Supporting Information

Molecular Nanofibers of Paclitaxel form Supramolecular hydrogel for

preventing Tumor growth in vivo

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Experimental Section

Materials and Methods

Fmoc-amino acids were obtained from GL Biochem (Shanghai). Taxol was purchased from Baoman Biotechnology (Shanghai). Succinic acid was purchased from Sigma. All other staring materials were obtained from Alfa. Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Gibco Corporation. CCK-8 kit was purchased from Beyuntian (Shanghai, China). Balb/c mice were purchased from HuaFuKang Biological technology Company (Beijing, China). All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in College of Life Sciences, Sichuan University.

The synthesized compounds were investigated by [1] H NMR (Bruker ARX-400) using DMSO-d6 as the solvent and ES-MS Spectrometric analyses were performed at the LCQ-Advantage System. HPLC was conducted by a HPLC system (Germany) using a C18 column with MeOH (0.1%TFA) and water (0.1%TFA) as the eluents. TEM samples were prepared as following: a copper grid coated with a thin layer of carbon was dipped into the hydrogel. And then, 20-30 μL of doubly distilled H_2O was used to wash the copper grid three times. A 5 μL solution of Uranyl acetate was used to stain the sample for 5 seconds, the staining solution was removed by a filter paper, and the copper grid was kept in a desiccator overnight. The dry sample was measured at the Tecnai G2 F20 system, operating at 100 kV.

Synthesis peptide RGD and RGE

The peptide derivative was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin, the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group. The resulting solid peptide was dissolved in DMSO for HPLC separation.

Prepare Taxol-SA-COOH

Briefly, 0.59 mmol of taxol was dissolved in pyridine and 7.6 mmol of succinic anhydride was added in. The reaction was kept at room temperature for 2-3 h and then the mixture was evaporated under reduced pressure. Subsequently, the mixture was treated with 20 mL of water, stirred for 20 min, and then filtered. The precipitate was dissolved in acetone, followed by addition of water. The tiny crystals were then collected.

Synthesis Taxol-SA-RGD and Taxol-SA-RGE

100 mg of Taxol–SA–COOH was dissolved in 15 mL of dichloromethane, followed by addition of 1.1 equiv.of N-hydroxysuccinimide (NHS), 1.2 equiv. of N,N-dicyclohexylcarbodiimide, and catalytic amount of 4-dimethylamiopridine. After being stirred 3 h at room temperature, the solution was filtered to remove precipitation, dried and evaporated under reduced pressure to yield a white power. The white powder was dissolved in 10 mL N,N-dimethylformamide. Then, 1.5 equiv. of RGD peptide or RGE peptide in 50 µL of Nethyldiisopropylamine was added. The reaction was stirred for overnight and the title products were purified by reverse phase HPLC.

Preparation of hydrogel

10 mg of a precursor of the gelator was dissolved in 2 mL of PBS buffer solution (pH= 7.4). And 2.0 equiv. of Na_2CO_3 (10.6 mg/ml) was then added to the above solution to adjust pH to 7.4. A gel would form after the solution was kept at room temperature 37 ℃ for 1 h.

