Electronic Supplementary Information

Electrospray based synthesis of fluorescent poly (D, L-lactide-co-glycolide) nanoparticle for the efficient delivery of anticancer drug and self-monitoring its effect in the drug-resistant breast cancer cells

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Calculation of yield of each step towards the synthesis of PLGA-PBA@MTX nanoparticles

Yield calculation after synthesis of EDA conjugated PLGA polymer :

To conjugate EDA with PLGA polymer, 1.0 gm PLGA was dissolved in 8.0 ml DCM. Then, DCC/NHS (1:1) having 10 times excess molar ratio than PLGA was added to the PLGA solution and stirred for 3 hours (h). EDA in DCM solution was added drop wise to the activated PLGA solution (molar ratio of PLGA: EDA was 1:2) under the continuous stirring condition at room temperature. After the overnight reaction, the amine-terminated PLGA polymer was precipitated.

Initial amount of precursor material = PLGA +EDA

= 0.4 + 0.00144

= 0.40144 g

After conjugation of EDA with PLGA polymer = 0.357 g

$$Yield\ (\%) = \frac{Amount\ of\ EDA\ conjugated\ PLGA\ polymer}{Initial\ amount\ of\ precursor\ material} X100$$

= 88.92 %

Yield calculation after synthesis of 1-pyrenebutyiric acid terminated PLGA polymer :

The EDA conjugated PLGA polymer was re-dissolved in DCM and then drop wise added into DCC/NHS activated PBA solution in DCM (molar ratio of PLGA: PBA was 1:5) under continuous stirring at room temperature. After overnight stirring, the PBA conjugated PLGA polymer (PLGA-PBA) was precipitated.

Initial amount of precursor material = EDA conjugated PLGA polymer +PBA

= 0.357+0.0145

= 0.3715 g

After PBA conjugation, amount of PLGA-PBA polymer = 0.32 g

$$Yield\ (\%) = \frac{Amount\ of\ PLGA-PBA\ polymer}{Initial\ amount\ of\ precursor\ material} X100$$

= 86.13 %

Yield calculation of PLGA-PBA@MTX nanoparticles after conjugation of Methotrexate with PLGA-PBA Nanoparticles:

EDA is first bonded to the PLGA-PBA nanoparticle by aminolysis of the ester linkage of PLGA backbone. For the aminolysis, the electrospray synthesized PLGA-PBA nanoparticles were incubated with 5.0 mM EDA for 8 min and immediately centrifuged at 18000 rpm for 10 min at 15°C to separate the excess EDA. The EDA conjugated PLGA-PBA

nanoparticle pellet was re-suspended in HPLC water. On the other hand, 7.0 mM aqueous MTX solution was activated by EDC/NHS (1:4). The activated MTX was added to the EDA conjugated PLGA-PBA nanoparticle suspension under continuous stirring for 5 h. This final conjugate was centrifuged at 18000 rpm for 10 min at 15°C to separate the unreacted MTX if any. The MTX conjugated PLGA-PBA nanoparticles (PLGA-PBA@MTX) were washed with HPLC water for three times.

Initial amount of precursor material = PLGA-PBA nanoparticles +EDA+MTX

= 2.5+0.601+1.3

= 4.401

After MTX conjugation, amount of PLGA-PBA@MTX nanoparticles = 3.3 mg

$$Yield\ (\%) = \frac{Amount\ of\ PLGA-PBA@MTX\ nanoparticles}{Initial\ amount\ of\ precursor\ material} X100$$

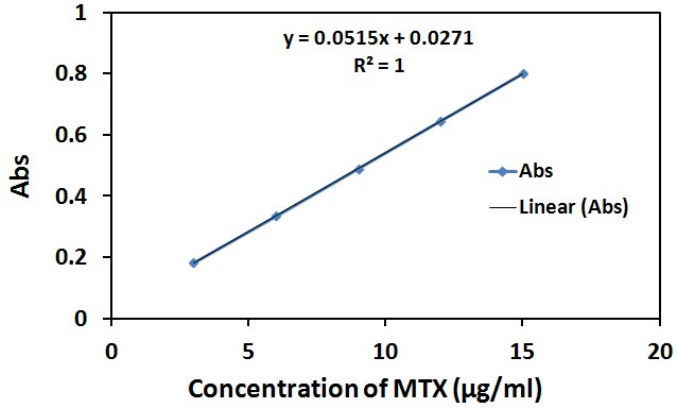
= 74.98%

Drug loading and Conjugation efficiency measurements

Theoretical amount of initial 1.30 mg MTX present in 3.3 mg PLGA-PBA@MTX nanoparticles and 1.188 mg MTXpresent in final 3.3 mg PLGA-PBA@MTX nanoparticles (after synthesis and washing)

Absorbance of MTX in PLGA-PBA@MTX nanoparticles = 0.6389

Calibration curve of MTX in HPLC water to determine the concentration of MTX from the corresponding absorbance



y=0.0515x+0.0271

x=1.88 µg/ml

10 µl contains 1.188 µg

$$Drug\ loading\ (\%) = \frac{Conjugated\ amount\ of\ MTX}{Weight\ of\ nanoparticles} X100$$

$$= \frac{1.188}{3.3} X100$$

=36%

$$Conjugation\ Efficiency\ (\%) = \frac{Conjugated\ amount\ of\ MTX}{Initial\ amount\ of\ MTX}\ X\ 100$$

$$= \frac{1.188}{1.30} X100$$

=91.38%

Table S1. Hydrodynamic size and zeta (ζ) potential of PLGA-PBA nanoparticle and PLGA-PBA@MTX nanoparticle.

