Electronic Supplementary Information (ESI)

Smart Multifunctional Polyurethane Microcapsules for the Quick Release of Anticancer Drugs in BGC 823 and HeLa Tumor Cells

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Synthesis and characterizations

Dihydroxyl terminated prepolymer PCL-diol.

Pre-polymer PCL-diol was prepared by transesterification between the purified PCL materials and 1,4-butanediol using *p*-toluenesulfonic acid as catalyst in our previous study[1-3] Typcially, purified PCL (10g) was dissolved in 100 mL of chloroform and refluxed for 30 min before *p*-toluenesulfonic acid (4.8 g) and 1,4-butanediol (20 g) were added in subsequently. The reaction was carried out under reflux for 3 h. The resultant solution was washed with distilled water for 3 times, concentrated and dried under reduced pressure. The yield was white waxy solid. ^{1}H NMR ($CDCl_3$, 400MHz, ppm): δ1.15 (d, J) 6 Hz, CH($C\underline{H}_3$)$_2$ end group), 1.3 (m, 2H, $C\underline{H}_2$ backbone), 1.6 (m, 4H, $C\underline{H}_2$ backbone), 2.2 (m, 2H, $C\underline{H}_2$ backbone), 3.6 (t, -$C\underline{H}_2$OH end group), 4.0 (m, 2H, O$C\underline{H}_2$ backbone), 4.9 (sept, J) 6 Hz $C\underline{H}(CH_3)_2$ end group).[1-3]The obtained PCL-diol structure is consistent with previous reports.[4] GPC: Mn=5887, Mw=7194, PDI=1.22. Hydroxyl Value: 52 mg KOH/g. Acid Value: 0.37 mg

KOH/g.

Synthesis of TPE-2OH

5.0 g (19.1 mmol) of 4-bromobenzophenone, and 2.5 g (38.2 mmol) zinc dust were placed into a 250 mL two-necked round-bottom flask with a reflux condenser. The flask was evacuated under vacuum and flashed with dry nitrogen three times. 100 mL of THF was then added. The mixture was cooled to -78 ℃ and 3.6 g (19.1 mmol) of $TiCl_4$ was added drop-wise with a syringe. The mixture was slowly warmed to room temperature. After stirring for 0.5 h, the mixture was refluxed for 24 h. The mixture was quenched with 10 % aqueous K_2CO_3 solution and filtered. The filtrate was extracted with dichloromethane three times. The organic layer was washed with purified water and dried over Na_2SO_4. After solvent evaporation, the obtained product was purified by silica gel column chromatography using petroleum ether as eluent to obtain the product 2 (white solid). To a stirred solution of 2 (2.0 g) in 50 mL anhydrous THF was added n-BuLi (2.7 mL, 1.6 M in hexane, 4.36 mmol) at -78 ℃ under nitrogen. The mixture was first stirred for 2 hrs at this temperature and then warmed to room temperature. After stirring for 1 h, the flask was cooled again to -78 ℃ and the *N*-formylpiperidine (0.62 g, 5.46 mmol) was injected in one portion. The solution was stirred overnight and warmed to room temperature gradually. The reaction was quenched by adding 100 mL of aqueous hydrochloric acid (2 M solution). The organic layer was separated and the aqueous layer was extracted with 100 mL ethyl ether three times. The organic layers were collected and dried over Na_2SO_4. After solvent evaporation, the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4/1, v/v) as eluent to give the desired product 3 (yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.02 (d, d, *J*=7.70, 1.59 Hz, 4 H) 7.09 - 7.14 (m, 6 H) 7.18 (d, *J*=8.31 Hz, 4 H) 7.58 - 7.68 (m, 4 H) 9.88-9.94 (m, 2 H). 50 mL THF solution of 3 (1.165g, 3mmol) was placed into a 100 mL round bottom flask, followed by addition of 20 mL of methyl alcohol solution of NaBH4 (0.227g, 6mmol) at room temperature. The mixture was stirred for 0.5 h and then the organic layer was separated and the aqueous layer was extracted with 100 mL dichloromethane. After solvent evaporation, the white solid was the final product 4. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.62 (s, 4 H),

4.69-4.72 (m, 1H), 7.02-7.05 (m, 6H), 7.07-7.17 (m, 12H).

Synthesis of TPE-PUs

Pre-polymer PCL-diol was prepared by transesterification between the purified PCL materials and 1,4-butanediol using *p*-toluenesulfonic acid as catalyst in our previous study.[2] Diisocyanate terminated prepolymer PEG-2NCO was prepared according to reported procedure.[2] Briefly, amount 0.002 mol PEG was dissolved in 10 ml 1,2-dichloroethane in a 50 mL two-neck flask at 105 ℃. Then, any trace of water in the system was removed through azeotropic distillation with amount 4 mL of 1,2-dichloroethane left in the flask, then transferred to a 25 mL isobaric drop funnel, this solution was added drop-wise to a 100 mL 4-neck flask in which a slight excess diisocyanate HMDI (1.155g, 0.0044 mol) was placed in advance, catalyst stannous octanoate (~ 0.005g) were injected sequentially. The reaction was carried out at 50 ℃ for 12 h under a nitrogen atmosphere. PCL/PEG block pre-copolymers were synthesized via a coupling reaction of terminal hydroxyl group of PCL-diol and terminal isocyanate group of PEG-2NCO at equal molar ratio. Amount 0.002 mol PCL-diol was dried by dissolution in 20 mL 1,2-dichloroethane and removed the water by azeotropic distillation, 10 mL solvent was removed. The reminder was transferred to an isobaric drop funnel. The reaction was started when the PCL-diol solution was added drop-wise to the above mentioned ready-prepared PEG-2NCO solution in the 4-neck flask under a nitrogen atmosphere at 70 ℃. After 24 h reaction, the PCL/PEG pre-copolymers were obtained, which is terminated with the isocyanate group. Then product 4 (0.157g, 0.0004 mol) was dissolved in 1,2-dichloromethane and then added into the pre-copolymer PCL/PEG-diisocyanate; the mixture was stirred for 48h at 70 ℃. The product was precipitated in diethyl ether, filtered, and then re-dissolved in dichloromethane, filtered to move the trace amount of insoluble byproduct. In order to eliminate the stannous octanoate residue and possible low molecular weight oligomers, the filtrate was again precipitated in a mixture of methanol and diethyl ether (1/20, v/v). Product was collected through filtration, washed by distilled water three times followed by drying under vacuum to constant weight at 40 ℃. ^{1}H NMR ($CDCl_3$,

400MHz): δ (ppm) 0.94-0.96 (t, C$\underline{H}_2$(Cy)$_2$), 1.03-1.10 (m, Cy), 1.34-1.46 (m, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 1.59-1.77 (m, -OCH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$C(O)O-), 2.25-2.37 (t, -OCH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$C(O)O-), 3.38 (s, CH-NH), 3.57-3.71(s, -C$\underline{H}_2$C$\underline{H}_2$O-), 3.76 (s, N$\underline{H}$), 4.0-4.09 (t, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 5.0 (s, C$\underline{H}_2$(OC(O)NH)), 7.0(m, aromatic backbone), 7.09 (m, aromatic backbone).

Characterization of TPE-PUs

The well-defined block structure of TPE-PU was obtained via the mutual coupling reaction of the corresponding terminal hydroxyl group from PCL-diol, and TPE-2OH with terminal isocyanate group from PEG-diisocyanate, as shown in *Scheme S1*. Through maintaining the stoichiometric ratio of molar ratio at 11:5:5:1 of HMDI, PCL-diol, PEG and TPE-2OH, we obtained a series of linear TPE-PUs. The chemical structure and composition of the TPE-PU was analyzed by ^{1}H NMR (Figure S1). All the proton signals belong to PCL-diol, PEG, TPE-2OH and urea linkages can be clearly confirmed. The molecular weight of the obtained TPE-PU samples was between 56.3 and 127.7 kDa with relatively narrow poly-dispersity indexes ranging from 1.39 to 1.59 (Table S1).

Characterization TPE-PU drug carriers.

