization of Ox-MWCNTs with PEGylated E2-hemisuccinate

For functionalization of MWCNTs with E2, acid-oxidized MWCNTs were converted to their corresponding acid-halide derivative by refluxing with thionyl chloride. Briefly, oxidized MWCNTs (100 mg) were dispersed in THF (10 ml) via ultrasonication for 1 minute. To the resultant dispersion, SOCl2 (15-20 ml) was added and the mixture was refluxed at 80°C for 24 h. Thereafter, solvents were removed using rotavapor and acylated MWCNTs were obtained. As determined by thermo-gravimetric analysis (TGA), carboxylic density on the surface of MWCNTs was determined to be 0.0018 mmoles/mg of MWCNTs. This value was necessary to calculate the exact amount of E2-PEG-NH2 that would be ideally required for complete interchange of surface carboxyl groups with the PEGylated steroid. Based on TGA results, around three fold molar excess of E2-PEG-NH2, dissolved in anhydrous DMSO, was added to a suspension of acylated MWCNTs in a 5:1 (v/v) mixture of DMSO and pyridine under ice cold conditions. The reaction mixture was kept for stirring for 24 h, following which the nanotubes were isolated by centrifugation. Supernatant was discarded and the pellet of functionalized MWCNTs was purified by washing it repeatedly with distilled water and acetone. Finally, the pellet was freeze dried using an optimized freeze drying cycle [3].

Optimization of oxidation time for pMWCNTs

To analyze the oxidized state of Oxidised MWCNTs, we optimized the oxidation time at different time points (1 to 6 h). Each time point was then characterized by SEM, DLS Sizing and zeta potential analysis to screen out the least toxic, easiest and the maximal cost effective oxidation parameters.

Characterization of Oxidized MWCNTs

Size and zeta potential measurement

The particle size and zeta potential of Oxidised MWCNTs were measured by dynamic light scattering (DLS) and the overall charge by zeta-potential measurements, using a particle analyzer (Zeta Nano Series, Malvern Instruments, Spain) in triplicate.

Table S1: Size and Zeta potential measurement of Ox-MWCNTs

Oxidation time	SEM Features	DLS based size (nm)	Zeta potential (mv)
Pristine CNT	Aggregates	2652±14.69	4.48±3.3
Ox-CNT 2hr	Aggregates	1653±12.85	-10.2±3.6
Ox-CNT 4hr	>1µ	635.5±9.3	-27.5±2.9
Ox-CNT 6hr	500nm	351.1±2.3	-37.9±2.8

Values are expressed as mean ± SD (n=3)

FTIR

FTIR spectra of Ox-MWCNTs represent the presence of surface -COOH groups, But in case of pristine MWCNTs no COOH peak was observed. Peak appeared at around 1040 cm^{-1} which increased in intensity with oxidation time due to increase in number of surface -COOH groups.

