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Doxorubicin Conjugated, Crosslinked, PEGylated Particles

Prepared via One-Pot Thiol-Ene Modification of a Homopolymer Scaffold: Synthesis and In vitro Evaluation

Lingjiun Wong,‡† Maria Kavallaris,§ Volga Bulmus*†^

‡Centre for Advanced Macromolecular Design (CAMD), School of Chemical Engineering, The University of New South Wales, Sydney 2052, NSW, Australia.
†School of Biotechnology and Biomolecular Sciences (BABS), The University of New South Wales, Sydney 2052, NSW, Australia.
§Children’s Cancer Institute Australia for Medical Research, Sydney 2031, NSW, Australia.

*CORRESPONDING AUTHOR. E-mail: volgabulmus@iyte.edu.tr ; Tel: +90-232-750 6660; Fax: +90-232-750 6645 ^Current address (VB): Izmir Institute of Technology, Department of Chemical Engineering, Gulbahce, Urla, Izmir 35430 Turkey.
Figure S-1: (a) $^1$H NMR spectrum and (b) GPC chromatogram of the RAFT synthesized poly(pyridyldisulfide ethylmethacrylate) (PPDSM) used in this study. The mobile phase for GPC: dimethylacetamide (DMAC). The RAFT polymerization was performed at 70°C, at a [PDSM]: [RAFT]: [Initiator] feed ratio of 0.4 M: 8.0 mM: 1.6 mM at a monomer conversion of 60mol% (by NMR). PPDSM has a number average molecular weight ($M_n$) of 8,900 g/mol and PDI of 1.18 (by GPC). Chemical shifts ($\delta = 8.4$ (1H), 7.6 (2H) and 7.1 (1H) ppm) and the methyl/methylene protons of the polymer backbone ($\delta = 4.2$ (2H), 3.0 (2H), 2.0-1.7 (2H), 1.1-0.7 (3H) ppm).
Scheme S-1: Synthesis of maleimide-Doxorubicin (mal-Dox) using EMCH crosslinker as reported in previous publications. (1-2)
Figure S-2: (a) $^1$H-NMR spectrum of maleimide-modified Dox (mal-Dox) in DMSO-$d_6$. (b) ESI-MS spectrum of mal-Dox with the exact molecular mass of 773.3 Da (the sample was prepared in HPLC-grade methanol at a concentration of 1 mg/ml).
Figure S-3: $^1$H-NMR spectrum (in CDCl$_3$) of mal-PEG (M$_n$ = 5000 g/mol) (repeating units of 110) used in the study. The purity of maleimide modified PEG is 41 mol%.
Figure S-4: (a) Representative HPLC traces of standard Dox solutions at increasing concentrations (5, 15, 30, 35, 40, 60, 70 and 80 µg/mL) in HPLC-grade acetonitrile (in triplicate measurements). (b) The calibration curve built by HPLC using standard Dox solutions: UV-absorption at 490 nm versus concentration in µg/ml. The HPLC analysis was performed using 150 mm x 4.60 mm Phenomenex (R) column packed with 5 µm of IB-SIL C-8 beads. The column was eluted with the gradient mixture of HPLC-grade acetonitrile and milli-Q water at a flow rate of 1 mL/min.
**Figure S-5:** HPLC traces of (a) filtered crude reaction mixture, (b) pure mal-Dox solution, (c) pure PPDSM solution, and (d) pure pyridine-2-thione solution. HPLC equipped with a UV-Visible photodiode array detector set at a wavelength of 490 nm was used for measurement. The HPLC analysis was performed using 150 mm x 4.60 mm Phenomenex (R) column packed with 5 µm of IB-SIL C-8 beads.
Figure S-6: $^1$H NMR spectrum of purified, Dox-conjugated and PEGylated PPDSM in DMSO-$d_6$. 
Figure S-7: Representative HPLC traces of Dox released from the PEGylated particles after incubation in (a) PBS pH = 7.4 and (b) Citrate-phosphate buffer saline pH = 5.0 for 2, 4, 6, 8, 12, 24, 48, 72 hours. The last chromatograms in both a and b were recorded after the complete release of Dox. The complete release was obtained by incubating the particles, that were already incubated for 72 hours in relevant buffer solution, in an acidic solution (pH = 3.0) for further 24 hours. All release experiments and following HPLC measurements were performed in duplicate.
References:
