Supporting information

Bleach Etches Nanosilver: HOCl-Responsive Drug Delivery System to Target Leukemic Cells

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Materials and Methods

Materials: Chemical reagents used in this study are of analytical grade and used as received. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), cetyltrimethylammonium bromide (CTAB) and Pluronic F127 were purchased from Sigma-Aldrich. Camptothecin, 3-aminopropyltriethoxysilane (APTES), tetraethyl orthosilicate (TEOS, 99.98%), hexane, rhodamin 6G, citric acid, sodium citrate, and absolute ethanol were purchased from Aladdin reagent Company.

Synthesis of citrate stabilized Silver Nanoparticles (Ag NPs): Ag NPs are produced using a newly developed synthetic protocol. Firstly, 3 g of F127 was dissolved in 100 mL of millipore water and then known amount of silver nitrate (130 mg, 0.75 mmol) was introduced into the surfactant solution. Temperature of the solution was maintained at 4°C using ice cold water. After 30 min stirring, 3 mL of NaBH₄ solution (40 mg/mL) was rapidly added into light yellow colored silver-F127 solution. Color of solution was rapidly transformed into brown after the addition of strong reducing agent. In order to provide linking sites, minute amount of citric acid and sodium citrate (300 µg + 1 mg) aqueous solutions were also rapidly introduced into as-synthesized silver hydrosol. Note: Compared to previous synthetic strategies, this method produce ultrasmall, water stable and 10-times more concentrated solution. Freshly prepared Ag NPs solution can be used within few hours, if long time stability is needed then dilution of solution can improve the stability for longer period of time. Note: Fresh solution of citrate stabilized Ag NPs should be used for MSNs capping, otherwise with passage of time quality of product will be deteriorated.

Synthesis of Mesoporous Silica Nanosphere: Mesoporous silica nanoparticles (MSNs) were synthesized using previously established protocol. CTAB surfactant (0.5 g, 1.35 mmol) was first dissolved in 240 mL of water. Then, aqueous solution of sodium hydroxide (2.00 M, 1.7 mL) was introduced into CTAB solution and temperature of the mixture was elevated to 78°C. After attaining the desired temperature, silica precursor TEOS (2.5 mL, 11.2 mmol) and amine source APTES (250 µL) were successively added into alkaline surfactant solution under vigorous stirring. The solution was stirred for 2 h to obtain a white precipitated product. The resulting product was filtered, washed with nanopure water, ethanol and then dried at 60°C.

Synthesis of Amine functionalized MSN (MSN-NH₂): In order to maximize the quantity of amine group, as-synthesized MSN were suspended in 50 mL of dry toluene containing 300 µL of
APTES. The solution was stirred under reflux for 12 h. The product was recovered by centrifugation and washed with ethanol trice. Later on, MSN-NH$_2$ nanoparticles were dispersed in 100 mL of ethanol containing 6 mL of HCl (37 %) and reflux the solution for 12 h to remove the surfactant template (CTAB).

**Loading, Capping and Release Experiments:** For loading hydrophobic camptothecin (CPT) and rhodamin 6G cargo molecules in the nanopores of MSNs, 50 mg of MSNs-NH$_2$ were introduced into DMSO solution of CPT (5 mL, 1 mg mL$^{-1}$) and ethanolic solution of rhodamine 6G and stirred the samples for 12 h. After 12 h stirring, both solutions were centrifuged and washed with ethanol and water. In order to cap these CPT and rhodamine 6G loaded MSNs, respective samples were dispersed separately in water and then 1mg EDC and 20 mL of freshly prepared aqueous solutions of citrate-functionalized Ag NPs were added into both MSNs solutions under sonication. Samples were quickly centrifuged and washed with water. To remove extra cargo molecules from uncapped nanopores, wet powders were stirred in water for one day and then centrifuged and dried at room temperature. The loading quantity of IBU (77 mg g$^{-1}$) was calculated by using UV/Visible spectroscopy, whereas the loading amount rhodamin 6G was found to be around 27.5 mg g$^{-1}$. Capping protocol was investigated by studying the release profiles of both Ag@MSNs nanoformulations in HOCl at various concentrations, using a dialysis bag diffusion technique. Briefly, 10 mg of CPT and/or Rhd 6G loaded Ag@MSNs samples were dispersed in 3 mL of water and different concentrations of HOCl solutions. Few minutes later, samples were sealed in a dialysis bag (molecular weight cutoff = 8000) and then dialysis bag was submerged in 20 mL of water and stirred for 3 days. The released cargo molecules were collected at predetermined time intervals and monitored by UV/Vis spectroscopy at 365nm and 525 nm respectively.

**Cell Culture:** Molm-13 cells were purchased from AddexBio (San Diego, CA, USA). The Molm-13 cell line was cultured in RPMI 1640 media with 20% fetal bovine serum (Gibco, invitrogen) and 2 mM L-glutamine, plus 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified atmosphere containing 5% CO$_2$/95% air at 37°C.

