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Supplementary Information

Acid-Breakable TPGS-Functionalized and Diallyl Disulfide-Crosslinked Nanogels for Enhanced Inhibition on MCF-7/ADR Solid Cancer

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1. MS spectrum of T-OE

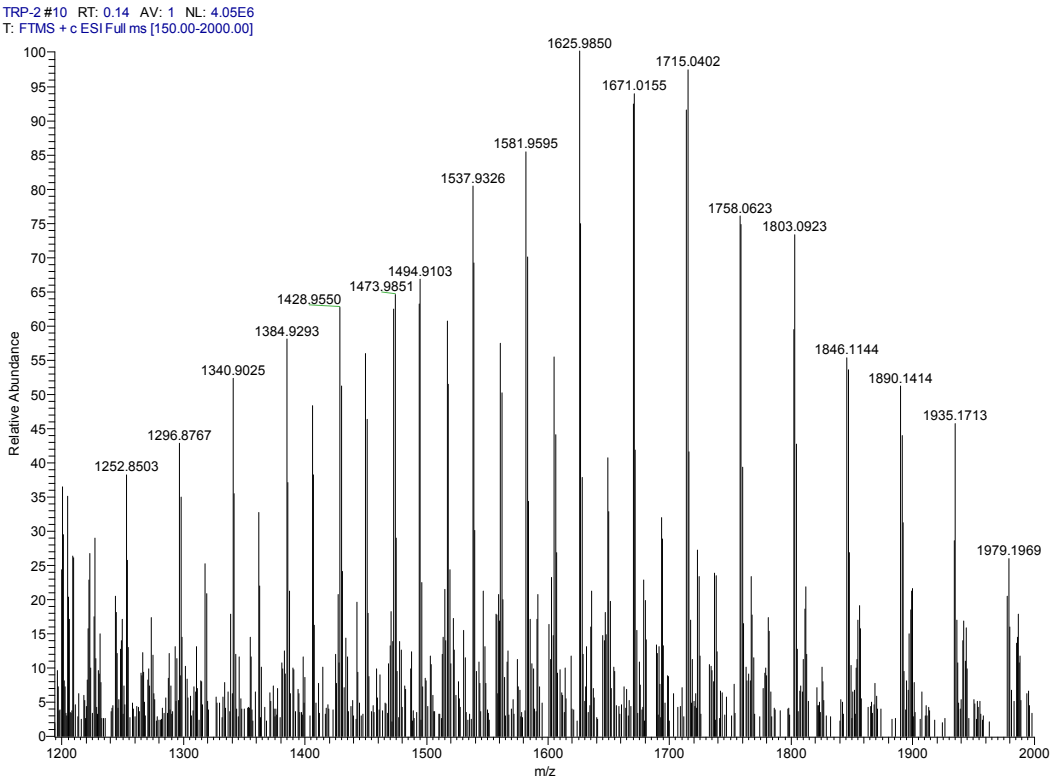

Fig. S1 ESI-MS spectrum of T-OE: $C_{37}H_{62}NO_8{\cdot}(C_2H_4O)_n$, $(C_2H_4O)_n$=1000, 1647.8; found m/z, 1671.0 (M + Na+).

Since tocopheryl polyethylene glycol succinate (TPGS) used in this experiment is composed of two parts, vitamin E (Fw = 530.8) and polyethylene glycol (PEG, average Fw = 1000), the TPGS conjugated with ortho ester (T-OE) is a narrowly distributed oligomer. And the theoretical molecular weight of T-OE is 1647.8 by calculating the molecular weight change during this reaction. Form Fig. S1, the ESI-MS spectrum of T-OE was determined by equal addition of Na^+.[1-2] It can be seen that the molecular weight of this substance is normally distributed and the average value is 1671.0, which is consistent with the theoretical results (1647.8 + 24.0 = 1671.8).

2. Investigation on the prepared method of NG

In order to prepare a nanogel with suitable particle size, we have carried out a series of research on the preparation method. In simple term, nanogels with various particle size and distribution were prepared by adjusting the feed ratio of reactive materials, such as $K_2S_2O_8$ and diallyl disulfide (DADS). All results were displayed in table. S1, when the ratio of MA-CMCS, DADS, and $K_2S_2O_8$ is 100:60:100 mg/mg/mg, the nanogels with optimal diameter (144.6 nm) and PDI (0.241) can be obtained, it was named as NG.

