Supporting information

MMP-9 Responsive PEG cleavable Nanovesicles for Efficient Delivery of Chemotherapeutics to Pancreatic Cancer

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SP1: Synthesis and characterization of Lipopeptide

The lipopeptide **LP** [$CH_3(CH_2)_{16}$CONH-GPQGIAGQR$(GPO)_4$GG-COOH] was synthesized by employing microwave assisted solid phase peptide synthesizer (Liberty, CEM Corporation, Matthews, SC) by following the protocol previously established in our laboratory[19]. The lipopeptide was purified by reverse phase HPLC (Shimadzu Scientific Instruments) using a diphenyl semi-preparatory column (Grace Vydac, 300 Å pore diameter silica, 5 μm particle size, 10 mm × 250 mm) as the stationary phase. A linear gradient (0−70%) of acetonitrile (with 0.1% trifluoroacetic acid) in water (with 0.1% trifluoroacetic acid) was used at a flow rate of 8 mL/min over 60 min. The chromatogram was recorded at 235 nm using a UV detector. After freeze drying the eluents, the peptide was characterized employing MALDI-TOF mass spectrometry with an AB 4800 MALDI TOF/TOF mass analyzer. An observed mass of 2332.3 Da in MALDI spectra confirmed the **LP** (calculated mass: 2332.2 Da). The collagen mimetic triple helical structure of the lipopeptide was assessed by CD spectrometry employing a Jasco J-815 CD spectrometer with a quartz cuvette of 1 mm path length. The positive peak at 222 nm and the negative peak at 198 nm confirmed the triple helical structure of collagen mimetic peptide. For the CD spectroscopic studies, 32 accumulations were recorded for each spectra.

SP2: Synthesis of POPE-SPDP derivative

To a stirred solution of POPE (100 mg, 0.139 mmol) in dichloromethane (10 mL), diisopropylethyl amine (33 µL, 0.167 mmol) was added followed by SPDP-OSu (46 mg, 0.1462 mmol). Upon stirring overnight under an inert atmosphere, the reaction mixture was washed with water, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was subjected to flash chromatography (R_f = 0.7 in 15% MeOH in CH_2Cl_2) to afford pure product as a waxy white solid (104 mg, 82%).

^{1}H NMR ($CDCl_3$,400 MHz): δ 0.81-0.89 (m, 6H), 1.2-1.4 (m, 41 H), 1.6 (br s, 4 H), 1.95- 2.05 (q, 4H), 2.25-2.35 (m, 4H), 2.6-2.8 (m, 6H), 3.0-3.1 (m, 2H), 3.41 (s, 2H), 3.8-3.95 (m, 4H), 4.3-4.4 (m, 2H), 5.2 (s, 1H), 5.3-5.4 (m, 2H), 7.12 -7.2 (t, 1H), 7.68-7.8 (m, 2H), 8.4 (d, 2H).

SP3: Synthesis of POPE-S-S-PEG

The product obtained in the previous reaction (35 mg, 0.038 mmol) was reacted with PEG-SH (MW: 5000, 191 mg, 0.038 mmol) in dichloromethane (8 mL) under inert condition for 12 h. The volume of the reaction mixture was reduced under reduced pressure and then subjected to PLC (R_f = 0.8 in 15% MeOH in CH_2Cl_2). The pure product was isolated as a white waxy solid (125 mg, 56%). ^{1}H NMR ($CDCl_3$,400 MHz): δ 0.81-0.89 (m, 6H), 1.19-1.42 (m, 45H), 1.51-1.62 (m, 4H), 1.95-2.05 (q, 4H), 2.24-2.32 (m, 4H), 2.57-2.67 (m, 2H), 2.85-2.9 (t, 1H), 2.91-2.96 (t, 1H), 3.01-3.09 (m, 2H), 3.4-3.5 (m, 2H),3.52-3.75 (m, 307 H)3.8-3.86 (m, 2H), 3.86-4.0 (m, 4H), 4.1-4.2 (m, 1H), 4.32-4.4 (m, 1H), 5.15-5.25 (s, 1H), 5.3-5.4 (m, 2H). ^{13}C NMR ($CDCl_3$,100MHz): δ 13.90, 14.31, 19.27, 22.87, 25.06, 27.43, 29.51, 29.56, 29.87, 29.93, 32.10, 34.3, 59.22, 70.75, 72.13, 129.87, 130.168. MALDI mass spectra also confirmed the conjugation of PEG (Figure S1).

