Supplementary Materials

Dimeric c(RGD) peptide conjugated nanostructured lipid carriers for efficient delivery of Gambogic acid to breast cancer

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Supplementary Method

Determination of conjugation efficiency

The extent of coupling of c(RGD) peptides with the DSPE-PEG_{2000} on the surface of NLC was evaluated indirectly by determining the unconjugated free peptide fraction after ultrafiltration of the sample (Amicon ultra, MWCO 10 kDa; Millipore Company) using HPLC method (Agilent 1260). A C_{18} column (Kromasil 100-5-C-18, 250 mm x 4. 6 mm i.d, 5 µm) was used with a mobile phase of 0.1% trifluoroacetic acid in water (eluent A) and 0.1% trifluoroacetic acid in acetonitrile (eluent B). The eluent gradient was set from 15-54% B in 13 minutes for E-[c(RGDfK)$_2$]. For c(RGDfK) peptide, a gradient of 0-50% solvent B in 14 minutes was used. The detection wavelength was 214 nm and 220 nm for c(RGDfK) and E-[c(RGDfK)$_2$], respectively. In order to confirm the sensitivity of the HPLC methods to detect the free peptides in the presence of the test samples (from conjugated), an excess of the free peptide was added to the test sample and analyzed by the HPLC.

Supplementary Tables

Table S1. The weight of excised tumor, ROI, TGI and IRBW of mice treated by the different formulations of GA (Mean ± SD, *n*=3).

Group	Weight of Tumor (g)	ROI (%)	TGI (%)	IRBW (%)
NS	1.18±0.45	-	-	-6.64
Cisplatin	0.54±0.03	54.24	55.95	-0.24
GA-Sol	0.71±0.19	39.83	34.16	-1.29
GA-NLC	0.64±0.19	46.05	44.45	3.71
c(RGDfK)-GA-NLC	0.41±0.07	64.97	52.27	2.05
E-[c(RGDfK)$_2$]-GA-NLC	0.49±0.12	58.76	70.84	7.17

Table S2. The visceral indexes in mice (Mean ± SD, *n*=3).

Group	Spleen (mg/g)	Liver (mg/g)	Kidney (mg/g)	Lung (mg/g)
NS	30.49±0.54	78.68±7.84	16.76±1.72	8.63±1.54
Cisplatin	20.59±1.60	72.90±1.38	15.47±1.19	9.45±0.71
GA-Sol	24.98±5.03	78.85±6.31	19.73±0.84	9.12±0.26
GA-NLC	25.30±5.90	86.66±10.02	19.54±1.71	12.27±2.30
c(RGDfK)-GA-NLC	24.91±2.73	78.28±2.95	18.49±1.65	8.78±2.01
E-[c(RGDfK)$_2$]-GA-NLC	25.02±1.56	81.74±11.72	18.31±6.02	11.21±0.99

