Supplementary Section

Supplemetary 1.1. In vitro serum stability study.

Stability of polymeric nanoparticles in the presence of serum proteins was evaluated by dispersing them in DMEM media supplemented with 10% FBS, at a final polymer concentration of 1 mg/mL, and determining the particle size. Briefly, 0.1 mL of a 1mg/mL nanoparticle suspension was resuspended in 3 mL of DMEM at 37 ± 1 °C. The particle size of the nanoparticles was determined periodically after 0.5, 1, 3, 6, 9, and 24 h by DLS using a Zetasizer.

R**esults:**

In vitro serum stability study Nanoparticle stability is an important factor for the clinical use of dual loaded PLA-PEG-PPG-PEG NPs because nanoparticles could aggregate and clog blood vessels, resulting in severe complications. The stability of tetrablock polymeric NPs and dual loaded PLA-PEG-PPG-PEG NPs was studied in DMEM containing 10% FBS at 37 °C to simulate nanoparticle stability in blood and in cell culture experiments. As shown in Table S1, the nanoparticles has not shown any significant increase in particle size in DMEM, with an average particle size remained below 160 nm up to 24 h, indicating the stability of the nanoparticles.

Table S1. *In vitro* serum stability studies

Time (h)	NPs		PTX-NuBCP9 dual NPs	
	Size (nm)	PDI	Size (nm)	PDI
0.5	110 ± 2.9	0.371 ± 0.010	142 ± 1.8	0.422 ± 0.046
1	115 ± 0.7	0.211 ± 0.007	144 ± 1.4	0.541 ± 0.031
3	118 ± 1.4	0.236 ± 0.041	149 ± 1.7	0.482 ± 0.041
6	115 ± 1.8	0.295 ± 0.014	151 ± 2.3	0.363 ± 0.092
9	110 ± 0.6	0.384 ± 0.037	157 ± 0.9	0.325 ± 0.012
24	110 ± 3.0	0.272 ± 0.009	162 ± 0.5	0.227 ± 0.016

Supplementary 1.2. Hemolysis Study.

Hemolysis study was carried out on RBC obtained after centrifuging whole blood at 1500 rpm for 10mins. The concentrated stock of RBC was washed three times with PBS (pH-7.4). A diluted stock of RBC was prepared by diluting concentrated stock of RBC to 10^3 times. 100 µl of PLA-PEG-PPG-PEG NPs stock (1 & 2 mg/ml) was incubated with 100 µl of RBC (diluted stock) at 37±1 °C for 1 h at 120 rpm in an incubator cum shaker. After 1 h, the mixture was centrifuged at 1500 rpm for 5 min, and the supernatant was analysed for the amount of released hemoglobin using an UV−Vis spectrophotometer by taking absorbance at 540 nm. The percent of hemolysis was calculated relative to hemolysis caused by positive (1% Triton X-100) and negative (PBS) controls using following equation.

$$\text{Hemolysis (\%)} = \left[\frac{\text{Sample}_{540} - \text{Negative control}_{540}}{\text{Positive control}_{540} - \text{Negative control}_{540}} \right] \times 100 \quad \ldots\ldots\ldots\ldots(1)$$

Results

Hemolysis Study. NPs on intravenous (I/V) injection will interact with various blood components including RBCs such interaction should not have any detrimental effects including hemolysis. Hemolysis study of PLA-PEG-PPG-PEG NPs was determined UV−visible spectrophotometry at 540 nm after incubating polymeric nanoparticles (at concentration 1 and 2 mg/mL) with RBCs. Hemolysis levels less than 20% are considered acceptable for nanoparticle formulations[1,2]. Polymeric nanoparticles exhibited low hemolytic activity for the concentrations studied. At a concentration of 1 mg/mL, 1.9% hemolysis was observed, while with 2 mg/mL, 4.5% hemolysis occurred as shown in Figure S2. Also, the treated samples were visualised under confocal light microscopy. Thus, the PLA based polymeric nanoparticles showed no significant blood hemotoxicity and hence are biocompatible with respect to

hemolysis.