Determination of release profile of Taxol from gels

1 mL of gels (0.5 wt %) was treated with 1 mL of fresh PBS buffer solutions (pH = 7.4) at 37 ℃. At different time points, 1 mL of the upper buffer solution was taken out to run HPLC and 1 mL of fresh PBS buffer solution was added back each time. The experiment was conducted in 3 parallel experiments.
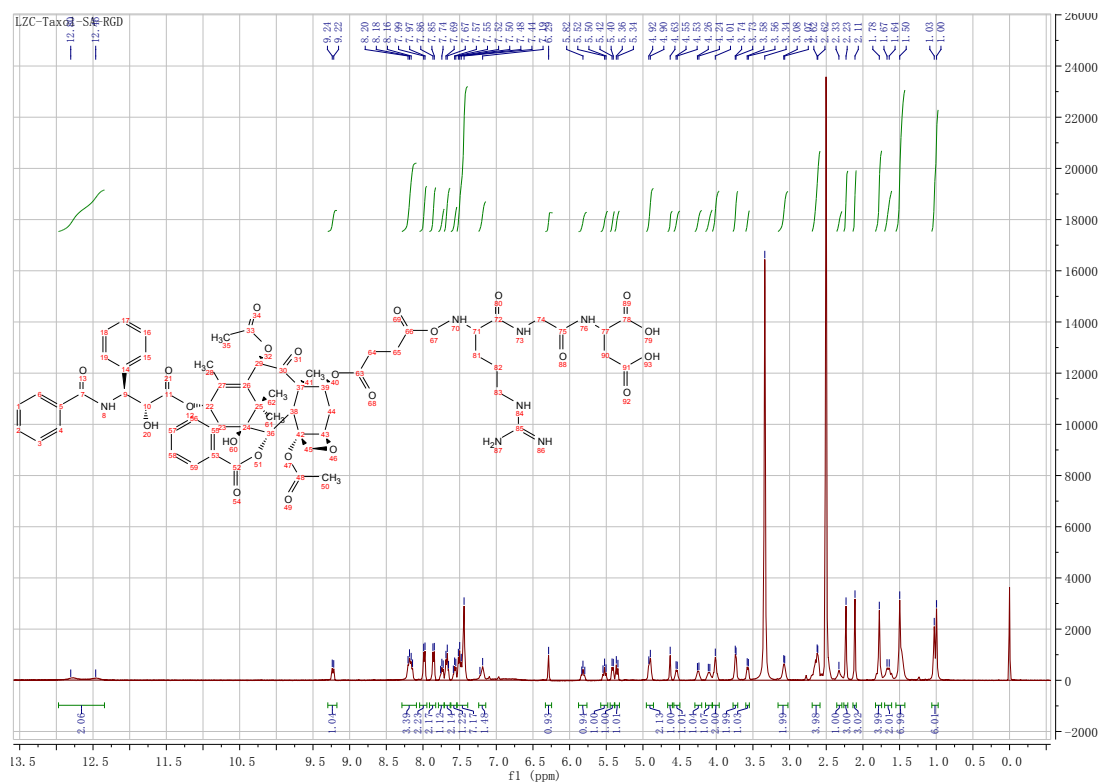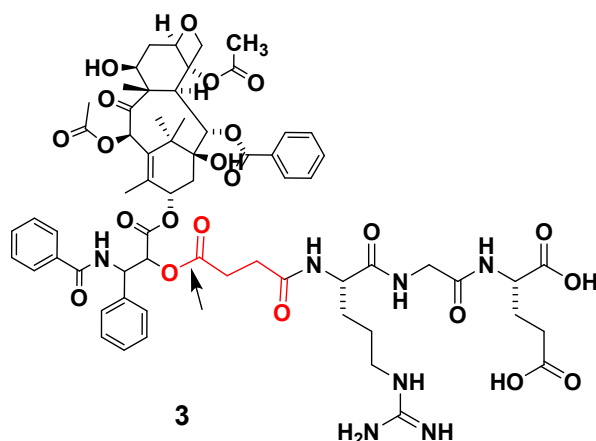
Scheme S1. Chemical structures of Taxol-SA-RGE (3)

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Figure S1. ¹H NMR spectrum of Taxol-SA-RGD in DMSO-d_6.

Taxol-SA-RGD ¹H NMR (400 MHz, DMSO) δ 9.23 (d, J = 8.1 Hz, 1H), 8.17 (dd, J = 16.8, 7.7 Hz, 3H), 7.98 (d, J = 7.2 Hz, 2H), 7.85 (d, J = 7.4 Hz, 2H), 7.74 (t, J = 7.0 Hz, 1H), 7.67 (t, J = 7.0 Hz, 2H), 7.62 – 7.54 (m, 1H), 7.53 – 7.39 (m, 7H), 7.20 (d, J = 14.8 Hz, 1H), 6.29 (s, 1H), 5.82 (t, J = 8.2 Hz, 1H), 5.52 (t, J = 8.2 Hz, 1H), 5.41 (d, J = 6.8 Hz, 1H), 5.35 (d, J = 8.7 Hz, 1H), 4.91 (d, J = 8.3 Hz, 2H), 4.63 (s, 1H), 4.54 (d, J = 6.4 Hz, 1H), 4.25 (d, J = 8.0 Hz, 1H), 4.10 (d, J = 8.3 Hz, 1H), 4.01 (s,