The TEM image of TPE-PU-1000 microcapsule appeared as hollow spheres and the shell thickness is around 90 nm (Figure 2 A and B). Since the pore size of the leaky vasculature ranges from 400 to 800 nm in tumor tissues, TPE-PU-1000 drug carriers can easily pass through the leaky vasculature pore to accumulate in tumor tissues.[5, 6] XRD patterns of free $NaHCO_3$, TPE-PU-1000 microcapsules and TPE-PU-1000 microcapsules containing $NaHCO_3$ is shown in Figure S2 C. The XRD peaks assigned to $NaHCO_3$ were observed in the XRD pattern of TPE-PU-1000 microcapsules, containing $NaHCO_3$, indicating the $NaHCO_3$ was encapsulated in the TPE-PU-1000 microcapsules successfully. SEM images revealed spherical TPE-PU-1000 microcapsules with smooth surface, which could be easily re-dispersed in water (Figure S2 D). AFM images showed that the pre-prepared TPE-PU-1000 microcapsules were spherical in shape and in the diameter size range between 200 and 700 nm (Figure S2 E). Further evidence of water soluble DOX incorporation and encapsulation inside the TPE-PU-1000 microcapsule was provided by CLSM. As shown in Figure S2 G-I,

the distribution of red fluorescence (represents DOX) arises from their aqueous core upon excitation at 488 nm, whereas blue fluorescence (represents TPE-2OH) in the TPE-PU microcapsules shell wall upon excitation at 340 nm. As hydrophilic DOX was mainly encapsulated in the aqueous core of the TPE-PU microcapsules, the lip soluble TPE-PU-1000 in the oil phase could only enter the capsule shell wall. A violet fluorescence was observed when merging the red and blue fluorescence (Figure S2 I). Hence, this system shows advantages of the red fluorescence (arising from DOX) in the aqueous core and the blue fluorescence (representing TPE adducts segments) in the shell wall, which could serve as fluorescent probes to facilitate us to simultaneously real-time trace these particulates and monitor their intracellular drug release dynamics.

The biocompatibility of the TPE-PU films

In Figure S3 A & B, the cell viability of the TPE-PU-1000 microcapsules was evaluated by treating with TPE-PU-1000 empty microcapsules (no DOX and $NaHCO_3$ included) with BGC 823 cells and Hela cells at different concentrations.[7] Typically, BGC 823 cells and Hela cells were seeded in 24-well flat-bottomed plates with a density of 5×10^4 cells per well and cultured for 24 hrs. After that, the medium was replaced by serum-free DMEM containing different concentrations of TPE-PU-1000 microcapsules. After co-culturing for 48 h. Then, CLSM and CCK-8 were employed for morphology observation and cell viability study.[8]

Platelet adhesion experiments were carried out to study the blood compatibility of the pre-prepared TPE-PU copolymer films.[9] Whole rabbit blood mixed with 3.8% sodium solution (ratio: 9/1, v/v) was centrifuged at 2000 rpm for 10 min at 4 ℃ to obtain platelet-rich plasma (PRP), which was used for platelet adhesion test. The polymer films in the glass dish were sterilized with 75 % ethanol, washed three times with PBS and equilibrated in PBS overnight. The samples were preheated to 37 ℃ before treatment. 1 mL RPR was added to the films, and films were then incubated at 37 ℃ for 1 h. The platelet-attached films were washed three times by PBS and immersed in PBS containing 2.5 % glutaraldehyde (pH=7.4) overnight for fixation. They were subsequently dehydrated in an ethanol-gradient series (from 30 %, 50 %, 70 %, 80 %, 90 %, 100 %, 100 %) for 15 min, respectively, and were dried under

vacuum. The morphologies of the platelet adhesion on the polymer film surfaces were observed by SEM. Three parallel films were performed for each polymer. Four different regions were randomly counted on each film, and result was taken as the average number of adhered platelets per square centimeter of surface.

Lactate dehydrogenase (LDH) activity of platelets was used to evaluate the platelet quantity.[5] After incubation with 1 ml PRP for 1 hr at 37 ℃, the film was washed with PBS to remove the platelets which were not attached on the film surface. 50 ml 1% Triton-X100 solution was treated on each sample for 5 min at room temperature to disrupt the platelets. The LDH activity of lysate was measured by LDH activity kit (GenMed Scientifics Inc., USA). The change of absorption at 340 nm immediately and at 1 min was used to calculate the LDH activity. A set of known concentrations of platelet lysates was used to determine their enzymatic activity and make a calibration curve under the same conditions as the film.

It has been demonstrated that covalently attached PEG chains to the polymeric drug carrier materials can provide stealthy surface when the carriers are injected into the blood stream. To verify whether the obtained TPE-PU materials have stealthy surface, platelet adhesion on the TPE-PU block copolymer films was employed to demonstrate their hemo-compatibility. SEM was used to quantify the platelet number and observe the morphology attached on the tested polyurethane copolymer film surfaces (Figure S3 C-F). The number of rabbit platelets adhered on neat PCL and TPE-PU-400 films was determined and the values were $(37.8 \pm 1.6) \times 10^4/cm^2$ and $(19.2 \pm 0.5) \times 10^4/cm^2$, respectively. Furthermore, the platelets adhering on the PCL and TPE-PU-400 films showed out their pseudopods (Figure S3 C & D), suggesting that platelets were activated, and thus, blood coagulation would be induced. However, hardly any platelets adhered to the surfaces of TPE-PU-1000 and TPE-PU-3400 films, and no pseudopod was observed (Figure S3 E & F), suggesting that the platelets were not activated, hence, not causing blood coagulation. The quantitative analysis of platelet activity and platelet adhesion numbers was based on the lactate dehydrogenase (LDH) activity assay. Consistent with the SEM observation results, all obtained TPE-PU films revealed a much weaker platelet adhesion than the raw PCL. This

result demonstrated that the polymer surface with a delicate hydrophilic/hydrophobic balance surface would reduce the aggregation and adhesion of platelet. In addition, a relatively delicate and flexible PEG (M_n=1 kDa) segments arrangement in the linear chains of TPE-PU-1000 can induce its film surface conformation change readily, which prevent blood plasma proteins cover on the TPE-PU-1000 surface.

Fluorescence characterization.

The AIE behaviour of TPE-PU-1000 was further studied in their solution state. As shown in Figure S4, as the phenyl rings of TPE-2OH undergo active intra-molecular rotations in THF solution, almost no PL signals were collected. Nevertheless, when large quantities of water (f_w > 60 vol %) are mixed with THF, the linear TPE-PU copolymer chains spontaneously aggregates to form nanoparticles in THF/water mixture-solvents, so the TPE fluorophores starts to radiate. When the THF/water mixture-solvents has a f_w of 90 vol %, its PL intensity (I) became ~1000 folds stronger than that in the THF solvent (I_0), indicating more and more TPE-PU chains are clustered together to form nanoparticles (Figure S4 C). When the linear TPE-PU chains aggregated together, the rigid hydrophobic block of PCL-diol and TPE-2OH cohesion formed solid shell, and the flexible hydrophilic block of PEG formed a hydrated shell on the surface of the shell, which hinder the intramolecular rotations of the aromatic rotors of the TPE. The restriction of the intramolecular rotation impedes non-radiative decay channels, forming radiative transitions, hence, contributing to the assembled fluorogenic molecules that emit strong fluorescence.

Fabrication of multifunctional TPE-PU microcapsules.

The TPE-PU microcapsules containing DOX was prepared by the emulsion-solvent evaporation method.[6, 10] Briefly, 1 mg DOX and pre-determined amount (0, 1.25, 2.5, or 5.0 mg) $NaHCO_3$ were added to 1 mL of 10 % (w/v) PVA aqueous solution, and they were thoroughly mixed to form the inner water phase (W_1). Then 2 mg TPE-PU-1000 was well dissolved in 2 mL DCM to form the oil phase (O). And the 6 mL of 20 % (w/v) PVA aqueous solution were used as the external water phase (W_2). Subsequently, 1 mL of the inner water phase (W_1) was emulsified with 2 mL of the oil phase (O), the emulsification was carried out by ultrasound treatment (at 35 W) in an ice-bath for 2 min to produce primary emulsion

(W_1/O). After emulsification, the primary emulsion (W_1/O) was added drop-wise to 6 mL of 20 % (w/v) PVA aqueous solution, and the mixture was homogenously dispersed at 8000 rpm for 15 min in water-ice bath with a Homogenizer (APV1000, Holland) to generate the double emulsion (W_1/O/W_2). Finally, the double emulsion was transferred into 30 ml de-ionized (DI) water and stirred overnight at room temperature to enable evaporation of all of the DCM. The solidified microcapsules were collected by centrifugation (1500 rpm, 20 min), washed three times with DI water, and re-suspended in 20 ml DI water.

In vitro drug release

To verify effectiveness of DDS for quickly drug release, TPE-PU-1000 microcapsules containing DOX and $NaHCO_3$ were incubated at different pH values and the release behavior of DOX were monitored by fluorescent spectroscopy. Fluorescent absorption spectra were taken from the supernatant of capsules-DOX-$NaHCO_3$ (with DOX loading amount as 660 μg DOX/mg inside TPE-PU-1000 microcapsules), after the TPE-PU-1000 microcapsules had been immersed in PBS (pH 5.0) medium for specified periods of time at body temperature. The released DOX in the supernatant has two characteristic absorptions at 550 and 590 nm, corresponding to the absorptions of monomer and dimer absorption of DOX, respectively (Figure S5A), suggesting the DOX had been encapsulated successfully into the TPE-PU-1000 microcapsules. Figure S5 B reveals the DOX release profiles from capsules-DOX-$NaHCO_3$ into a PBS buffer at different pH values. When these microcapsules were immersed in pH = 5.0 for 24 h, approximately 76 % of the DOX formerly loaded in the TPE-PU-1000 microcapsules was released into the PBS, indicating the sample of Capsules-DOX-$NaHCO_3$ were highly sensitive to endo-/lysosomal pH. Incubation of Capsules-DOX-$NaHCO_3$ at pH= 6.0 resulted in a declined cumulative release of DOX within the same period (Figure S5B and S6A). Conversely, when immersed in pH 7.4, only 7.21 ± 0.89 % of DOX were released into the PBS (Figure S5B and S6B).