**Cell Viability:** In vitro cell viability of AML (Molm-13) cells were measured by using MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazoliumbromide, Sigma-Aldrich) assays. Briefly, the cells were treated with variable concentrations of Ag@ MSNs and Ag@CPT@MSNs nanoformulations for 48 h in the presence and absence of vitamin C. MTT were added to a final
concentration of 1 mM and cells were incubated for 4 hours at 37°C. The cells were lysed overnight using 10% SDS in 10 mM HCL and plates were read at 590 nm using a microplate reader. IC_{50} values were calculated as drug concentrations necessary to inhibit 50% growth compared to vehicle control treated cells. The cell line IC_{50} values are presented as mean values ± standard errors from at least three independent experiments.

Assessment of Apoptosis: Molm-13 cell lines was treated with different concentrations of Ag@MSNs and Ag@CPT@MSNs nanoformulations in the presence and absence of vitamin C and subjected to flow cytometry analysis to determine drug-induced apoptosis using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Kit(Beckman Coulter; Brea, CA). Results were shown as percent of Annexin V+ cells. Experiments were performed 3 independent times in triplicate. Data presented are from one representative experiment.

Western Blot Analysis: Cells were lysed in tris buffer (10 mM, pH 8.0) containing protease inhibitors (Roche Diagnostics). Whole cell lysates were subjected to SDS-polyacrylamide gel electrophoresis, electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Inc., Rockford, IL) and immunoblotted with γ-H2AX(2577), Caspase 3 (9661), Bax and β-actin antibody. Immunoreactive proteins were visualized using the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE), as described by the manufacturer. Western blots were repeated at least 3 times and one representative blot is shown.

Confocal Laser Scanning Microscopy (CLSM): To evaluate the cellular uptake and release of both drugs, Molm-13 cells were cultured in a 12-well chamber slide with one piece of cover glass at the bottom of each chamber in incubation medium (DMEM) for 24 h. CPT and Rhd 6G loaded Ag@MSNs formulations were introduced into the incubation medium at the concentration of 50 µg mL\(^{-1}\) for 5 h in 5% CO\(_2\) at 37°C. After the removal of medium, cells were washed twice with PBS (pH = 7.4) and cover glass was visualized under a laser scanning confocal microscope (Carl Zeiss, LSM 710).

Characterization:
The powder XRD patterns were obtained using Rigaku D/Max 2550 X-ray diffractometer with Cu-Kα radiation (λ = 1.5418 Å). The morphologies and detailed structure of the samples were analyzed via JEOL JSM-6700F field-emission scanning electron microscope (SEM) and FEI Tecnai G2 F20 S-TWIN transmission electron microscope (TEM). (λ=1.5418 Å). Elemental
analysis was carried out on Perkin-Elmer ICP-OES Optima 3300DV. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 410 FTIR spectrometer in the range of 400-4000 cm\(^{-1}\). X-ray photoelectron spectroscopy (XPS) was done using an ESCALAB 250 spectrometer. The nitrogen adsorption and desorption isotherms were measured at liquid N\(_2\) temperature by using a Quantachrom Autosorb-iQ, after degassing the sample for 12 h at 80°C. Surface area was determined with conventional BET method and the adsorption branches of the isotherms were employed for the estimation of the pore parameters using the BJH method.

**Figure S1.** (a) FTIR spectra of MSNs-NH\(_2\), Rhd@MSNs-NH\(_2\) and CPT@MSNs-NH\(_2\). (b) Ag-CA and Ag@MSNs.
Figure S2. Low-angle X-ray diffraction (XRD) patterns of MSNs-NH$_2$ and Ag@CPT@MSNs. The PXRD indicates an obvious reduction in the intensity of the MCM-41 type peaks after drug loading and Ag capping.

Figure S3. Nitrogen adsorption–desorption isotherms for MSNs and Ag@CPT@MSNs samples, revealing the clogging of nanopores after the drug loading and pore capping.
Figure S4. EDX spectrum of Ag@MSNs which suggests the conjugation of Ag NPs onto the surface of MSNs.

Figure S5. (a) XPS survey scan spectrum of Ag@MSNs. (b) Spectrum of silver of Ag@MSNs. (c) Spectrum of silicon of Ag@MSNs. (d) XPS survey scan spectrum of HOCl treated Ag@MSNs. (e) Spectrum of silver of HOCl treated Ag@MSNs. (f) Spectrum of chlorine of HOCl treated Ag@MSNs.
**Figure S6.** Molm-13 cells were treated with various concentrations of Ag@MSNs and Ag@CPT@MSNs in the presence and absence of vitamin C for 24 h. Apoptotic events were determined by annexin V/PI staining and flow cytometry analyses.

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<thead>
<tr>
<th>Materials</th>
<th>Zeta-Potential</th>
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<tbody>
<tr>
<td>MSNs</td>
<td>-12.4 mV</td>
</tr>
<tr>
<td>MSNs-NH2</td>
<td>28.7 mV</td>
</tr>
<tr>
<td>Ag-CA</td>
<td>-10.2 mV</td>
</tr>
<tr>
<td>Ag@MSNs</td>
<td>25.7 mV</td>
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**Table S1.** Zeta Potential of various synthesized nanoparticles to reveal surface modifications.