2H), 3.74 (d, J = 4.2 Hz, 2H), 3.57 (d, J = 6.3 Hz, 1H), 3.07 (d, J = 5.1 Hz, 2H), 2.62 (d, J = 3.4 Hz, 4H), 2.33 (s, 1H), 2.23 (s, 3H), 2.11 (s, 3H), 1.78 (s, 4H), 1.66 (d, J = 12.0 Hz, 2H), 1.50 (s, 7H), 1.01 (d, J = 12.7 Hz, 6H).
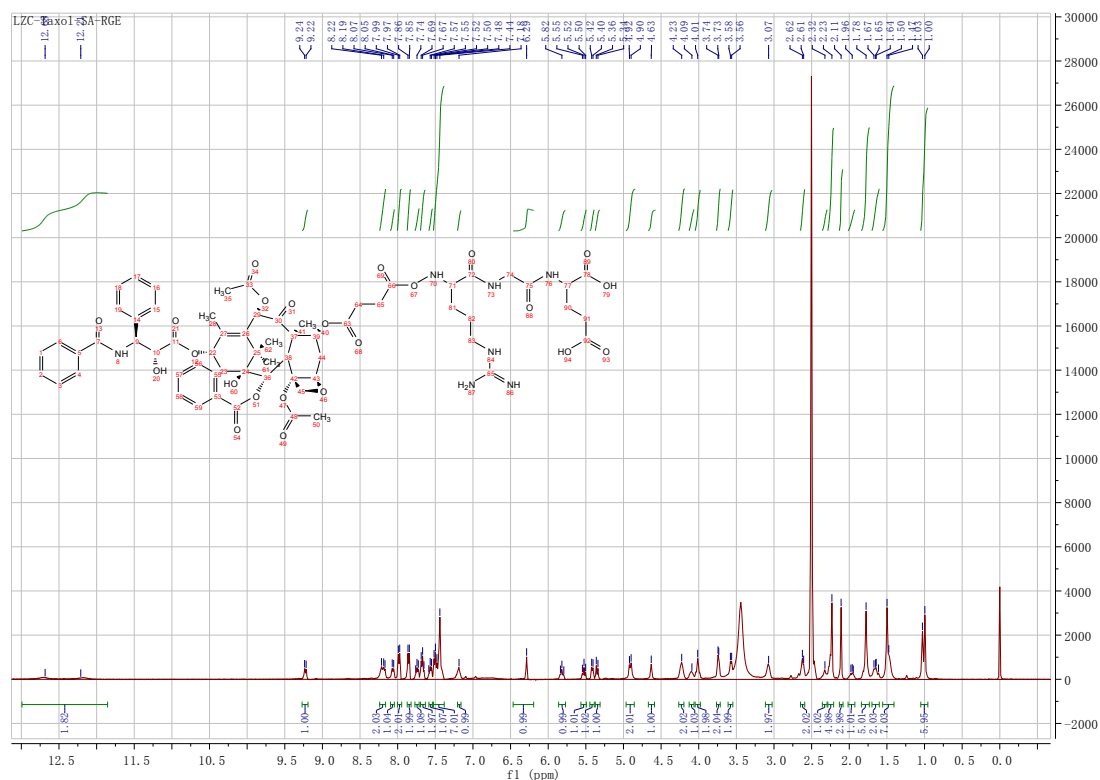

Figure S2. ^{1}H NMR spectrum of Taxol-SA-RGE in DMSO-d_6.

