Hierarchical porous polycaprolactone microspheres generated in a simple pathway combining nanoprecipitation and hydrolysis

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Content

1. Experimental Section

1.1 Materials

Polycaprolactone (Mw 65,000; Mn 42,500) was purchased from Sigma-Aldrich Inc. and used without further purification. Analytical grade tetrahydrofuran (THF) and sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. Doxorubicin hydrochloride (DOX·HCl, 90%) was purchased from Beijing Zhongshuo Pharmaceutical Technology Development Co., Ltd. and used as received.

1.2 Fabrication of PCL microspheres

To fabricate porous microparticles

8 mL PCL/THF (1 mg/mL) solution and 40 mL NaOH aqueous solution (0.22 M) were prepared and kept in 40 ˚C water bath. Then, NaOH aqueous was added into polymer solution (V_{NaOH} : V_{THF} = 5 : 1) at 40 ˚C, the mixture was shaken vigorously to produce uniform emulsion. The turbid PCL suspension was kept in 40 ˚C water bath for 24 h. Then, 20 mL DI water was added to dilute the suspension. Next, the mixture was poured into a beaker and kept over 3 days to evaporate the volatile organic solvent at 30 ˚C. The resulting suspension was centrifuged at 10,000 rpm for 3 minutes, the sediment PCL particles were washed with DI water several times and collected to conduct further characterizations. The final pH of suspension is ~7.

To fabricate solid microparticles (conventional way)

40 mL DI water was added into 8 mL PCL/THF (1 mg/mL) solution, the mixture was shaken vigorously to produce uniform emulsion. Then, the turbid PCL suspension was poured into a beaker and kept over 3 days to evaporate the volatile organic

solvent at 30 ˚C. The resulting suspension was centrifuged at 10,000 rpm for 3 minutes, the sediment PCL particles were washed with DI water several times and collected to conduct further characterizations.

1.3 General Characterizations

Scanning electron microscopy (SEM, JEOL 7401) was performed at an accelerating voltage of 5 kV. The samples were coated with a thin layer of gold before SEM characterization. The transmission electron microscope (Hitachi TEM, H-7650B) was operated at an accelerating voltage of 80 kV. A droplet of microspheres suspension in water was placed onto copper grids for TEM analysis. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer (Switzerland) and the chemical shifts were reported in parts per million (δ) relative to internal standard TMS (0 ppm) for $CDCl_3$. Differential Scanning Calorimetry (DSC) analysis was performed on a TA Instruments Q100 (TA Instruments New Castle, DE) under N_2. The samples were heated from room temperature to 120 °C with a heating rate of 10 °C/min. The zeta potential values of porous PCL microspheres in aqueous solution at different pH values were measured using a Zetasizer (Nano-ZS, Malven Instruments) at 25 °C. Measurements were performed five times for each sample. The optical microscope images of double-emulsion-like suspension were captured with Olympus BX51 microscope. Fluorescent images of the porous microspheres containing DOX were captured with Olympus BX51 microscope in the green field.

1.4 Gel Permeation Chromatography measurement

Gel Permeation Chromatography (GPC) analyses of polymers were performed on

GPCmax VE-2001 (Viscotek) equipped with a Viscotek TriSEC model 302 triple detector array [refractive index detector, viscometer detector, and laser light scattering detector (7° and 90°)] using two I-3078 polar organic columns. THF was used as the eluent at a flow rate of 1.0 mL/min. Calibration of the molecular weight of polymer was based on polystyrene standards.

1.5 Particle size measurement

qNano Analyzer (Izon Sciences, Christchurch, New Zealand) was used for microspheres size determination with NP2000 nanopore and 2000 nm calibration particles. qNano's working principle and detailed experimental setup have been reported elsewhere.[1] First, the nanopore and cells were cleaned with KCl aqueous solution (0.2 M) and a baseline current (70–140 nA) was developed. Diluted sample or calibration particles (40 μL) were loaded in the upper fluid cell and the lower fluid cell was filled with 80 μL of KCl aqueous solution. All samples were run under the same applied voltage (0.04 V) and stretch (44 mm). Each recorded measurement was based on at least 500 particles. Data was analyzed using Izon control suite 3.0 software.

