ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.201301376

Dual Surface-Functionalized Janus Nanocomposites of Polystyrene/Fe_3O_4@SiO_2 for Simultaneous Tumor Cell Targeting and Stimulus-Induced Drug Release

Feng Wang, Giovanni M. Pauletti, Juntao Wang, Jiaming Zhang, Rodney C. Ewing, Yilong Wang, and Donglu Shi**

Submitted to ADVANCED MATERIALS

Supporting Information for DOI: 10.1002/adma.201301376

Dual Surface-functionalized Janus Nanocomposites of Polystyrene/Fe_3O_4@SiO_2 for Simultaneous Tumor Cell Targeting and Stimulus-induced Drug Release

By Feng Wang, Giovanni M. Pauletti, Juntao Wang, Jiaming Zhang, Rodney C. Ewing,

Yilong Wang* and Donglu Shi*

(Dr. Yilong Wang and Dr. Donglu Shi are co-corresponding authors for this paper)

Feng Wang,
The Materials Science and Engineering Program, College of Engineering & Applied Science, University of Cincinnati, Cincinnati, OH 45221-0012 (USA)
Dr. Donglu Shi
The Materials Science and Engineering Program, College of Engineering & Applied Science, University of Cincinnati, Cincinnati, OH 45221-0012 (USA)
The Institute for Biomedical Engineering & Nano Science, Tongji University School of Medicine, Shanghai 200092 (China)
E-mail: shid@ucmail.uc.edu
Dr. Giovanni M. Pauletti
James L. Winkle College of Pharmacy, University of Cincinnati,
Cincinnati, Ohio 45267 (USA)
Dr. Jiaming Zhang, Dr. Rodney C. Ewing
Departments of Earth & Environmental Sciences, Nuclear Engineering & Radiological Sciences and Materials Science & Engineering, University of Michigan,
Ann Arbor, Michigan 48109-1005 (USA)
Dr. Yilong Wang, Juntao Wang
The Institute for Biomedical Engineering & Nano Science, Tongji University School of Medicine, Shanghai 200092 (China)

Keywords: (Janus, dual-functional, targeting, drug delivery)

Experimental

1. Materials and Chemicals

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), oleic acid (OA), styrene (St), tetraethoxysilane (TEOS), sodium dodecyl sulfate (SDS), hexadecane (HD), 4,4'-azobis(4-cyanovaleric acid) (ACVA), triethylamie (TEA), folic acid (FA), 3-(triethoxysilyl)propyl isocyanat, adipic acid dihydrazide, trifluoroacetic acid (TFA), Hank's Balanced Salts, sodium bicarbonate, D-(+)-glucose, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (USA). Diethyether, tetrahydrofuran (THF), ammonium hydroxide (NH_4OH, 29.79 wt%), phosphate buffered saline (PBS), and the DMEM media with high glucose (Hyclone) were purchased from Fisher Scientific (USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Thermo Fisher Scientific (USA). Poly(ethylene glycol) bis-amine with molecular weights of 1000 Da (NH_2-PEG-NH_2) was obtained from YareBio (China). Doxorubicin hydrochloride (DOX) was purchased from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). Acetate buffer solutions (10mM) were prepared in our laboratory. The human breast cancer cell line MDA-MB-231 was obtained from American Type Culture Collection (ATCC) and cultured in DMEM with high glucose supplemented with 10% fetal bovine serum, 1% MEM-Non Essential Amino Acids , 1% Penicillin Streptomycin, and 1% l-glutamine. HBSS buffer was made by dissolving Hank's Balanced Salts (0.98 %), sodium bicarbonate (0.037 %), D-(+)-glucose (0.35 %), and HEPES (0.286 %) in autoclaved water and titrating pH to 7.35 with NaOH. Assay material CellTiter-Glo™ Luminescent Cell Viability Assay Kit was purchased from Promega (Madison, WI). Styrene monomer was washed with 5 wt% sodium hydroxide solution and stored at -20 °C before use. All other chemical were used as received. Deionized (DI) water was used through the entire experiment. Ultra high purity nitrogen gas was obtained from Wright Brother Gas (Cincinnati, OH).

