Supplementary information

Polymer-augmented liposomes enhancing antibiotic delivery against intracellular infections

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Fig. S5 Representative fluorescence microscopy images of RAW 264.7 incubated with rhodamine-labeled PALs or PEGylated liposomes (400 μg/mL) for 1 h (100X magnification).

Reference

Supplementary method

1. RAFT synthesis of poly[(DEAEMA-*co*-BMA)-*b*-ManEMA]

A macro chain transfer agent (macroCTA) is the initial polymer block of a diblock copolymer that functions as the CTA for subsequent polymerizations. Here macroCTA was synthesized *via* RAFT using 2,2'-Azobis(4-methoxy-2.4-dimethyl valeronitrile) (V 40) as the initiator, ECT as the CTA, with 50 wt% monomer in dioxane. The initial monomer ($[M]_o$) to CTA ($[CTA]_o$) to initiator ($[I]_o$) ratios for the macroCTA synthesis was 25:1:0.1. Feed ratios of diethylaminoethyl methacrylate (DEAEMA) and butyl methacrylate (BMA) can be found in Fig. 1. Polymerization solutions were vortexed, transferred to septa-sealed round bottom flasks, purged under N_2 for 30 min and incubated in a 90 °C water bath for 8 h. The resultant polymers were purified by dialysis against acetone for two days and then isolated by further dialysis against water in Spectra/Por dialysis tubing (MWCO 6-8 kDa). The final polymers were then lyophilized for further use.

To synthesize the mannose-containing block, mannose functionalized with ethyl methacrylate (ManEMA) was synthesized and fully characterized as described previously[1]. The synthesized ManEMA monomers with acetyl protecting groups (1g, 480 mM) were added to the macroCTA (1.1g, 38.4 mM) and dissolved in dioxane. 4,4-azobis(4-cyanovaleric acid) (ABCVA) was used as the radical initiator. The initial monomer to macroCTA molar ratio ($[M]_0$:$[macroCTA]_0$) was 12.5:1, and the initial macroCTA to initiator molar ratio ($[macroCTA]_0$:$[I]_0$) was 5:1. A stock solution of ABCVA (9.79 mg, 7.68 mM) dissolved in dioxane was added to the reaction solution. Following the addition of ABCVA the solution was purged with nitrogen for 30 min and allowed to polymerize at 70 °C for 18 h. The resulting diblock copolymer was obtained via precipitation in diethyl ether (6x) and placed in vacuum overnight. To deprotect acetyl groups on ManEMA monomers, the obtained diblock copolymer was added to a solution of 1 wt% sodium methoxide in methanol at a copolymer concentration of 50 mg/mL. After 1 h incubation at room temperature, the solution was quenched and neutralized with acetic acid to a pH 7 and dialyzed against deionized water. The solution was then lyophilized to obtain the final deprotected glycopolymer, poly[(DEAEMA-*co*-BMA)-*b*-ManEMA].

Characterization of diblock copolymer by SEC-GPC and NMR

The compositions of the macroCTA and the diblock copolymer were determined by ^{1}H-NMR (Bruker DRX499) in deuterated dimethylsulfoxide (DMSO-d_6) at 25 °C. The number average molecular weight (M_n) and dispersity (Đ) were measured by GPC using Tosoh SEC TSK GEL α-3000 and α-4000 columns (Tosoh Bioscience, Montgomeryville, PA) connected in series to a 1200 Series liquid chromatography system (Agilent, Santa Clara, CA) and a miniDAWN TREOS three-angle light scattering instrument with an Optilab TrEX refractive index detector (Wyatt Technology, Santa Barbara, CA).

2. High-performance size exclusion chromatography (HPSEC)

Incorporation of poly[(DEAEMA-*co*-BMA)-*b*-ManEMA] with liposomes was examined using HPSEC[2]. This analysis was performed with a HPLC (Agilent 1260 series HPLC,

USA) equipped with an Acclaim™ SEC-1000 LC Column (7.8 mm × 150 mm, 7 μm ThermoFisher). The eluent was PBS and the flow rate was 1 mL/min. Diluted polymer solution or rhodamine-labeled PALs in 10-μL aliquots were injected. The elution was followed by absorbance (λ= 310 nm) and fluorescence measurement (Ex. 560 nm, Em. 580 nm) to detect the polymer and rhodamine-labeled liposomes, respectively.