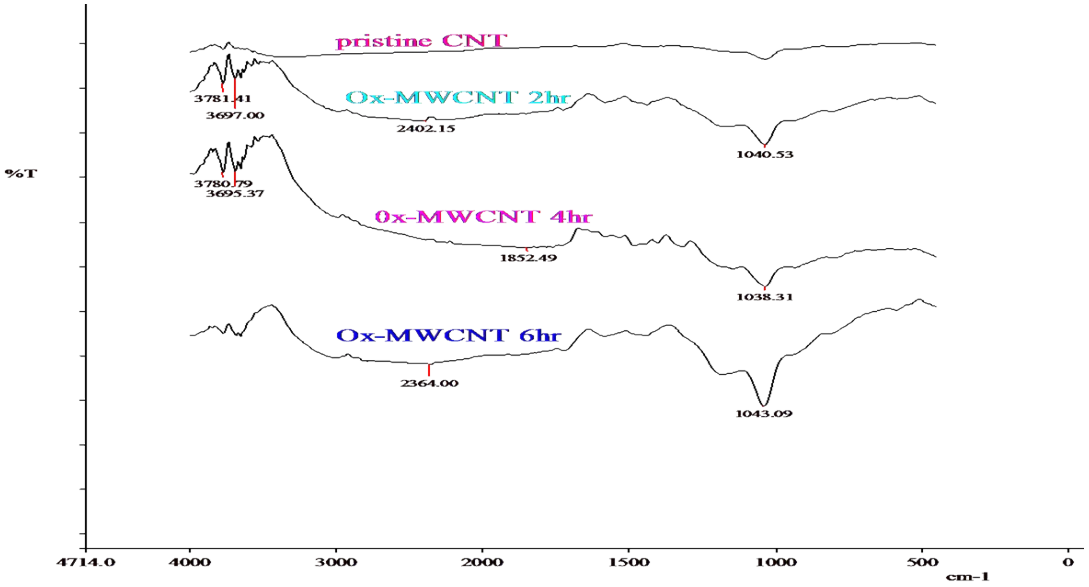

Figure S4: FTIR Spectra of Oxidised MWCNTs

- Pristine CNTs have poor solubility in most of the solvents.
- Oxidation of CNT causes generation of surface -COOH groups & shortening of CNTs. This was confirmed by FTIR, SEM & DLS Studies.

- Zeta potential became more negative with oxidation time up to 6h due to generation of more -COOH groups , where size decreased owing to enhanced dispersion , shortening and debundling of CNTs.
- Six hour oxidation time was optimized for producing better oxidized product.

NMR

The ^{1}H NMR Spectrum of Es-PEG-NH_2 revealed the presence of characteristic proton signals of steroid aromatic ring at 7.9-7.6, 6.6-6.2 ppm. In addition to these, distinctive proton signals of PEG (-O-CH_2-CH_2) unit were observed over the range of 3.6-3.1 ppm, owing to successful derivatization of Es with PEG.

HR-NMR Spectrum of Es-PEG-MWCNT conjugate contains, characteristic doublets of phenyl ring appeared at 7.6 and 6.6 ppm. The chemical shift values at 3.2 and 3.4 ppm were assigned to the (-O-CH_2-CH_2) units of PEG chain. The various signals ranging between 2.7-1.0 ppm were representative of the various methylene and methane protons of the steroid rings. The sharp singlet at o.868 ppm was ascribed to 11-methyl –CH_3protons of the steroid moiety.