1.6 Porous measurement

The porosity of cage-like PCL microspheres was determined by the ethanol replacement method.[2] The frozen-dried microspheres were put into a cylindrical shape container and sample's volume was calculated. After measuring its initial weight, the sample was immersed in ethanol to fully absorb the ethanol. The ethanol-absorbed sample was weighed after wiping with tissue paper. Experiments were

performed in triplicate. The porosity of scaffolds was calculated using the following equation:

$$Porosity = \frac{(W_e - W_d) \times 100\%}{\rho_{et} \times V}$$

where W_e is the weight of ethanol-absorbed sample, W_d is the weight of dry sample, ρ_{et} is ethanol density at 20 °C, V is volume of the sample.

1.7 Carboxyl group concentration measurement

The conductometric titration method were performed to determine the concentration of carboxyl on the particle surface.[3] The excess amount of HCl aqueous solution was added to a particle dispersion (30 mg in 20 mL) to pH 2. After 10 min of stirring, the titration was started by slow addition of the standard 0.1 M NaOH solution into the suspension using conductivity meter (DDS-307A, Shanghai INESA Scientific Instrument Co., Ltd., China). The measurements of conductivity were performed over the range of pH from 2 to 11. The amount of carboxyl groups per gram of particles was calculated from the conductivity curve as a function of consumed NaOH solution.

1.8 Loading DOX into PCL microspheres

Loading DOX into porous PCL microspheres

Drug loading was performed by incubating 1.0 mg of porous PCL microspheres with 1.0 mL of 1.0 mg/mL DOX stock solution prepared in PBS solution (pH = 6.5). After stirring at 30 °C for 24 h, DOX-loaded microspheres were collected by centrifugation, and then washed several times with PBS solution to remove the physically adsorbed DOX residues on the surface.

To fabricate DOX-loaded solid microspheres

40 mL DI water was added into 8 mL PCL/DOX/THF (concentration of both PCL and DOX are 1 mg/mL) solution, the mixture was shaken vigorously to produce uniform emulsion. Then, the turbid PCL suspension was poured into a beaker and kept over 3 days to evaporate the volatile organic solvent at 30 ˚C. The DOX-loaded microspheres were collected by centrifugation, and then washed several times with DI water to remove the free DOX in suspension.

The drug content was assayed by UV−vis spectrophotometer (Cary 50 UV–vis instrument) at 479 nm. The loading capacity of DOX in microspheres was calculated according to the following equation:

$$Loading\ efficiency\ (\%) = \frac{Amount\ of\ DOX\ in\ microsphere\ (mg)}{Amount\ of\ DOX\ in\ microsphere + microsphere\ weight\ (mg)} \times 100\%$$

1.9 In Vitro Drug Release

The release kinetics of DOX from porous or solid PCL microspheres (1.0 mg) were measured in 25 mL of PBS buffer with different pH values (pH = 5.0, 7.4, respectively). The samples were incubated at 37 °C under mild stirring. At certain time intervals, 3 mL of the release medium was taken out and centrifuged at 3000 rpm for 3 minutes, and the supernatant was taken out to measure the released drug concentration. Then the supernatant and precipitate was returned to the original release medium. For the measurement of released DOX concentration, the absorbance of the release medium at 479 nm was recorded on UV−vis spectrophotometer. The drug release were tested in three replicates.

