Supporting Information

Coumarin Polycaprolactone Polymeric Nanoparticles: Light and Tumor microenvironment activated Cocktail Drug Delivery.

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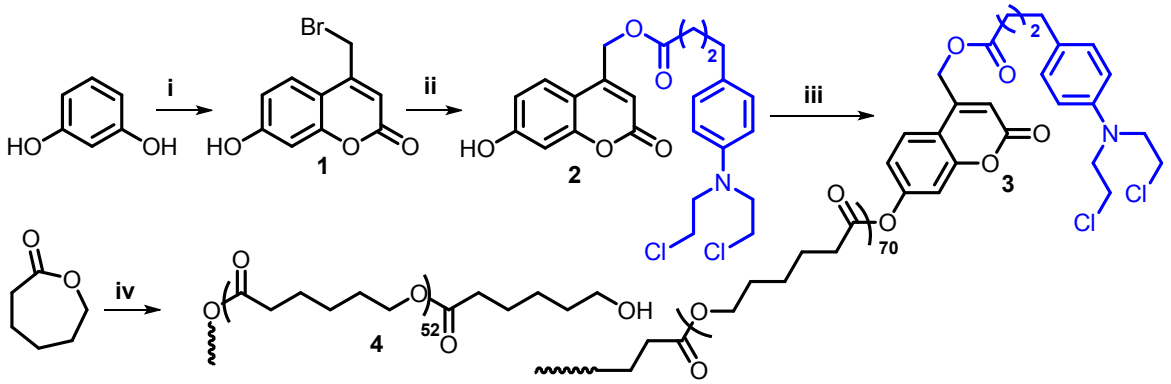
Supporting Information

1. General Information: ^{1}H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. MALDI-ToF were recorded on VOYAGER- DE PRO Applied Biosystems, The molecular weight and its distribution were examined by size-exclusion chromatography. The molecular weights of the polymers were determined at ambient temperature using a Visco tek gel permeation chromatograph equipped with a VE 1122 solvent-delivery system, a VE 3580 RI detector and two Visco GEL mixed-bed columns (17392-GMHHRM), which were preceded by a guard column. HPLC-grade THF was used as the eluent at a flow-rate of 1.0 mL min^{-1} and calibration was carried out using low polydispersity poly(methyl methacrylate) standards. The bulk morphology of the PMs was studied by HRTEM using a JEOL 2000 instrument operated at an accelerating voltage of 200 kV. The hydrodynamic size of the PMs was determined by DLS using a Malvern Nano ZS instrument.Photolysis of the conjugates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. RP-HPLC was taken using mobile phase acetonitrile, at a flow rate of 0.6mL / min (detection: UV 254 nm).

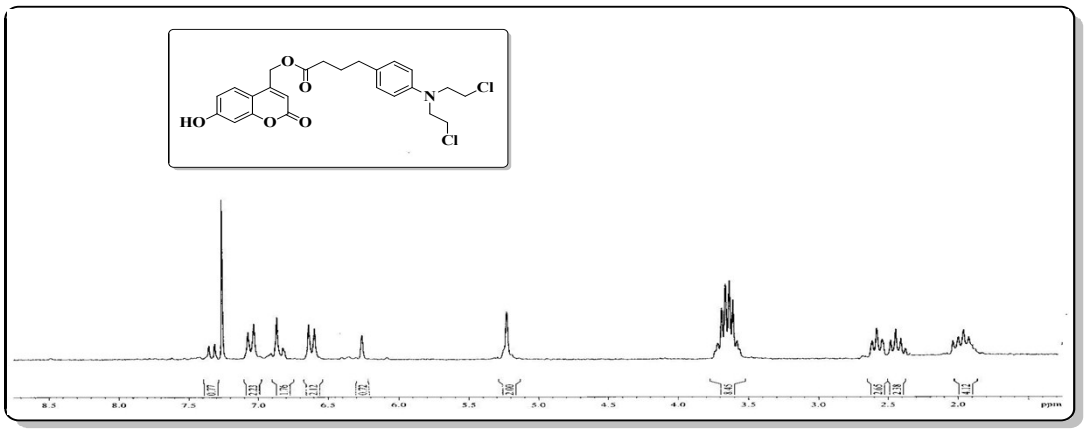
2) Synthesis of polycaprolactone tagged Coumarin chlorambucil