The SEM images show that all microcapsules of Capsules-DOX-$NaHCO_3$ exhibited a dense morphology and smooth surface at pH 7.4 (Figure S5C). However, when the carriers immersed in the acidic milieus (pH 5.0), the hydrophilic PEG chains in the shell of the TPE-PU-1000 microcapsules become transparent and flexible. Subsequently, the protons (H^+)

in the acidic medium can readily penetrate into the TPE-PU-1000 shell, and then react with the gas-foaming agent $NaHCO_3$ in the aqueous core to produce lots of CO_2 bubbles. The continuous formed CO_2 bubbles will lead the TPE-PU-1000 shell to rupture because of the increasing internal pressure generated inside the particles. It creates many micro pores in the TPE-PU-1000 microcapsules shell, or alternatively breaks the TPE-PU-1000 microcapsules in a way similar to opened and emptied breakfast eggs (Figure S5D). Therefore, the pores in the TPE-PU-1000 shells cause the free DOX quickly release. After immersed in the weakly acidic medium (pH 6.0 and 5.0) for 24 h, about 30 % and 64 % of the burst microcapsules were observed, respectively (Figure S5C). From this prospect, the sample of Capsules-DOX-$NaHCO_3$ can avoid premature DOX release during circulation, specifically enhancing intracellular drug release.

Intracellular drug release characteristics of TPE-PU microcapsules.

The intracellular distribution of the TPE-PU-1000 microcapsules and the DOX release from the Capsules-DOX-$NaHCO_3$ was monitored by CLSM in Figure S7, after 0.5 h of incubation, Capsules-DOX-$NaHCO_3$ (violet fluorescence) were rapidly internalized in the BGC 823 cell in compared with free DOX, and mainly localized in the LysoTracker labeled endocytic organelles, with rare DOX observed in the nucleus. However, from 2 to 6 hrs, a gradual increasing red fluorescence (represents DOX) accumulated in the nucleus, indicating the effective release of DOX from the Capsules-DOX-$NaHCO_3$ (Figure S8 & S9). After 12 hrs incubation, DOX were completely released from Capsules-DOX-$NaHCO_3$ and resided in the cell nuclei, and the microcapsules showed remarkable enhanced separation of red fluorescence from the blue. Through 24 h observations, DOX have completely released from Capsules-DOX-$NaHCO_3$ and quickly enters the cell nucleus to exert its cytotoxicity, as we can observe the cancer cell membrane fragments and nuclear chromatin psychosis and organelles dissolve (Figure S10). However, in the sample of Capsules-DOX, although a large number of violet carriers were endocytosed by BGC 823 cell and entrapped in the LysoTracker labeled endocytic organelles, DOX was seldom observed in the cell nuclei even after 24 h of incubation.

References

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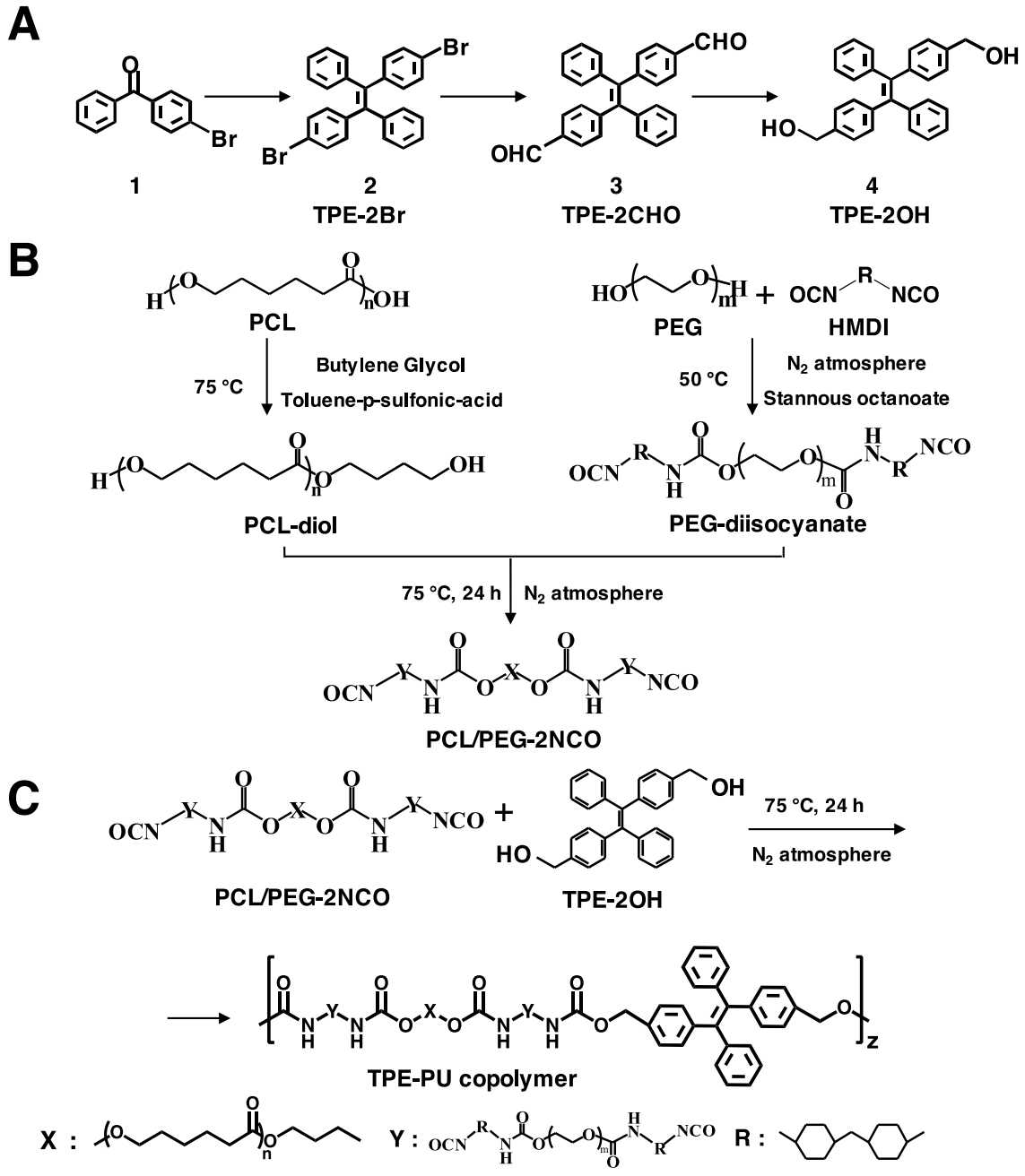
Figure



***Scheme* S1. Synthesis of linear block fluorescent polyurethane copolymers based on TPE-2OH, PCL-diol, and PEG.** Synthetic route of (A) TPE-2OH adducts, (B) Pre-polymer of PCL/PEG/2NCO, and (C) polyurethane copolymer TPE-PU.