2. Supplementary Figures

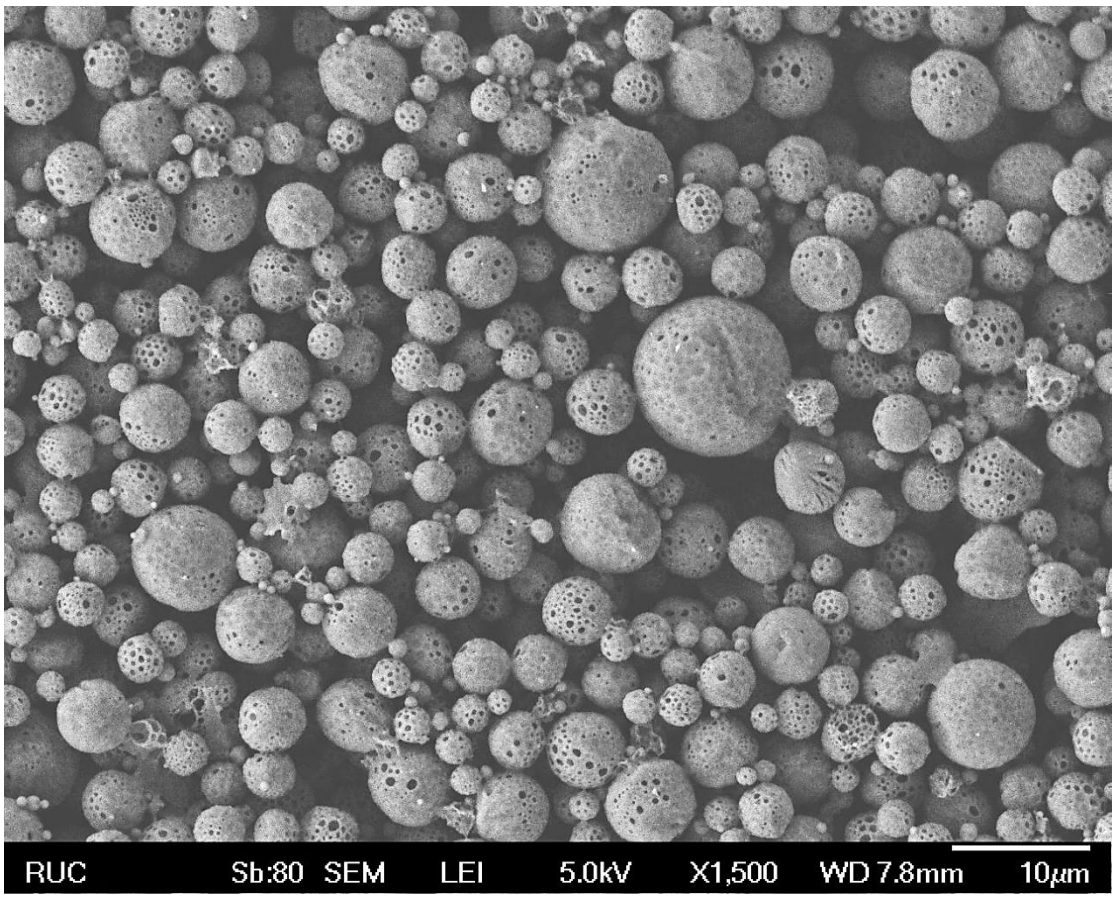

Fig. S1 SEM image of PCL porous microspheres. The concentration of PCL/THF is 1.0 mg/mL, 0.22 M NaOH aq. ($V_{NaOH\ aq.}$: V_{THF} = 5 : 1), they were mixed at 40 ˚C, and kept for 24 hours at 40 ˚C then THF was evaporated at 30 ˚C.

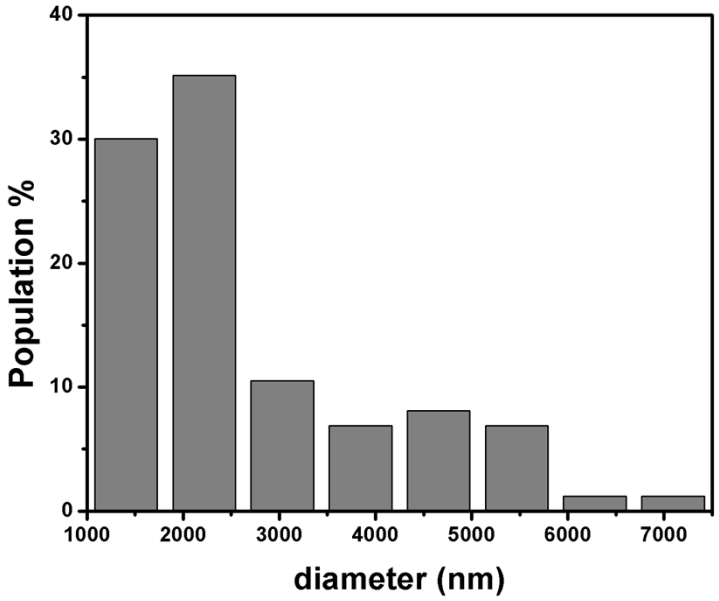

Fig. S2 Size distribution of porous PCL microspheres determined by qNano size analyzer.

