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Research article

Chemotherapeutic loading via tailoring of drug-carrier interactions in poly (sialic acid) micelles

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Supplementary

Table S1. ^{1}H-NMR peak shifts of PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
HO-CH_2-CHOH-CHOH-C HO-C_2O_2Na-CH_2-*CH*OH-CH-CONH-CH_3	4.18	c	HO-CH_2-CHOH-*CH*OH-*C**H**O*-C_2O_2Na-CH_2-CHOH-CH-CONH-CH_3	3.65	d f h’
HO-CH_2-CHOH-CHOH-C HO-C_2O_2Na-CH_2-CHOH-*CH*-CONH-CH_3	4.11	e	HO-CH_2-CHOH-CHOH-C HO-C_2O_2Na-*CH_2*-CHOH-CH-CONH-CH_3	2.68	a
HO-CH_2-*CH*OH-CHOH-C HO-C_2O_2Na-CH_2-CHOH-CH-CONH-CH_3	3.90	g	HO-CH_2-CHOH-CHOH-C HO-C_2O_2Na-CH_2-CHOH-CH-CONH-*CH_3*	2.09	i
HO-*CH_2*-CHOH-CHOH-C HO-C_2O_2Na-CH_2-CHOH-CH-CONH-CH_3	3.83	h	HO-CH_2-CHOH-CHOH-C HO-C_2O_2Na-*CH_2*-CHOH-CH-CONH-CH_3	1.75	b

Table S2. ^{1}H-NMR peak shifts of PSA + EDC/NHS in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
CH_3CH_3N-CH_2-CH_2-C H_2-NHCO-CH_3<u>CH_2</u>N-C O-PSA	3.20	g	<u>CH_3CH_3</u>N-CH_2-CH_2-C H_2-NHCO-CH_3CH_2N-C O-PSA	2.90	e
CH_3CH_3N-CH_2-CH_2-<u>C H_2</u>-NHCO-CH_3CH_2N-C O-PSA	3.15	h	CH_3CH_3N-CH_2-<u>CH_2</u>-C H_2-NHCO-CH_3CH_2N-C O-PSA	1.90	i
CH_3CH_3N-<u>CH_2</u>-CH_2-C H_2-NHCO-CH_3CH_2N-C O-PSA	3.10	k	CH_3CH_3N-CH_2-CH_2-C H_2-NHCO-<u>CH_3</u>CH_2N-C O-PSA	1.09	f

Table S3. ^{1}H-NMR peak shifts of PEA-*g*-PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH_2-CH_2-<u>C_6 $H_{5\text{-}META}$</u>	7.44	d_1	PSA-NH-<u>CH_2</u>-CH_2-C_6 H_5	3.02	a_1
PSA-NH-CH_2-CH_2-<u>C_6 $H_{5\text{-}ORTHO/PARA}$</u>	7.36	c_1/e_1	PSA-NH-CH_2-<u>CH_2</u>-C_6 H_5	2.78	b_1

Table S4. ^{1}H-NMR peak shifts of PPA-*g*-PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH_2-CH_2-CH_2-<u>$C_6H_{5\text{-}META}$</u>	7.40	e_2	PSA-NH-CH_2-CH_2-<u>CH_2</u>-C_6H_5	2.75	c_2
PSA-NH-CH_2-CH_2-CH_2-<u>$C_6H_{5\text{-}ORTHO/PARA}$</u>	7.32	d_2/f_2	PSA-NH-CH_2-<u>CH_2</u>-CH_2-C_6H_5	2.00	b_2
PSA-NH-<u>CH_2</u>-CH_2-CH_2-C_6H_5	3.02	a_2			

Table S5. ^{1}H-NMR peak shifts of PBA-*g*-PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH_2-CH_2-CH_2-CH_2-<u>$C_6H_{5\text{-}META}$</u>	7.38	f_3	PSA-NH-CH_2-CH_2-CH_2-<u>CH_2</u>-C_6H_5	2.68	d_3
PSA-NH-CH_2-CH_2-CH_2-CH_2-<u>$C_6H_{5\text{-}ORTHO/PARA}$</u>	7.30	e_3/g_3	PSA-NH-CH_2-<u>CH_2-CH_2</u>-CH_2-C_6H_5	1.70	b_3/c_3
PSA-NH-<u>CH_2</u>-CH_2-CH_2-CH_2-C_6H_5	3.02	a_3			