Supplementary figures

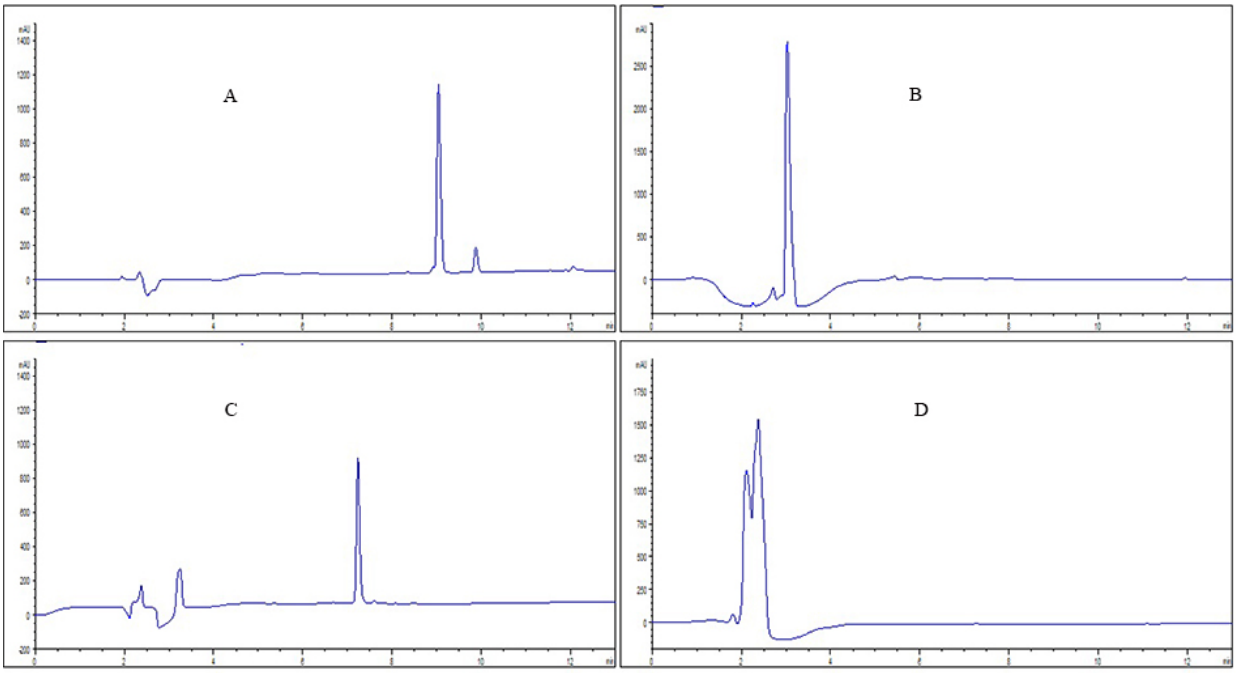

Figure S1: HPLC confirmation of conjugation efficiency c(RGD) to the NLC. (A) Free c(RGDfK), and (B) sample from c(RGDfK) coupled NLC. (C) Free E-[c(RGDfK)$_2$], (D) sample from E-[c(RGDfK)$_2$] coupled NLC.