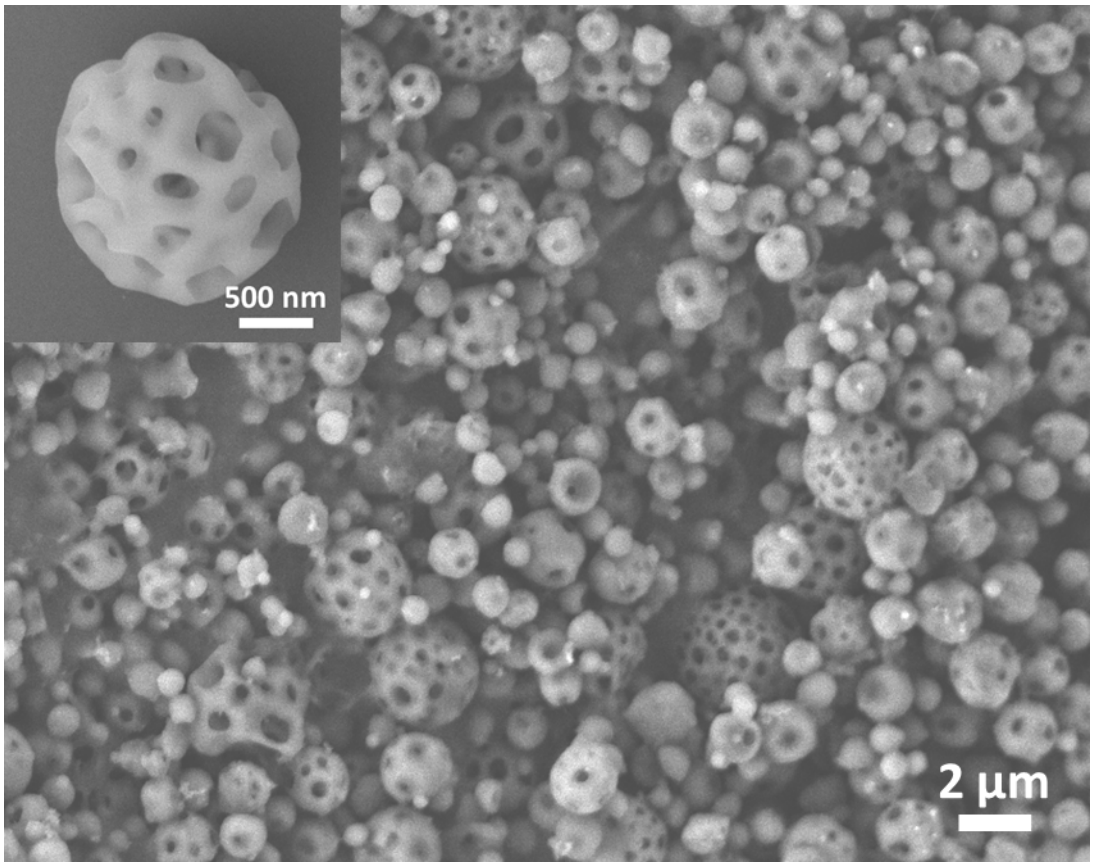

Fig. S3 SEM image of PCL porous microspheres. The concentration of PCL/THF is

0.2 mg/mL, 0.5 M NaOH aq. ($V_{NaOH\ aq.}$: V_{THF} = 5 : 1), they were mixed at 25 ˚C, kept for 12 hours at 25 ˚C then THF was evaporated at 25 ˚C.