Nanoparticle type	Size (nm)	Charge (mV)
PLGA-PBA nanoparticles	71.0	-13.7
PLGA-PBA@MTX nanoparticles	252.1	-38.5

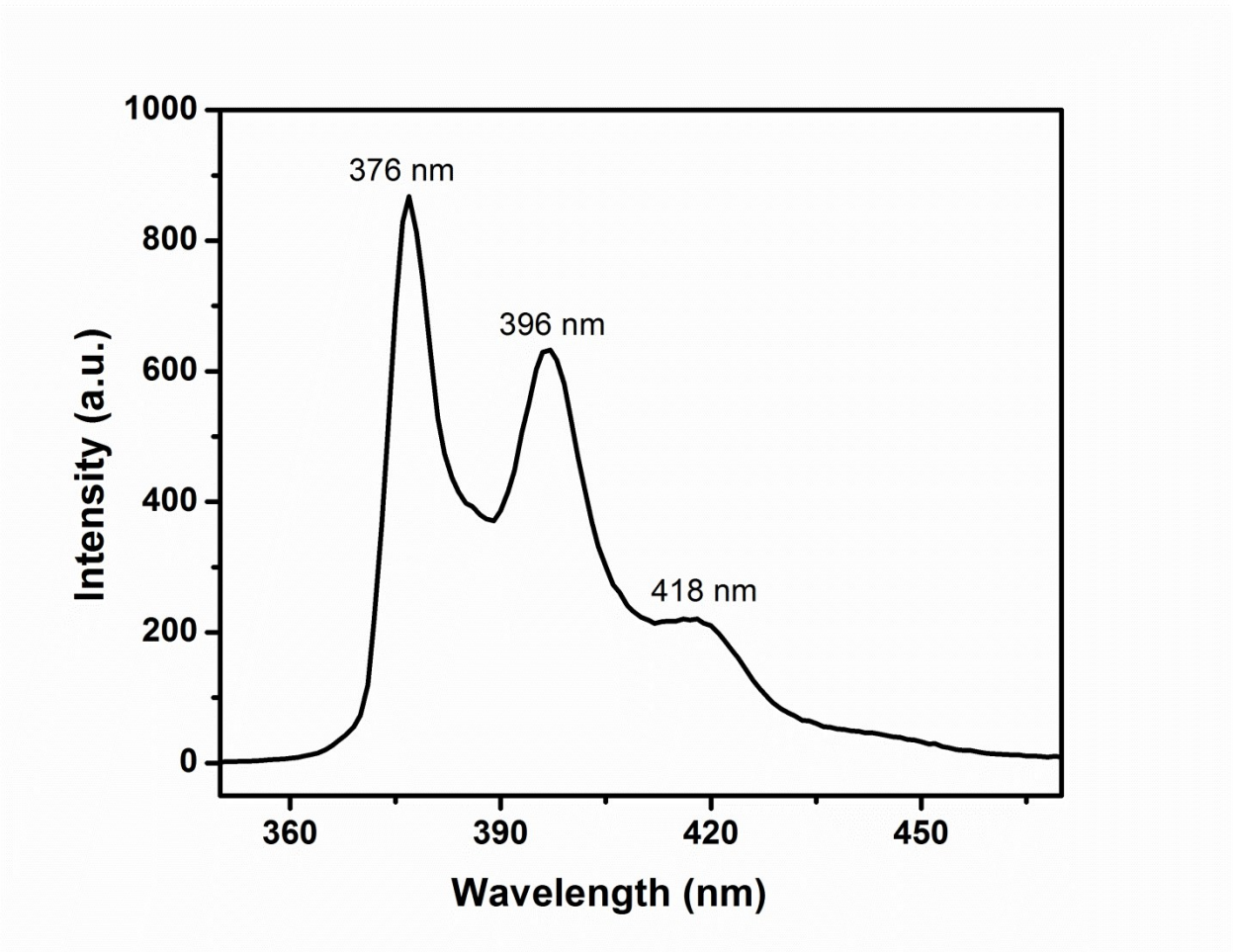


Fig. S1 Fluorescence spectrum of 1-Pyrenebutyiric acid (PBA) exhibited three emission peaks at 376 nm, 396 nm and 418 nm when excited at 240 nm.