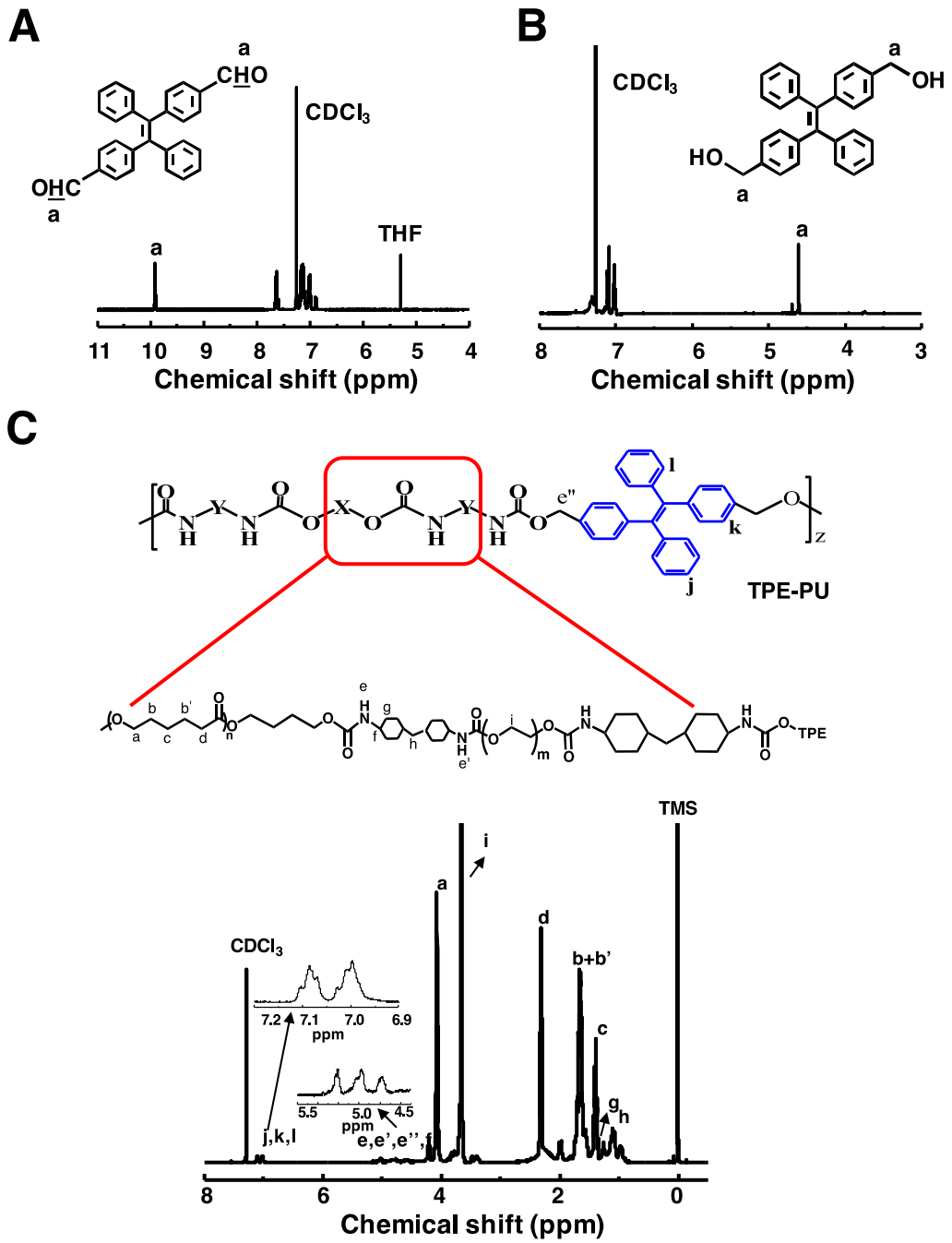


Figure S1. ^{1}H NMR spectra of (A) TPE-2CHO, (B) TPE-2OH and (C) TPE-PU-1000 in $CDCl_3$.

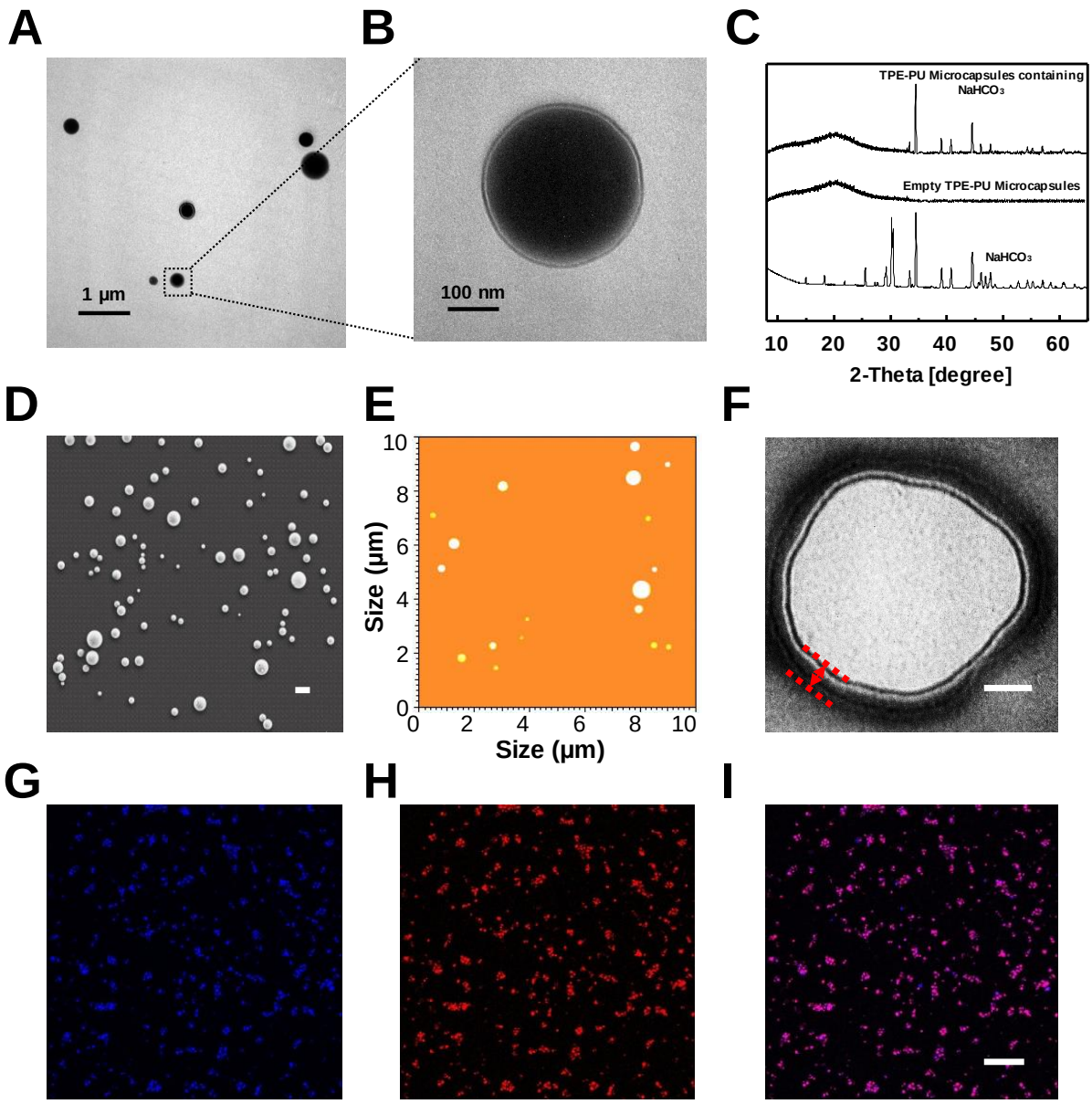


Figure S2. Material characterization. (A)(B) TEM images of the TPE-PU nanoparticles in THF/water mixture with fraction of water (f_w=60 %) under different magnification. (C) XRD patterns of $NaHCO_3$, empty TPE-PU-1000 microcapsules and the TPE-PU-1000, microcapsules containing $NaHCO_3$. (D) SEM and (E) AFM image of the TPE-PU microcapsules. Scale bar, 1 μm; (F) TEM photograph showing the spacious interior hollow structure of one TPE-PU microcapsule. Scale bar, 100 nm; (G)–(I) CLSM micrographs of the TPE-PU microcapsules, revealing the fluorescence images of (G) TPE-2OH in the shell wall, (H) DOX in the aquoeus core, and (I) their superposition images. Scale bar, 20 μm.