Taxol-SA-RGE ^{1}H NMR (400 MHz, DMSO) δ 12.45 (d, J = 189.4 Hz, 2H), 9.23 (d, J = 8.2 Hz, 1H), 8.24 – 8.16 (m, 2H), 8.06 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 7.3 Hz, 2H), 7.85 (d, J = 7.2 Hz, 2H), 7.77 – 7.71 (m, 1H), 7.67 (t, J = 7.3 Hz, 2H), 7.56 (d, J = 7.1 Hz, 1H), 7.48 (dd, J = 19.2, 11.4 Hz, 7H), 7.18 (s, 1H), 6.29 (s, 1H), 5.82 (t, J = 8.9 Hz, 1H), 5.52 (t, J = 8.5 Hz, 1H), 5.41 (d, J = 7.1 Hz, 1H), 5.35 (d, J = 8.8 Hz, 1H), 4.91 (d, J = 8.7 Hz, 2H), 4.63 (s, 1H), 4.23 (s, 2H), 4.09 (s, 1H), 4.01 (s, 2H), 3.74 (d, J = 3.9 Hz, 2H), 3.57 (d, J = 6.6 Hz, 2H), 3.07 (s, 2H), 2.61 (d, J = 6.6 Hz, 2H), 2.32 (s, 1H), 2.23 (s, 5H), 2.11 (s, 3H), 2.02 – 1.93 (m, 1H), 1.78 (s, 5H), 1.64 (dd, J = 13.7, 10.7 Hz, 2H), 1.49 (d, J = 9.5 Hz, 7H), 1.01 (d, J = 12.8 Hz, 6H).
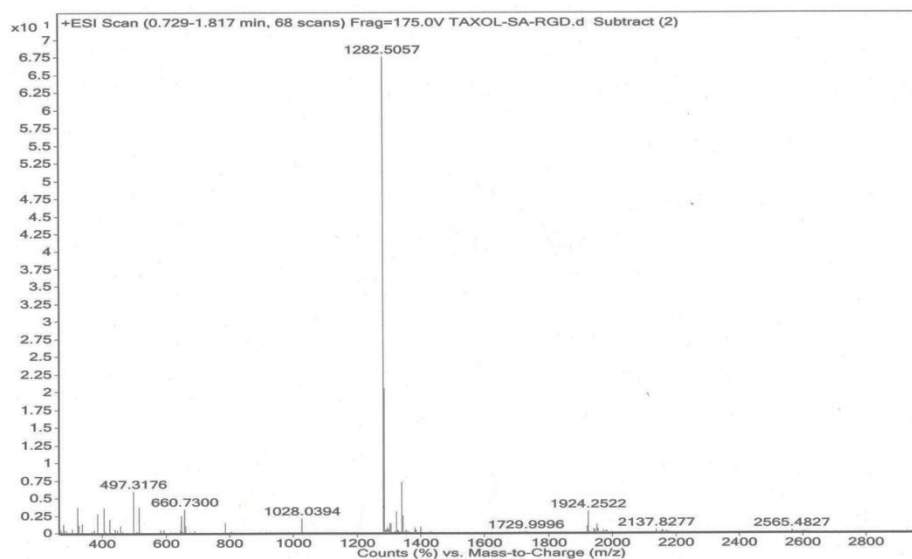
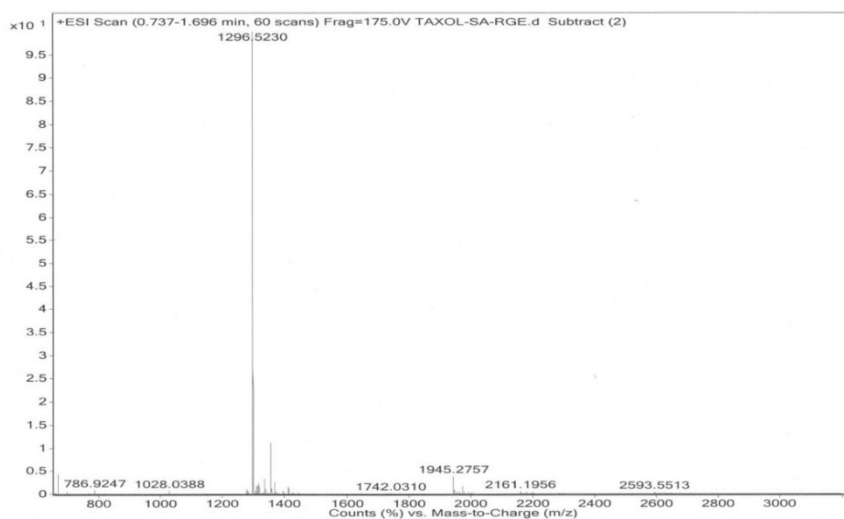**Figure S3.** MS spectrum of Taxol-SA-RGD