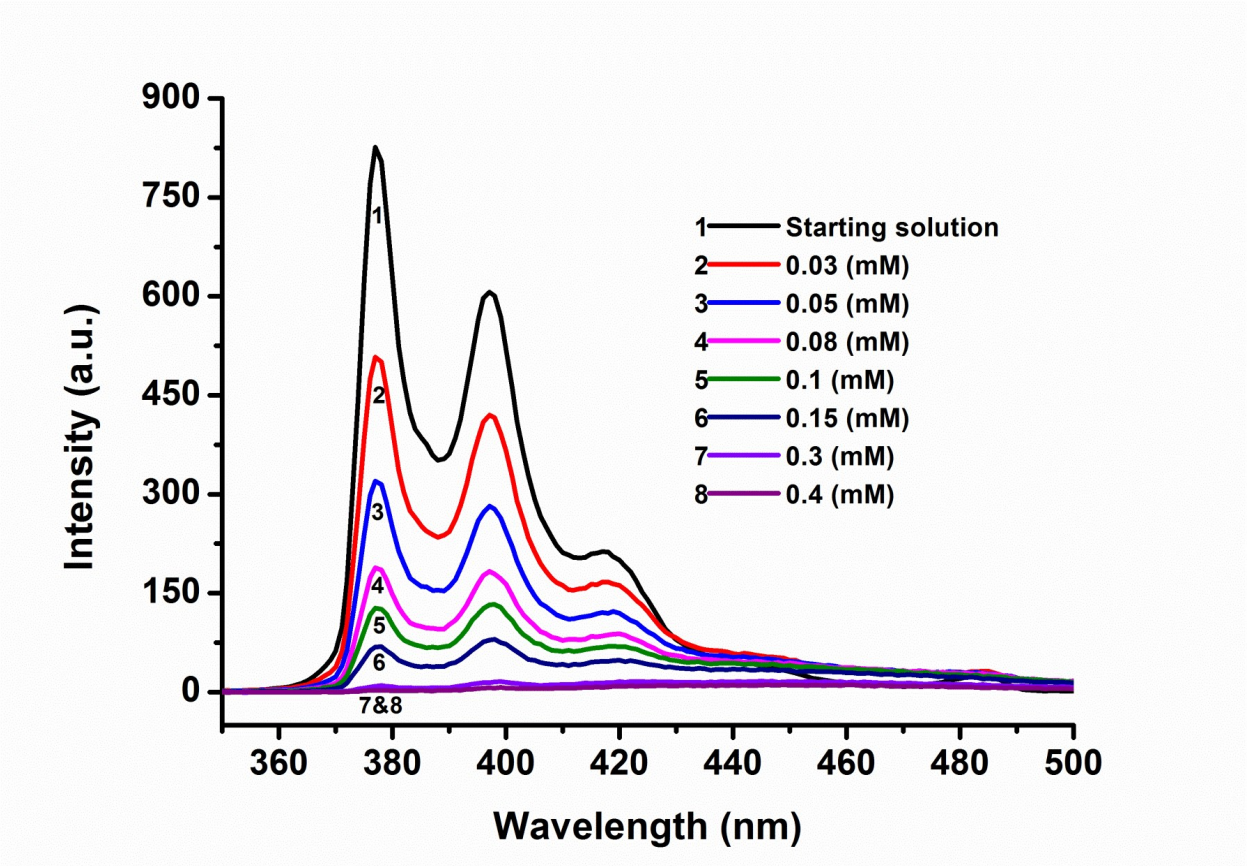


Fig. S2 Quenching of fluorescence of PBA with the increasing concentration of MTX from 0.03 mM to 0.4 mM.

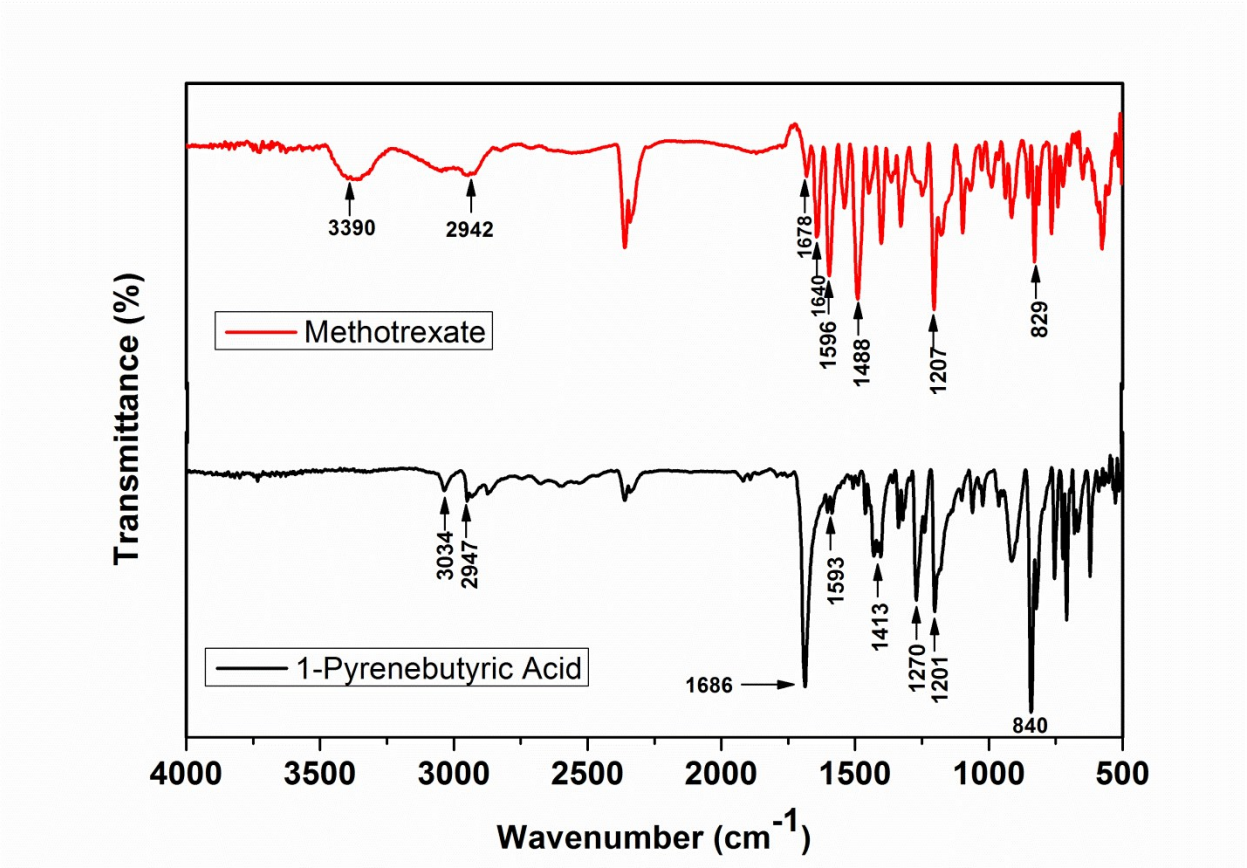


Fig. S3 FTIR spectrum of methotrexate (MTX) molecule shows characteristic stretching vibration of two amine groups, an aromatic pteridine ring and a p-amino benzoic acid at 3390 cm^{-1}, 1640 cm^{-1} and 1678-1488 cm^{-1} respectively, and 1-Pyrenebutyiric acid (PBA) shows an intense peak for the carbonyl group of PBA–COOH at 1686 cm^{-1} and the structure.