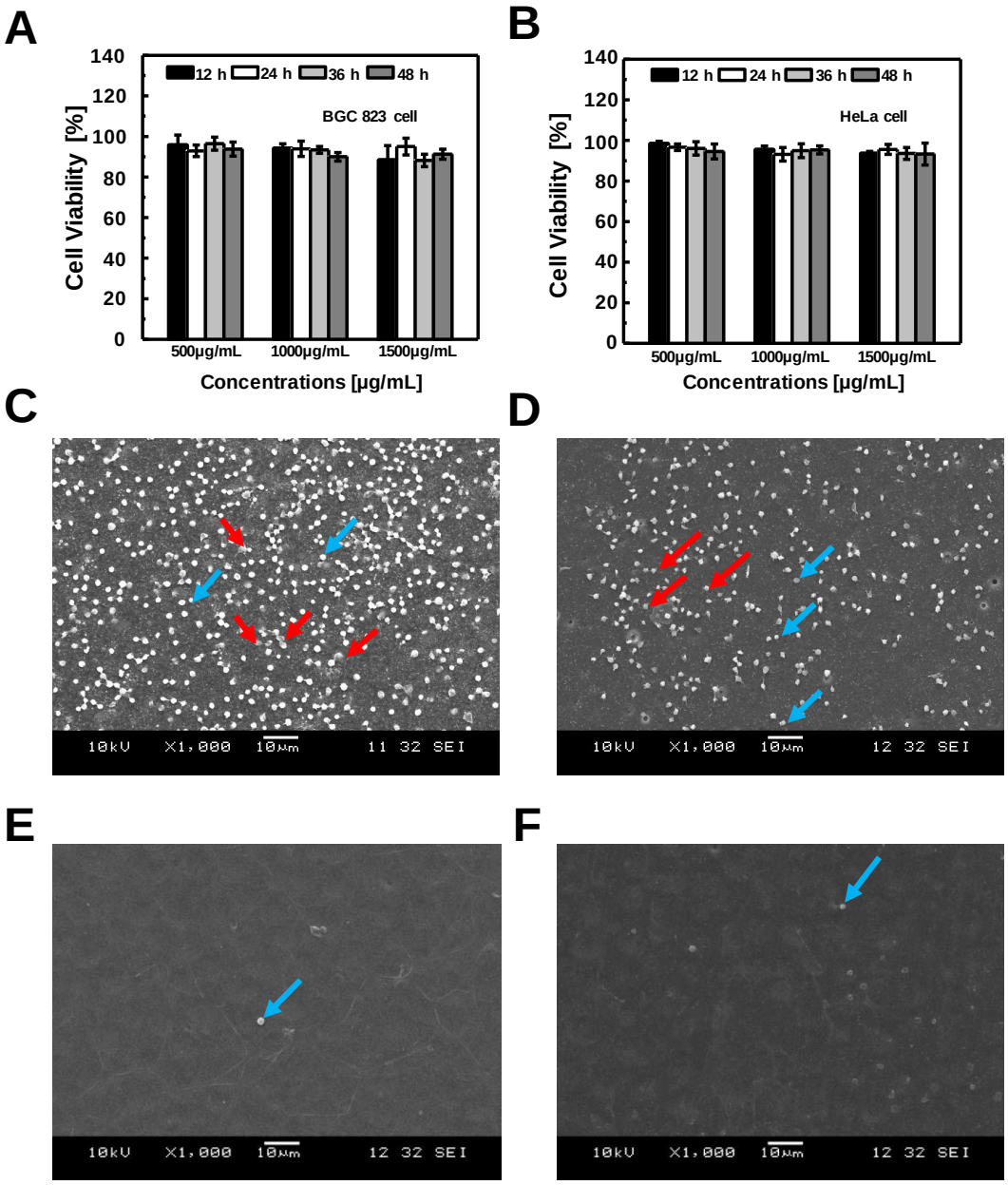


Figure S3. Biocompatibility test of material. Cell viability of (A) BGC 823 and (B) HeLa cells incubated with various concentrations of the TPE-PU-1000 microcapsules for 12, 24, 36 and 48h. Error bars are standard deviation. n=3. Characterization of platelet adhesion and activation on the surfaces of PCL and the TPE-PU films: the morphology of rabbit platelets cultured on the film surfaces of (C) PCL; (D) TPE-PU-400; (E) TPE-PU-1000; (F) TPE-PU-3400. Film thickness=0.2mm. Red arrows: platelets with pseudopod, blue arrows: platelets without pseudopod.

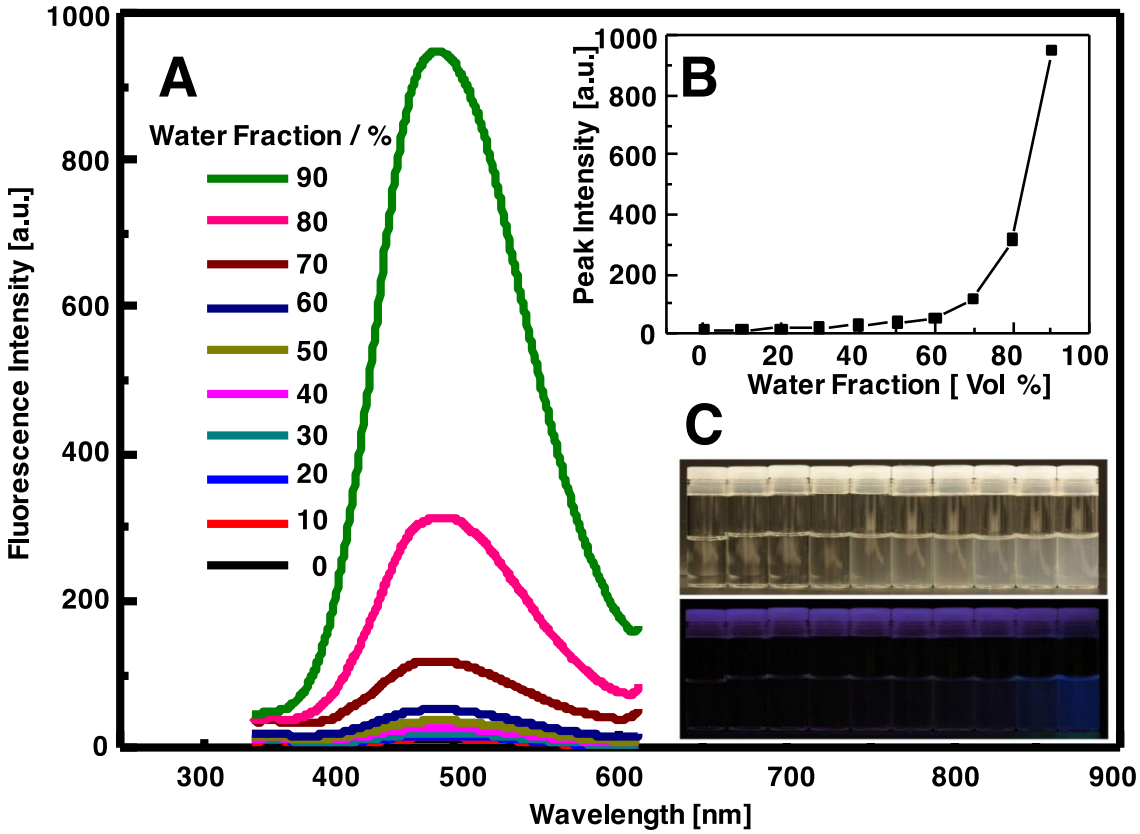


Figure S4. (A): PL spectrum of TPE-PU-1000 (TPE segment：10^{-5} mol/L) in THF/water (v/v) mixtures with different fractions of water (f_w). Excitation wavelength: 340nm. (B): Plot of the relative PL intensity (I/I_0) of TPE-PU-1000 at 475 nm vs the composition of the THF/water mixture (f_w). I_0 = PL intensity of TPE-PU-1000 in THF solution. (C): Photographs of TPE-PU-1000 different solutions taken under room lighting and UV illumination.

