Active-targeting and acid-sensitive pluronic prodrug micelles for efficiently overcoming MDR in breast cancer

Xu Cheng, Jiaxi Xu, Yan Zheng, Qin Fang, Xiaodong Lv, Xin Wang, Rupei Tang*

Engineering Research Center for Biomedical Materials, Anhui Key Laboratory of Modern Biomanufacturing, School of Life Sciences, Anhui University, 111 Jiulong Road, Hefei, Anhui Province, 230601, P. R. China

* Corresponding author.

Email: tangrp99@iccas.ac.cn (R. Tang)
Fig. S1. Synthetic route of pluronic copolymer prodrug (P123-CAD); Reaction conditions: (i) anhydrous dichloromethane; (ii) anhydrous dimethyl sulfoxide; (iii) 0.1 M PB solution, pH 8.0, 0℃; (4) EDC/NHS, TEA, 25℃.

Fig. S2. ¹H NMR spectra of P123, P123-CDI and P123-NH₂, and CDCl₃ was used as the solvent.
Fig. S3. $^1$H NMR spectra of DOX and CAD, and DMSO$_{d6}$ was used as the solvent.

Fig. S4. $^1$H NMR spectra of pluronic copolymer prodrugs (P123-CAD), and DMSO$_{d6}$ was used as the solvent.

Fig. S5. XRD pattern of free DOX, P123, P123-NH$_2$ and P123-CAD.
Fig. S6. Synthetic route of active-targeting pluronic copolymer (F127-PBA); Reaction conditions: (i) anhydrous dichloromethane; (ii) dimethyl sulfoxide, 0.2% DMAP, at room temperature for 48 h.

Fig. S7. $^1$H NMR spectra of F127, F127-CDI and F127-PBA, and CDCl$_3$ was used as the solvent.
Fig. S8. FT-IR spectra of (a) P123, P123-NH$_2$ and P123-CAD; (b) DOX and CAD; (c) F127 and F127-PBA.

Fig. S9. The CMC value of FP-CAD and FBP-CAD.
Fig. S10. The degradation mechanism of CAD in acid conditions.

Fig. S11. Morphology change of prodrugs particles in pH 5.0 (a) and pH 7.4 (b), scale bar = 200 nm.

Fig. S12. Sialic acid (SA) content in different types of cells.
Fig. S13. MTT method evaluated the cytotoxicity of four DOX formulations in MCF-7 cells (a) and MCF-7/ADR cells (b); Anti-proliferation ability of pluronic copolymer in MCF-7 cells (c) and MCF-7/ADR cells (d); Data are represented as mean ± SD (n = 6).

Fig. S14. In vitro cytotoxicity of different DOX formulations in 3T3 cells.
Fig. S15. Intracellular ROS level assessment by DCFH-DA probe in MCF-7 (a) and MCF-7/ADR cells (b), scale bar = 10 μm; Semi-quantitative analysis of ROS fluorescence intensity (c).

Fig. S16. Cytochrome C (brown colour) release from mitochondrial, scale bar = 10 μm.
Fig. S17. Cells apoptosis after treatment with free DOX, P123-CAD, FP-CAD and FBP-CAD in MCF-7 (a) and MCF-7/ADR (b) cells.
Fig. S18. MCF-7/ADR-bearing mice images after treatment with free DOX, FP-CAD and FBP-CAD for 7 days, scale bar = 2 cm.

Fig. S19. H&E staining of heart, liver, spleen, lung, kidney, scale bar = 5 μm.
Fig. S20. DOX fluorescence staining in heart tissue after treatment for 24 h (a); Fluorescence intensity statistics (b).

Fig. S21. Mice body change during treatment for 7 days.
Table S1. IC\textsubscript{50} value, resistance and reversal index of different formulations against MCF-7 and MCF-7/ADR cells.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>IC\textsubscript{50} (µg/mL)</th>
<th>Resistance index</th>
<th>Reversal index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7</td>
<td>MCF-7/ADR</td>
<td></td>
</tr>
<tr>
<td>Free DOX</td>
<td>8.34</td>
<td>203.16</td>
<td>24.36</td>
</tr>
<tr>
<td>FP-DOX</td>
<td>6.12</td>
<td>8.17</td>
<td>1.33</td>
</tr>
<tr>
<td>FBP-DOX</td>
<td>4.5</td>
<td>5.29</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Resistance index: the ratio of IC\textsubscript{50} (MCF-7/ADR) against IC\textsubscript{50} (MCF-7).

Reversal index: the ratio of IC\textsubscript{50} (free drug) to IC\textsubscript{50} (drug-loaded micelles) against MCF-7/ADR cells.