Table S6. ^{1}H-NMR peak shifts of POE-*g*-PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH_2-CH_2O-$\underline{C_6H_{5\text{-}META}}$	7.41	d_4	PSA-NH-CH_2-$\underline{CH_2}O$-C_6H_5	4.30	b_4
PSA-NH-CH_2-CH_2O-$\underline{C_6H_{5\text{-}ORTHO}}$	7.10	c_4	PSA-NH-$\underline{CH_2}$-CH_2O-C_6H_5	3.40	a_4
PSA-NH-CH_2-CH_2O-$\underline{C_6H_{5\text{-}PARA}}$	7.06	e_4			

Table S7. ^{1}H-NMR peak shifts of 33DPP-*g*-PSA in D_2O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH_2-CH_2-$CH\underline{C_6H_{5\text{-}META}C_6H_{5\text{-}META}}$	7.41	e_5	PSA-NH-$\underline{CH_2}$-CH_2-$CHC_6H_5C_6H_5$	2.95	a_5
PSA-NH-CH_2-CH_2-$CH\underline{C_6H_{5\text{- ORTHO/PARA}}C_6H_{5\text{-ORTHO/PARA}}}$	7.29	d_5/f_5	PSA-NH-CH_2-$\underline{CH_2}$-$CHC_6H_5C_6H_5$	2.48	b_5
PSA-NH-CH_2-CH_2-$\underline{CH}C_6H_5C_6H_5$	4.12	c_5			

Table S8. LD_{50} Values of PTAG-*g*-PSA micelles towards LBC3 GMB cell type are proportional to PTAG alkyl chain length and composition. LD_{50} values will decrease as increased hydrophobicity is added to the PTAG group either by longer chain length or added grafting (DOS) as a result of interaction with the cell membrane.

PTAG-g-PSA	LD_{50} (μg/mL)		
	20–30%	50–60%	90–100%
PEA-*g*-PSA	1033 ± 99	751 ± 2	640 ± 7
PPA-*g*-PSA	763 ± 26	556 ± 30	576 ± 44
PBA-*g*-PSA	702 ± 78	699 ± 14	418 ± 155
POE-*g*-PSA	>1000	>1000	>1000
33DPP-*g*-PSA	278 ± 34	118 ± 2	-

Table S9. LC/EC of DOX Loaded POE-*g*-PSA and 33DPP-*g*-PSA Micelles with Varying DOS. Differences between formulations of different DOS were compared for both POE-*g*-PSA and 33DPP-*g*-PSA formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

POE-g-PSA			33DPP-g-PSA		
Group	DOS	p-value	Group	DOS	p-value
P_1	20–30% vs. 50–60%	n.s.	D_1	20–30% vs. 50–60%	<0.0001
P_2	20–30% vs. 90–100%	<0.01	D_2	20–30% vs. 90–100%	<0.0001
P_3	50–60% vs. 90–100%	<0.05	D_3	50–60% vs. 90–100%	<0.0001

Table S10. LC/EC of POE-*g*-PSA and 33DPP-*g*-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

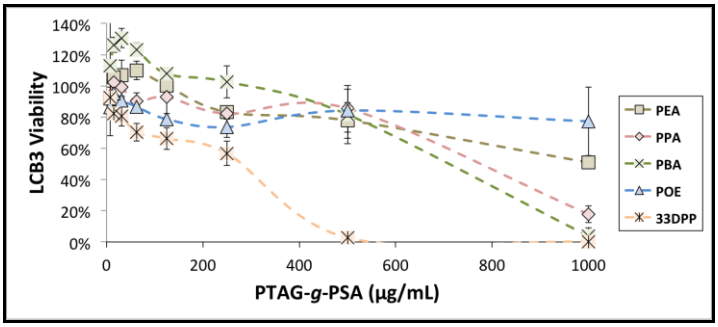
POE-g-PSA			33DPP-g-PSA		
Group	DOX_{FEED}	p-value	Group	DOX_{FEED}	p-value
P_1	5% vs. 10%	<0.05	D_1	5% vs. 10%	<0.01
P_2	5% vs. 15%	n.s.	D_2	5% vs. 15%	n.s.
P_3	10% vs. 15%	n.s.	D_3	10% vs. 15%	<0.01

Table S11. Sizes of POE-*g*-PSA and 33DPP-*g*-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test. 33DPP D_0; D_5; D_{10}; D_{15}: post-hoc Tukey HSD test not performed based upon ANOVA results.