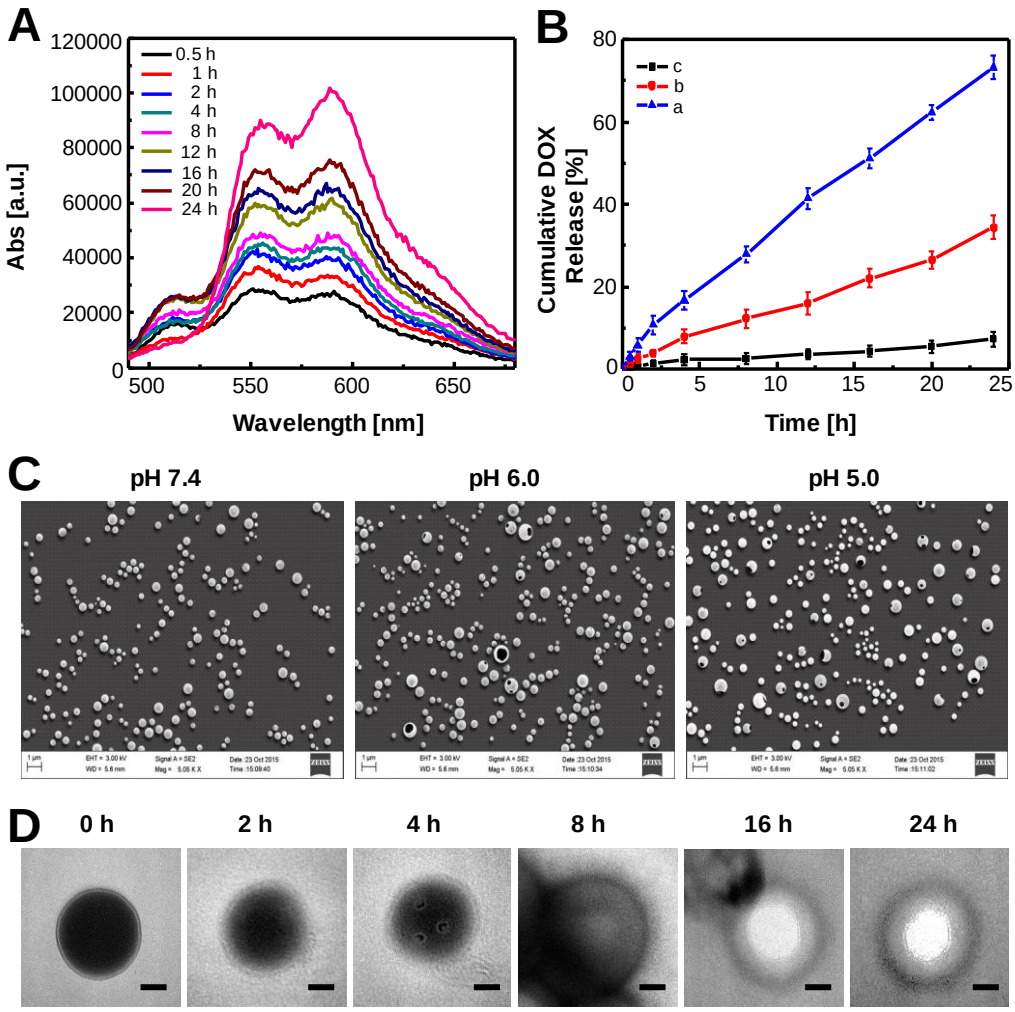


Figure S5. *In vitro* drug release behavior of the Capsules-DOX-$NaHCO_3$. (A) The supernatant after the TPE-PU microcapsules have been immersed in PBS of pH 5.0 at 37 ºC for 0.5, 1, 2, 4, 8, 12, 16, and 24 h; (B) The release profiles of DOX from TPE-PU microcapsules incubated in PBS with different pH environments at 37 ºC. Error bars are standard deviation. n=8. a, pH=7.4; b, pH=6.0; c, pH=5.0; (C) SEM images of TPE-PU microcapsules after incubation in PBS with different pH values to mimic the extra-cellular milieu (pH 7.4), early endosomal (pH 6.0), and late endo-/lysosomal compartment milieu (pH 5.0); (D) TEM images of one TPE-PU microcapsule after incubation in PBS with pH 5.0 for 0, 2, 4, 8, 16, and 24 h. Bar scale=100 nm.

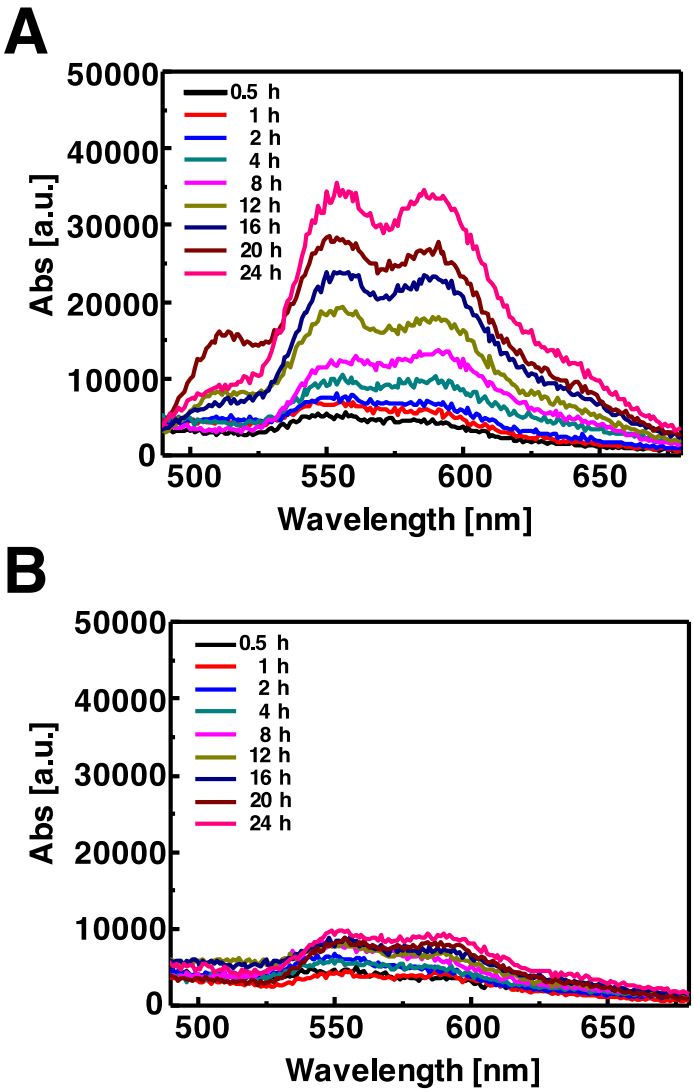


Figure S6. The supernatant after the sample of Capsules-DOX-$NaHCO_3$have been immersed in PBS of (A) pH 6.0 and (B) pH 7.4 at 37 ℃ for 0.5, 1, 2, 4, 8, 12h, 16h, and 24 h.

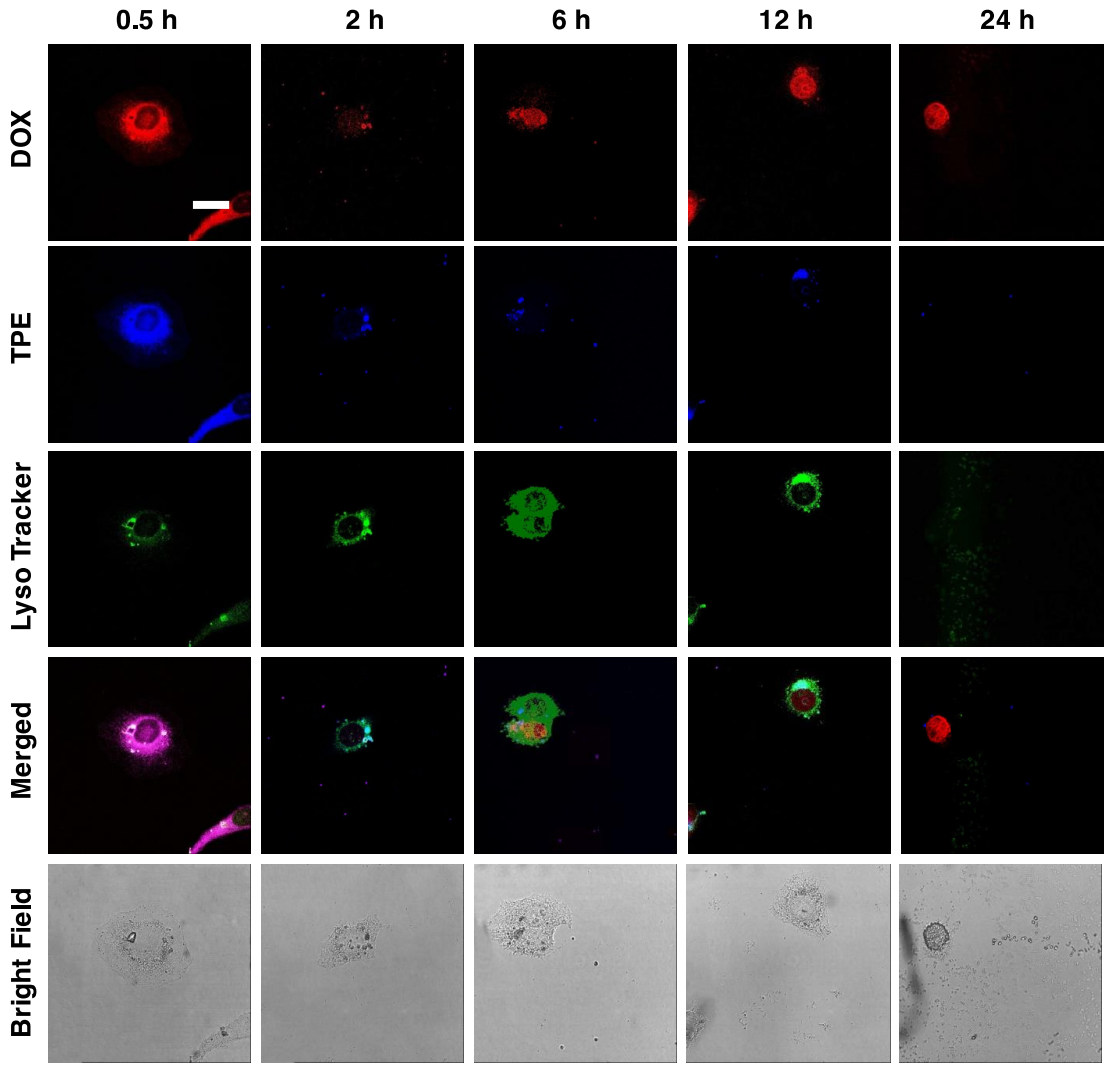


Figure S7. CLSM micrographs of BGC 823 cells treated by Capsules-DOX-$NaHCO_3$ at 37 ºC for 0.5-24 h. The red channel fluorescence emission was originated from DOX in the aqueous core of the microcapsules, the blue channel fluorescence emission was originated from TPE in the shell wall. Late endo-/Lysosomes were stained with LysoTracker Green (green). Scale bar = 20 μm.