2. Methods

2.1. Synthesis of Oleic Acid Functionalized Iron Oxide Nanoparticles (OAIOs)

Oleic acid surface functionalized iron oxide nanoparticles (~10 nm) (OAIOs) were fabricated using a reported method in our previous paper[1]. Briefly, $FeCl_2 \cdot 4H_2O$ (7.72 g) and $FeCl_3 \cdot 6H_2O$ (24g) were dissolve in 100 ml of DI water. The dispersion was heated to 80 °C with nitrogen bubbling for 0.5 h followed by addition of NH_4OH (50g). Then 3.76 g of oleic acid was added and the dispersion was kept at 80 °C for 3 h. The dispersion was cooled to room temperature and OAIOs were obtained after washing with ethanol and DI water. The OAIOs were dispersed in octane and dried under rotary evaporation for further use.

2.2. Fabrication of Polystyrene/Fe_3O_4@SiO_2 Superparamagnetic Janus Nanocomposites (SJNCs)

The water phase was formed by dissolving 0.092 g of SDS in 39 g of DI water. The oil phase was prepared by dissolving 25 mg of OAIOs, 0.4 g of HD and 2 g of TEOS in 8 g of St monomer. Then the oil phase was injected dropwise into the water phase under sonication, followed by mechanical stirring at 250 rpm for 0.5 h. The miniemulsion droplets were formed by sonicating the emulsions for 10 min at 500 W at a duty cycle of 50 % using a Scientz-IID sonifier (Ningbo, China) in an ice bath before the miniemulsion system was transferred to a three-neck flask. After 0.12 g of ACVA was neutralized by 1ml of NaOH solution (0.856 M) and added into the flask, the system was deoxygenized by nitrogen bubbling for 0.5 h with 250 rpm mechanical stirring. Then the flask was moved into an 80 °C water bath with a condenser under nitrogen protection to initiate St monomer polymerization. After 60min, 50 ul of NH_4OH was added into the reaction. After another 5 h, the reaction system was taken out of water bath and stirred at room temperature overnight. Finally, Polystyrene/Fe_3O_4@SiO_2 superparamagnetic Janus nanocomposites (SJNCs) were purified by washing with DI water using an external magnetic field and dried under vacuum at room temperature. The SJNCs

were readily dual functionalized with carboxyl groups on polystyrene (PS) surface and hydroxyl groups on silica surface.

2.3 Synthesis of NH_2-PEG-FA and further conjugation onto Polystyrene Surface of SJNCs

Folic acid was conjugated to the NH_2-PEG-NH_2 through a carbodiimide-mediated coupling reaction. In a typical reaction, 50 mg of NH_2-PEG-NH_2 was dissolved in 8 ml of DMSO. Then 22 mg of folic acid, 47.8 mg of EDC and 15 mg of NHS were dissolved in 10ml of DMSO followed by addition of 20 ul of TEA and the mixture was magnetically stirred for 1 h. The resulting yellow solution was added dropwise (one drop per 2 secs) into the NH_2-PEG-NH_2 solution under vigorous stirring and the reaction mixture was stirred overnight before DMSO was removed under reduced pressure at 80 °C. The yellow oily residue was dissolved in THF and NH_2-PEG-FA was obtained by precipitation after the addition of diethyether.

For conjugation of NH_2-PEG-FA onto the PS surface of SJNCs, 40 mg of SJNCs were dispersed in 10 ml of DI water, followed by dissolution of 80 mg of EDC. The suspension was stirred for 15 min. Then, 12 mg of NHS and 15 mg of NH_2-PEG-FA were added. The reaction system was stirred overnight and subjected to magnetic wash until no absorbance can be detected at 270 nm in the supernatant by UV-Vis spectroscopy. The resulting FA-PS/Fe_3O_4@SiO_2 nanocomposites (FA-SJNCs) were dried under vacuum at room temperature.