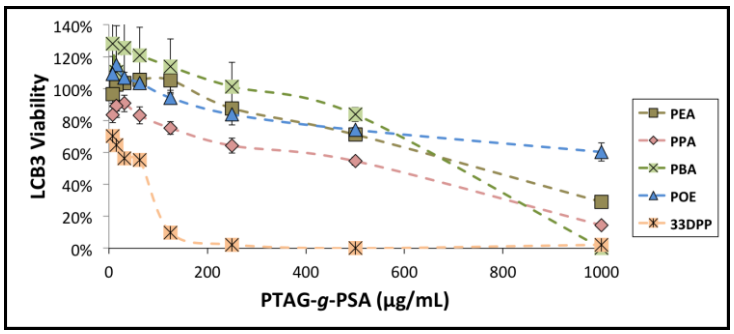
POE-g-PSA					
Group	DOX_{FEED}	p-value	Group	DOX_{FEED}	p-value
P_0	0% vs. 5%	<0.01	P_{10}	10% vs. 15%	<0.01
P_0	0% vs. 10%	<0.01	P_{15}	15% vs. 0%	<0.01
P_5	5% vs. 10%	<0.01			

Table S12. Stability of POE-*g*-PSA and 33DPP-*g*-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

POE-g-PSA			33DPP-g-PSA		
Group	DOX_{FEED}	p-value	Group	DOX_{FEED}	p-value
P_0	0% vs. 5%	n.s.	D_0	0% vs. 5%	n.s.
P_0	0% vs. 10%	<0.01	D_0	0% vs. 10%	n.s.
P_5	5% vs. 10%	<0.01	D_5	5% vs. 10%	n.s.
P_{10}	10% vs. 15%	<0.01	D_{10}	10% vs. 15%	<0.01
P_{15}	15% vs. 0%	<0.01	D_{15}	15% vs. 0%	<0.01



(a)



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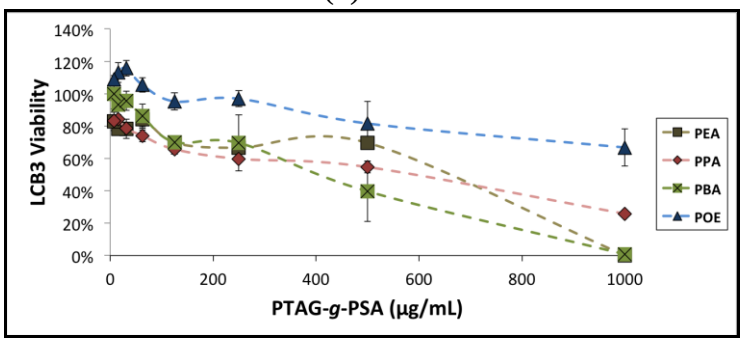

(c)

Figure S1. Cytotoxicity of PTAG-*g*-PSA micelles towards LBC3 GMB cell type is highly dependent upon PTAG composition and DOS. Cell toxicity increases with higher DOS of each PTAG group; (a) 20–30%; (b) 50–60%; (c) 90–100%. Decreasing the alkyl chain length or enhancing the polarity of the PTAG group effectively decreases the cytotoxic response of LBC3 cells.

