Supporting information

Amphiphilic copolymer and TPGS mixed magnetic hybrid micelles for stepwise targeted co-delivery of DOX/TPP-DOX and image-guided chemotherapy with enhanced antitumor activity in liver cancer

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Characterization of TPP-DOX and PPG2L

Fig.S1 shows the $^1$H NMR spectra of free DOX and TPP−DOX. In comparison, after TPP was conjugated with DOX, it can be seen in Fig. S1b that besides the dominant DOX signals, the occurrence of the signals ranging from 7.60 to 7.87 ppm belong to protons of phenyl rings of TPP. The $^1$H NMR spectrum of TPP-DOX exhibited the following absorptions (ppm): 8.02 (m, 1H) H3, 7.68-7.80 (m, 15H) TPP aromatic protons, 7.32 (d, 1H) H2, 5.5 (s, 1H) amide proton, 5.3(s, 1H) 3.14(d, 1H), 2.15(m, 1H), 1.78(m, 2H)1.25(d, 3 H), 3.4(m, 2 H), 2.5 (m, 2H), 2.0 (m, 2H)

The structural characterization of TPP−DOX was confirmed by FT-IR as show in Fig.S2. The spectrum of TPP−DOX shows characteristic absorption peaks at 3428 cm$^{-1}$ (stretching of OH and NH of the amide groups); 1722 cm$^{-1}$ (stretching of C=O in the ketone groups); 1637 cm$^{-1}$ (stretching of C=O in the carbonyl of amide group); and 1577, 1438, and 1386 cm$^{-1}$ (stretching of C=C of aromatic rings). Comparing the IR spectra of DOX and TPP−DOX revealed a new peak at 1637 cm$^{-1}$ (C=O stretching of amide) in the TPP−DOX spectrum. This absorption band confirmed the formation of amide bond (−CO−NH−) between DOX and TPP.

Fig.S3 shows the $^1$H NMR spectra of LA, PPG2 and PPG2L. Typical signals of PPG2 remain and in the meantime new signals appear. The signals at 1.28, 1.52, 2.6, and 3.98 ppm represent methylene protons of −(CH$_2$)$_3$−, −(CH$_2$)$_2$−, −OCCH$_2$−, and −CH$_2$OOC− in the PCL blocks, along with PEG block protons at 3.51 ppm and the peaks at 2.5-2.8 ppm are assigned to the methane protons of −CH$_2$NH−, which belong to G2.0 dendrimer. And after the conjugation of LA to PPG2 copolymer, we can see that the chemical shift of 3.24-3.37, 4.3 and 4.56 ppm was assigned to LA, which indicated the formation of PPG2L polymer. And on the basis of the integrated values of the protons in LA at 4.3 ppm of pyran ring and the protons of PCL at 1.28 ppm, each molecule of copolymer was found to have approximately three LA moieties bonded to it.

Subsequently, the structure of the polymer was characterized by FT-IR, as shown in Fig. S4. Compared with the spectrum of PP and G2.0, we could find the
enhancement of stretching of I and II bands belong to G2.0 PAMAM (-CO-NH): 1652 cm\(^{-1}\) and 1558 cm\(^{-1}\) in PPG2 polymer. And C-H stretching at 2945 cm\(^{-1}\) and 2866 cm\(^{-1}\) can be assigned to ester bonds of PCL and –OCH\(_2\)-CH\(_2\) of PEG, which overlap with the peak of G2.0 to form the new peak at 2887 cm\(^{-1}\) in PPG2 polymer, proved the successful synthesis of PPG2. By comparison with the infrared spectra of LA, it was found that the absorption peaks at 3446 cm\(^{-1}\) (stretching of OH and NH of the amide groups) were significantly enhanced in the PPG2L, which confirmed the successful preparation of PPG2L.

![Fig. S1. 1H NMR spectra of (a) DOX in DMSO-D6, (b) TPP-DOX in CDCl\(_3\).](image)
Fig. S2. FT-IR spectra of (a) DOX, and (b) TPP-DOX.

Fig. S3. $^1$H NMR spectra of (a) LA, (b) PPG2, and (c) PPG2L. All samples were dissolved in DMSO-D6.
Fig. S4 FT-IR spectra of (a) PP, (b) G2.0, (c) PPG2, (d) LA, (e) PPG2L.

Table S1. The nanoparticle diameters were determined by DLS dynamic light scattering (DLS) for OA@IONPs in cyclohexane and for others in water.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DLS (nm)</th>
<th>PDI</th>
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<tbody>
<tr>
<td>OA@IONPs</td>
<td>11.03 ± 2.546</td>
<td>0.118</td>
</tr>
<tr>
<td>TPGS@IONPs</td>
<td>15.06 ± 4.05</td>
<td>0.457</td>
</tr>
<tr>
<td>PPG2L@IONPs</td>
<td>37.12 ± 7.9</td>
<td>0.303</td>
</tr>
<tr>
<td>TPGS/PPG2L@IONPs</td>
<td>26.2 ± 3.5 nm</td>
<td>0.259</td>
</tr>
<tr>
<td>TPGS/PPG2L@IONPs@DOX/TPP-DOX</td>
<td>29.01+7.2</td>
<td>0.437</td>
</tr>
</tbody>
</table>
Fig. S5. Atomic force microscope (AFM) image of TPGS/PPG2L@IONPs@DOX/TPP-DOX hybrid micelles.
Fig. S6. High performance liquid chromatogram (HPLC) of (a) free DOX and (b) TPP-DOX.

Fig. S7 The drug release profiles of (a) DOX- and TPP-DOX-loaded micelles (b) DOX/TPP-DOX drug-loaded hybrid micelles, over time in buffers at two pH values indicated (pH 7.4 and pH5.0). Significantly accelerated drug release was detected at a slightly acidic condition. Error bars indicated the standard deviations of triplicated samples.

Fig. S8 Thermal gravimetric analysis (TGA) data was obtained for TPGS/PPG2L@IONPs.
Fig. S9 The cytotoxicity of drug-loaded hybrid micelles and LA-hybrid micelles on A549 cells. Data are presented as mean ± SD (n = 3).