2.4. Synthesis of Silane-hydrazide Crosslinker and Surface Modification of Silica Shell of SJNCs

In a typical reaction, 52.26 mg of adipic acid dihydrazide was dissolved in 4 ml of DMSO. Then 74.2 ul of 3-(Triethoxysilyl)propyl isocyanat was dissolved in 500 ul of DMSO and added into the adipic acid dihydrazide solution dropwise (one drop per 10 secs) under vigorous stirring. After reaction overnight, DMSO was removed under reduced pressure at 80 °C and the waxy residue was dissolved in 2 ml of EtOH.

A standard silane coupling method was employed to modify the silica surface. 40 mg of FA-NPs were dispersed in 16 ml of EtOH/H_2O (95:5) mixture. The 60 ul of the above silane-hydrazide solution in EtOH was added into the FA-SJNCs dispersion under stirring. The pH was tuned to 5 by HCl and the dispersion was stirred for 4 h at room temperature. Then the pH was tuned to neutral by adding NaOH and stirred overnight. The surface modified FA-SJNCs-$NHNH_2$ nanocomposites were washed by magnetic separation with EtOH and DI water for four times respectively and dried at room temperature under vacuum for drug loading.

2.5. Doxorubicin loading onto FA-SJNCs-$NHNH_2$ Nanocomposites

DOX was conjugated to the surface of silica half shell through the formation of hydrazone bonds. Briefly, 35 mg of FA-SJNCs-$NHNH_2$ nanocomposites were dispersed in 15 ml of DOX solution in anhydrous methonal (0.33 mg ml^{-1}) and 3 ul of TFA was added to tune the pH to 5. The mixture was stirred in darkness for 40 h at room temperature and intense magnetic wash with anhydrous methanol was carried out till no absorbance at 480 nm was detected in the supernatant on a UV-Vis spectrophotometer. The FA-SJNCs-DOX conjugates were dried at room temperature under vacuum.

2.6. Evaluation of pH sensitivity

The pH-responsive DOX release profile from the FA-SJNCs-DOX conjugates was studied using UV-Vis spectrophotometer. First, 10mg of FA-SJNCs-DOX conjugates were dispersed in 5 ml of buffer solutions of different pH conditions [PBS buffer, pH 7.4 and acetate buffer (pH 5.0 and 6.0)] in glass vials and placed on an electric rotator in an incubator set at 37 °C. At selected time intervals, 1 ml of supernatant was removed from the vial after magnetic wash and replaced by fresh buffer solution with the same volume. The absorbance of released DOX was measured by a UV-Vis spectrophotometer at 480 nm and the amount was calculated according to a pre-established DOX calibration curve.

2.7. In-vitro Cell Cytotoxicity Assay

The cytotoxicity of SJNCs-DOX conjugates against the MDA-MB-231 cell was studied using the luminescent ATP-based assay kit (CellTiter GLO, Promega, Madison, WI) according to the manufacturer's instructions. First, MDA-MB-231 cells were seeded (2500 cells per well) in 96-well plates for 24 h. Then, the media was replaced with HBSS buffer solution containing free DOX, FA-SJNCs-$NHNH_2$, FA-SJNCs-DOX, and SJNCs-DOX. For competition experiments, 1 mM of folic acid was included as inhibitor into the HBSS buffer solution. Cells were incubated for 4 h, washed with warm HBSS buffer twice and further incubated with serum-containing media for another 48 h. Luminescence was measured using a BMG PolarSTAR microplate reader. All experiments were performed at least in triplicate. Results are expressed as mean ± standard deviation. Statistical comparison between experimental groups was performed using Students *t*-test at a significance level of $p < 0.05$ (GraphPad Prism 5).