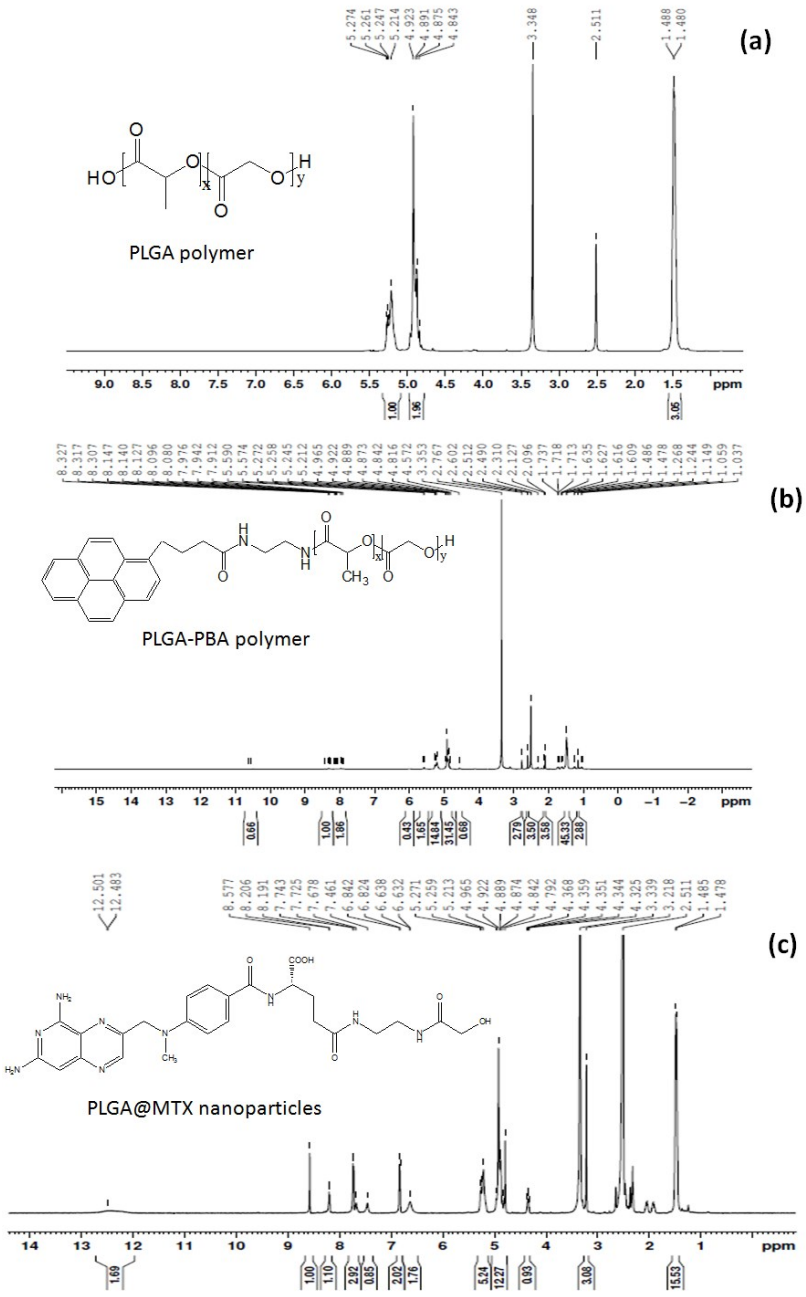


Fig. S4 ^{1}H NMR analysis of all the conjugation steps i.e. (a) PLGA polymer, δ (ppm) =1.48 (3H, CH_3), 4.89 (2H, CH_2), 5.24 (1H, CH).
(b) PLGA-PBA polymer, δ (ppm) =1.48 (CH_3 of PLGA), 2.09, and 2.6, 2.7 (CH_2 of PBA), 4.89 (CH_2 of PLGA), 5.24 (CH of PLGA), 5.57-5.59 (CH_2 of EDA), 7.91-8.42 (CH of PBA), 10.56-10.61 (NH of EDA).
(c) PLGA-PBA@MTX nanoparticles, δ (ppm) = 1.48 (CH_3 of PLGA), 3.21 (N-CH_3 of MTX), 4.79 (CH_2 of EDA and MTX), 4.89 (CH_2 of PLGA), 5.24 (CH of PLGA), 6.63 (NH_2 of MTX), 7.46-7.67 (NH of EDA), 7.67-8.2 (CH of aromatic pteridine ring and p-aminobenzoic acid), 8.57 (1CH of pteridine ring), 12.48-12.5 (COOH of MTX).