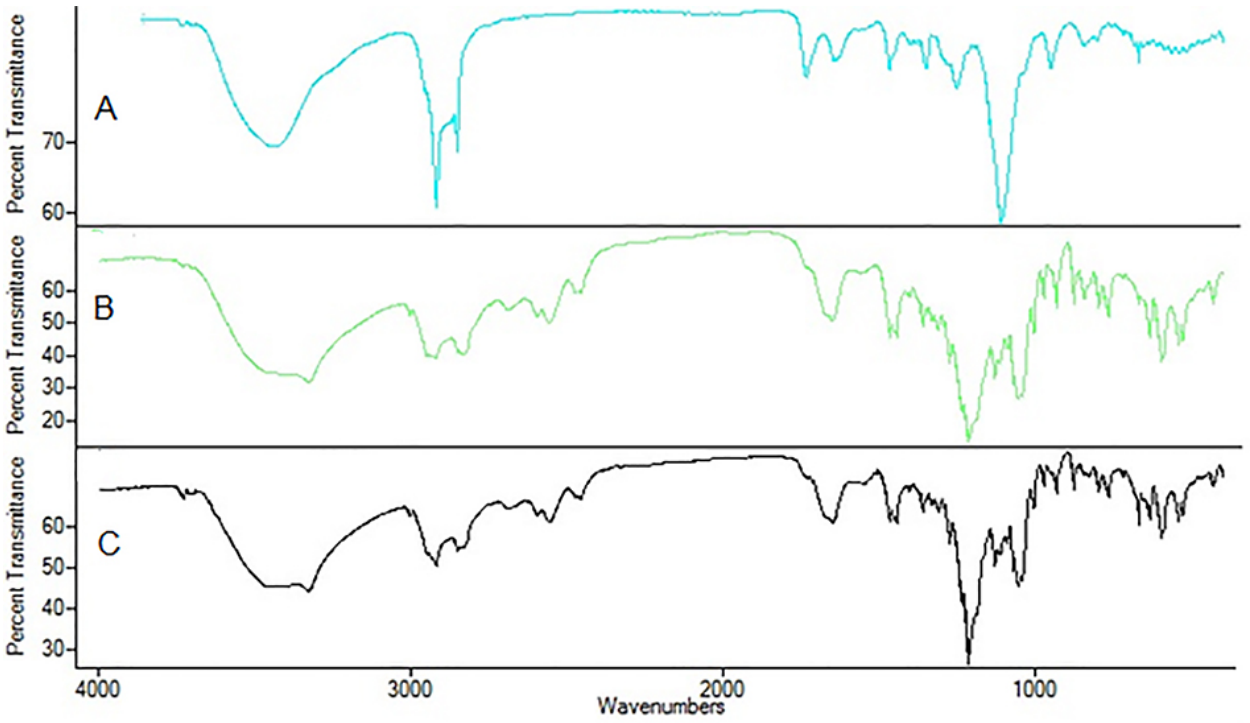

Figure S2: FT-IR spectrum of DSPE-PEG-COOH and c(RGD) peptides conjugated. (**A**) FT-IR spectrum of DSPE-PEG-COOH containing GA-NLLC, (**B**) c(RGDfK) conjugated with DSPE-PEG, (**C**) E-[c(RGDfK)$_2$] conjugated to DSPE-PEG on the surface of GA-NLC. The band at 1736 cm^{-1} belongs to the carboxy (-COOH) group of the DSPE-PEG-COOH on the surface of NLC. The band at 1651cm-1 is corresponding to C=O stretching of the amide bond formed between the –COOH group of DSPE-PEG-COOH and the –NH_2 of c(RGD).