Table. S1 Diameter and distribution (PDI) of prepared nanogels

Named	MA-CMCS (mg)	DADS (mg)	$K_2S_2O_8$ (mg)	Size (nm)	PDI
NG1	100	80	200	643.9	0.623
NG2	100	60	200	398.2	0.403
NG3	100	40	200	214.6	0.318
NG4	100	80	100	558.3	0.714
NG5 (NG)	**100**	**60**	**100**	**144.6**	**0.241**
NG6	100	40	100	301.4	0.216

3. Investigation on the prepared method of TNG

For further fabrication of T-OE grafted nanogel (TNG), we also study its feed ratio in the same way. All variables and results were shown in Table. 2. Besides, the degree of substitution (DS) of T-OE was measured using ninhydrin reaction.[3-4] In brief, TNG were mixed with 1.0 mL of ninhydrin solution (1%, glycerol/H2O=1:2 v/v) and 1.0 mL of acetic acid/sodium acetate buffer (pH 6.0). The mixture was incubated at 100 °C for 20 min and then diluted with 6.0 mL of ethanol solution (60%). Glycine was used as the standard amino acid and this sample was detected by a UV spectrum at 570 nm. In addition, DS of T-OE was calculated using the formulas: $DS_{T\text{-}OE} = (OD_{NG} - OD_{TNG}) / OD_{NG} \times 100\%$, where OD_{NG} represented the absorbance of NG group; OD_{TNG} represented the absorbance of TNG group.

Table S2 Diameter index and degree of substitution (DS) of prepared nanogels

Named	NG (mg)	T-OE (mg)	DS (%)	Size (nm)	PDI
TNG1	100	150	21.1	213.9	0.213
TNG2 (TNG)	**100**	**100**	**13.0**	**171.2**	**0.197**
TNG3	100	50	9.8	161.4	0.416

When the ratio of NG and T-OE is 100:100 mg/mg, TNG with optimal diameter (171.2 nm) and PDI (0.197) can be obtained, it was named as TNG. And the DS of TNGs was further examined. For TNG, the amino group content of free or T-OE modified nanogel was 1.46 μM/mg or 1.27 μM/mg, respectively. Thus, the degree of substitution of T-OE was 13.0 %.

3. Verification of T-OE detachment

In order to confirm the abscission of grafted T-OE, TVG was suspension in PBS (pH 5.0), then, the solution was put into dialysis devices and dialyzed against corresponding buffers for 12 hours. The dialysate was collected and freeze-dried to obtain degraded product. Finally, its chemical composition was proved by ^{1}H NMR and these spectra were exhibited in Fig. S2.

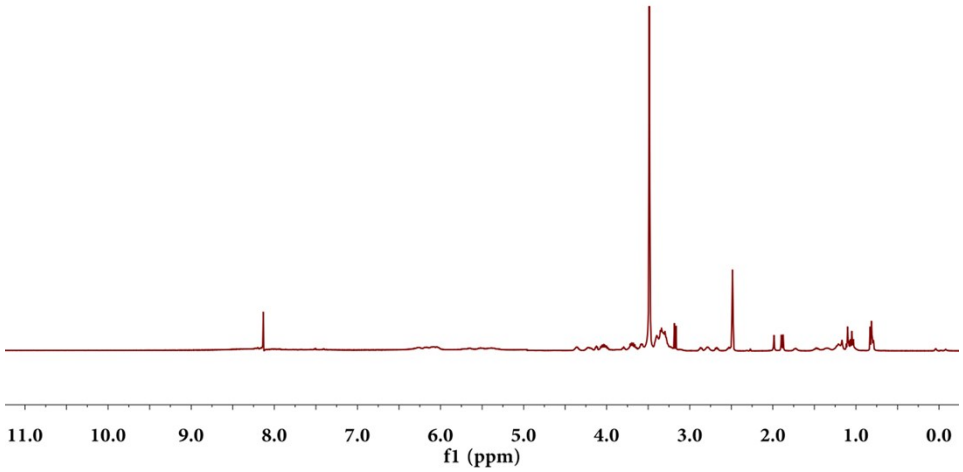

Fig. S2 ^{1}H NMR spectra of degraded production of TNG using deuterated dimethyl sulfoxide (DMSO-d6) as a solvent.