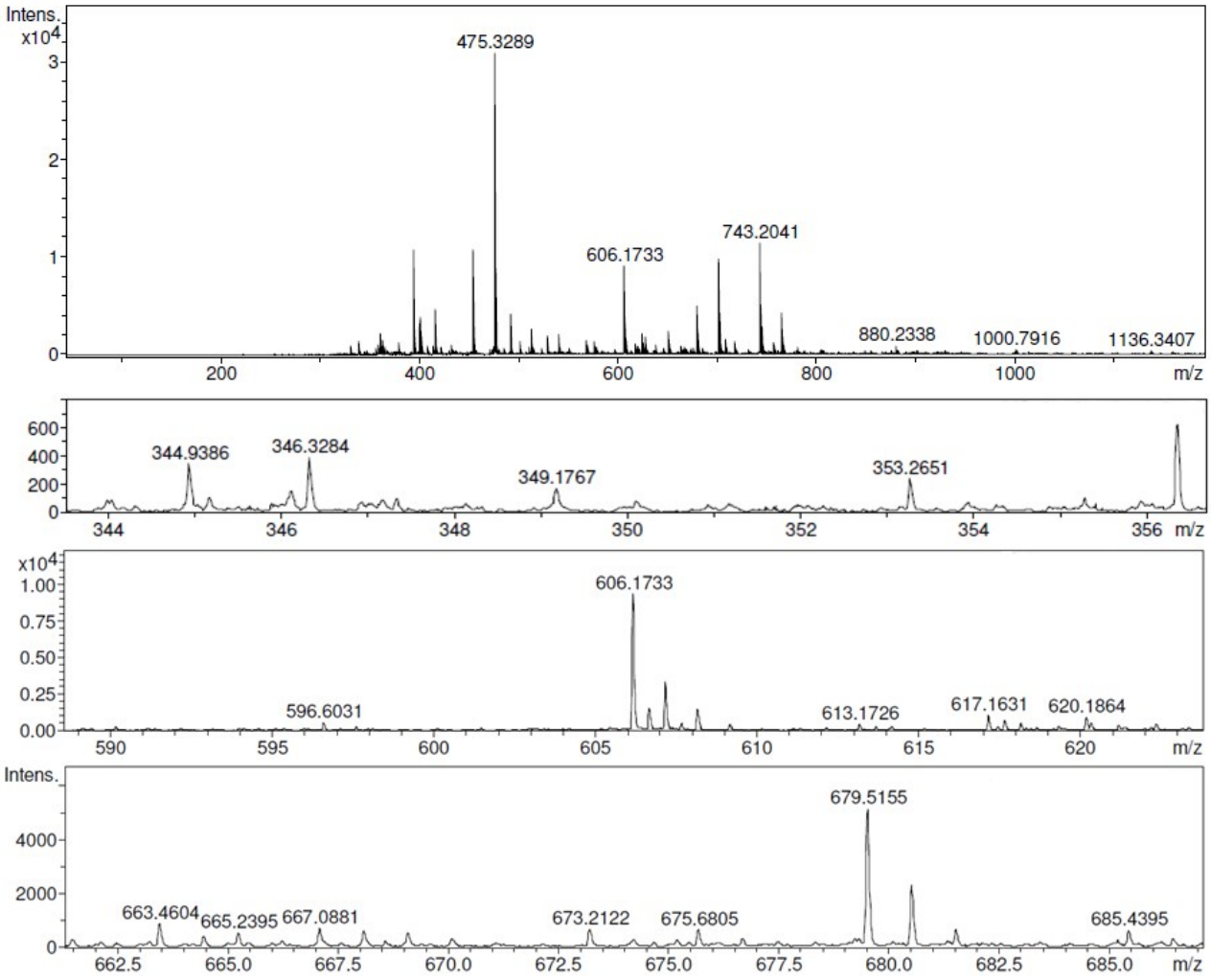


Fig. S5 Mass spectrum of PLGA-PBA polymer shows peak of $[PBA\text{-}EDA]^+$, $[PBA\text{-}EDA\text{-}(LA)_1\text{-}(GA)_1]^+$, $[PBA\text{-}EDA\text{-}(LA)_2\text{-}(GA)_2]^+$ and $[PBA\text{-}EDA\text{-}(LA)_3\text{-}(GA)_2]^+$ ions at 346.32, 475.32, 606.17 and 679.51 m/z respectively.