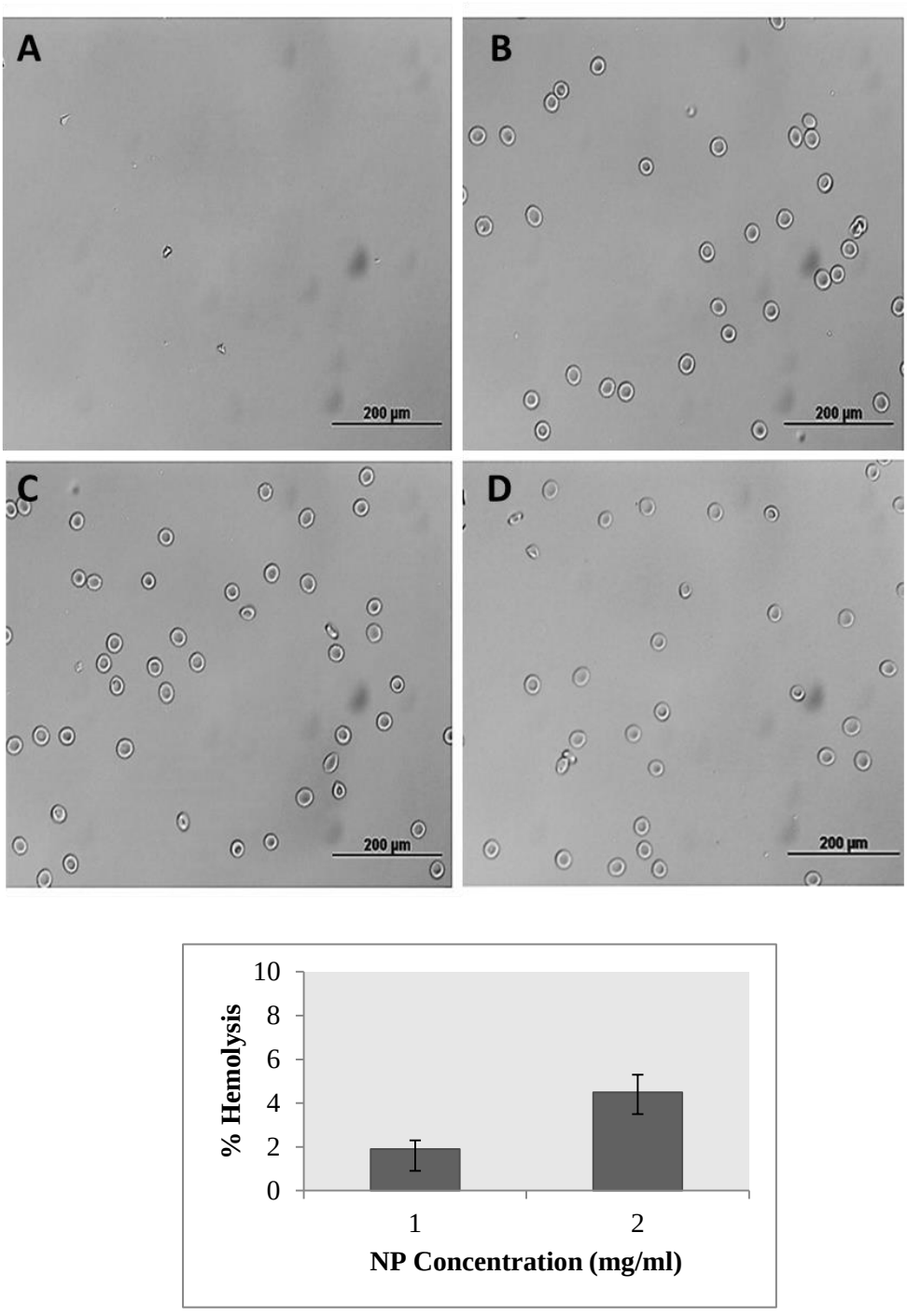

Figure S1 Hemolytic studies. Bright field microscopic images (A) Triton X (control) (B) RBC (PBS) (C) 1mg/ml PLA-EG-PPG-PEG NPs (D) 2mg/ml PLA-EG-PPG-PEG NPs. (E) Percent hemolysis with different concentrations of polymeric nanoparticles. (Mean ± SD, n = 3.)

Table S2 Flow cytometry assessment of apoptosis and necrosis of MCF7 cells after treatment with different nanoformulations

Samples	% Live cells (Annexin V^- /PI^-)	% Necrotic damage cells (Annexin V^+/PI^+)	% Late apoptosis/ necrotic cells (Annexin V^+/PI^+)	% Early apoptois (Annexin V^+/PI^-)
PTX-NuBCP9 NPs	8.86	1.94	71.35	17.85
NuBCP9 NPs	36.1	2.1	19.4	42.4
PTX- NPs	12.11	6.09	24.35	57.45
NPs- NPs	98.6	0.69	0.25	0.46

References

1. Amin K, Dannenfelser R. M. In Vitro Hemolysis: Guidance for the Pharmaceutical Scientist. *J. Pharm. Sci.* 2006; **95**: 1173−1176.

2. Krzyzaniak J.F, Nuunnez F.A.A, Raymond D.M, Yalkowsky S.H. Lysis of human red blood cells. Comparison of *in vitro* and *in vivo* hemolysis data. *J. Pharm. Sci.* 1997; **86**:1215−1217.