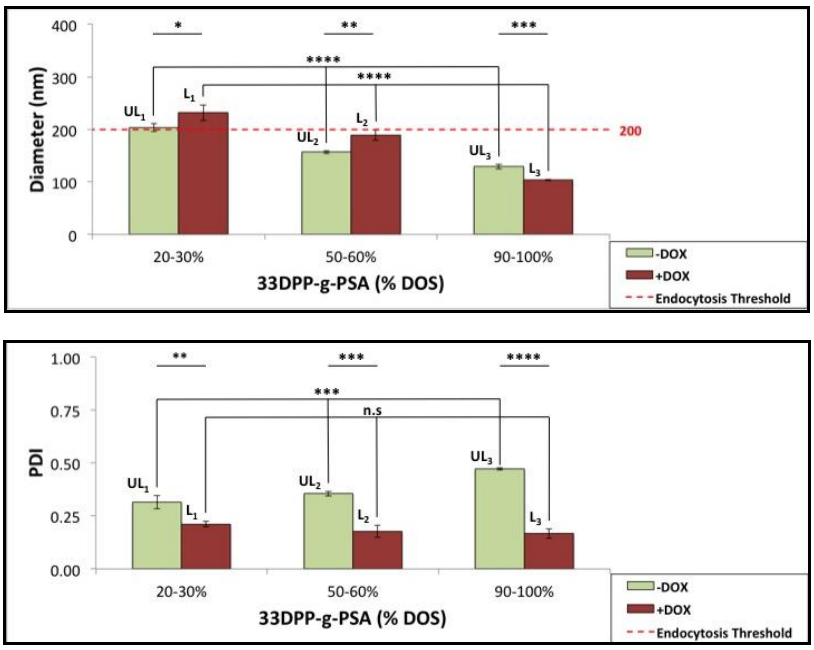

Figure S2. Size and stability of 33DPP-*g*-PSA/DOX Micelles with Varying DOS. Variation in the DOS (20–30%, 50–60%, 90–100%) of 33DPP-*g*-PSA results in tailorable sizes of both unloaded and DOX-loaded micelles. Stability was greatly increased as a result of DOX loading within the micelle core.

Differences between loaded and unloaded samples at each DOS were statistically analyzed by an F-Test ($p < 0.05$) followed by a Student's *t* test assuming equal or unequal variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; n.s. no significance. Differences between formulations of different DOS were compared for both unloaded and DOX-loaded formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

Size: L_1: 20–30% (DOX) vs. 50–60% (DOX) $p < 0.01$; L_2: 20–30% (DOX) vs. 90–100% (DOX) $p < 0.01$; L_3: 50–60% (DOX) vs. 90–100% (DOX) $p < 0.01$; UL_1: 20–30% vs. 50–60% $p < 0.01$; UL_2: 20–30% vs. 90–100% $p < 0.01$; UL_3: 50–60% vs. 90–100% $p < 0.01$. Data presentation as mean ± SD (n = 3).

PDI: L_1: 20–30% (DOX) vs. 50–60% (DOX) n.s; L_2: 20–30% (DOX) vs. 90–100% (DOX) n.s.; L_3: 50–60% (DOX) vs. 90–100% (DOX) n.s.; UL_1: 20–30% vs. 50–60% n.s.; UL_2: 20–30% vs. 90–100% $p < 0.01$; UL_3: 50–60% vs. 90–100% $p < 0.01$. Data presentation as mean ± SD (n = 3).

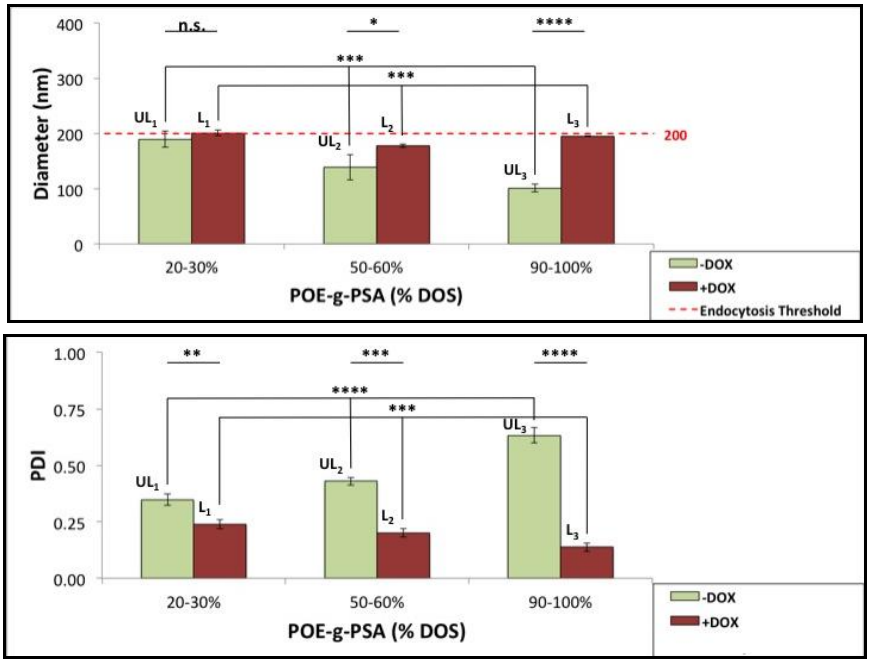

Figure S3. Size and stability of POE-*g*-PSA/DOX Micelles with Varying DOS. Variation in the DOS (20–30%, 50–60%, 90–100%) of POE-*g*-PSA results in tailorable sizes of both unloaded and DOX-loaded micelles. Stability was greatly increased as a result of DOX loading within the micelle core.