Form Fig. S2, after 12 hours of incubation with PBS (pH 5.0), the special peaks of TPGS were obviously observed in the ^{1}H NMR spectrum of degradation of TNG, and the peak at δ 8.12 ppm belonging to the signal of ortho ester degradation peak.[5] These phenomena indicate that the T-OE functional nanogels can trigger the breakage and release of TPGS under weak acidic conditions.

5. DOX efflux quantification

All treated MCF-7/ADR cells (in the section 2.8.1) was further sami-quantified the cell efflux level. Briefly, after fixing with 4% paraformaldehyde for 10 min and staining the nucleus for another 10 min, the of red fluorescence (DOX) intensity of each sample was analyzed by Image J, and all results was exhibited in Fig. S3.

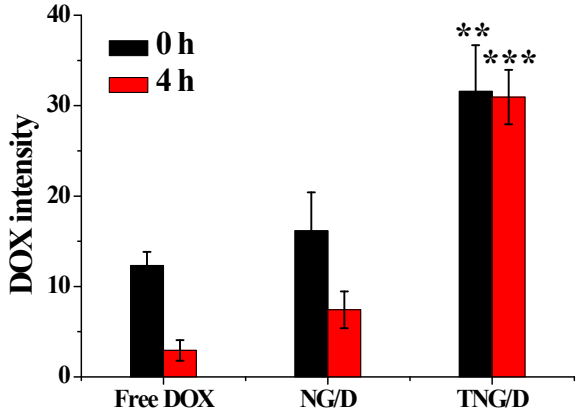

Fig. S3 Intracellular red fluorescence intensity analysis after incubation with fresh medium for 0 hours or 4 hours; ** Represents $p < 0.01$, *** Represents $p < 0.05$, as compared to free DOX group at same time point.

Form Fig. S3, after incubation with various DOX formulation, MCF-7/ADR cells shown a powerful drug signal in TNG/D group, and this intensity was 31.58, which can be attributed to the excellent enrichment and retention. Then, cells in each group were further incubated with fresh medium to observe drug efflux. There was strong cell efflux in groups without TPGS, for example, the fluorescence intensity was change from 12.31 and 16.16 to 2.93 (free DOX) and 7.43(NG/D), respectively. In contrast, the TNG group still showed high fluorescence intensity after continuing to culture for 4 h. In general, these results indicate that TNG/D can effectively increase the retention time of DOX via effectively inhibiting cell efflux.

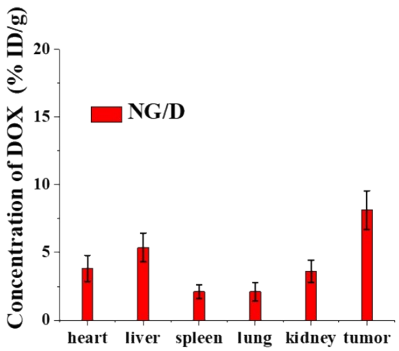

Fig. S4 *In vivo* drug distribution in major organs after 12 h of treatment with NG/D.

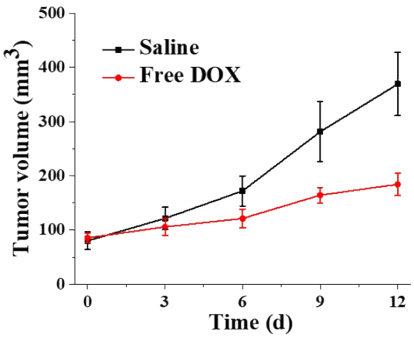

Fig. S5 The volume change of MDR-7/ADR tumor treated with saline and free DOX during 12 days.

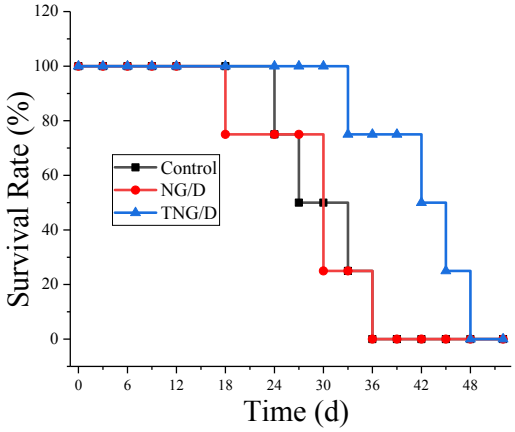

Fig. S6 The survival rate curve after treatment with various DOX formulations.

Reference

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