Development of GE11-functionalized Liposomes for Doxorubicin and Curcumin Delivery in Breast Cancer Treatment

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Introduction

Liposomes are versatile and promising nanoplatforms for the delivery of antineoplastic agents and other bioactive compounds. The co-encapsulation of doxorubicin (DOX) and curcumin (CUR) within these nanosystems may help overcome chemoresistance while enhancing drug bioavailability, therapeutic efficacy, and synergistic antitumor activity. Furthermore, liposomal functionalization with DSPE-PEG2000-mal conjugated to the GE11 peptide (YHWYGYTPQNVI) — a selective ligand for the epidermal growth factor receptor (EGFR), which is frequently overexpressed in solid tumors — enables targeted drug delivery. This strategy promotes the selective accumulation of therapeutic agents in tumor cells, potentially enhancing antitumor efficacy while minimizing damage to healthy tissues.

Methodology

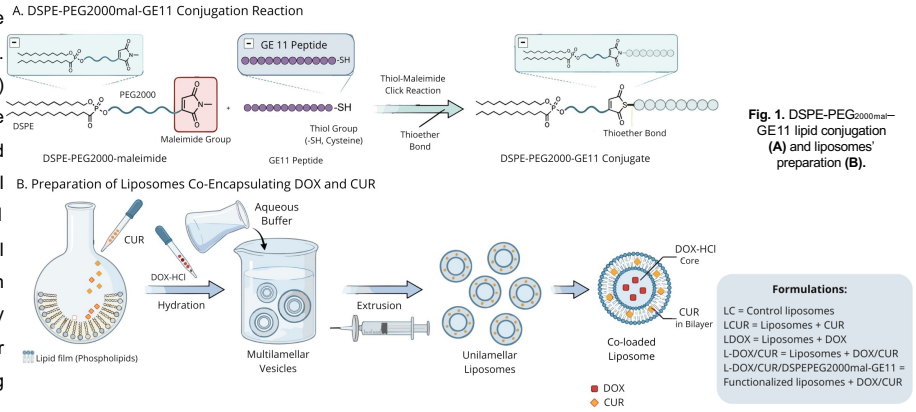


Fig. 1. DSPE-PEG$_{2000mal}$–GE11 lipid conjugation **(A)** and liposomes' preparation **(B).**

Results

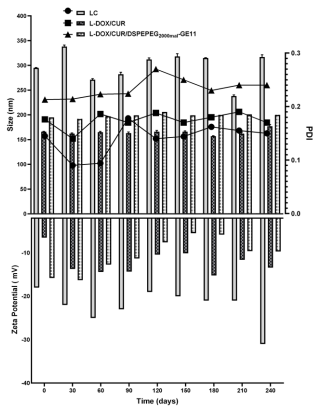


Fig. 2. DLS data: average diameter (Size, bars), polydispersity index (PDI, lines) and Zeta potentials (bars) of LC, L-DOX/CUR and L-DOX/CUR/DSPEPEG$_{2000mal}$-GE11, during 240 days of storage at 5 ± 3 ºC.

Table 1. Nanotracking Analysis: size, polydispersity (Span index) and concentration of liposomes/mL, during 240 days of storage at 5 ± 3 ºC.

Time (days)	Control Liposome (LC)			L-DOX/CUR			L-DOX/CUR/DSPEPEG2000mal-GE11		
	Size (nm)	Particle/mL x10^13	SPAN	Size (nm)	Particle/mL x10^13	SPAN	Size (nm)	Particle/mL x10^13	SPAN
0	180.4±4.2	0.48±0.02	1.01	144.6±2.2	1.61±0.41	0.97	153.6±3.8	1.63±0.21	1.08
30	212.0±3.2	0.39±0.03	1.03	170.2±5.1	8.26±0.31	0.75	187.3±4.4	1.12±0.33	1.01
60	217.2±1.5	0.69±0.05	1.21	164.5±3.4	0.25±0.01	0.84	168.4±2.5	1.17±0.29	0.79
90	196.2±2.9	0.77±0.03	0.82	154.6±1.9	0.66±0.03	0.62	185.6±2.9	1.65±0.12	0.80
120	201.4±3.9	0.34±0.02	0.90	148.1±2.1	1.61±0.15	0.83	148.0±3.4	1.60±0.17	0.87
150	215.9±5.6	0.41±0.07	0.95	144.5±2.1	3.44±0.27	0.87	177.1±3.2	1.15±0.22	0.92
180	201.1±6.7	0.39±0.01	0.89	163.1±2.1	4.12±0.33	0.91	181.3±2.1	1.95±0.37	0.95
210	211.5±2.2	0.47±0.09	0.93	155.3±2.1	2.21±0.25	0.89	160.9±5.4	1.23±0.12	0.93
240	209.2±3.9	0.31±0.02	0.90	151.2±2.3	1.63±0.11	0.90	157.0±3.7	1.53±0.13	0.91

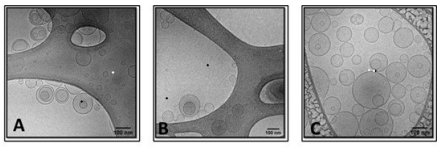
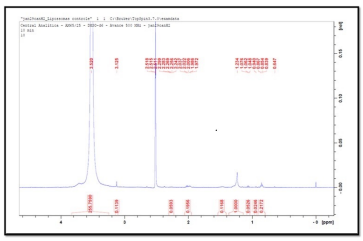


Fig 3. Cryo-EM: Representative images of: A) LC, B) L-DOX/CUR and C) L-DOX/CUR/DSPEPEG$_{2000mal}$-GE11 liposomes; scale bar – 100 nm.