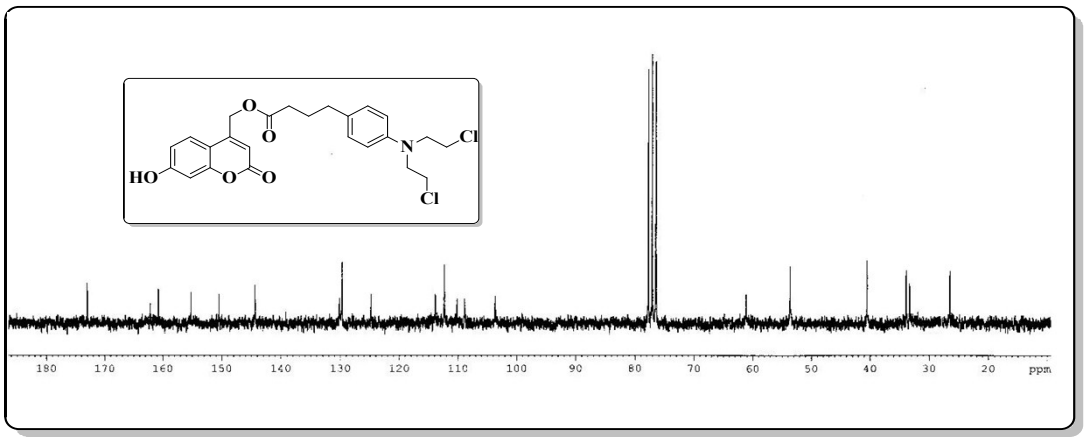


Reagents (a) = i) Bromoethylacetoacetate/ H_2SO_4/H_2O (7:3), rt, 12h, ii) Chlorambucil, K_2CO_3, KI, DMF, rt, 12h, iii) Caprolactone, $Sn(Oct)_2$, 110 °C, 24h, iv) $Sn(Oct)_2$, 110 °C, 24h

Scheme S1: Synthesis of Polycaprolactone tagged coumarin chlorambucil

2.a Synthesis of 7-hydroxy coumarin-chlorambucil (2) :





2.b *Polycaprolactone tagged coumarin chlorambucil (PCL-CC (3))*:

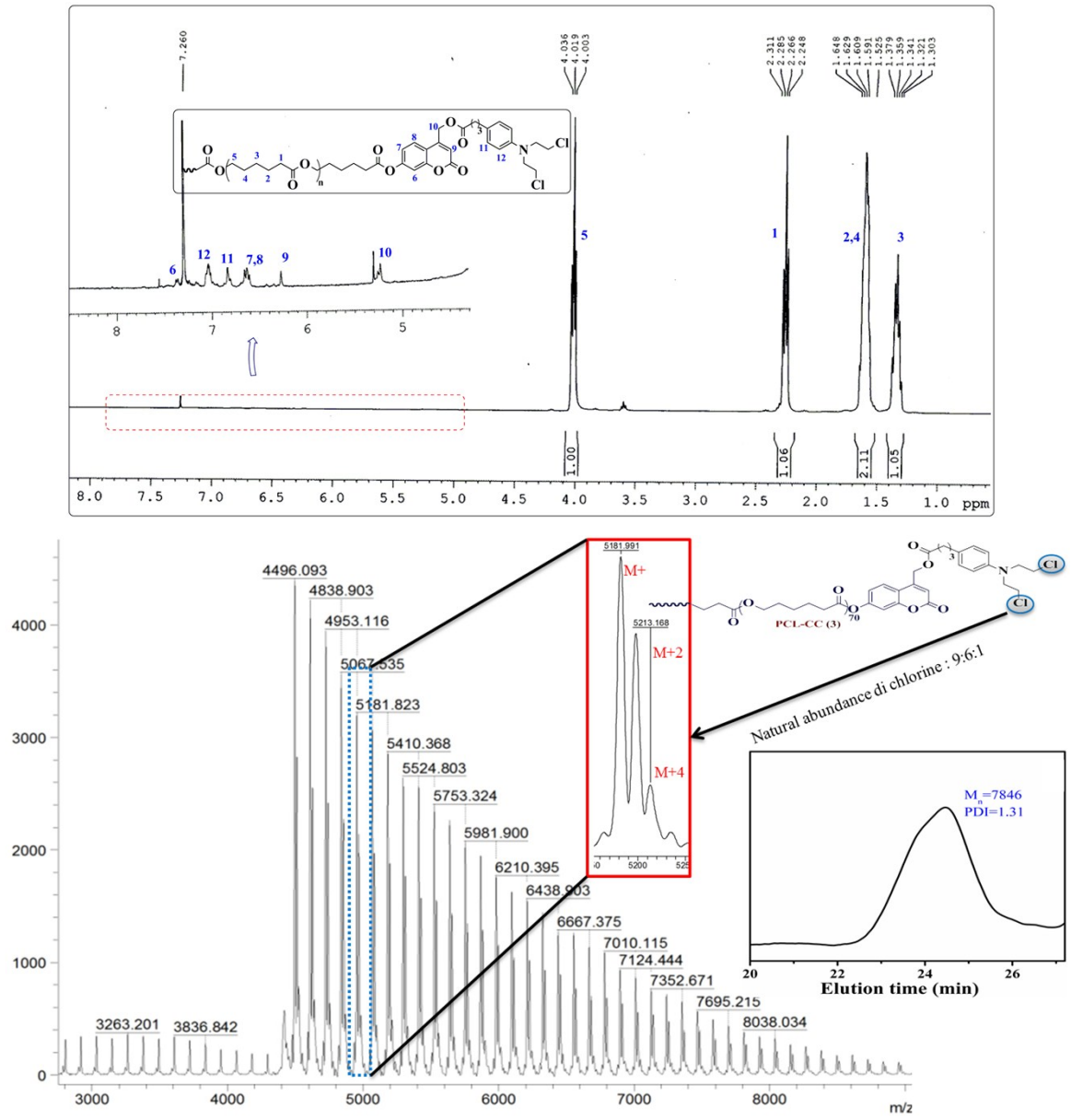


Figure S1 : ^{1}H NMR , MALDI-ToF spectra and inset GPC spectra of PCL-CC

The resulting polymer (PCL-CC) had a numer average molecular weight and poly dispersity index of M_n=7846 g/mol (n=70) and PDI=1.31respectively, as calculated from GPC experiment. From MALDI-ToF its clearly shows that coumarin chlorambucil is tagged with polycaprolactone. The specta clearly shows that (9:6:1) peak intensity because of dichlorine present in the chlorambucil moity and regular dissociation of 114. 36 caprolactone unit.