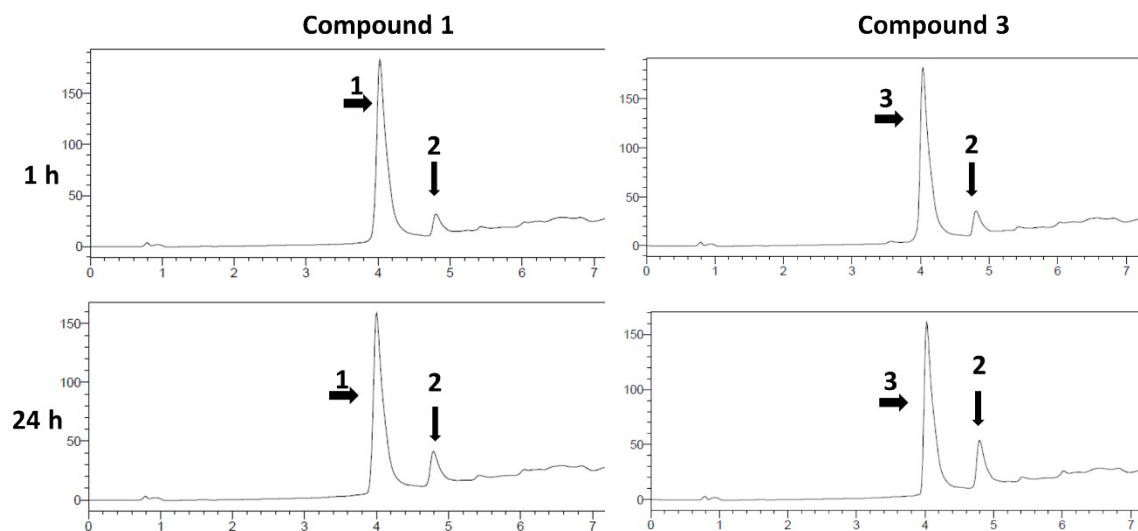
Figure S4. MS spectrum of Taxol-SA-RGE

Figure S5. HPLC indicates the hydrolysis of compound 1 or 3 in PBS buffer at the concentration of (0.5 wt%)
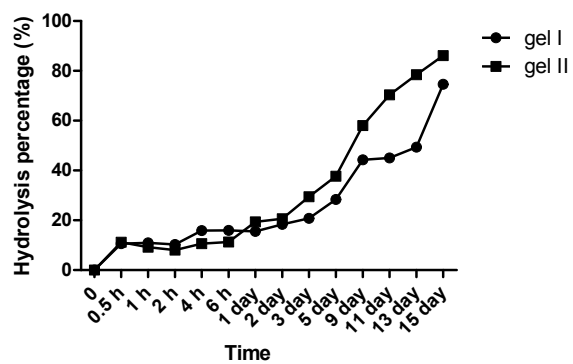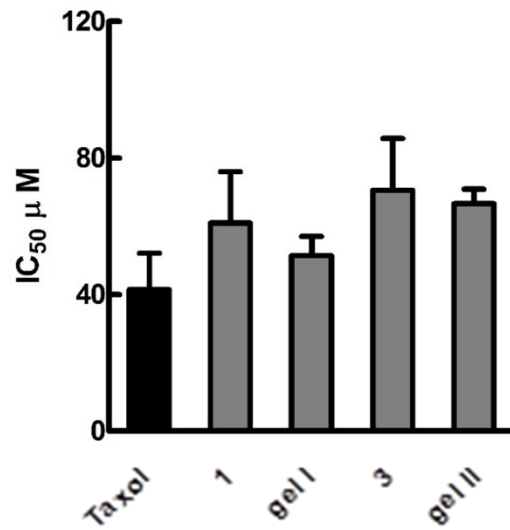

Figure S6. Hydrolysis percentage of gel I and gel II.

Figure S7. Summary of IC_{50} of different group against 4T-1 cell line.
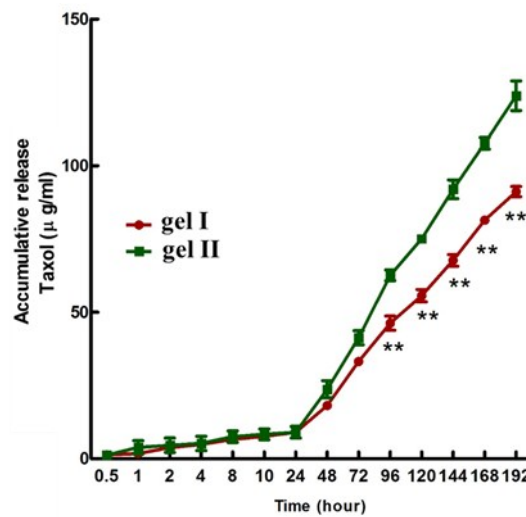

Figure S8. Accumulative release profile of paclitaxel from gel I and II at the concentration of 0.5 wt % (pH=7.4) at 37℃ for 192 h. Each experiment was performed by 3 times. Bars shown are mean ± SE, and differences between two groups are determined using t-test analysis. **: p<0.01.