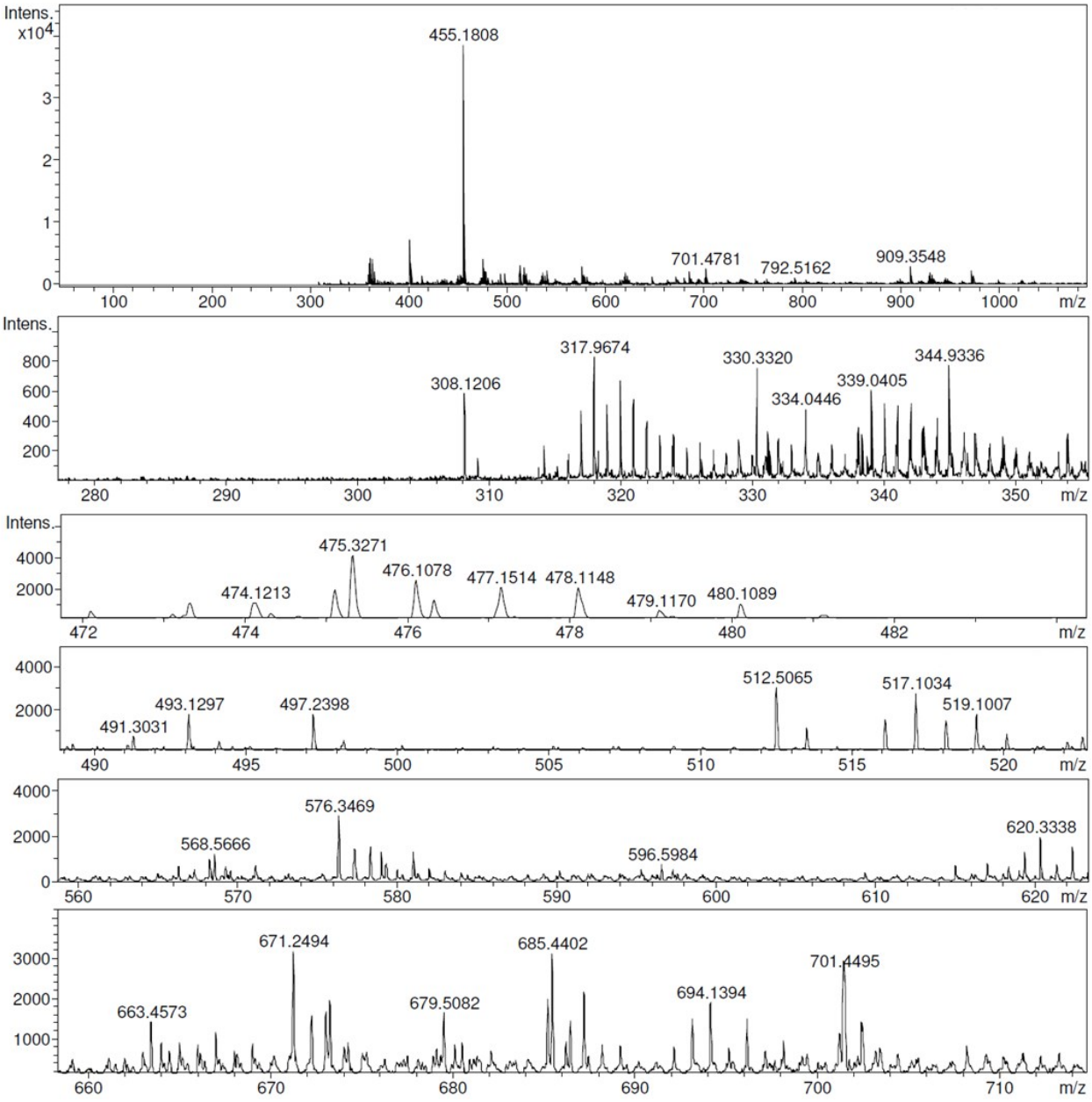
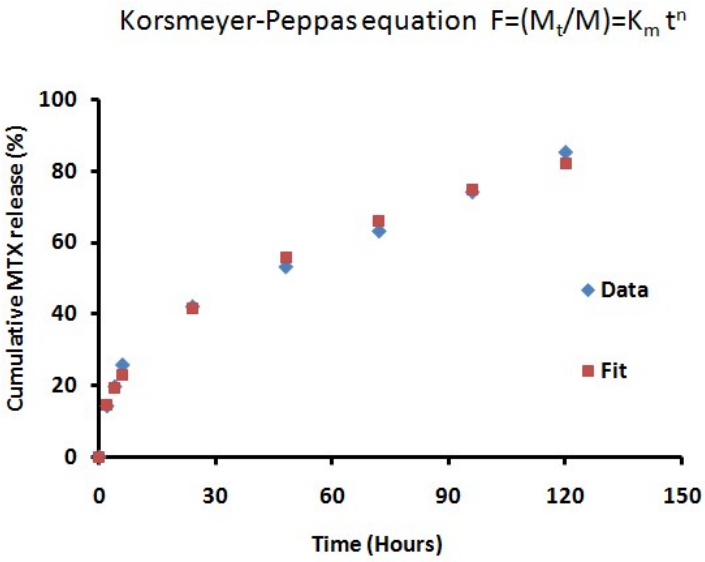


Fig. S6 Mass spectrum of PLGA-PBA@MTX nanoparticles shows ionization peak of $[MTX]^+$, $[MTX\text{-}EDA]^+$, $[MTX\text{-}EDA\text{-}(LA)1]^+$, $[MTX\text{-}EDA\text{-}(GA)_2\text{-}(LA)_1]^+$, $[PBA\text{-}EDA]^+$, $[PBA\text{-}EDA\text{-}(LA)_1\text{-}(GA)_1]^+$ and $[PBA\text{-}EDA\text{-}(LA)_3\text{-}(GA)_2]^+$ at 455.1, 497.23, 568.56, 685.44, 346.32, 475.32 and 679.51 m/z respectively.



Parameters	Value
R^2	0.997616
n	0.423879
Mechanism of drug release	Fickian diffusion

Fig. S7 The Korsmeyer-Peppas kinetic model demonstrates the mechanism of the MTX release from PLGA-PBA@MTX nanoparticles. The regression coefficient (R^2) and release exponent (n) values are 0.997616 and 0.423879 respectively. Since the release exponent value remain <0.5, the fickian diffusion-based release mechanism is mainly followed.

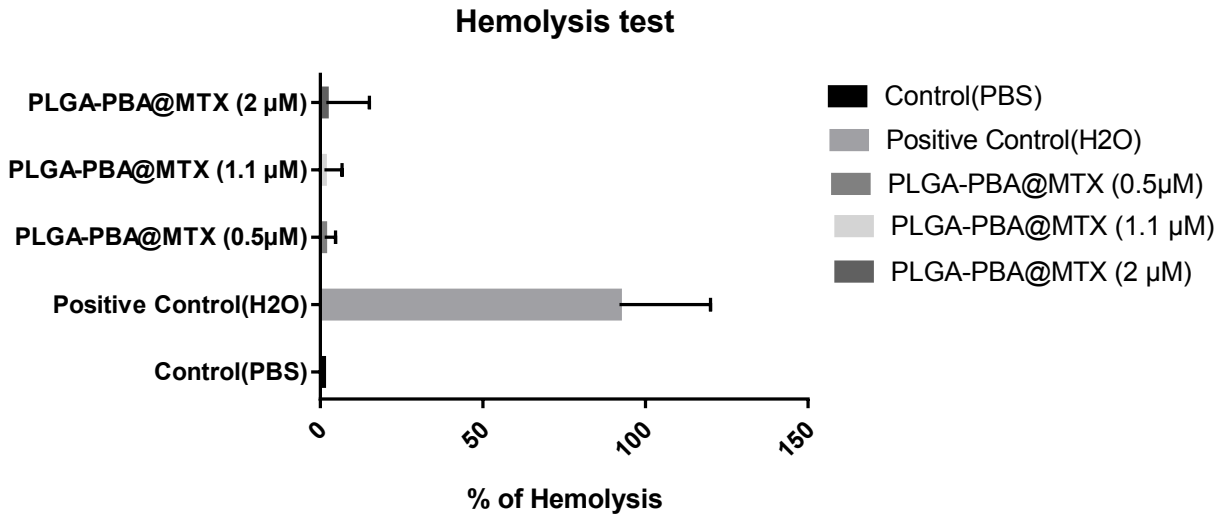


Fig. S8 The hemolysis rate analyses where x-axis represent % of hemolysis. Error ranges are standard deviations over n = 3 samples.

