Supporting Information

Matrix metalloproteinase triggered size-shrinkable gelatin-gold fabricated nanoparticles for tumor microenvironment sensitive penetration and diagnosis of glioma

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1. Methods

1.1 Synthesis

The detail synthetic route of SH-R-Hyz-Cy5.5 was showed in Figure S1, the synthetic procedures was elucidated as followed.

a): S-3-oxopropyl ethanethioate: 6.5 g Potassium thioacetate (57 mmol) in component solvent containing pyridine and glacial acetic acid (6.5 : 3.5), dichloromethane was added until the potassium thioacetate dissolve at room temperature. Then 2.54 mL acrolein (38 mmol ) was added to the reaction mixture with constant stirring at the same temperature for 12 h. After the completion of the reaction (monitored by TLC), the mixture was evaporated in vacuo. Residue was purified by column chromatography on silica gel (ethyl acetate : hexanes) to afford the corresponding preliminary outcome.

b): 112.7 mg 3-(acetylyhio)propanoic aldehyde (0.854 mmol) in 5 mL anhydrous methanol was added with 46.4 µL hydrazine hydrate (0.939 mmol, 1.1 eq), a catalytic amount of glacial acetic acid was added to accelerate the reaction, the reaction mixture was stirred at room temperature for 3 days with nitrogen protection. After the completion of the reaction (monitored by TLC), the mixture and overdose hydrazine hydrate were evaporated in the vacuo. The following procedures were generally same as the described above.

c): The compound 2 (1.5 mg, 0.0103 mmol, 1.5 eq) and Cy5.5 NHS ester (4.7 mg, 0.0067 mmol, 1 eq) was dissolved in 5 mL of dichloromethane and stirred in the dark at room temperature for 8 h. After reaction (monitored by TLC), the mixture was evaporated to remove the dichloromethane and afford the compound 3.

e): Deacetylation reaction: the compound 3 (2.5 mg, 3.4 µmol) was dissolved in 5 mL of
anhydrous methanol was added 1.9 mg potassium carbonate (32.7 µmol, 4 eq), the reaction mixture was stirred in the dark at room temperature for 4-30 h. After the completion of the reaction (monitored by TLC), the solvent was evaporated in the vacuo to give the final compound 4. However, the residue did not carry a further purification owing to the activity of thiol and the instability of hydrazone bond.

1.2 Preparation of AuNPs-DC-PEG and AuNPs-DC-RRGD

The SH-R-Hyz-DOX (25 µg) and SH-R-Hyz-Cy5.5 (50 µg) was incubated with 5 mL of AuNPs solution at 37 °C for 8 h to anchored DOX and Cy5.5 onto AuNPs. Following incubation with 50 µg of SH-PEG-COOH, the solution was incubated at 150 rpm and under 37 °C for 8 h to obtain AuNPs-DC-PEG.

The preparation procedure of AuNPs-DC-RRGD was based on AuNPs-DC-PEG. After completion of AuNPs-DC, the solution was added with 25 µg of SH-PEG-COOH. The solution was incubated at 150 rpm and under 37 °C for 8 h to obtain AuNPs-DC-PEG, followed by 25 µg of SH-PEG-RRGD. Finally, the AuNPs-DC-RRGD was obtained.
2. Results

Figure S1. Syntheses of thiol-alkyl-hydrazone bond-Cy5.5 (SH-R-Hyz-Cy5.5). a): Pyridine, glacial acetic acid, Dichloromethane. b): Hydrazine hydrate, methanol, glacial acetic acid. c): Cy5.5 NHS ester, dichloromethane. d): Potassium carbonate, anhydrous methanol.
Figure S2. NMR $^1$H spectra of SH-R-Hyz-Cy5.5 in deuterated chloroform.

Table S1. Physicochemical characterization of different formulations (data represent mean data ± SD, n=3)

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
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<tbody>
<tr>
<td>AuNPs</td>
<td>19.25</td>
<td>0.225</td>
<td>-20.34±0.95</td>
</tr>
<tr>
<td>GNPs</td>
<td>147.5</td>
<td>0.158</td>
<td>-10.21±0.52</td>
</tr>
<tr>
<td>AuNPs-DC-PEG</td>
<td>30.38</td>
<td>0.263</td>
<td>-14.58±1.21</td>
</tr>
<tr>
<td>AuNPs-DC-RRGD</td>
<td>33.60</td>
<td>0.281</td>
<td>-12.77±0.89</td>
</tr>
<tr>
<td>G-AuNPs-DC-PEG</td>
<td>179.3</td>
<td>0.232</td>
<td>-5.62±0.47</td>
</tr>
<tr>
<td>G-AuNPs-DC-RRGD</td>
<td>188.2</td>
<td>0.251</td>
<td>-4.32±0.61</td>
</tr>
</tbody>
</table>
Figure S3. DLS data of different formulations. A: AuNPs. B: GNPs. C: AuNPs-DC-RRGD. D: G-AuNPs-DC-RRGD. E: G-AuNPs-DC-RRGD treated with MMP-2 for 24 h.

Figure S4. In vitro release of DOX and Cy5.5 after incubated in different pH condition at 37 °C for 10 min.
Figure S5. Cellular uptake and subcellular localization. Confocal microscope images of cellular uptake after 2 h incubation with G-AuNPs-DC-RRGD, G-AuNPs-DC-PEG, AuNPs-DC-RRGD, AuNPs-DC-PEG and Free DC and bar represented 20 µm.
Figure S6. 3D reconstruction imaging of C6 xenograft bearing mice at 24 h after treatment with G-AuNPs-DC-RRGD, AuNPs-DC-RRGD, G-AuNPs-DC-PEG, AuNPs-DC-PEG and Free DC.

Figure S7. Ex vivo imaging of C6 xenograft-bearing mice. A: Ex vivo imaging of tissues at 24 h after intravenous injection with G-AuNPs-DC-RRGD, AuNPs-DC-RRGD, G-AuNPs-DC-PEG, AuNPs-DC-PEG and Free DC. B: Corresponding semi-quantitative fluorescent intensity of tissues.