SC1: Calculation for percent entrapment of gemcitabine

To calculate percent drug entrapment, absorbance of liposomes was measured at 268nm (λmax of gemcitabine) before passing through Sephadex column (A1) and after collecting the eluent (A2). Dilution factor (d) was taken into consideration while calculating percent entrapment of the drug.

$$Percent\ Entrapment = \frac{A1 - A2d}{A1} \times 100$$

SC2: Calculation for amount of gemcitabine entrapped in nanovesicles

Gemcitabine was loaded in nanovesicles by pH gradient method. Citrate buffer (pH 4) encapsulated nanovesicles were incubated with gemcitabine, maintaining lipid:drug ratio of 10:1 (for example, 1 mg lipid containing vesicles were incubated with 0.1 mg of gemcitabine). Percent drug entrapment was calculated by using equation given in SC1. Percent entrapment of 50% indicated that 50 µg of gemcitabine is encapsulated in nanovesicles containing 1 mg equivalent of lipid.

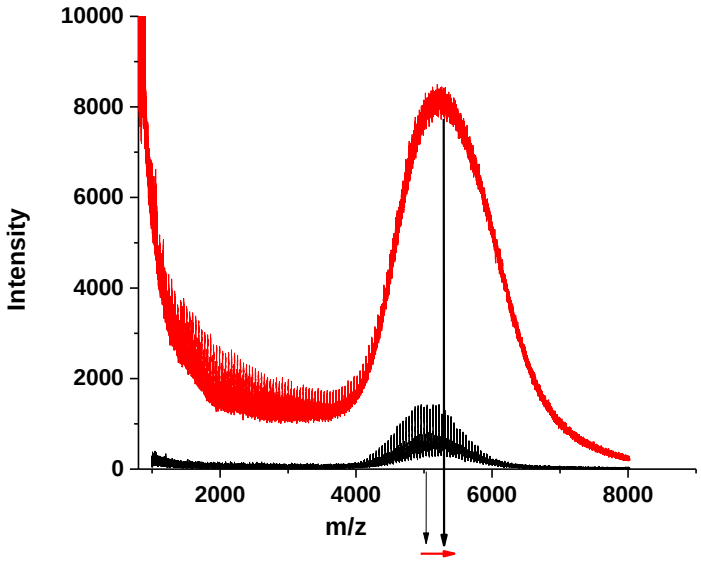

Figure S1. Overlay plot of MALDI spectra indicating increase in mass of PEG_{5000} (black) after successful synthesis of POPE-SS-PEG_{5000} (red)

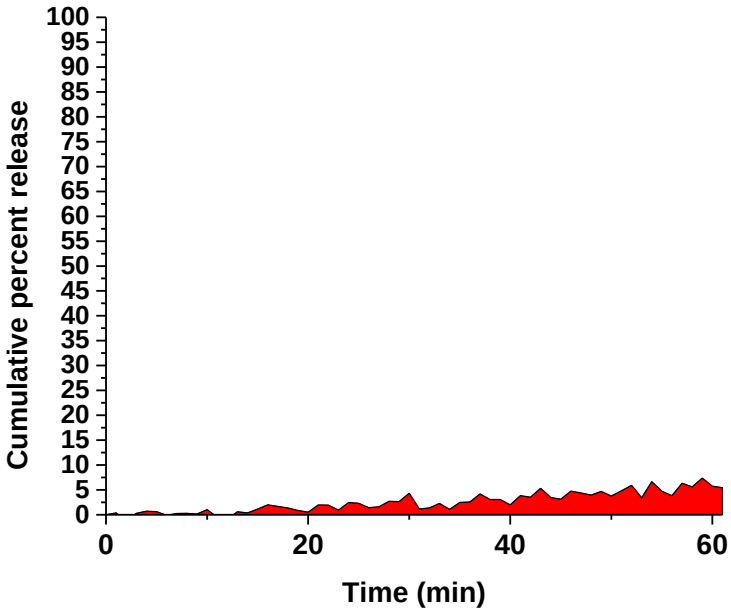

Figure S2. Cumulative percent release of carboxyfluorescein from nanovesicles was observed to be less than 5 (area represented in red) in 60 min in the presence of 10% human serum which was suggestive of stability of nanovesicles in circulation.