Differences between loaded and unloaded samples at each DOS were statistically analyzed by an F-Test ($p < 0.05$) followed by a student's *t* test assuming equal or unequal variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; n.s. no significance. Differences between formulations of different DOS were compared for both unloaded and DOX-loaded formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

Size: L_1: 20–30% (DOX) vs. 50–60% (DOX) $p < 0.01$; L_2: 20–30% (DOX) vs. 90–100% (DOX) n.s.; L_3: 50–60% (DOX) vs. 90–100% (DOX) $p < 0.01$; UL_1: 20–30% vs. 50–60% $p < 0.05$; UL_2: 20–30% vs. 90–100% $p < 0.01$; UL_3: 50–60% vs. 90–100% n.s.

PDI: L_1: 20–30% (DOX) vs. 50–60% (DOX) n.s; L_2: 20–30% (DOX) vs. 90–100% (DOX) $p < 0.01$; L_3: 50–60% (DOX) vs. 90–100% (DOX) $p < 0.05$; UL_1: 20–30% vs. 50–60% $p < 0.05$; UL_2: 20–30% vs. 90–100% $p < 0.01$; UL_3: 50–60% vs. 90–100% $p < 0.01$.

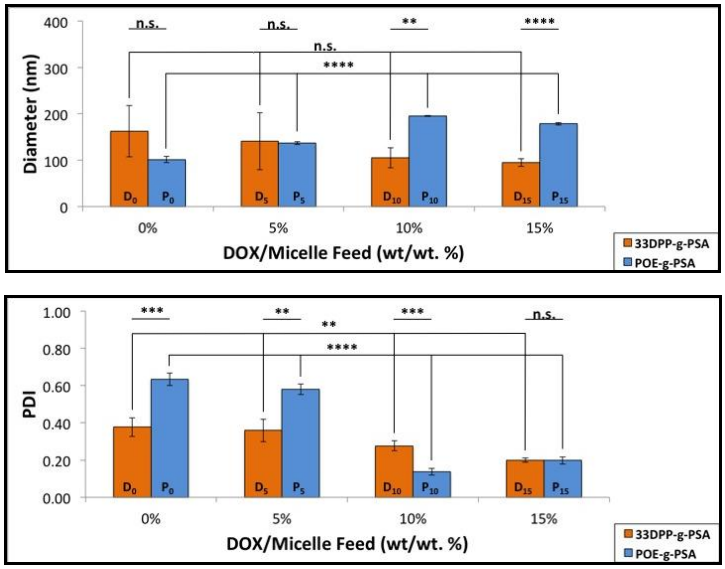

Figure S4. Sizes of POE-*g*-PSA and 33DPP-*g*-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Micelles form at different sizes based upon the composition of the core. 33DPP-*g*-PSA formulations already contain sufficient hydrophobicity can form smaller micelles at higher feed rates due to earlier micelle formation. POE-*g*-PSA micelles are more polar within their cores, allowing for more DOX to be entrapped on a per micelle basis and leading to larger sizes upon DOX encapsulation based upon later micelle formation. The stabilizing of the micelle core is initiated only at a feed rate greater than 10% DOX. POE micelles stabilize greater than 33DPP formulations at 10% while both reach saturation at 15%.

Differences between POE-*g*-PSA and 33DPP-*g*-PSA micelles at each feed rate were statistically analyzed by an F-Test ($p < 0.05$) followed by a student's *t* test assuming equal or unequal variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; n.s. no significance. Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

Size: POE P_0: 0% vs. 5% (DOX) $p < 0.01$; P_0: 0% vs. 10% (DOX) $p < 0.01$; P_5: 5% vs. 10% (DOX) $p < 0.01$; P_{10}: 10% vs. 15% (DOX) $p < 0.01$; P_{15}: 15% vs. 0% (DOX) $p < 0.01$. 33DPP D_0; D_5; D_{10}; D_{15}: Post-hoc Tukey HSD test not performed based upon ANOVA results. Data presentation as mean ± SD (n = 3).

PDI: POE P_0: 0% vs. 5% (DOX) n.s.; P_0: 0% vs. 10% (DOX) $p < 0.01$; P_5: 5% vs. 10% (DOX) $p < 0.01$; P_{10}: 10% vs. 15% (DOX) $p < 0.01$; P_{15}: 15% vs. 0% (DOX) $p < 0.01$. 33DPP D_0: 0% vs. 5% (DOX) n.s.; D_0: 0% vs. 10% (DOX) n.s.; P_5: 5% vs. 10% (DOX) n.s.; P_{10}: 10% vs. 15% (DOX) $p < 0.01$; P_{15}: 15% vs. 0% (DOX) $p < 0.01$. Data presentation as mean ± SD (n = 3).