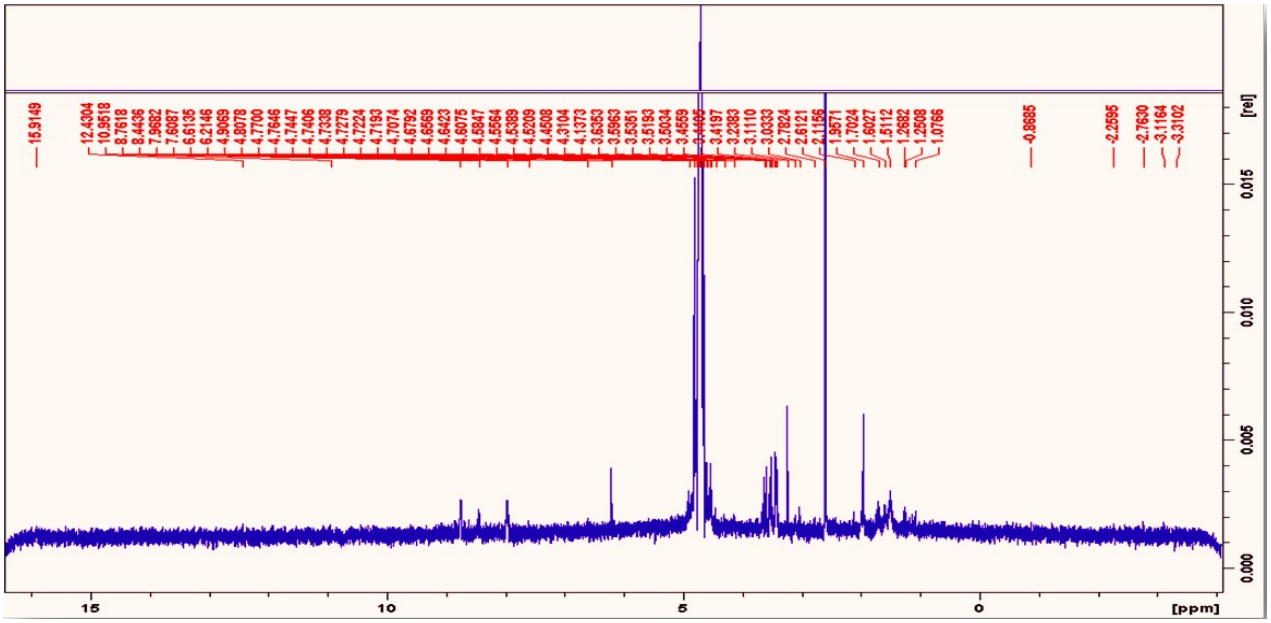

Figure S5: ^{1}H NMR Spectrum of Es-EDBE-MWCNT Showing characteristic proton signals

TGA analysis of Estradiol conjugated polyethylene glycol grafted MWCNTs

TGA weight loss curves of (A) MWCNT-EDBE (5.06%), (B) ES-PEG-g-MWCNT (9.22%), and (C) ES-EDBE-MWCNT (D) Pure EDBE (E) MWCNT-COOH (F) Pure PEG

