Electronic Supplementary Information (ESI)

Graphene quantum dots conjugated albumin nanoparticles for targeted drug delivery and imaging of pancreatic cancer

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1. Chemicals

Human serum albumin (HSA), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), Gemcitabine hydrochloride, Glutaraldehyde, Dimethyl Sulfoxide (DMSO), dimethyl maleic anhydride (DMMA) , MTT (3-[4, 5-dimethylthiazol-2-yl]-3, 5-

diphenyltetrazolium bromide), Graphite powder, Hyaluronic acid (HA) and Lawsone (2-hydroxy 1,4 naphthoquinone) were obtained from Sigma-Aldrich and used as received. CD-44 expressing pancreatic cancer cell line (Panc-1) was procured from National Centre from Cell Science (NCCS-India) Cell Repository and was cultured in DMEM media (Himedia-India) supplemented with 10 % FBS (Gibco, Life Technologies, India).

2. Experimental details

(i) Synthesis of HSA, Gemcitabine loaded HSA, Hyaluronic acid (HA) functionalized HSA nanoparticles

In this study HSA and Gem-HSA nanoparticles were prepared via desolvation method described earlier[1, 2] and their efficacy as drug delivery vehicles was observed. Different parameters like albumin concentration, pH, rate of addition of ethanol, amount of glutaraldehyde as crosslinker and duration of crosslinking were optimized before synthesis. Briefly, in 3% HSA solution (pH=9), ethanol was added drop wise with flow rate of 1.7 ml/min while stirring at 800 rpm (Magnetic Stirrer, Tarsons-India) and as soon as solution attained turbidity, 50 µL glutaraldehyde (8%) was added into the solution and left for 24 hrs for proper cross linking.

Two approaches were adopted concurrently for preparing gemcitabine loaded particles. (i) Drug encapsulation and (ii) drug adsorption on the surface of nanoparticles. For direct drug encapsulation, 2 ml gemcitabine solution (20 mg/ml) was added into the HSA solution (pH=9.0) with drop-wise addition of ethanol at room temperature under magnetic stirring. Subsequently 2 ml gemcitabine solution (15 mg/ml) was also added to the mixture after turbidity achieved for its physical adsorption onto the surface of nanoparticles so as to increase the loading efficiency of NPs. Glutaraldehyde solution was added to crosslink HSA nanoparticles and again kept under stirring for 24 hrs. After ethanol elimination under reduced pressure, centrifugation was done at 13500 rpm for 25 min to remove free HSA, gem and glutaraldehyde. Resulting pellets were collected and freeze dried for further use. HSA-NPs were functionalized by conjugation of surface amines of HSA with hyaluronic acid via EDC-NHS coupling. In brief, 30 mg HA was incubated at room temperature for 3 hrs in a 3:1 molar ratio solution of EDC-NHS for activation of its carboxylic group. Later 120 mg HSA-NPs (in 15 ml PBS 50mM, pH= 7.0) were added to it and left overnight.

(ii) Synthesis of graphene quantum dots

Graphene Oxide (GO) was synthesized by modified Hummers process[3] and subsequently, Chemical reduction of GO solution was achieved using Lawsone (2-hydroxy 1,4 naphthoquinone) as reducing agent under a hydrothermal environment. Typically 100ml of above GO solution was sonicated for half an hour and then 2.87 mM of Lawsone was added with magnetic stirring for 15 min. The solution was then transferred to oil bath and heated at 200^0 C for 4h. The reduced graphene were collected after centrifugation followed by washing with deionised water several times and then purified using dialysis membrane for 24h to reduce size into quantum dots. GQDs were covalently linked to albumin nanoparticles via EDC-NHS coupling same as HA.

(iii)Characterization of Nanoparticles

All the above synthesized nanoparticles were further analyzed for morphology, size and surface charge and physiochemical characteristic. SEM (Quanta 2003D, FEI, The Netherlands), Dynamic light scattering (DLS) (90 plus Brook Havens Instruments, USA), FT-IR (Shimadzu, Japan & Nicolet iS5, Thermo scientific, USA), Photo Luminescence (LS 55, Perkin Elmer, USA), XRD (Bruker D8 Advance, USA), Thermo Gravimetric analysis (TGA) (DTG 60 Shimadzu) with 10^0 C/min rate upto 1000^0 C for thermal degradation studies and UV-Vis spectroscopy (Cary50 Bio, Varian, The Netherlands) were performed to characterize the nanoparticles.

(iv)Encapsulation efficiency (EE) and Drug release profile

To determine the encapsulation efficiency of nanoformulation, supernatant after centrifugation of the nanoparticles solution was assessed via UV-Vis spectroscopy at 275 nm and encapsulation efficiency was calculated by the following formula:

$$\textbf{\% EE} = ([C_{td}] - [C_{ds}]) / [C_{td}] \times 100$$

$[C_{ds}]$ is concentration of drug in supernatant and $[C_{td}]$ is concentration of total drug used for encapsulation in HSANPs.