3. MTS assay

Cell viability of the polymer alone or PALs with streptomycin encapsulation was evaluated by MTS assay in a murine macrophage cell line, RAW 264.7. Culture condition of RAW 264.7 cells was DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a 37°C, 5% CO_2 incubator. The RAW 264.7 cells were seeded into 96-well plates at a density of 50,000 cells per well in 100 μL DMEM supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 incubator at 37 °C. After 16-18 h, cells were incubated with the polymer or PALs (100 μL per well) at a series of PAL concentrations (polymer + lipid). After continued incubation for 24 h, the effect of different treatments on cell viability was assessed by the MTS assay. The absorbance at 490 nm in each well was determined by a microplate reader (Tecan Safire2). Untreated cells were used as a control with 100% viability. The relative cell viability (%) compared to control cells was calculated by $(A_{sample}/A_{control}) \times 100$. All treatments were done in triplicates.

2. Supplementary data

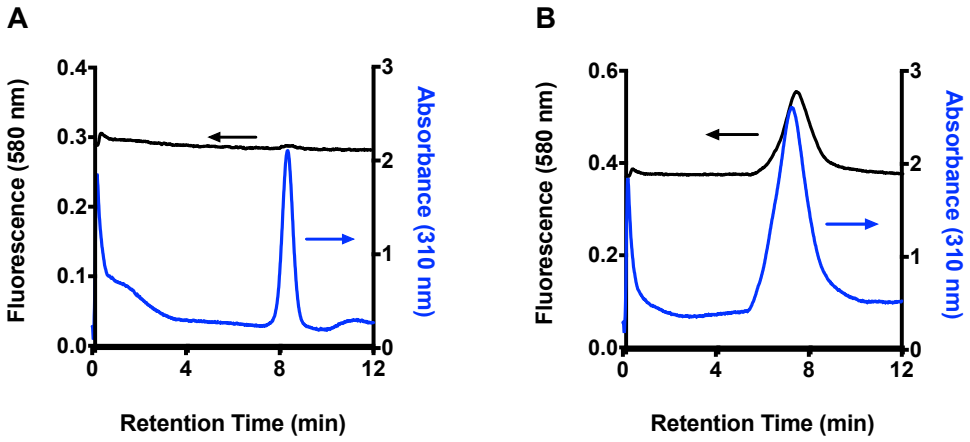

Fig. S1 High performance size exclusion chromatography showing the incorporation of poly[(DEAEMA-*co*-BMA)-*b*-mannose ethyl methacrylate] with liposomes. (a) Polymer alone; (b) polymer-augmented liposomes. Absorption of the polymer at 310 nm was contributed by the thiocarbonyl bond of RAFT agent on the polymer terminal. Fluorescence-labeled PALs were prepared using rhodamine B conjugated lipids (fluorescence at 580 nm). DEAEMA, diethylaminoethyl methacrylate; BMA, butyl methacrylate.