***2.c Synthesis of Polycaprolactone (PCL) 4*:**

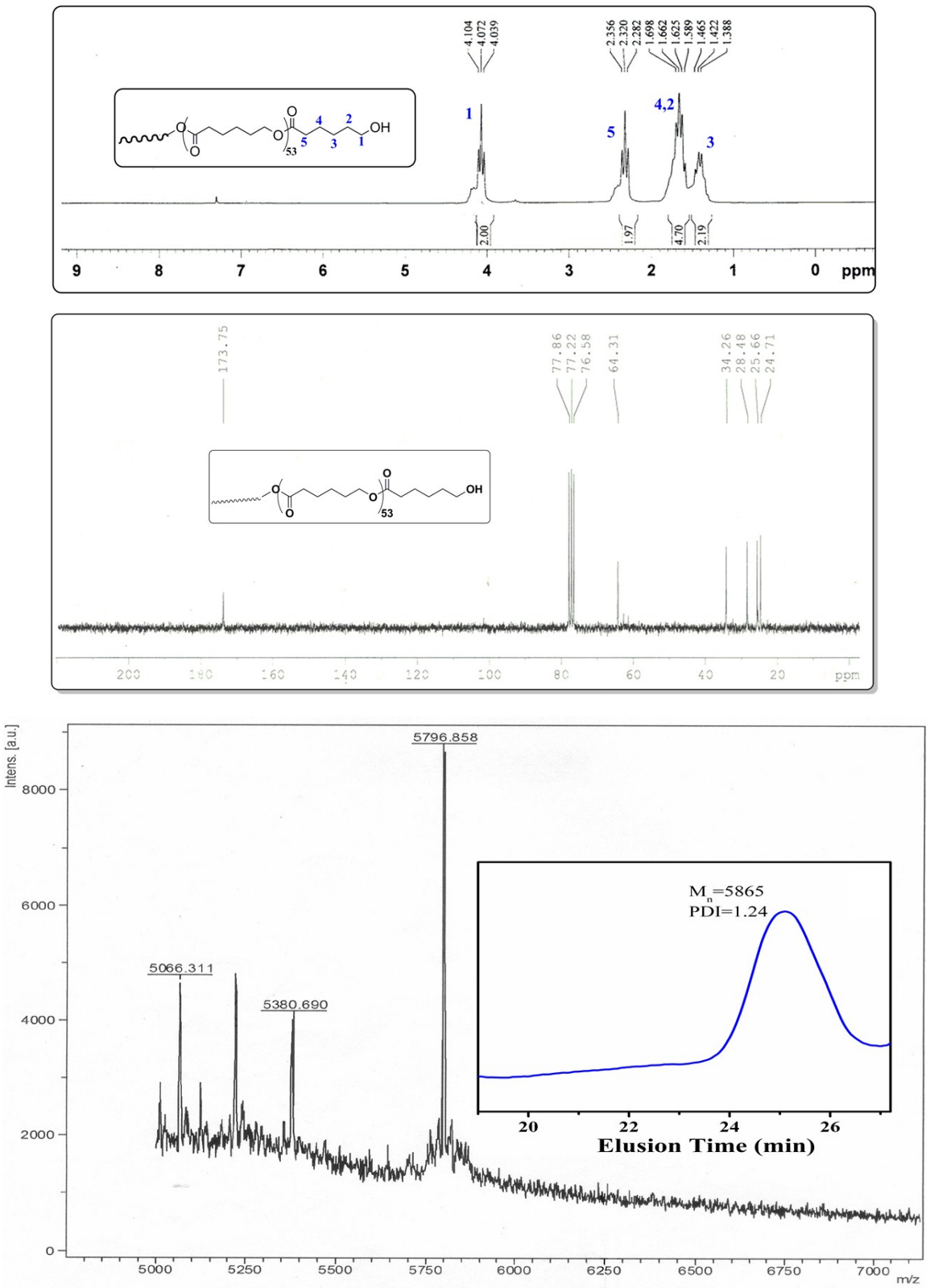


Figure S2 : ^{1}H, ^{13}C NMR, MALDI-ToF spectra and inlet GPC spectra of PCL

The resulting polymer (PCL) had a numer average molecular weight and poly dispersity index of M_n=5865 g/mol (n=52) and PDI=1.24respectively, as calculated from GPC experiment. From molecularweight was further confirmed by MALDI-ToF.

we synthesized different polymer derivatives by varying polymer condition like time of polymerization reaction and feed ratio of 7 hydroxy coumarin (Table S1 and Table S2). All synthesized polymer were characterized by GPC, and DLS. It clearly shows that

1) As we increase the reaction time the molecular weight of the polymer gets increased and after 24 h the increase in molecular weight of the polymer gets stagnated with a PDI of 1.3.

