Supplementary data

pH-triggered delivery of Pirarubicin-Gemcitabine Duo via polymeric nanoparticles for synergistic breast cancer therapy

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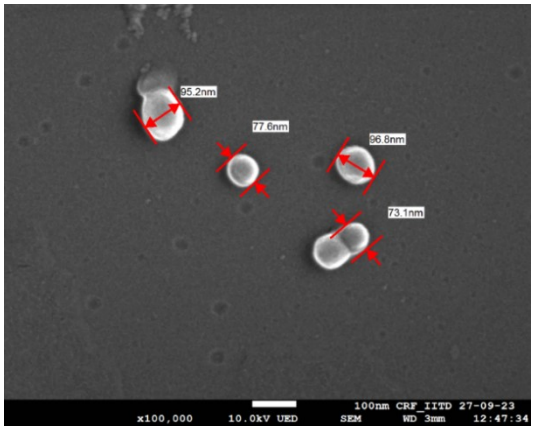


Fig. S1 FE-SEM image of mPEG-b-PLA-LA nanoparticles

Table S1. Calculation of Number Average Molecular weight (M_n) and Degree of polymerization (DP) of mPEG-b-PLA via ^{1}H NMR spectroscopy

Polymer	Feed Ratio LA/EO	^{1}H NMR Ratio LA/EO	DP	Mn
mPEG-b-PLA	0.30	0.21	18	6296

DP_{PLA} of mPEG-b-PLA = DP_{PEG} × (4× Integral of 1.6 ppm /3× Integral of 3.6 ppm)

DP_{PEG} = 113

Mn of mPEG-b-PLA = Mn of mPEG + (72 × DP_{PLA})

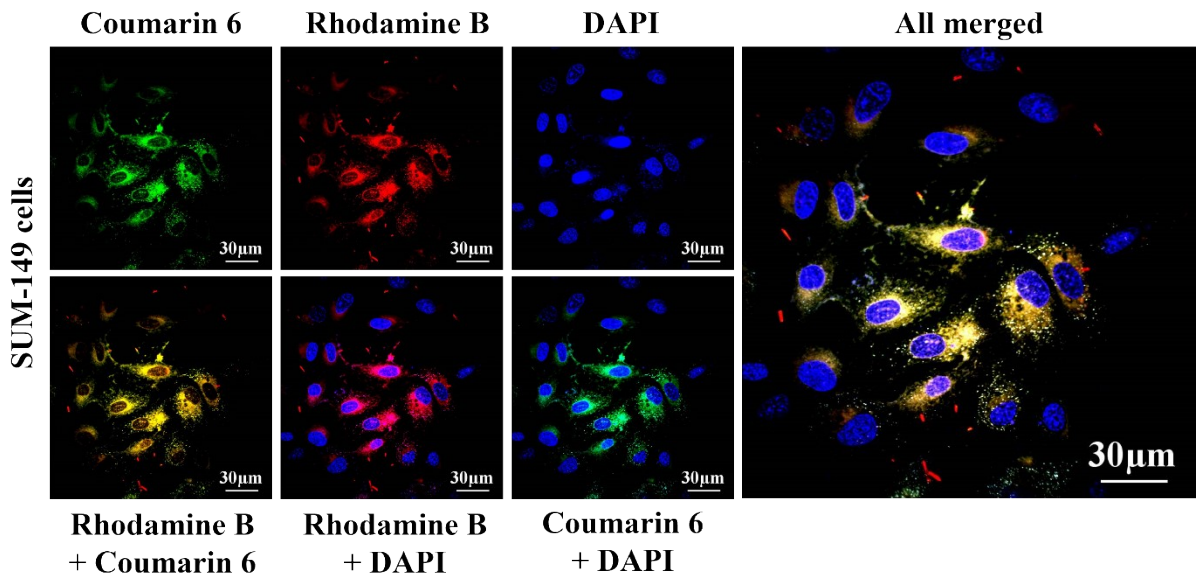
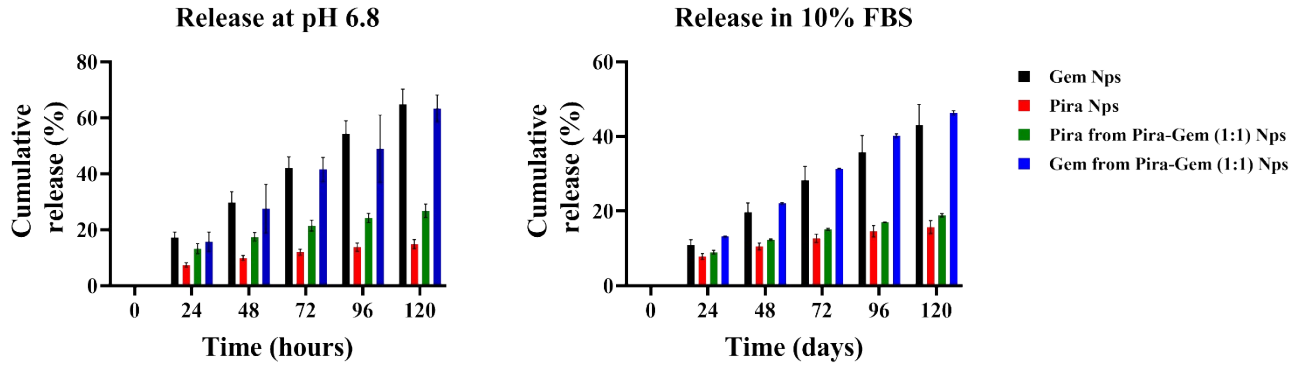