The drug release profile of gemcitabine was determined by dispersing 25 mg of gem loaded HSA nanoparticles in 20 mL of phosphate-buffered saline (PBS; pH 7.4) under constant shaking at 150 rpm/min at 37°C. Sampling was done in predefined time intervals and the amount of free gemcitabine was determined via UV spectrophotometer. This analysis was performed thrice to minimize experimental errors.

(v) Cytotoxicity and Imaging analysis

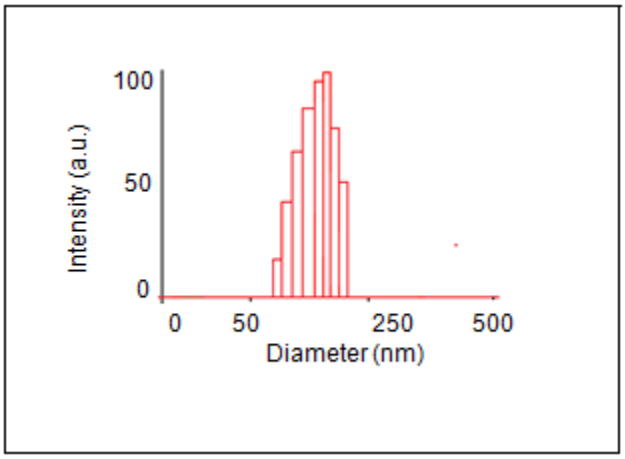
MTT (3-[4, 5-dimethylthiazol-2-yl]-3, 5-diphenyltetrazolium bromide) assay was performed to determine the efficacy of our nanoformulation. Panc-1 (pancreatic cancer cell line) was incubated in DMEM media with 10% FBS and 50 µL gentacyin at 37^0 C in 5 % CO_2 environment. Cells were seeded in 96 well plates for 24 hrs and HSA-NPs, HSA-HA, HSA-Gem, HSA-Gem-HA and free Gem with concentration of 0.10, 0.5, and 1.0, 10 and 50 µg/ml was added to cells and further incubated for 24 hrs into the same environment. After second incubation, MTT (10 µl, 10mg/ml) was added and kept for 4 hrs. Subsequently, culture media was removed and DMSO (150 µl) was added and after 15 minutes incubation with shaking, optical density (OD) was taken at 570 nm. All the analysis was done in triplicate to avoid experimental errors.

For imaging, GQDs labelled HSA and HA-HSA-NPs were injected into the Panc-1 (Pancreatic cancer cell line) and incubated for 12 h. Panc-1 cell lines were maintained in DMEM media supplemented with 10% fetal bovine serum in 5% CO_2 atmosphere at 37^0 C. Cellular uptake of nanoformulation was visualized by Floid cell imaging system (Invitrogen).

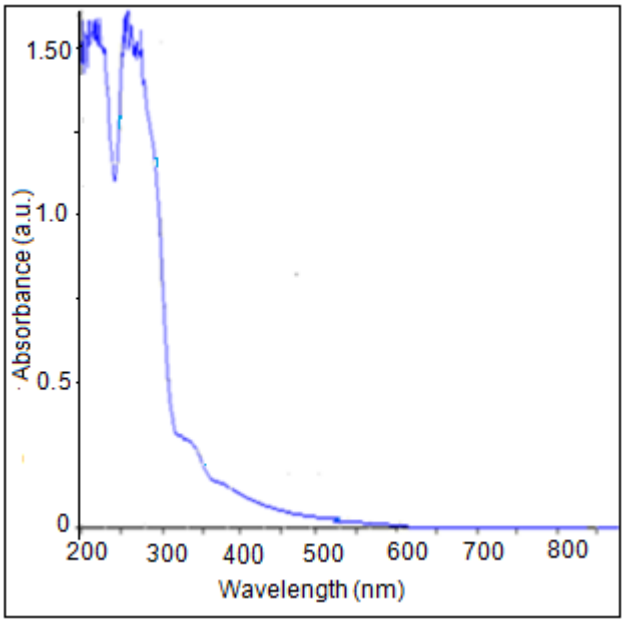
3. Table S1: Physicochemical properties of HSA-NPs, HSA-HA-NPs, GQDs and HSA-HA-GQDs

	HSA-NPs	HSA-HA NPs	GQDs	HSA-HA-GQDs
Size	150 nm		5 nm	
Zeta Potential	-27.78	-31.45	-21.19	-33.21
Drug encapsulation efficiency	92%	90%	-	90%
Release Time	20 hrs	20hrs	-	20 hrs
Photoluminescence emission wavelength (nm)	-	-	530	-
Quantum yield(at 360 nm)	-	-	14%	-