2) Next, we study the nanoparticles formation under controlled conditions and analyzed by dynamic light scattering (DLS). In order to drive the ring opening polymerization of caprolactone to completion, the refluxing time was increased from 4 to 48 h, which significantly improve the yield of polymer. It was observed clearly that when time increases the particle size decrease. In 2 h the molecular weight of polymer is low, so its loosely bound particle was formed with 825 nm. After 24 h, the molecular weight increase 3fold, the nanoparticles were formed with 68 nm. It clearly shows that size of the nanoparticles will be controlled by monomer feed ratio and temperature.

Table S1: Effect of time of the polymerisation reaction on the size of the nanoparticles

No	Sample name	Time (h)	$M_{n\ (GPC)}$	PDI	DLS (nm)
1	PCL-CC (P1)	2	2002	1.8	825 nm
2	C (P2)	6	3125	1.6	712 nm
3	PCL-CC (P3)	12	6511	1.4	141 nm
4	PCL-CC (P4)	24	7846	1.3	68 nm
5	PCL-CC (P5)	48	7984	1.3	72 nm

Table S2: Effect of feed ratio of the polymerisation reaction on the size of the nanoparticles

No	Name	Feed ratio	$M_{n\ (GPC)}$	PDI	DLS (nm)
1	PCL (P6)	0	5865	1.2	120 nm
2	PCL-CC (P7)	0.5 eq	6113	1.4	91 nm
3	PCL-CC (P8)	1 eq	7846	1.3	68 nm
4	PCL-CC (P9)	1.5 eq	8020	1.4	76 nm

3a.Encapsuation of Nile Red as model hydrophobic compound for CMC calculation of PCL-CC:

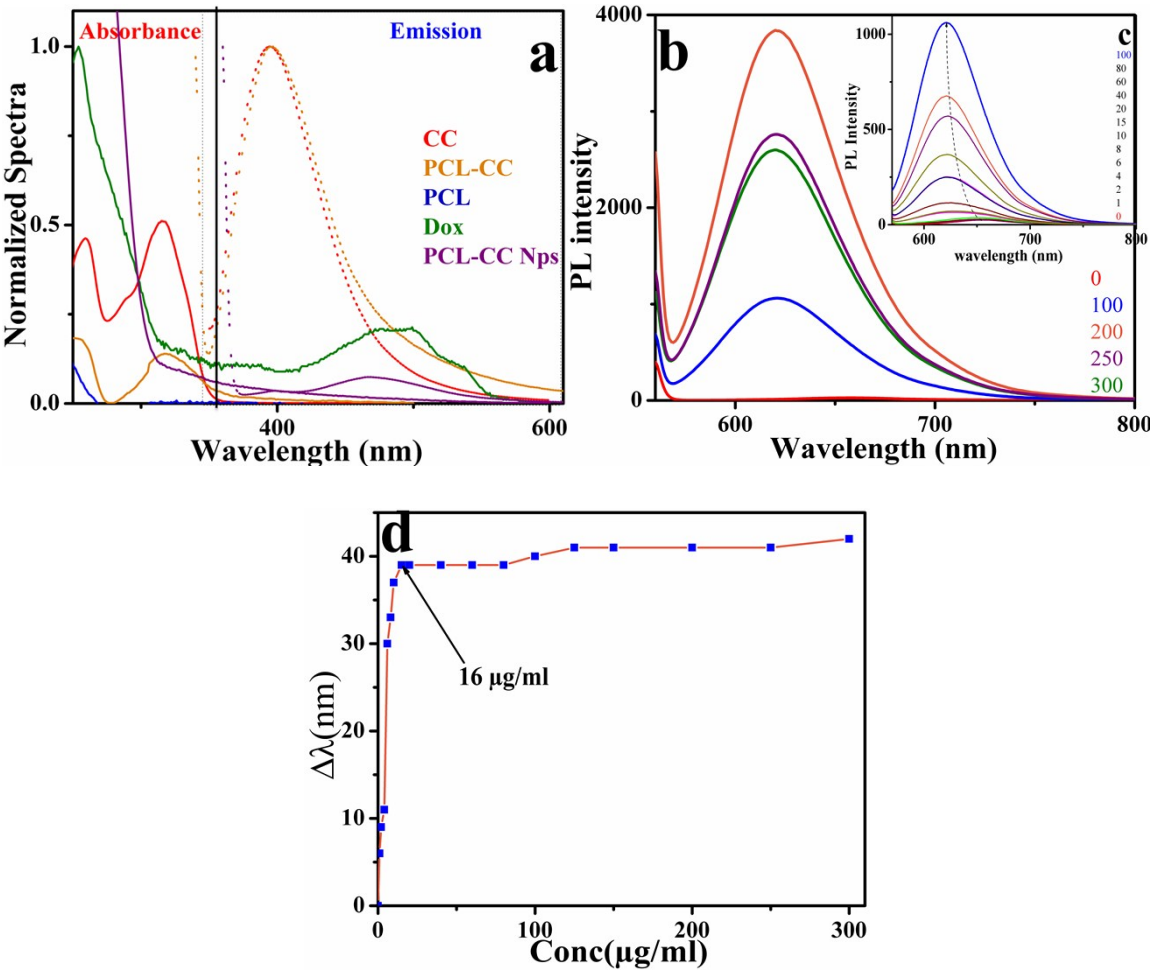


Figure S3. (a) Absorption and emission spectra of Coumarin chlorambucil (CC), polycaprolactone tagged Coumarin chlorambucil (PCL-CC), polycaprolactone (PCL), Doxorubicin (Dox), polycaprolactone tagged Coumarin chlorambucil nanoparticle (PCL-CC NPs), (b-c) Emission spectra of Nile Red (λ_{exc} =550 nm) in solutions of (1mg/1mL) concentrations of PCL-CC in water, (d) plot of spectral shift ($\Delta\lambda$ nm) as a function of concentration of polymer in (1mg/mL) of PCL-CC.

3b. Doxorubicin (Dox) encapsulation efficiency of PCL-CC NPs and PCL NPs:

The amount of drug in the nanoparticles measured by UV–vis spectroscopy by dissolving drug-loaded nanoparticles in methanol. UV absorbance was monitored at a wavelength of 470 nm for doxorubicin. Drug concentration was determined by calibration with a series of standards of known concentration of drugs in the same solvent. The total weight of the

nanoparticles is determined by removing water from the aqueous solution and weighing the sample.

3c. Physicochemical properties of PCL-CC NPs and Dox –PCL-CC NPs

a) Dynamic Light Scattering (DLS) of PCL-CC NPs , and doxorubucin loaded PCL-CC NPs

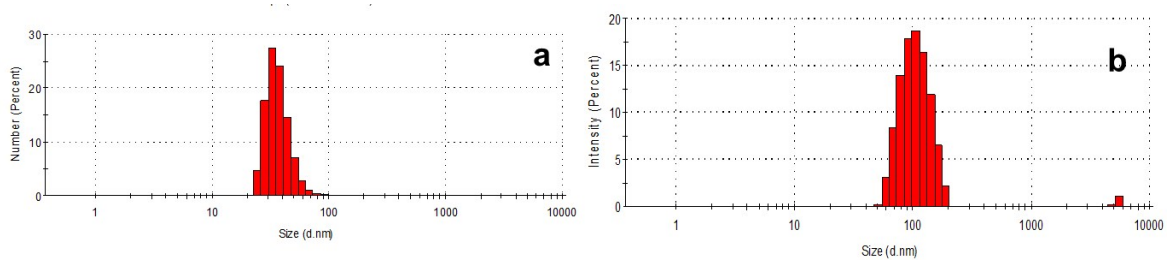


Figure S4: Particle size distribution graph of (a) PCL-CC NPs (b) Dox loaded PCL-CC NPs (Dox-PCL-CC NPs) as revealed by DLS.

Table S3: The Dox encapsulation efficiency (% EE) in the nanoparticles.

Sample	Dox loading content (%)	Dox loading efficiency (%)	Mean diameter (DLS)
PCL-CC NPs	-	-	73.1±0.64
PCL-CC NPs	13.04%	5.35%	110.5±1.6
PCL NPs	8.67%	3.45%	121.3±1.35

pH effect of the polymer :

we studied the pH effect of our polymer. We incubate our polymer in different pH 7.2 and 5.5 (Figure S5). This result showed that the Nile red loaded PCL NPs have ideal stability under physiological pH conditions 7.2. The release of Nile red was significantly accelerated at the pH 5.5. After 48 h, 67.6 % of Nile red was released, likely due to pH-induced hydrolysis of ester bonds in the polymer backbone.