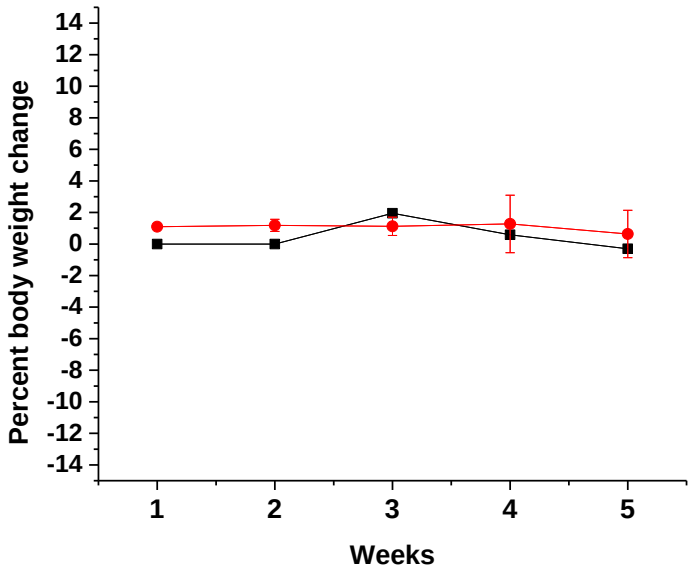

Figure S3. Body weight changes for mice under study were monitored over 5 weeks during the treatment. Weight loss of more than 15% was set as reference for toxicity. However, no significant weight loss was observed in control (black) as well as gemcitabine nanovesicles treated group (red).

Table S1 Release studies at 37 ºC

Treatment	Time (min)	Percent release
GSH (2 µM)	60	15
GSH (50 µM)	60	22
MMP-9 (2 µM)	60	43
MMP-9 (2 µM) and GSH (50 µM)	60	58

Table S2 Release from liposomes in spent media of cells

Conditioned media of cell line	Percent release in 1 hour
Brain endothelial cell line (does not secret MMP-9)	15
PANC-1	28

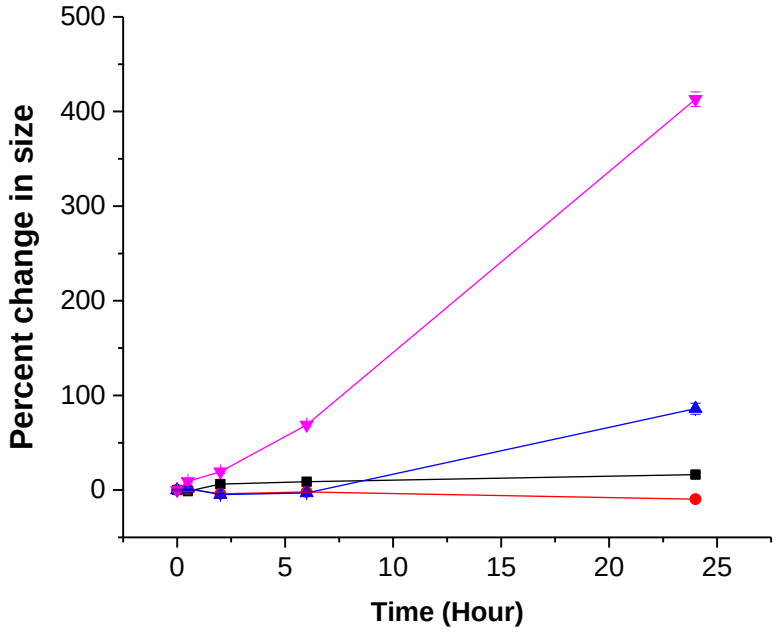

Figure S4. Effect of MMP-9 and GSH treatments on the size of nanovesicles at 37 ºC. Nanovesicles treated with MMP-9 (2 µM) and GSH (50 µM) showed significant increase in size in 24 hours (magenta triangles). Nanovesicles receiving only MMP-9 (2 µM) treatment also showed some increase in size within 24 hours (blue triangles). No substantial change in size was observed when nanovesicles received no treatment (black squares). Treatment with GSH (50 µM) showed a slight decrease in size over 24 hours (red circles).

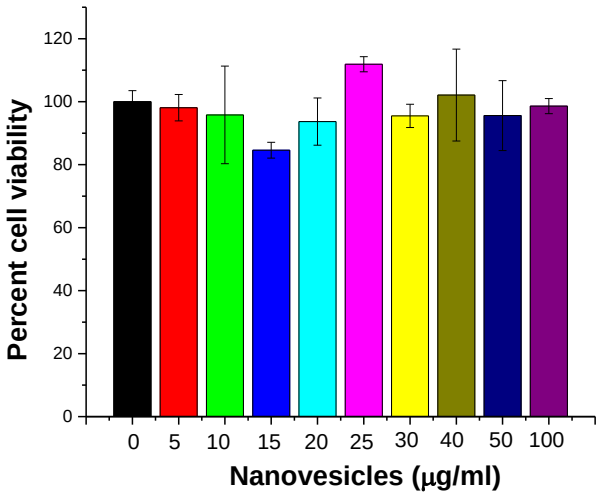

Figure S5. Toxicity of nanovesicles. Nanovesicles did not show any toxicity when incubated with MIAPaca-2 cells for 72 hours