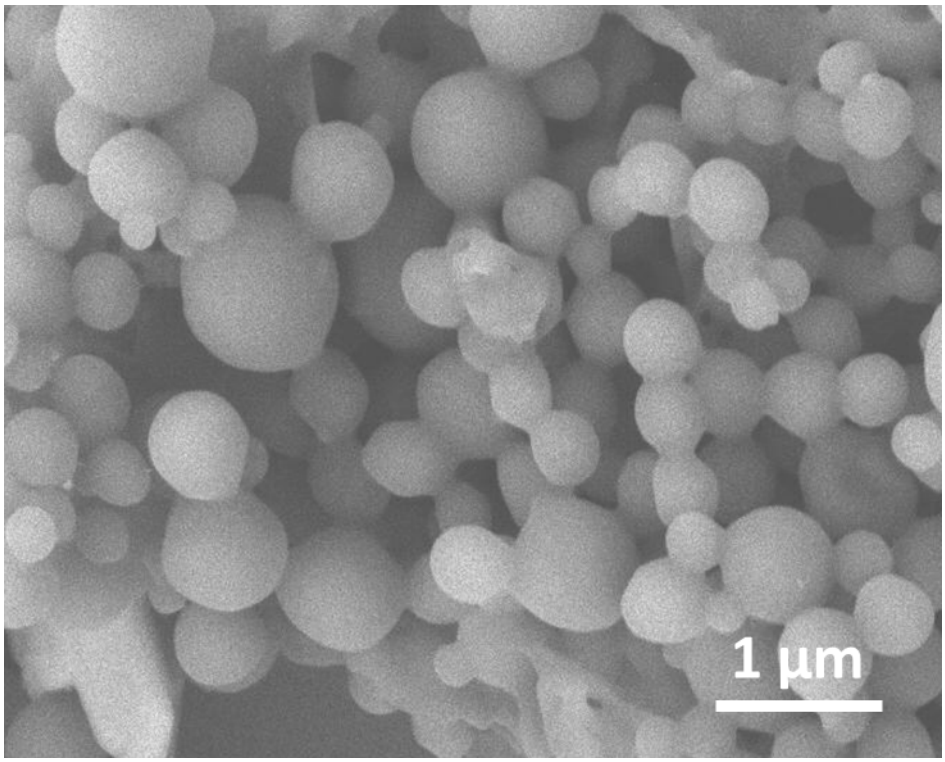

Fig. S4 SEM image of PCL microspheres obtained using water as nonsolvent. PCL concentration is 1.0 mg/mL, V_{water} : V_{THF} = 5 : 1.

Table S1. The molecular weights of PCL in raw material and porous microspheres measured by GPC

	PCL raw material	porous PCL microspheres
Mw (Daltons)	6.97×10^4	2.59×10^4
Mn (Daltons)	5.39×10^4	1.76×10^4
Mw/Mn	1.29	1.47