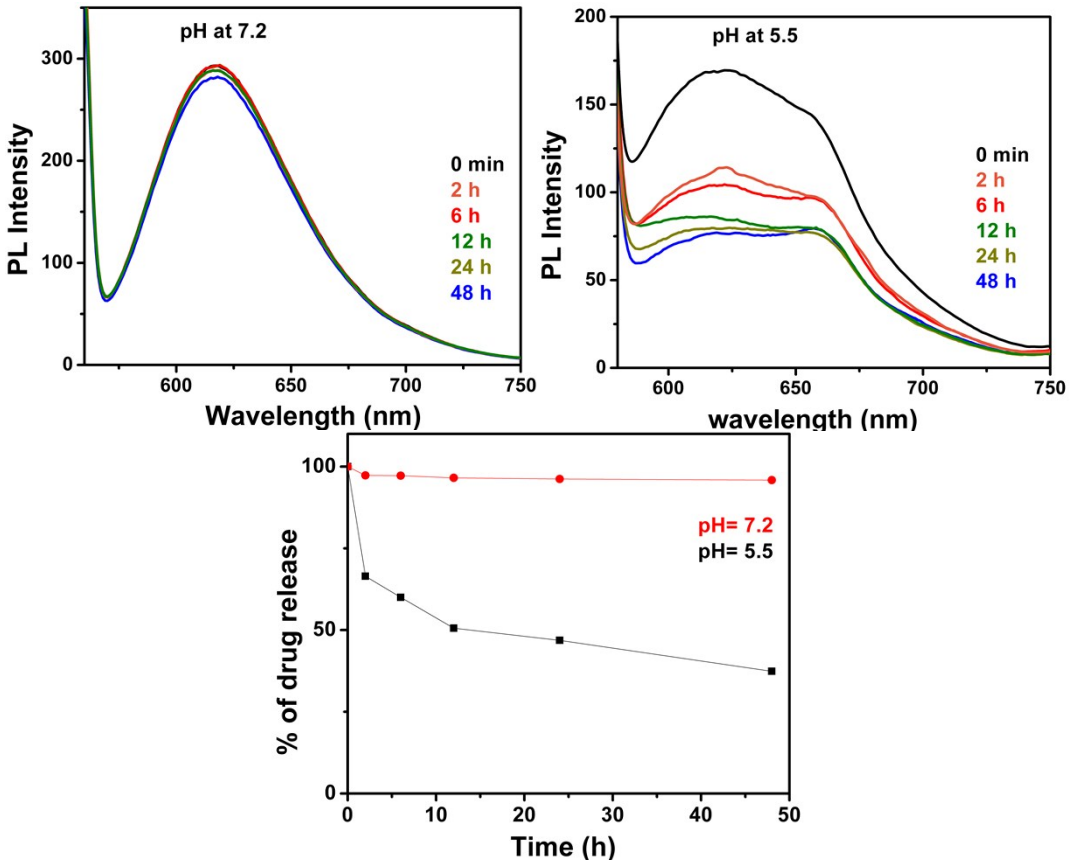


Figure S5. Drug release behavior of the NR-loaded PCL NPs under neutral (pH = 7.2) and acidic (pH = 5.5) conditions.

DLS study

The pH responsive nanoparticle was investigated by using DLS and fluorescence spectra. We incubate the NR-PCL-CC NPs in different pH 7.2 and 5.5 (**Figure S5-S6**). The result indicated that the NR-PCL-CC NPs have quit stable under physiological pH conditions. After incubation for 48 h, the aggregated average size of nanoparticles ultimately increased to over 3000 nm. The size increase could be attributed to the cleavage of ester bonds in the nanoparticles this clearly shows that the pH-induced hydrolysis of ester bonds in the nanoparticles.

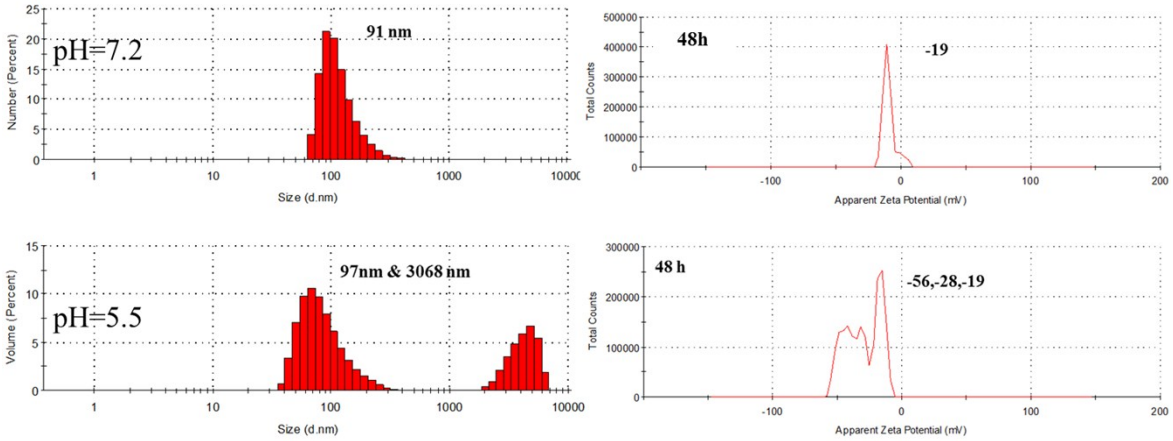


Figure S6. Size change of NR-loaded PCL NPs in response to the pH stimuli determined by DLS measurements.

3. Studies of encapsulated NR release as a model hydrophobic compound from NR-PCL-CC NPs: The dissociation of polymeric organic nanoparticles and the encapsulated dye release from the NR-PCL-CC NPs was studided under three different conditions. (i) soft UV irradiation (≥ 365 nm), (ii) 1mM (10µL) H_2O_2 and (iii) 1mM (10µL) H_2O_2 in the presence of soft UV irradiation (≥ 365 nm).