3. Characterization

The morphology of SJNCs was observed on a Philips Tecnai 20 transmission electron microscope with an accelerating voltage of 200 kV. The STEM images and elemental mapping were obtained on a Zeiss Ultra55 equipped with Oxford Aztec X-Max 50 EDS system operated at an accelerating voltage of 1 kV. Size distribution of SJNCs dispersed in DI water was measured using a Malvern Zetasizer (Nano ZS). Thermal gravimetric analysis (TGA) analysis was performed on a thermogravimetric analyzer (NETZSCH TG209) under nitrogen atmosphere. The magnetic properties were measured at room temperature using a Vibrating Sample Magnetometer (VSM 7407, Lake Shore, USA). FTIR spectra were recorded with a Nicolete 6700 FTIR from Thermo Scientific using the KBr method. UV-Vis spectra were recorded on a Hitachi U-3000 spectrophotometer using a cuvette with the path length of 1 cm. Luminescence was obtained on a POLARstar OPTIMA microplate reader (BMG Labtechnologies, Chicago, USA).

[1] Wang, Y. L.; Xu, H.; Ma, Y. S.; Guo, F. F.; Wang, F.; Shi, D. L. *Langmuir* **2011**, *27*, 7207.

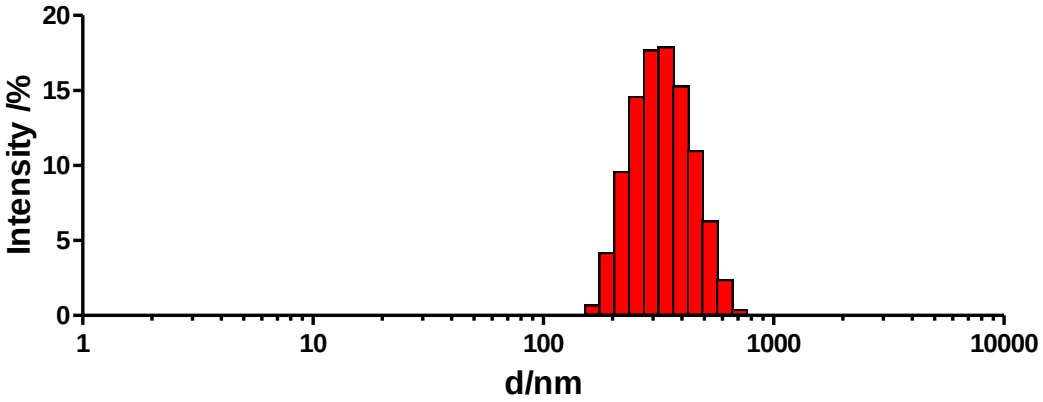

Figure S1. DLS data of SJNCs in DI water.

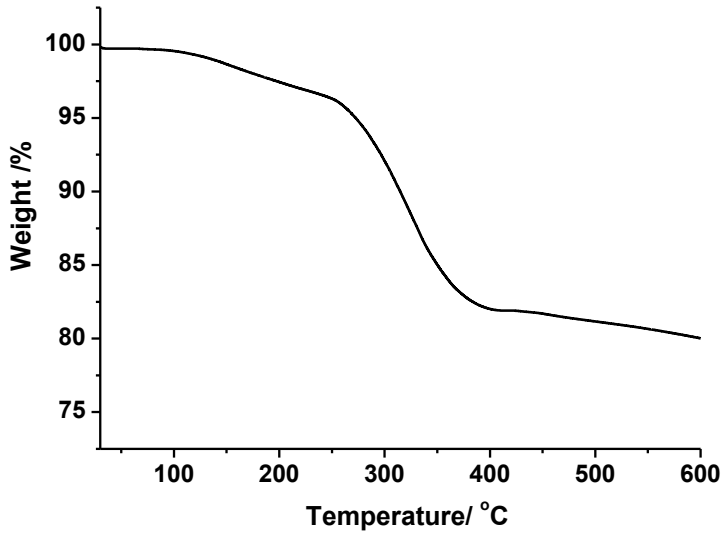

Figure S2. TGA curve of SJNCs.