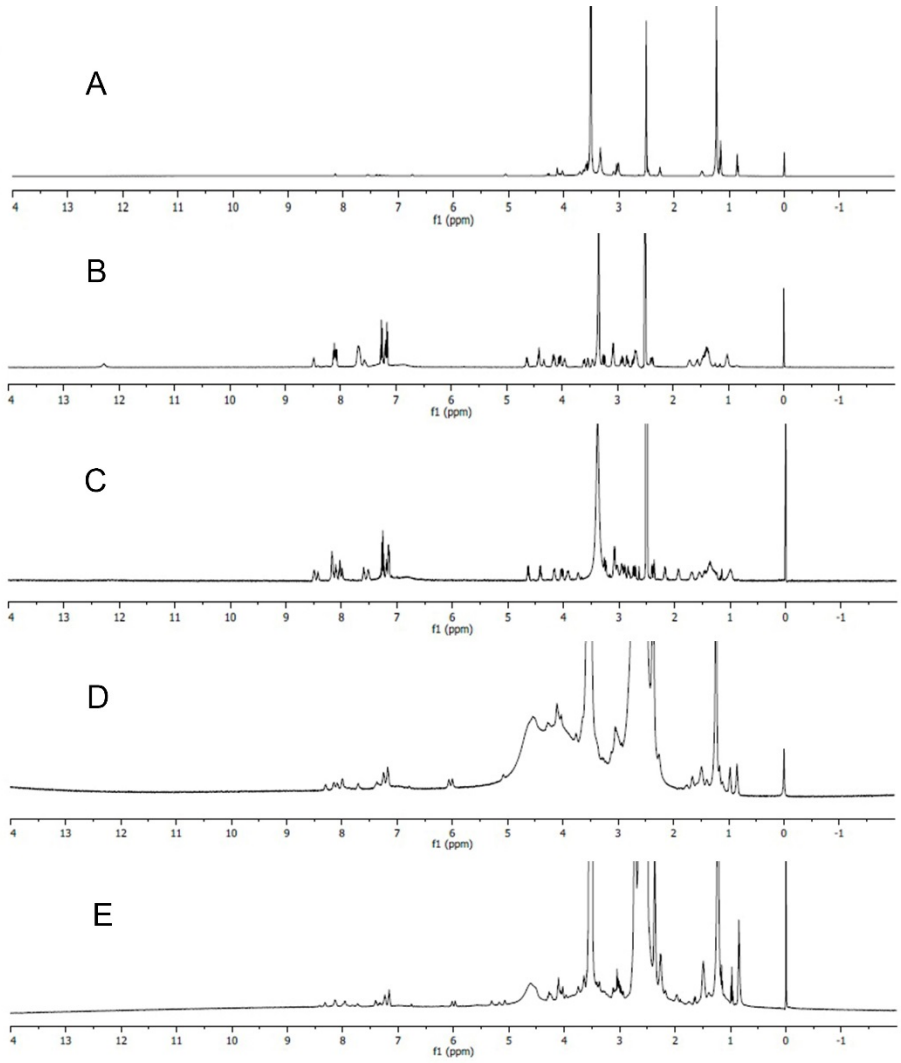

Figure S3: A: The H-NMR spectra of DSPE-PEG-COOH, the proton peaks at 1.23 and 3.52 are corresponding to the DSPE and methylene protons of PEG unit of DSPE-PEG-COOH, respectively. The peak at 2.5ppm was attributed to the solvent (DMSO-d6). **B** and **C** are the H-NMR spectra of c(RGDfK) and E-[c(RGDfK)$_2$], respectively. The chemical shift as a benzene ring and secondary amide (C-NH) of the peptides are observed between 7.1 and 8.6 ppm. **D** and **E** are c(RGDfK) and E-[c(RGDfK)2] conjugated to DSPE-PEG-COOH, respectively. The chemical shift as a benzene ring and secondary amide (C-NH) of the peptides are observed between 7.1 and 8.6 ppm, indicating the successful conjugation of the peptides to DSPE-PEG-COOH.

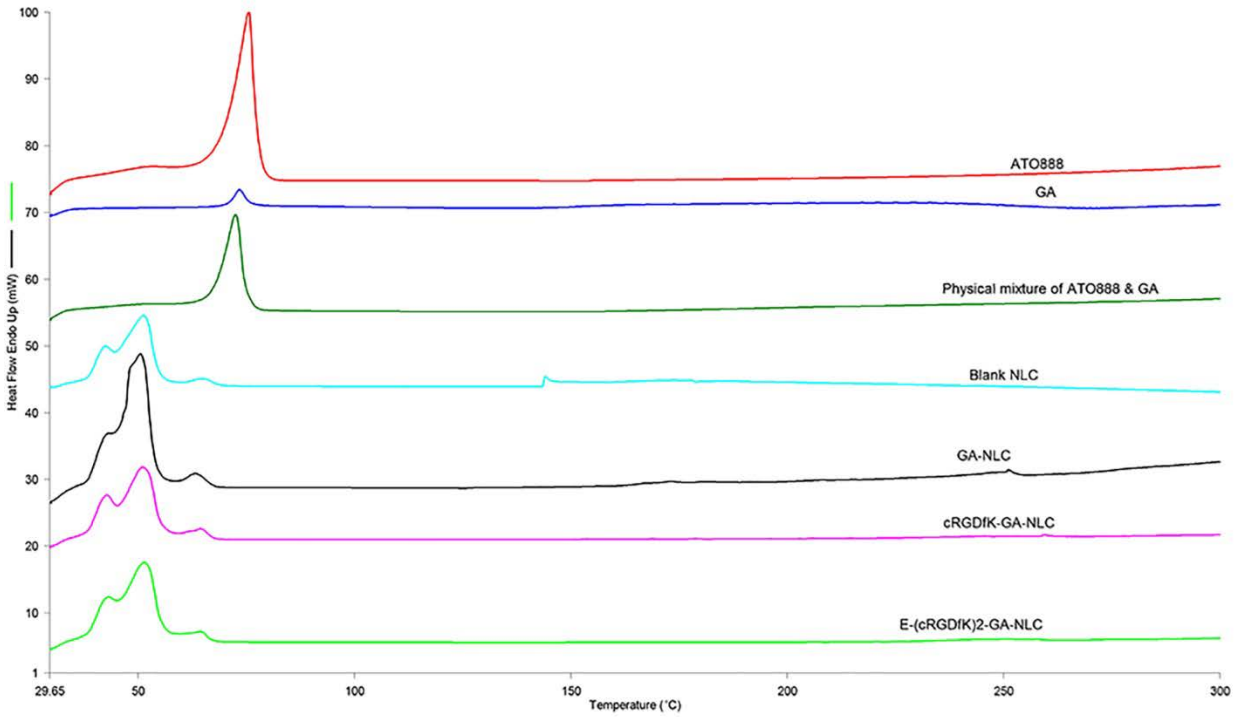

Figure S4: DSC curve of GA, ATO888, physical mixture and GA-NLC. The melting peak of GA is 73.4℃. However, the disappearance of this peak in NLC formulations suggest the successful encapsulation of GA in NLC.