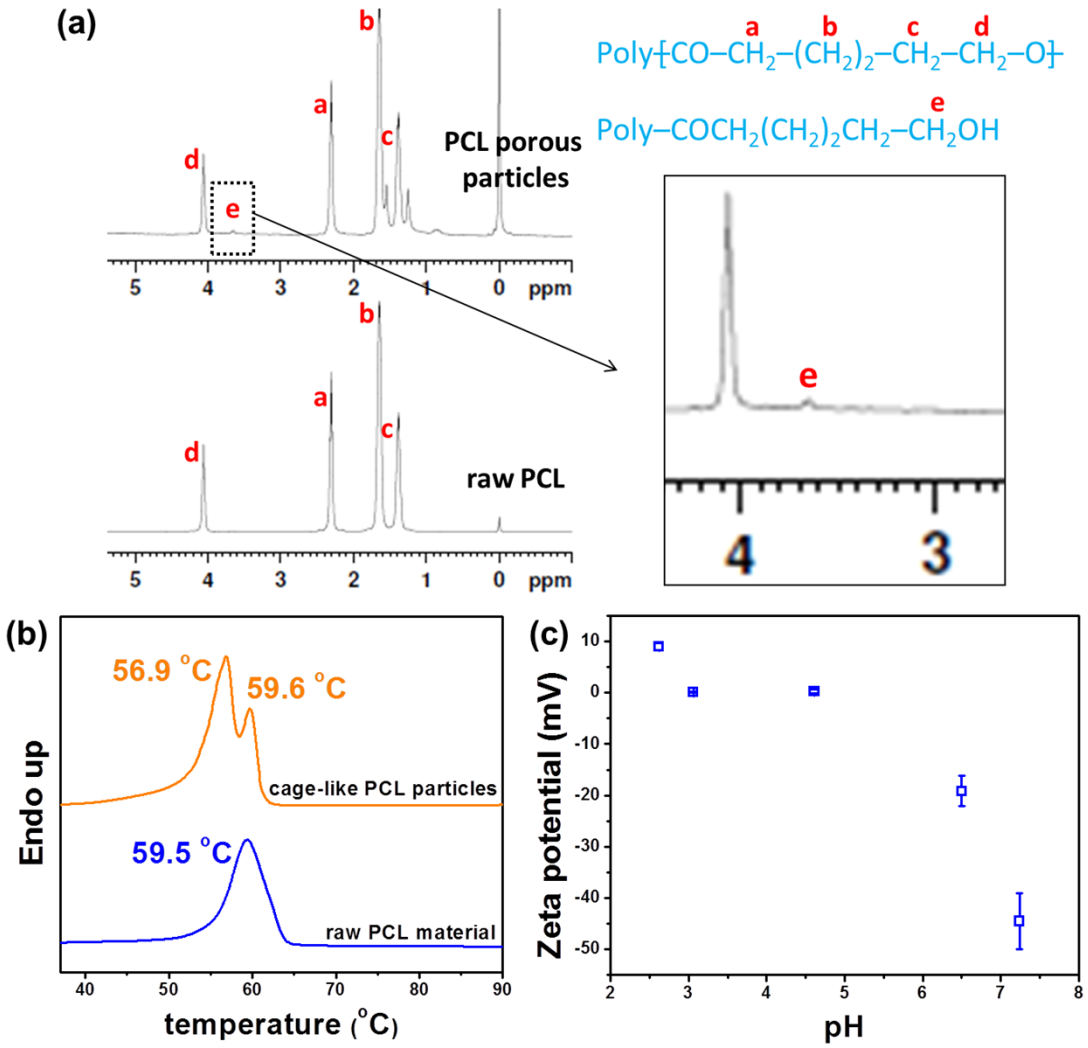

Fig. S5 (a) ^{1}H NMR spectra of raw PCL material and porous microparticles. (b) DSC endotherms of cage-like and raw PCL material. (c) Zeta potential of PCL porous microspheres suspended in aqueous solution at different pH values.

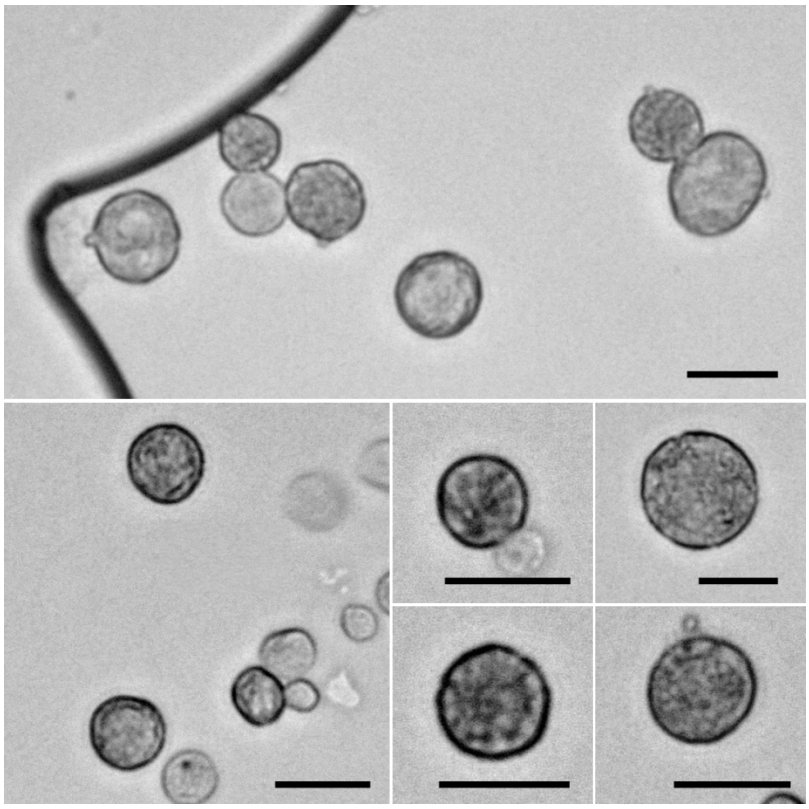

Fig. S6 The optical microscope images of suspension (kept at 40 ˚C for 1 day before dilution and THF evaporation), the scale bars are 10 μm.

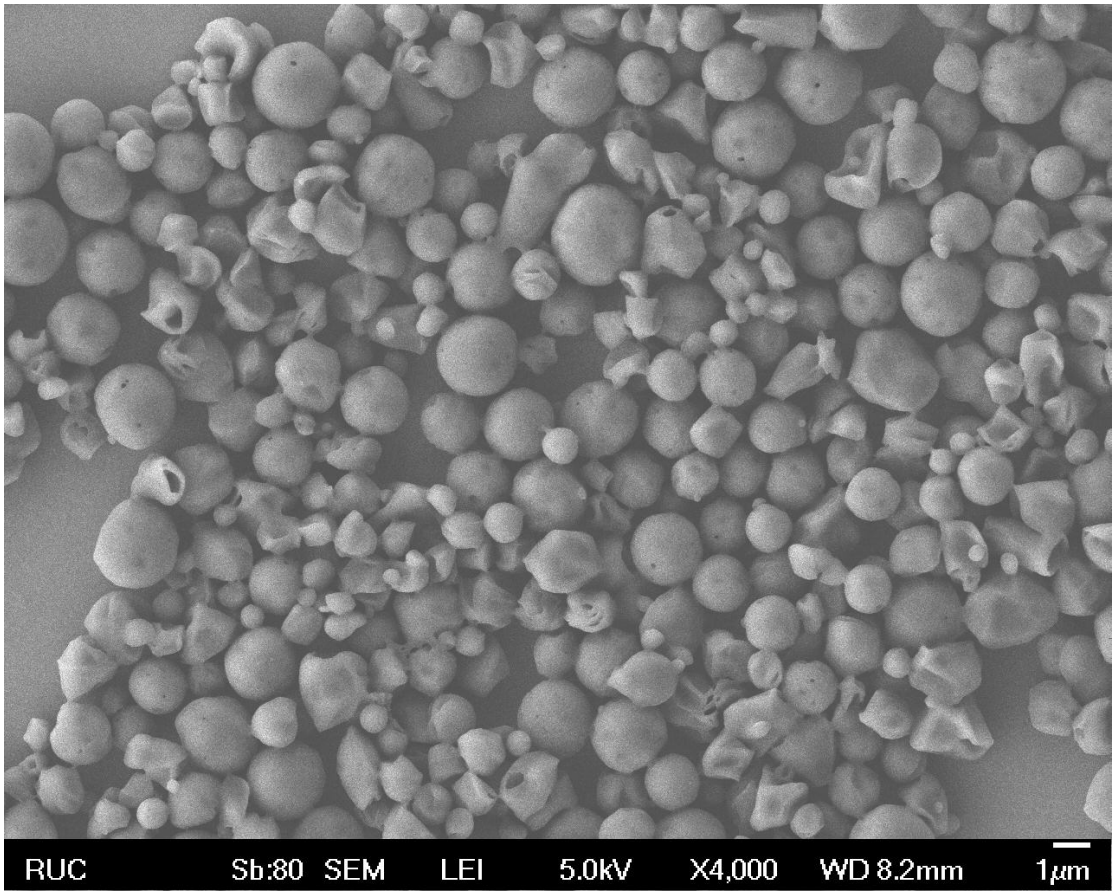

Fig. S7 SEM image of PCL microspheres obtained without keeping in 40 ˚C water bath for 1 day. The concentration of PCL/THF is 1.0 mg/mL, 0.22 M NaOH aq. ($V_{NaOH\ aq.}$: V_{THF} = 5 : 1), they were mixed at 40 ˚C, then THF was evaporated immediately at 30 ˚C.