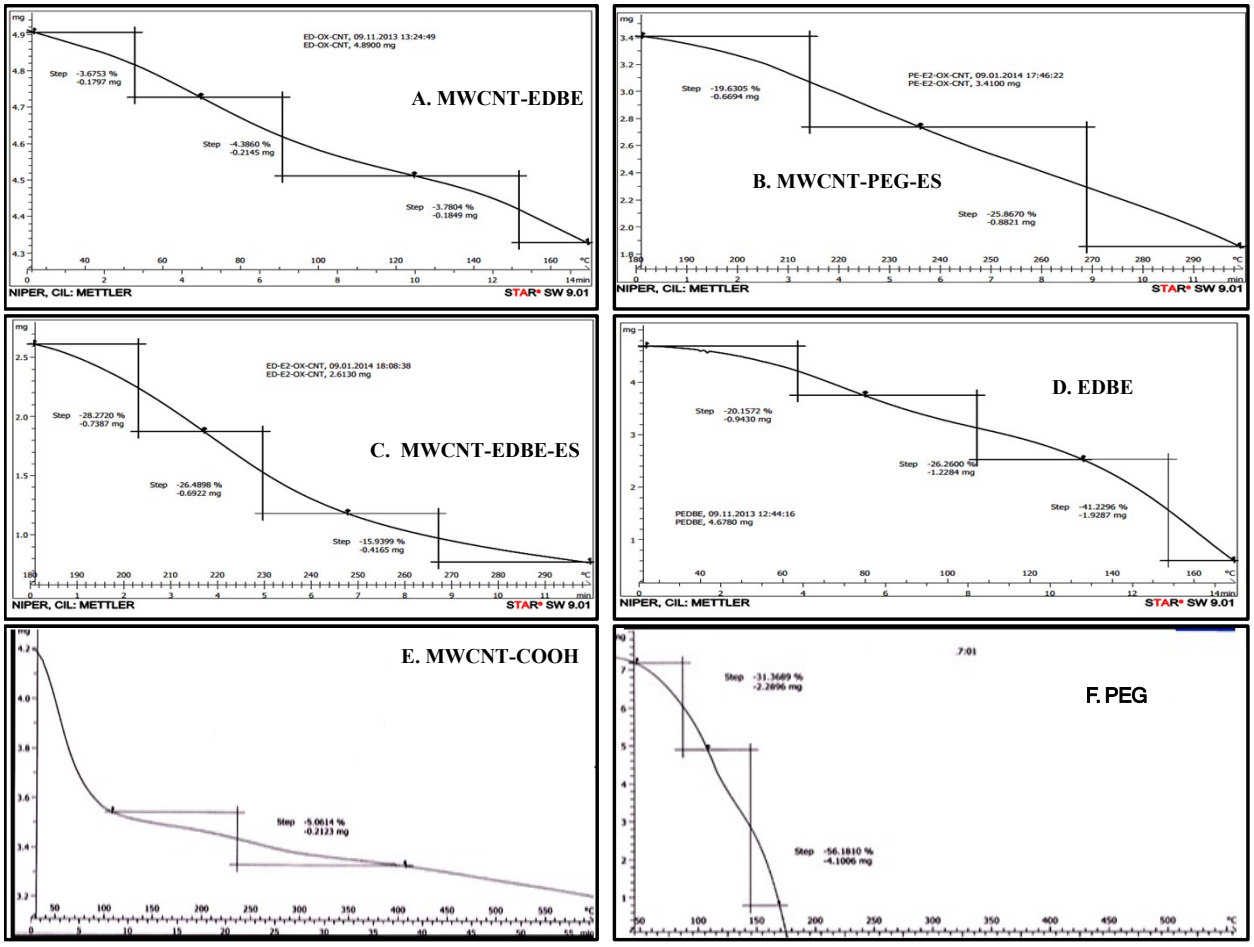

Figure S6: TGA Curves for quantification of functional groups on the surface of MWCNT

Authentication of extracted pDNA

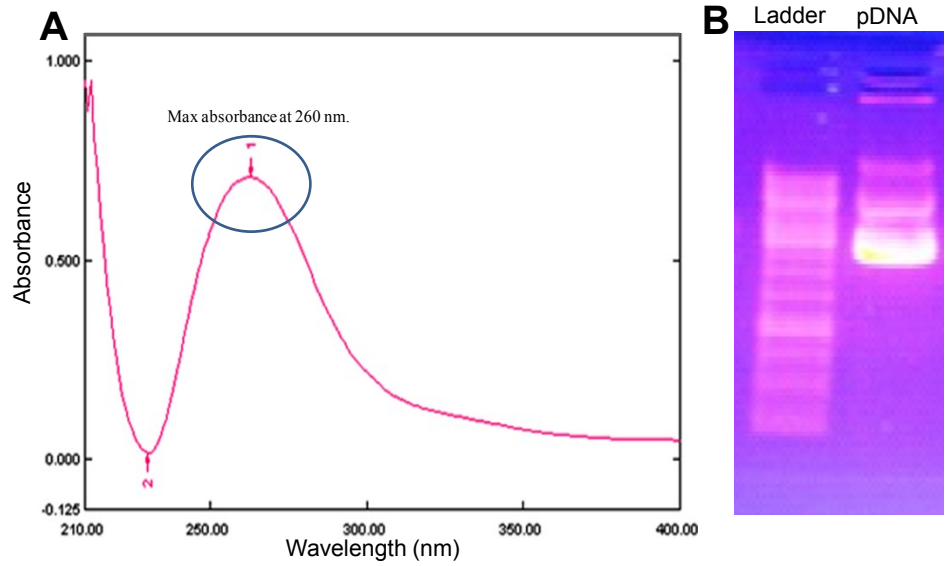

Figure S7: (A) UV spectrum and (B) gel retardation of extracted pDNA

[1] A. Osorio, I. Silveira, V. Bueno, C. Bergmann, H 2 SO 4/HNO 3/HCl—Functionalization and its effect on dispersion of carbon nanotubes in aqueous media, Applied Surface Science, 255 (2008) 2485-2489.
[2] F. Pompeo, D.E. Resasco, Water solubilization of single-walled carbon nanotubes by functionalization with glucosamine, Nano letters, 2 (2002) 369-373.
[3] M. Das, R.P. Singh, S.R. Datir, S. Jain, Intranuclear Drug Delivery and Effective in Vivo Cancer Therapy via Estradiol–PEG-Appended Multiwalled Carbon Nanotubes, Molecular pharmaceutics, 10 (2013) 3404-3416.