Supporting Information

Fibril aggregates of the poly(glutamic acid)-drug conjugate

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Figure S1 GPC results of PGA and a typical PGA-Dox sample with drug content 30 wt%. The experiment was performed using a Shimadzu system equipped with a refractive index detector (Shimadzu RID-10A). Samples were separated on three 300×7.5mm PL Aquagel-OH MIXED-H 8 μm columns (with a guard column) in series at 25°C using water with 0.05% NaN₃ as the mobile phase at a flow rate of 1 ml/min. The elution time became a little longer after conjugation. It can be explained by the unimolecular micelle model, in which the Dox moieties aggregate and result in a more compact structure. Thus the size of the polymer conjugates could be smaller than the un-conjugated PGA chains, and results in the delay of the elution time.
Figure S2. Diffusion ordered spectroscopy (400 MHz, DMSO-\textsubscript{d6}) of the PGA-Dox conjugate. The band with average diffusion coefficient of $5.3 \times 10^{-11}$ m$^2$/s corresponds with the polymer conjugate, in which the peaks with chemical shifts around 1.0 and 8.0 ppm indicate the Dox moieties in the conjugate. The band with diffusion coefficient around $1.1 \times 10^{-10}$ m$^2$/s corresponds with some remaining free Dox molecules in the sample.
Figure S3 Fluorescence intensity of (A) pH 7 PGA-Dox solution, drug content: ~20 wt%; (B) Dox hydrochloride salt, excitation: 350 nm, emission: 562 nm. When the concentration of PGA-Dox was higher than 1 mg/ml, a significant decrease of the fluorescence intensity was observed. The Dox hydrochloride salt solution showed similar profile, indicating that the decrease should be resulted from the self-quenching of the aggregating Dox moieties.
Figure S4 The diameter distribution of fibrils. The samples are obtained from 2 mg/ml PGA-Dox solution (drug content: ~20 wt%), incubated at pH 4.7, 60°C for 24 hours. Scale bar: 2 μm. The fibrils seemed uniform in dimension that most of the fibrils have diameters of about 30-45 nm.
Figure S5 TEM images of the collected precipitates from PGA-Dox solution, incubated at pH 4.7, 60°C for 24 hours, (A), (B) 8 mg/ml, drug content: ~20 wt%; (C), (D) 2 mg/ml, drug content: ~10 wt%. Similar fibrils were observed in all samples. Some of the fibrils were larger (arrows), probably because the secondary aggregates or the aggregates of detached Dox adsorbed on the primary fibril surface.
Figure S6: $^1$H NMR (400 MHz, D$_2$O) spectrum of the dissociated fibrils. $\delta = 4.35-4.05$ (α-H), 2.30-2.00 (γ-H) and 2.00-1.65 ppm (β-H) are assigned to PGA. $\delta = 1.20$ ppm (H$_3$C-C) are assigned to Dox. The chemical shift and the ratio of Dox/PGA did not significantly change from the freshly synthesized conjugates (Figure 1).
Figure S7 Diffusion ordered spectroscopy (400 MHz, DMSO-d$_6$) of the released species. The peaks in $^1$H NMR spectrum have two groups of diffusion coefficients. The smaller one is about 4.8×10$^{-11}$ m$^2$/s, which is corresponding to the polymer conjugate. And the peaks with chemical shifts around 1.0 and 8.0 ppm indicate the doxorubicin moieties in the conjugate. The larger one is about 1.1×10$^{-10}$ m$^2$/s, which is corresponding to the detached Dox.
Figure S8. UV-vis spectra of Dox and PGA-Dox samples, tested in pH 7 aqueous solutions. Doxorubicin has very low solubility in pH 7 water, and even the saturated Dox shows very weak absorption. The adding of PGA does not significantly increase the solubility. In contrast, the fresh conjugates and the released species from the fibril aggregates all show strong absorption at about 485 nm, proving that the released species still contain the conjugate. The peak broadens after dissociation, which may because the dissociated conjugates are still in some smaller aggregates.
**Figure S9** (A) $^1$H NMR (400 MHz, DMSO) spectrum of the particle precipitates appeared on the bottom of the solution after 40 days of incubation; (B) $^1$H NMR (400 MHz, D$_2$O) spectrum of Dox hydrochloride salt. The peak assignments were shown above. Different from the dissociated fibrils, no obvious peaks from PGA were observed, indicating that the particle precipitates after 40 days were mostly consisted of the detached Dox molecules.
Figure S10 TEM images of 1 mg/ml PGA solution, incubated at pH 4.0, 60°C for 24 hours. Fibrils were also formed by PGA but much different from the PGA-Dox fibrils in morphology. These fibrils fit well with the reported amyloid-like PGA fibrils, and was able to increase the fluorescence intensity of ThT when adding to ThT solution (data not shown), indicating its amyloid-like structure.