Fig. S2 Confocal laser scanning microscopy (CLSM) images showing cellular uptake of dual dye-loaded mPEG-b-PLA-LA nanoparticles (Rhodamine B and Coumarin-6) in SUM-149 cells. Rhodamine B (red) and Coumarin-6 (green) indicate nanoparticle distribution, while DAPI (blue) stains the cell nucleus. Images were acquired at 60× magnification

Fig. S3 *In-vitro* release of Pira/Gem single vs dual-loaded mPEG-b-PLA-LA nanoparticles at pH 6.8 and in 10% FBS solution. n = 3, p value > 0.05 = ns



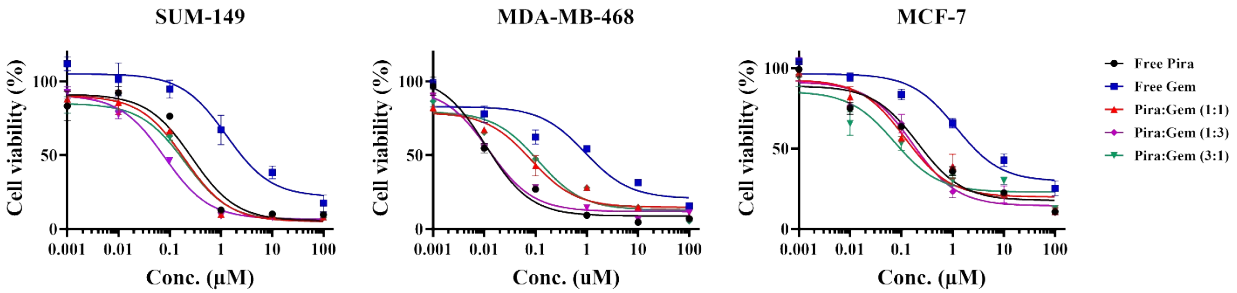


Fig. S4 IC_{50} sigmoidal curves of free Pira/Gem and Pira-Gem combination on breast cancer cells plotted using GraphPad Prism V9.0, n=3

Table S2. IC_{50} values of free Pira/Gem and their combinations with respect to Pira concentration.

Drug/Drug combination	SUM-149	MDA-MB-468	MCF-7
Free Pira	0.2690	0.0108	0.2093

Free Gem	1.2770	0.9262	1.1730
Pira-Gem (1:1)	0.1943	0.0818	0.1190
Pira-Gem (3:1)	0.0793	0.0121	0.0723
Pira-Gem (1:3)	0.0186	0.1126	0.1671

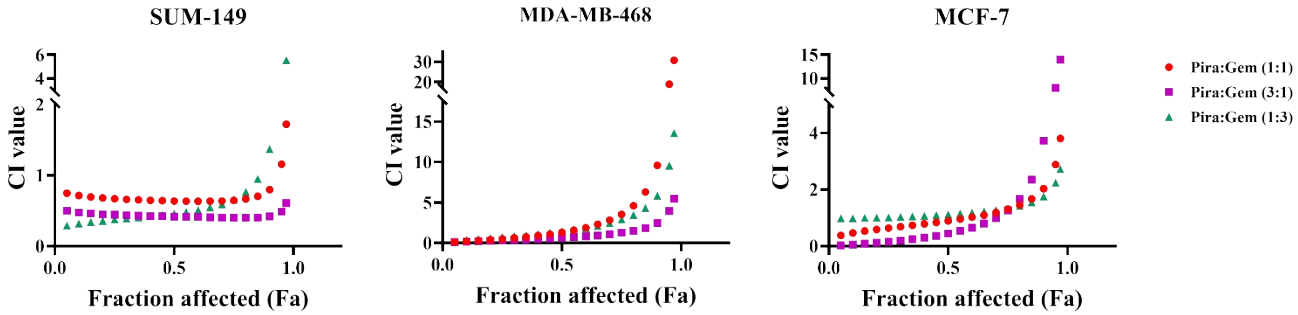


Fig. S5 Fraction affected (Fa) vs CI values scatter plots of free Pira/Gem combinations on different breast cancer cells

Table S3. Combination Index (CI values) of free Pira/Gem drug combinations

Drug combination	SUM-149	MDA-MB-468	MCF-7
Pira-Gem (1:1)	0.638	1.310	0.890
Pira-Gem (3:1)	0.418	0.620	0.440
Pira-Gem (1:3)	0.459	1.410	1.110