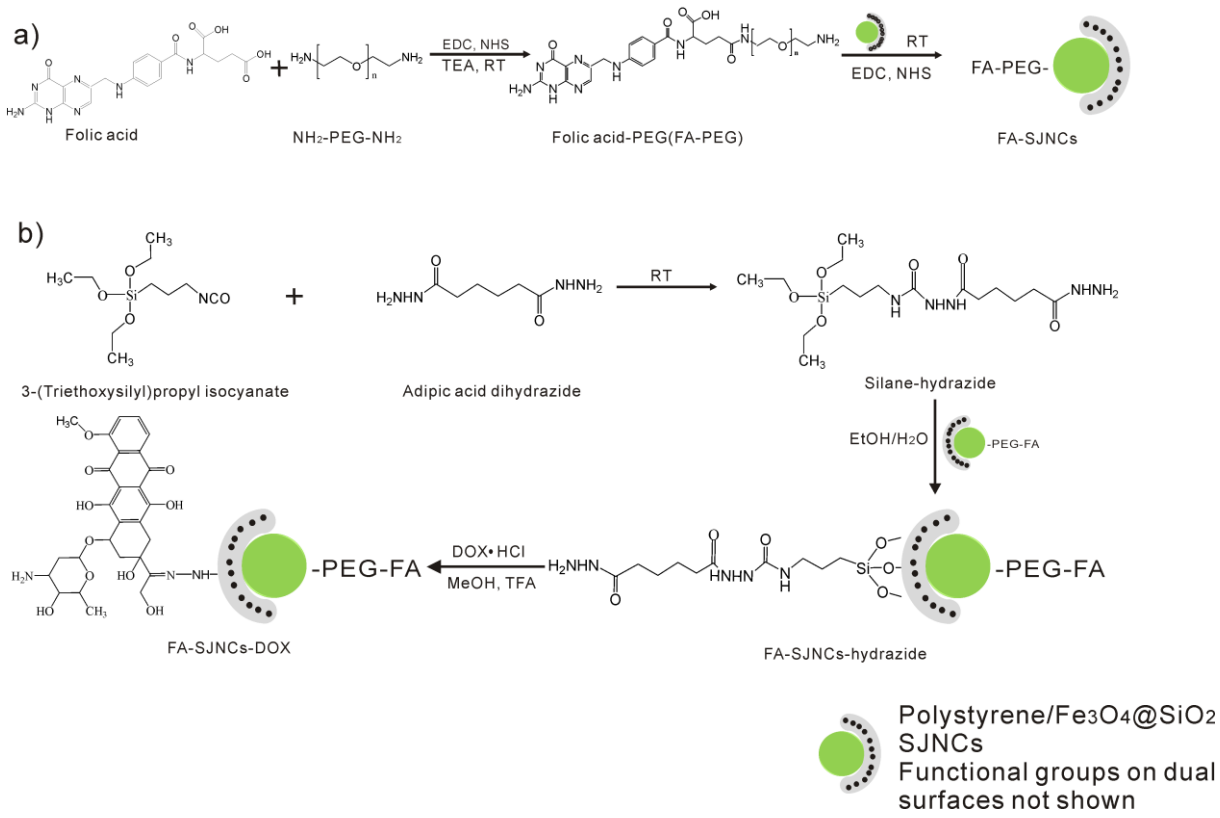

Figure S3. Schematic drawing of (a) folic acid (FA) conjugation and (b) doxorubicin (DOX) loading onto SJNCs dual surfaces.

Table S1. Estimated IC50 values for free DOX, FA-targeted SJNCs-DOX in the presence and absence of 1 mM FA, and non-targeted SJNCs-DOX using human MDA-MB-231 breast cancer cells.

	DOX	FA-SJNCs-DOX	FA-SJNCs-DOX-FA Competition	SJNCs-DOX
IC50 [μg ml^{-1}]	3.3 ± 0.3	255.3 ± 55.1	781.2 ± 163.0	1030.2 ± 416.1