1. Photolysis of NR-PCL-CC NPs (≥ 365 nm): 3 ml water containing 0.5 ml of NR-PCL-CC NPs was irradiated with ≥ 365 nm (125 W medium-pressure Hg vapor lamp using a suitable filter 1 M $CuSO_4$ solution in 0.1 N H_2SO_4, the transmittance of the above filter = 365 to 500 nm) UV light and the photolysis was monitored by recording emission spectra. At every 10 minutes of time interval, 0.1 ml of aliquots was taken and then analyzed by emission spectra. With an increase in the irradiation time emission intensity of nile red at 640 nm decreased steadily and from the decrease in emission intensity it was found that almost 44% of encapsulated dye was released after 1 h of irradiation.

Similarly experiment was adopted to release Dox from Dox-PCL-CC NPs and it was analyzed by MALDI-TOF, TEM and DLS.

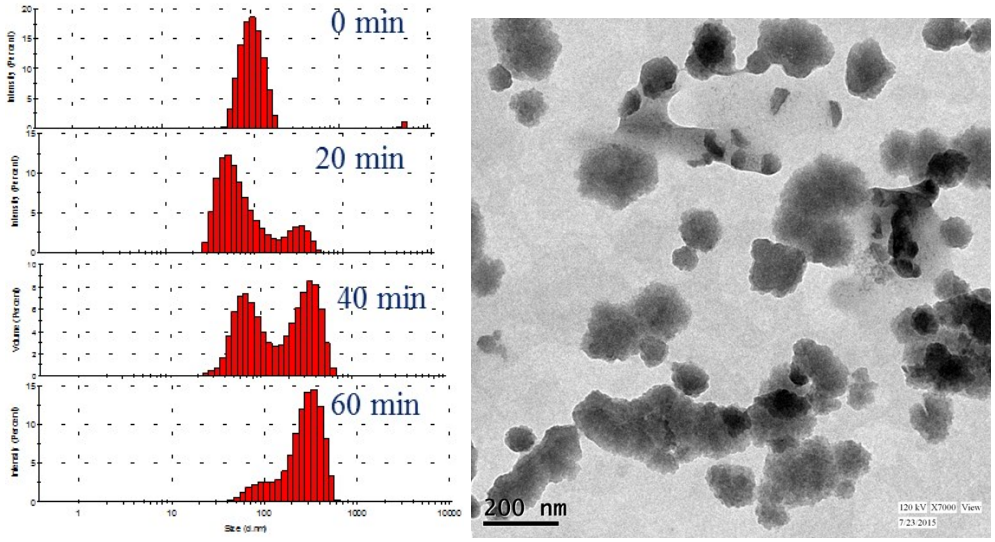


Figure S7: Photolysis of Dox-PCL-CC NPs at ≥ 365 nm. The course of photolysis was followed by DLS with different time intervals.

2. In presence of 1 mM (10 µL) H_2O_2: 3 ml water containing 0.5 ml of NR-PCL-CC NPs was digested with 1 mM (10 µL) H_2O_2, and the encapsulated dye release was monitored by emission spectra. At every 10 minutes of time interval, 0.1 ml of the aliquots were taken and analyzed by emission spectra. While increasing irradiation time the emission intensity of nile red at 640 nm decreased steadily, from the decrease in emission intensity it was found that almost 14 % of encapsulated dye was released after 1 h of digestion.

Similarly experiment was adopted to release Dox from Dox-PCL-CC NPs and it was analyzed by MALDI-TOF, TEM.

3. In presence of 1 mM (10 µL) H_2O_2/ soft UV Irradiation (≥ 365 nm): 3 ml water containing 0.5 ml of NR-PCL-CC NPs was irradiated with ≥ 365 nm (125 W medium-pressure Hg vapor lamp using a suitable filter 1 M $CuSO_4$ solution in 0.1 N H_2SO_4, the transmittance of the above filter = 365 to 500 nm) UV light in presence of 1 mM (10 µL) H_2O_2 and the photolysis was monitored by emission spectra. At every 30 seconds, 0.1 ml of the aliquots were taken and analyzed by emission spectra. The emission peak of nile red decreases sharply and shifted to 660 nm, which indicates ~ 97% of encapsulated dye was released within 10 min of irradiation.

Similarly experiment was adopted to release Dox from Dox-PCL-CC NPs and it was analyzed by MALDI-TOF, TEM and DLS.

Invitro Cell Imaging and Cytotoxicity Dox loaded PCC NPs (Dox-PCL-CC NPs) on HeLa cell line : we performed the control experiment were cells were treated with UV

light and H_2O_2 for 30 min. The cells treated with UV light are not affected, but Cell morphology has changed because of the cells treated with H_2O_2.