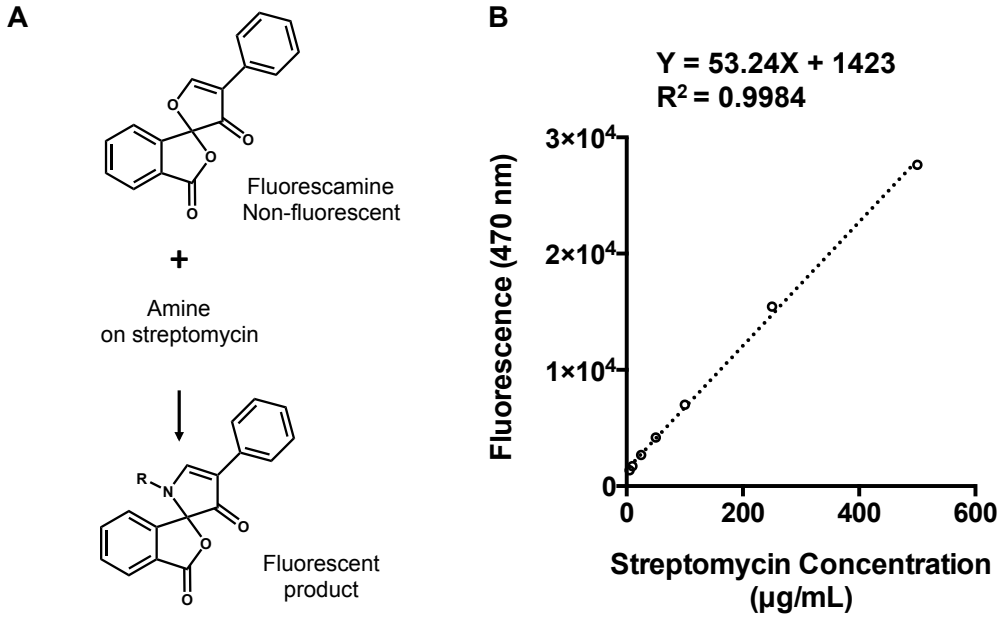

Fig. S2 Fluorescamine assay for quantifying streptomycin concentrations. (a) Scheme showing the reaction between fluorescamine and amine groups on streptomycin; (b) representative calibration curve of streptomycin concentration (µg/mL) versus fluorescence intensity of fluorescamine derivative at 470 nm. (λ_{ex}= 390 nm, λ_{em}= 470 nm) (mean ± SD, n=3).

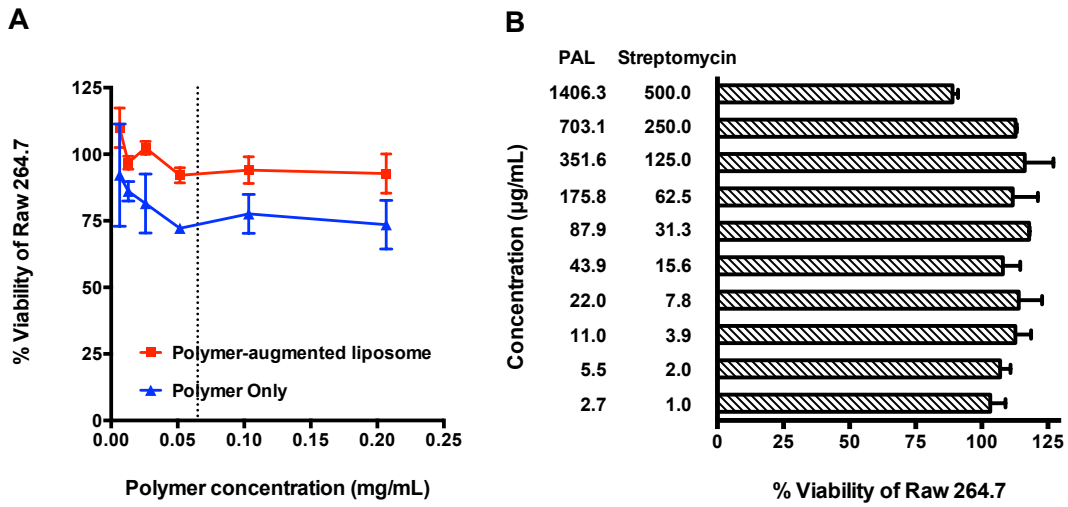

Fig. S3 Cell viability of RAW 264.7 cells treated with poly[(DEAEMA-*co*-BMA)-*b*-ManEMA] and streptomycin-loaded polymer-augmented liposomes (PAL). RAW 264.7 cells were treated with the polymer or PAL suspension with a series of 1:1 dilution for 24 h. Cell viability numbers were relative to untreated controls as determined by MTS. Data represent the average of a single experiment conducted in triplicate. (A) Viability was present as a function of polymer concentrations. The dash line indicates the highest polymer concentration in PALs used in the co-culture studies (Fig. 6). Formulating the polymer with liposomes mitigated the polymer toxicity. (B) Viability was present as a function of streptomycin and PAL (polymer and lipid) concentrations. No notable toxicity was observed in the dose range used in the *in vitro* assays (115 µg/mL to 1.8 µg/mL streptomycin).