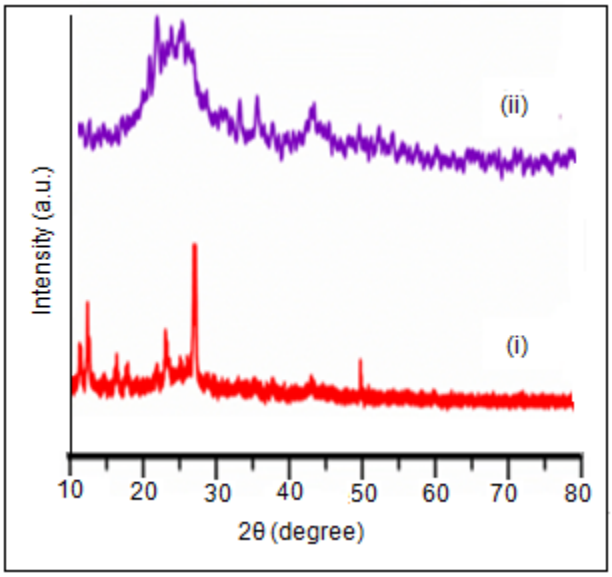
4. Figure S1: DLS histogram of albumin nanoparticles size distribution



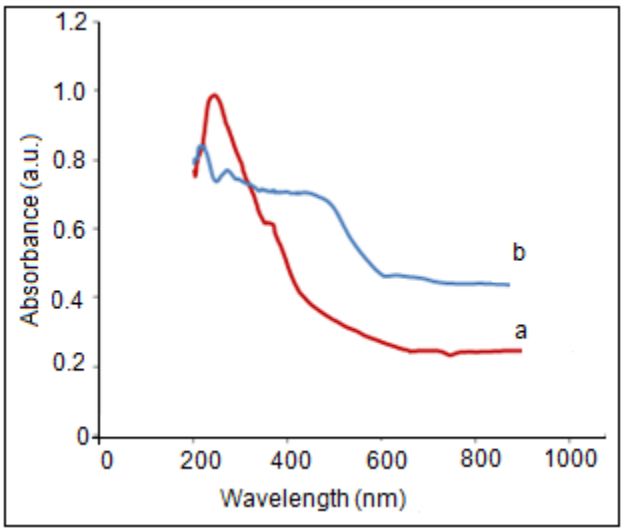
5. Figure S2: UV-Vis spectra of aqueous solution of graphene quantum dots at room temperature (~25 ^{0}C).



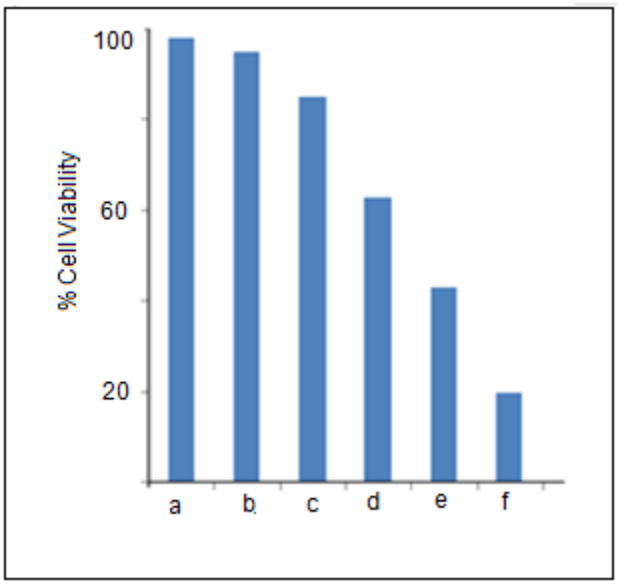
6. **Figure S3:** XRD pattern of (i) Graphene Oxide (ii) Graphene quantum dots.



7. **Figure S4:** UV Vis spectra of (a) Gemcitabine (5 mg/ml), (b) Gemcitabine loaded HSA nanoparticles dispersed in miliQ water (at room temperature ~25 ^{0}C).



8. Figure S5: % cell viability after MTT assay a) HSA NPs; b) HSA-HA; c) Graphene quantum dots; d) Free Gemcitabine; e) HSA-Gem; f) HSA-HA-Gem (Cells were incubated in DMEM media with 10 % FBS at 37 ^{0}C and 5% CO_2 environment in incubator).



References

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2. C. Weber, C. Coester, J. Kreuter and K. Langer, *Int. J. Pharm*., 2000, **194**, 91-102.

3. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.,* 1958, **80**, 1339.