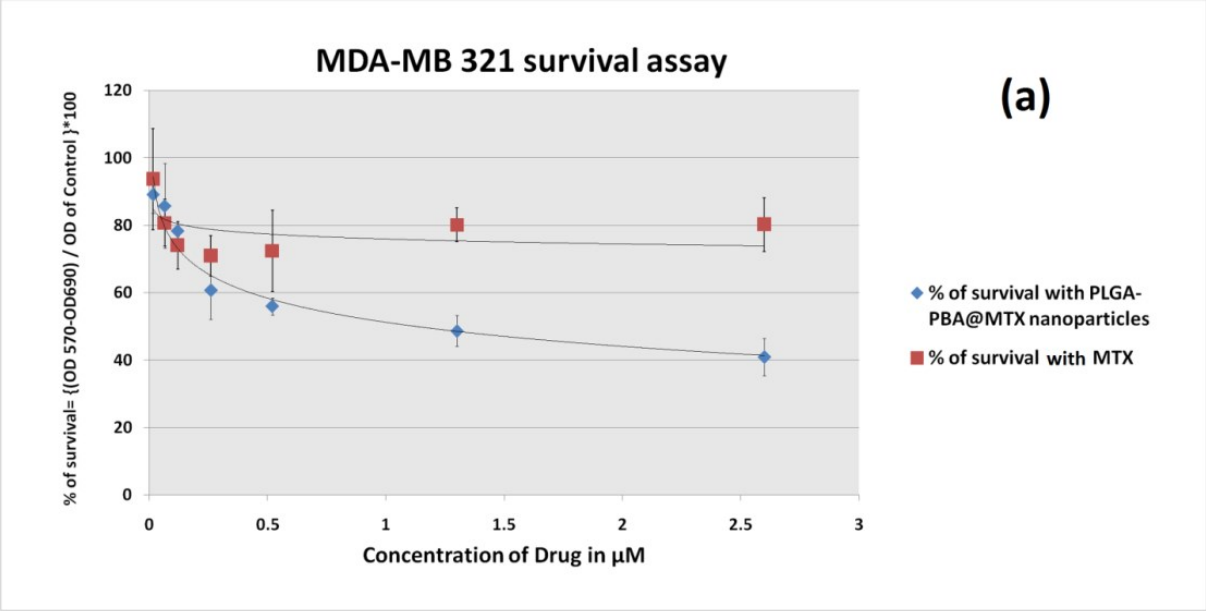


Fig. S9 Survival assay of breast cancer cells treated with PLGA-PBA@MTX nanoparticles and MTX alone by MTT assay in (a) MDA-MB-231 cells and (b) MCF-7cells shows PLGA-PBA@MTX nanoparticles can significantly inhibit MDA-MB-231 and MCF-7 cells in a dose dependent manner compared to free MTX treatment.