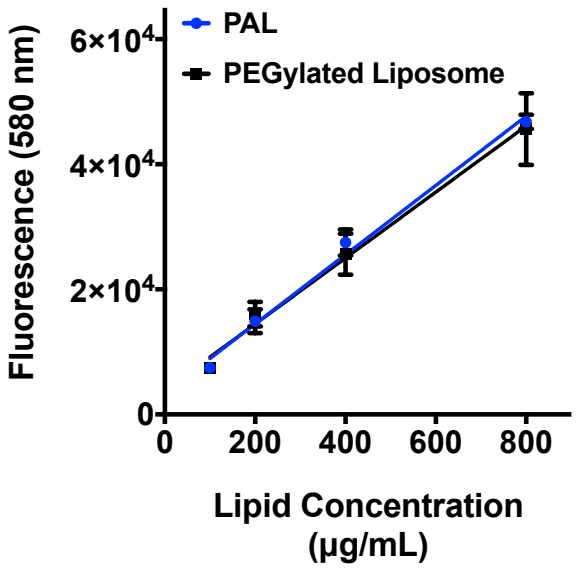

Fig. S4 Similar fluorescence intensity of rhodamine-labeled polymer-augmented liposomes (PAL) and PEGylated liposomes used in the uptake study (Fig. 5 & Fig. S5) (mean ± SD, n=3).

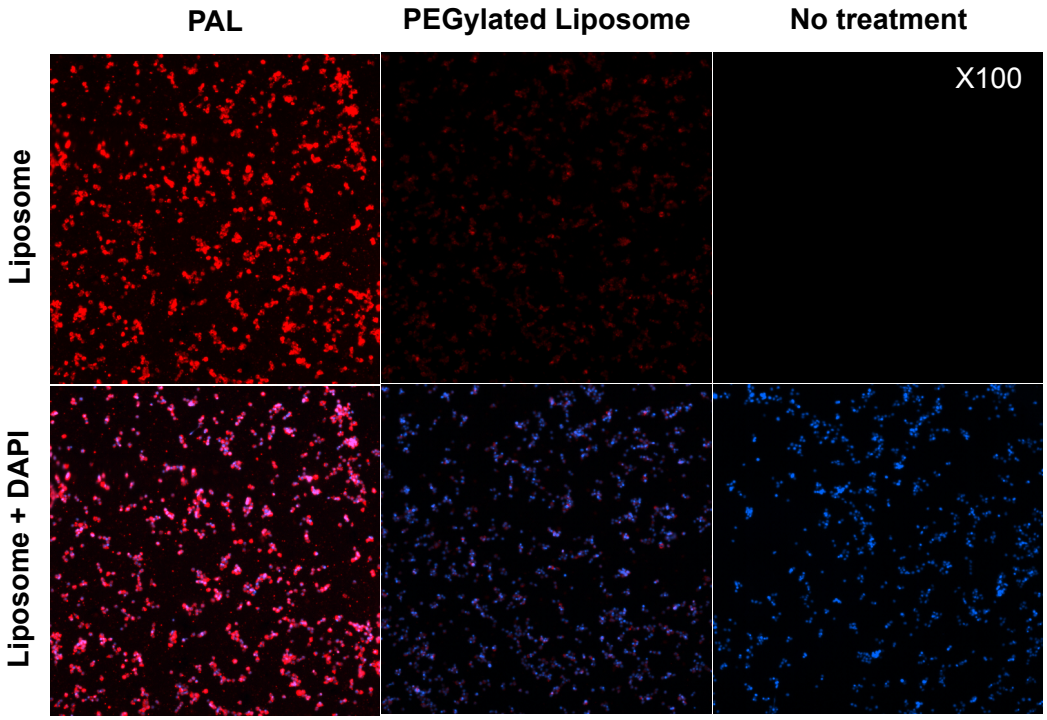

Fig. S5 Representative fluorescence microscopy images of RAW 264.7 incubated with rhodamine-labeled polymer-augmented liposomes (PALs) or non-targeted PEGylated liposomes (400 μg/mL) for 1 h (100X magnification).

3. Reference

1 J. Chen, H.-N. Son, J. J. Hill, S. Srinivasan, F.-Y. Su, P. S. Stayton, A. J. Convertine and D. M. Ratner, *Nanomedicine*, 2016, **12**, 2031–2041.

2 C. Grabielle-Madelmont, S. Lesieur and M. Ollivon, *Journal of Biochemical and Biophysical Methods*, 2003, **56**, 189–217.