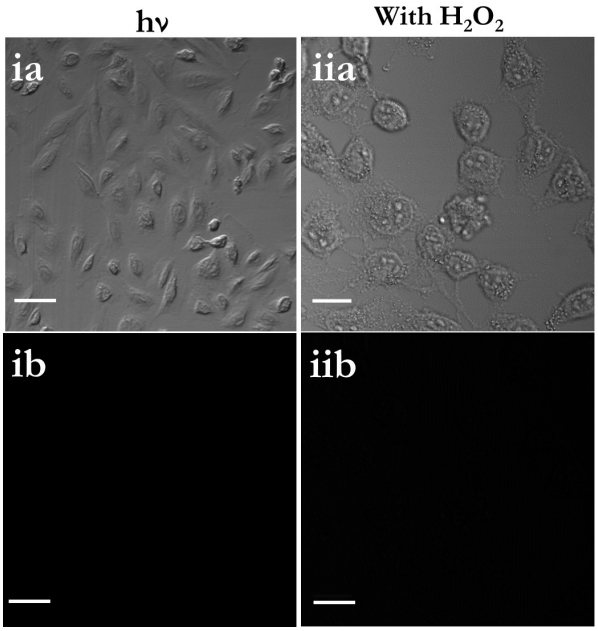


Figure S8: Cells were treated with light and 1mM H_2O_2 (10 µL) for 30min. (ia-b) and (iia-b) cells were treated with and 1mM (10µL) H_2O_2 respectively. Scale bars: 20 µm.

Optcal image of nanoparticles (PCL-CC)

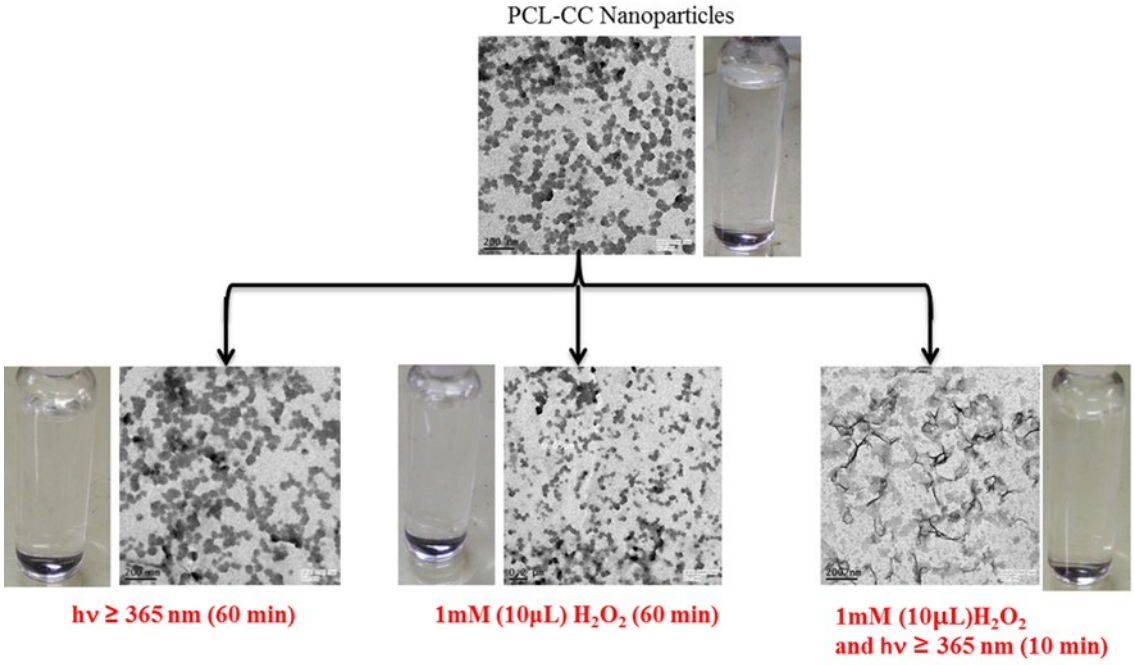
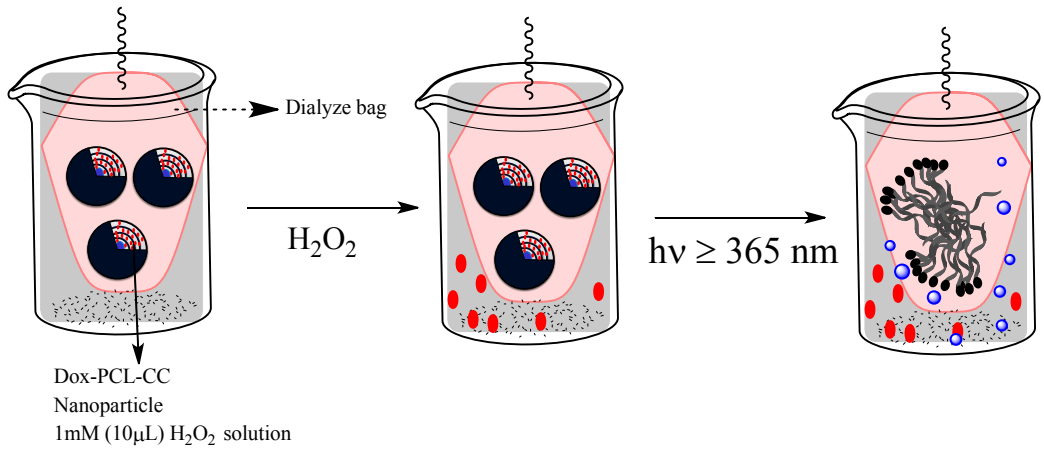


Figure S9: optical and TEM image of PCL-CC nanoparticles.

Release of Dox and Cbl from Dox-PCL-CC Nps:



References

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