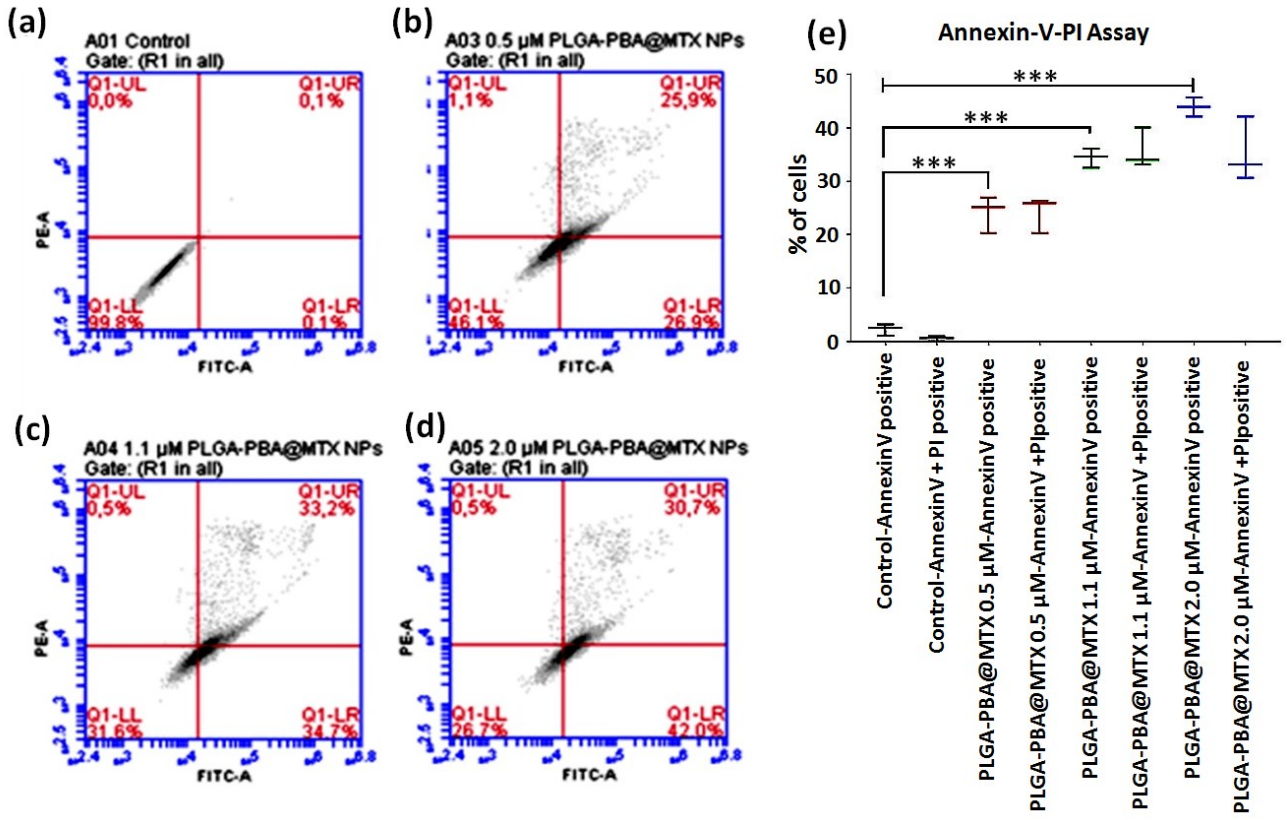


Fig. S10 Flow cytometric analysis of apoptosis in MDA-MB-231cells with a contour plot of Annexin-V-alexafluor 488 -fluorescence (x-axis) versus PI (y-axis). The figure represents best of three independent experiments. The percentage of apoptosis analyzed in (a) untreated (control) and PLGA-PBA@MTX nanoparticle treated cells having MTX concentration of (b) 0.5µM (c) 1.1µM (d) 2.0µM.(e) Quantitative analysis of apoptosis percentage for each concentration against control cells.

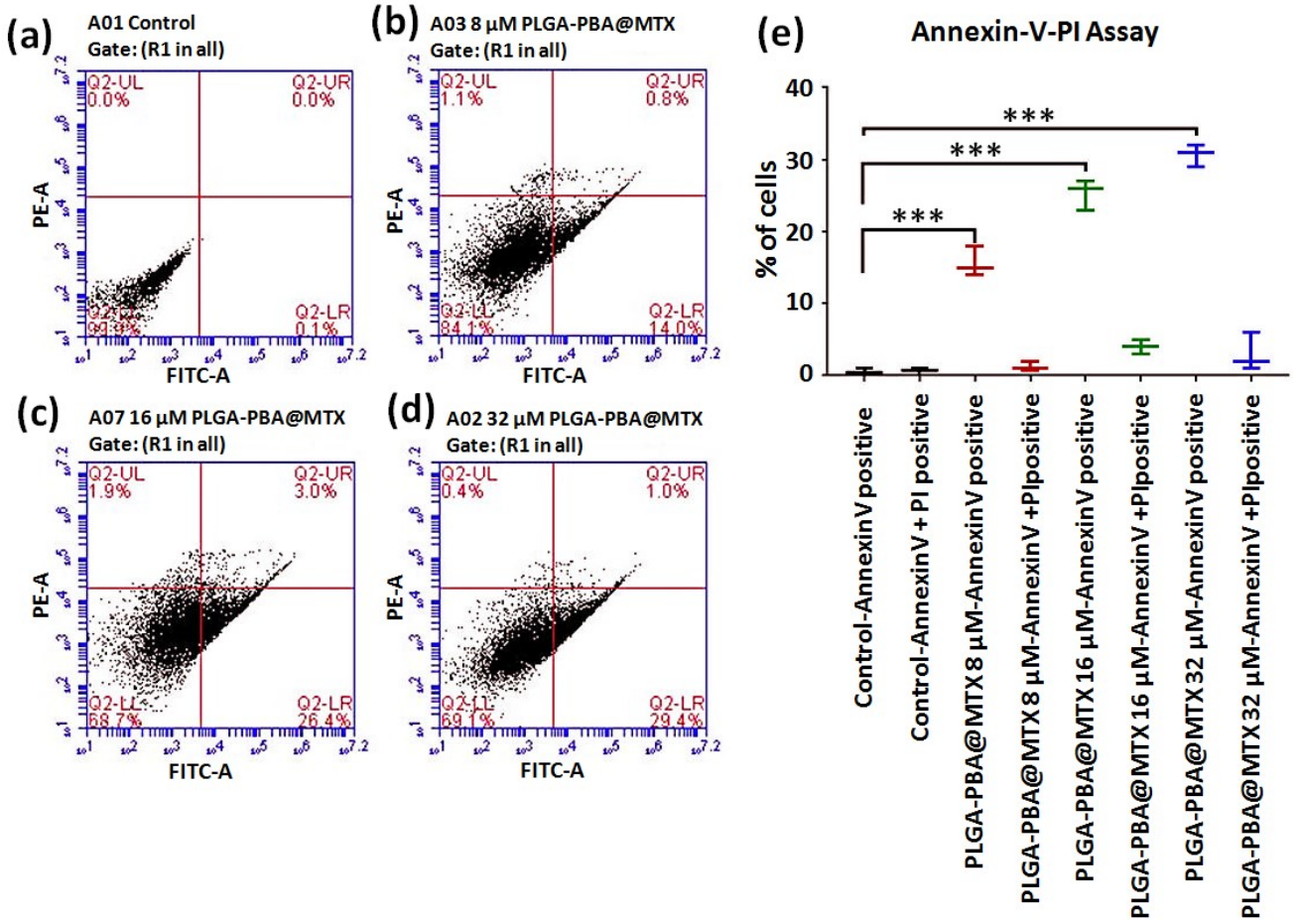


Fig. S11 Flow cytometric analysis of apoptosisin MCF-7 cells with a contour plot of Annexin-V-alexafluor 488 -fluorescence (x-axis) versus PI (y-axis). The figure represents best of three independent experiments. The percentage of apoptosis analyzed in (a) untreated (control) and PLGA-PBA@MTX nanoparticles treated cells having MTX concentration of (b) 8 µM (c) 16 µM (d) 32 µM. (e) Quantitative analysis of apoptosis percentage for each concentration against control cells.