Signal (ppm)	Assignment / Description
2.50	Deuterated DMSO
1.23	Triplet of methyl groups from the aliphatic chains of DSPE
3.56	Protons of the repeating unit OCH_2CH_2 of DSPE-PEG
0.83	Methyl group of threonine residue in the GE11 peptide
1.06	Acyl chains of the phospholipid
3.12	Choline and glycerol group (O_2PO-CH_2)

Fig 4. / Table 2. ^{1}H-NMR peaks and assignment of DSPE-PEG$_{2000mal}$–GE11 conjugate signals in the liposomes. 500 MHz, chemical shifts referenced to the D_2O peak.

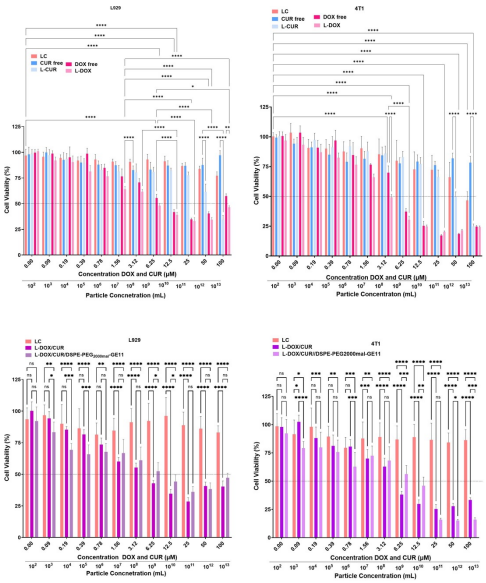


Fig. 6. Viability (%) of L929 and 4T1 cells treated with LC, L-DOX/CUR, and L-DOX/CUR/DSPEPEG$_{2000mal}$-GE11 (A, B), or with LC, CUR free, L-CUR, DOX free, and L-DOX (C, D), as determined by the MTT assay. Statistical analysis: two-way ANOVA followed by Tukey's multiple-comparisons post hoc test (*p < 0.05; **p < 0.01; ***p < 0.005, ****p < 0.001).

L929 = fibroblast cells derived from the subcutaneous connective tissue of a C3H/An mouse.
4T1 = mouse mammary carcinoma cells (model for human stage IV triple-negative breast cancer.

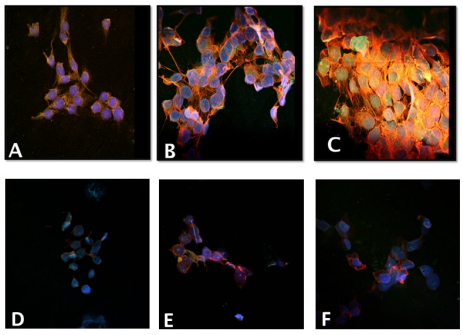


Fig. 7. Confocal microscopy: internalization of L-DOX/CUR/DSPEPEG$_{2000mal}$-GE11 in tumor (4T1 cells, A–C) and normal (L929 cells, D–F) after 30 min (A, D), 1 h (B, E), and 4 h (C, F) of incubation. Cells were stained with DAPI – blue - and phalloidin – red - to visualize nuclei and actin filaments, respectively.

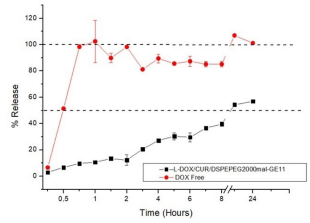


Fig. 5. *In vitro* release kinetics of DOX, free or encapsulated in L-DOX/CUR/DSPEPEG$_{2000mal}$-GE11, as determined at 37 ºC.

Conclusions

- Liposomal formulations remained stable for up to 240 days at 5 ± 3 °C, with particle sizes of 200–300 nm, low polydispersity (PDI < 0.30; Span ≤ 1), negative zeta potentials (−10 to −30 mV), and particle concentrations of 10^{12}–10^{13} vesicles/mL.
- Cryo-TEM analysis confirmed the spherical morphology of the vesicles and revealed the presence of both unilamellar and oligolamellar structures.
- The formulation significantly sustained DOX release (>24 h), with a biphasic profile consistent with Weibull kinetics (R^2=0.97).
- ^{1}H-NMR confirmed the presence of hydrogen signals corresponding to the GE11 peptide conjugate in the liposomes.
- Confocal microscopy demonstrated preferential liposome uptake by 4T1 cancer cells, with pronounced nuclear localization, consistent with the primary site of DOX action.
- *In vitro* cytotoxicity assays demonstrated that the L-DOX/CUR/DSPE-PEG$_{2000mal}$-GE11 exhibited enhanced cytotoxicity toward 4T1 cancer cells, especially at the higher concentrations tested. No synergistic effect between DOX and CUR could be demonstrated.

GE11-functionalized liposomes co-encapsulating doxorubicin and curcumin represent a promising targeted nanotherapeutic strategy for cancer treatment, combining enhanced antitumor efficacy, reduced chemoresistance, improved drug bioavailability, and lower systemic toxicity through selective EGFR-mediated delivery to tumor cells.

References

Acknowledgments

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