Supplementary information

Poly(N-isopropylacrylamide) Derived Nanogels Demonstrated Thermosensitive Self-assembly and GSH-triggered Drug Release for Efficient Tumor Therapy

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Table S1. Characteristics of DOX-loaded H-SS-P nanogels. Data are represented as mean ± standard deviation (SD) (n = 3).

<table>
<thead>
<tr>
<th>Drug/polymer (w/w)</th>
<th>Mean diameter (nm)</th>
<th>Zeta potential (mV)</th>
<th>Encapsulation Efficiency (%)</th>
<th>Drug Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>119.4 ± 1.1</td>
<td>-17.1 ± 0.5</td>
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</tr>
<tr>
<td>0.2</td>
<td>130.4 ± 1.9</td>
<td>-23.9 ± 0.6</td>
<td>81.71 ± 0.55</td>
<td>13.62 ± 0.23</td>
</tr>
<tr>
<td>0.4</td>
<td>134.3 ± 3.2</td>
<td>-20.8 ± 0.3</td>
<td>75.53 ± 0.55</td>
<td>21.58 ± 0.23</td>
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<tr>
<td>0.6</td>
<td>141.3 ± 2.7</td>
<td>-15.2 ± 0.7</td>
<td>63.17 ± 1.07</td>
<td>23.69 ± 0.58</td>
</tr>
</tbody>
</table>
Fig. S1. FT-IR spectra of HA, HA-CYS and HA-SS-PNIPAAm in the wavenumber region of 400-4000 cm\(^{-1}\).
Fig. S2. $^1$H NMR of a simple mixture of HA, CYS, and PNIPAAm. All compounds were dissolved in D$_2$O.
Fig. S3. $^{13}$C NMR of HA, CYS, HA-CYS and HA-SS-PNIPAAm. All polymers were dissolved in D$_2$O.
**Fig. S4.** a) Size distribution of H-SS-P nanogels at 37 °C determined by DLS. b) Size distribution of H-SS-P nanogels in response to GSH at 37 °C determined by DLS. (MW$_{\text{PNIPAAm}}$ = 2000 Da, DS 40, C = 0.2 mg/mL)
**Fig. S5.** Critical micelle concentration (CMC) determination of H-SS-P nanogels. a) MW$_{\text{PNIPAAm}}$ = 2000, DS20. b) MW$_{\text{PNIPAAm}}$ = 2000, DS30. c) MW$_{\text{PNIPAAm}}$ = 2000, DS40.
Fig. S6. The results of the lower critical solution temperature (LCST). a) Temperature-transmittance curves of H-SS-P (MW$_{\text{PNIPAAm}}$ = 2000, DS30). b) Temperature-transmittance curves of H-SS-P (MW$_{\text{PNIPAAm}}$ = 2000, DS40). c) Changes in the LCST with the concentration (MW$_{\text{PNIPAAm}}$ = 2000, DS40). d) Changes in the LCST with the number average molecular weight of PNIPAAm-COOH (MW$_{\text{PNIPAAm}}$ = 2000, DS40, C = 0.5 mg/mL)
Fig. S7. *In vitro* release behavior of DOX from H-SS-P nanogels triggered by GSH. Data are presented as means ± SD (n = 3).
Fig. S8. *In vitro* cytotoxicity of a) blank nanogels against A549 cells and LO2 cells. *In vitro* cytotoxicity of free DOX and H-SS-P@DOX nanogels against b) A549 cells and c) LO2 cells. * and ** represent $p < 0.05$ and $p < 0.01$ vs H-SS-P@DOX group, respectively. Data are presented as means ± SD (n = 3).
Fig. S9. Quantification of DOX signal for the *in vivo* fluorescence microscopy images of the major organs (heart, liver, spleen, lung and kidney) and tumors in 4T1 tumor-bearing mice after intravenous injection of DOX (control), H-SS-P@DOX with free HA pretreatment and H-SS-P@DOX.
**Fig. S10.** Histological analysis of a) major organs (heart, liver, spleen, lung and kidney) and b) tumors from 4T1 tumor-bearing mice on the 11th day after they are treated with saline, H-SS-P, free DOX, H-SS-P@DOX and H-SS-P@DOX with BSO treatment.