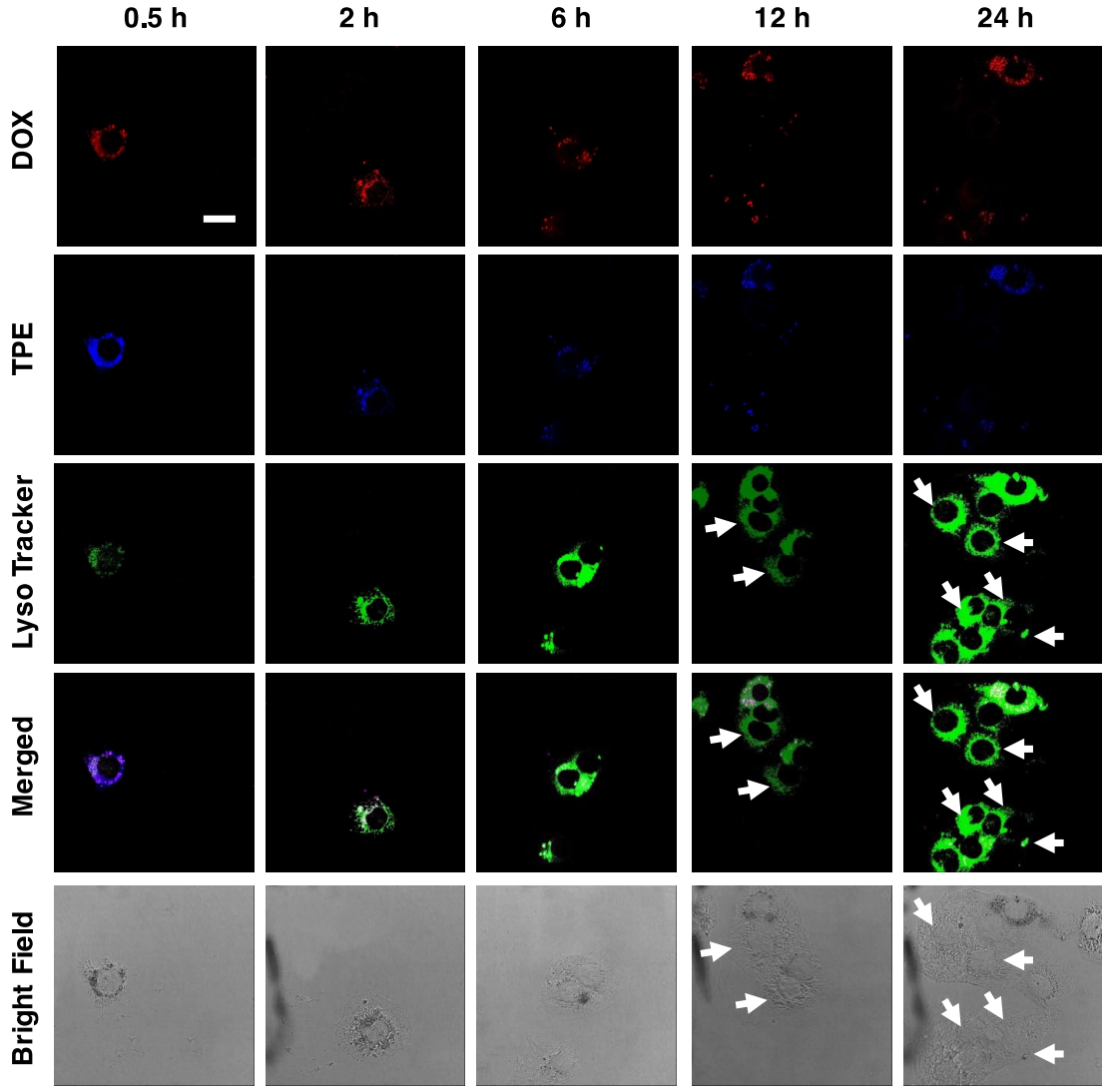


Figure S8. CLSM images recorded for BGC 823 cells after co-incubation with Capsules-DOX (DOX concentrations: 5μg/mL) at 37 ℃ for 0.5, 2, 6, 12, and 24 h. The blue channel fluorescence emission was originated from TPE in the shell wall, the red channel fluorescence emission was originated from DOX in the aqueous core of the microcapsules. Late endo-/Lysosomes were stained with LysoTracker Green (green). White arrows: newly proliferating cancer cells. Scale bar = 20 μm.

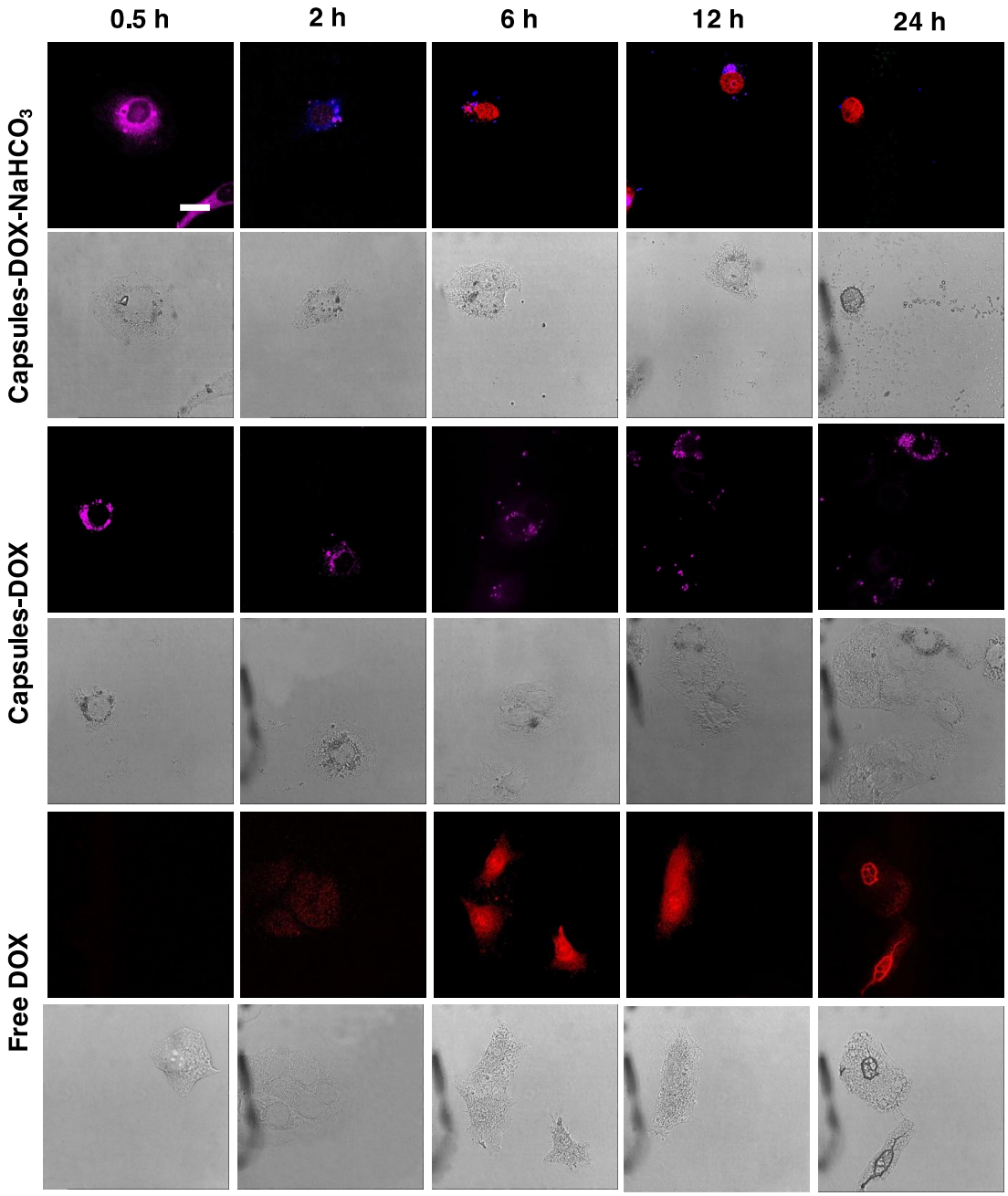


Figure S9. CLSM micrographs of the intracellular accumulation of DOX in BGC 823 cells treated with Capsules-DOX-$NaHCO_3$, Capsules-DOX and free DOX at 37 ℃ for 0.5, 2, 6, 12, and 24 h. (Top) merged images and (bottom) in the bright filed. Scale bar =20 μm.