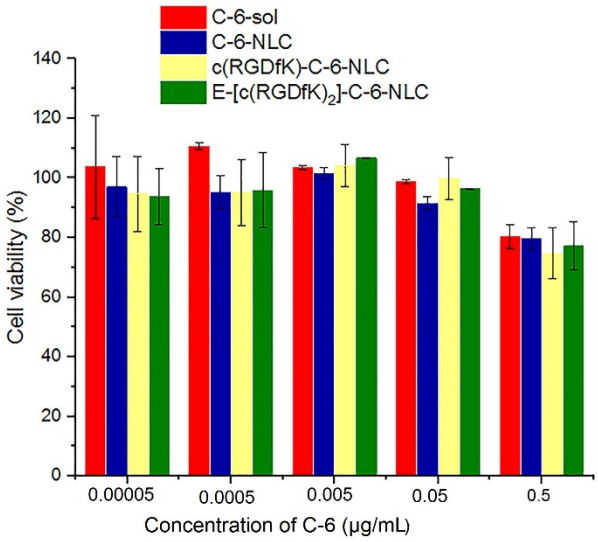

Figure S5: Cytotoxicity study of the different concentration of C-6 labeled NLC formulations. At 0.05µg/mL and below concentration of C-6, no considerable cytotoxicity was observed. Therefore, cell uptake study was conducted at 0.05 µg/mL of C-6.

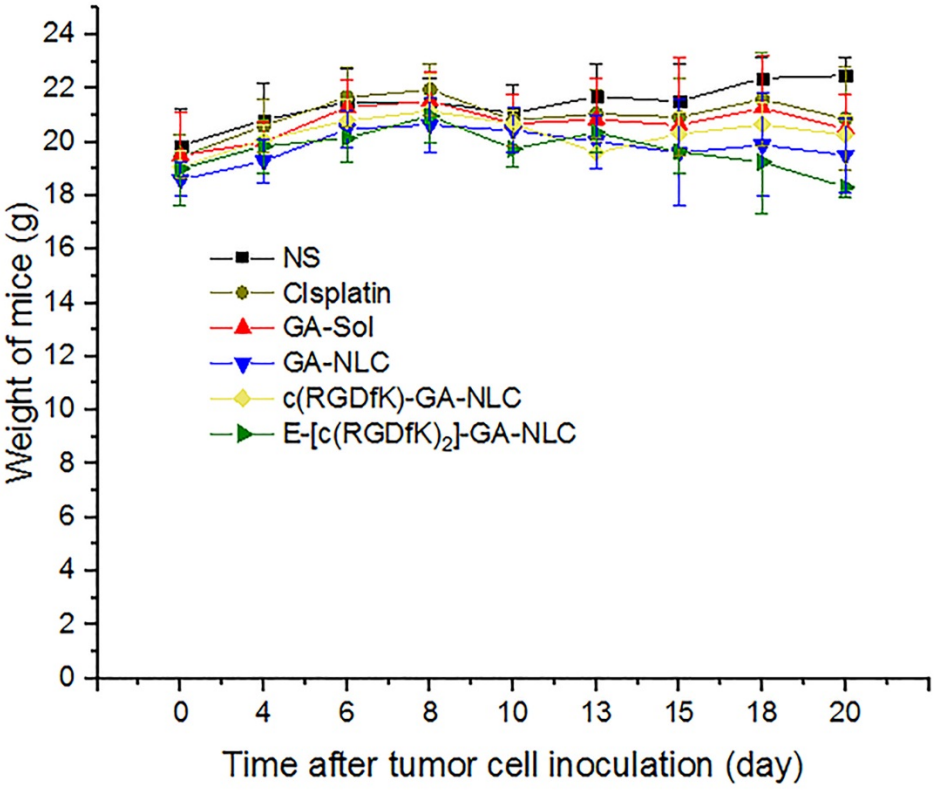

Figure S6: Changes of mice body weight over the treatment course (n=6)