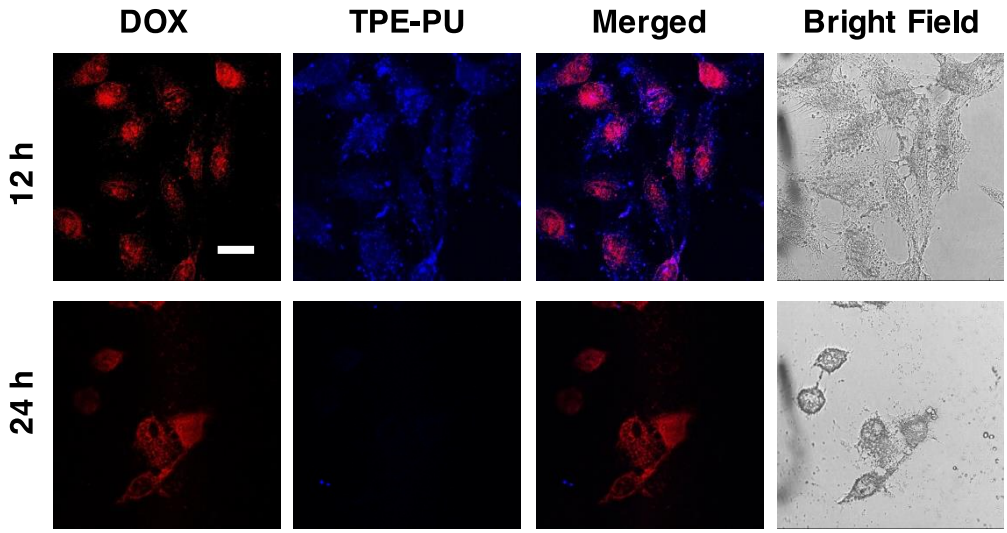
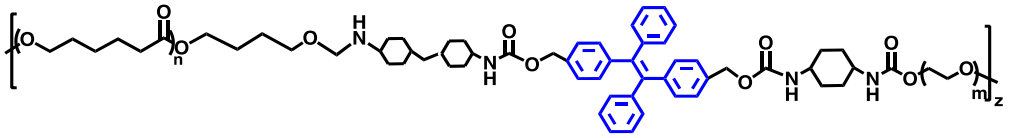


Figure S10. CLSM micrographs of HeLa cells treated by Capsules-DOX-NaHCO$_3$ at 37 ºC for 12,and 24 h. The red channel fluorescence emission was originated from DOX in the aqueous core of the microcapsules, the blue channel fluorescence emission was originated from TPE in the shell wall. Bar scale 20 μm.



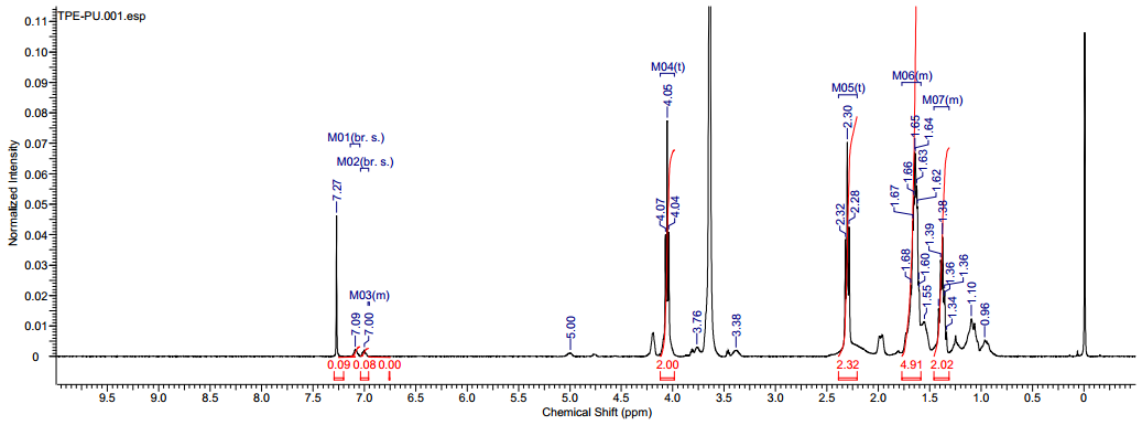


Fig. S11. ^{1}H NMR spectrum of TPE-PU copolymer. ^{1}H NMR ($CDCl_3$, 400MHz): *d* (ppm) 0.94-0.96 (t, C$\underline{H}_2$(Cy)$_2$), 1.03-1.10 (m, Cy), 1.34-1.46 (m, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 1.59-1.77 (m, -OCH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$C(O)O-), 2.25-2.37 (t, -OCH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$C(O)O-), 3.38 (s, CH-NH), 3.57-3.71(s, -C$\underline{H}_2$C$\underline{H}_2$O-), 3.76 (s, N$\underline{H}$), 4.0-4.09 (t, -OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C(O)O-), 5.0 (s, C$\underline{H}_2$(OC(O)NH)), 4.81 (s, N$\underline{H}$), 7.0(m, aromatic backbone), 7.09 (m, aromatic backbone).

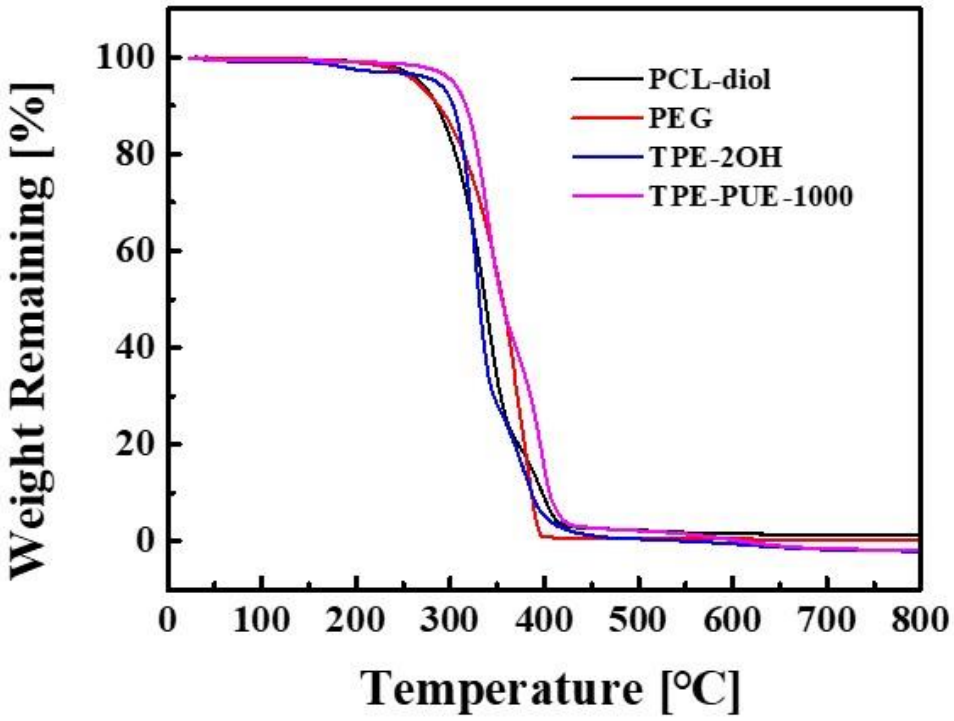


Fig. S12. TGA of TPE-PUE-1000 block polyurethane and the PCL-diol, PEG, TPE-2OH prepolymers.

Table S1

Sample	R[a]	R'[b]	W_e[c]/%	W_e'[d]/%	W_t[e]/%	$W_{t'}$[f]/%	M_w[g]	PDI[h]
TPE-PUE-400	11:5:5:1	11.2:5:5.2:0.94	10.76	11.25	2.108	2.201	56300	1.39
TPE-PUE-1000	11:5:5:1	10.5:5:4.35:1.15	31.48	27.36	1.645	2.145	127700	1.59
TPE-PUE-3400	11:5:5:1	11:4.95:5:1.05	51.02	52.09	1.176	1.235	87600	1.40

[a] R : HMDI/PCL-diol/PEG/TPE molar ratio in feed.

[b] R' : HMDI/PCL-diol/PEG/TPE molar ratio in product calculated from NMR integration.

[c] W_e : The mass percentage of PEG content in feed.

[d] W_e': The mass percentage of PEG in product detected by TGA.

[e] W_t : The mass percentage of TPE-2OH content in feed.

[f] $W_{t'}$: The mass percentage of TPE-2OH in product detected by TGA.

[g] M_w: Weight average molecular weight, determined by GPC using THF as mobile phase.

[h] PDI: M_w/M_n, determined by GPC using THF as mobile phase.

[i] Sample abbreviation TPE-PUE-1000 means that the feeding PCL-diol segment M_n=2000, PEG segment M_n=1000. TPE-PUE-400 and TPE-PUE-3400 were used the same reactive weight ratios of PCL-diol, M_n of PCL-diol was 2600. PEG segment was M_n=400 and M_n=3400, respectively.