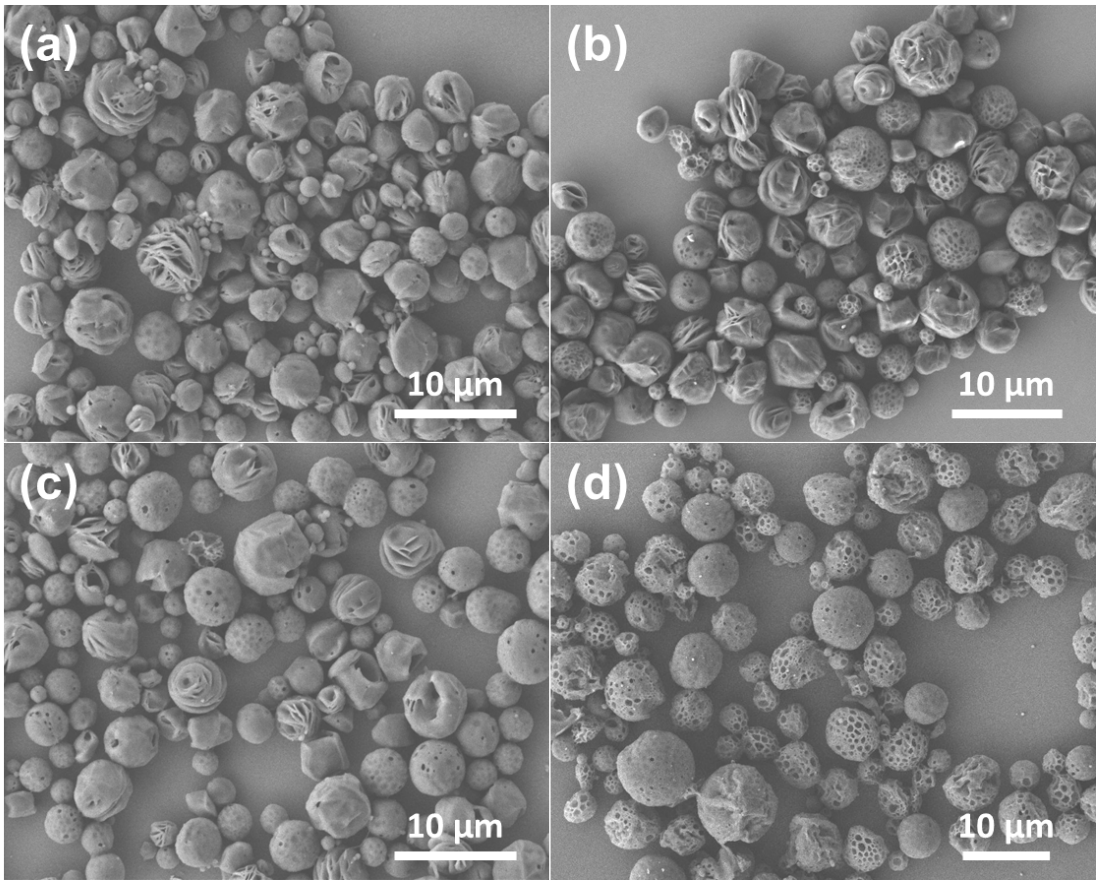

Fig. S8 SEM images of PCL porous microparticles under different mix temperature. The concentration of PCL/THF is 1.0 mg/mL, 0.22 M NaOH aq. ($V_{NaOH\ aq.}$: V_{THF} = 5 : 1), they were mixed at (a) 4 ˚C, (b) 20 ˚C, (c) 30 ˚C, (d) 40 ˚C, kept for 24 hours at 40 ˚C then THF was evaporated at 30 ˚C.

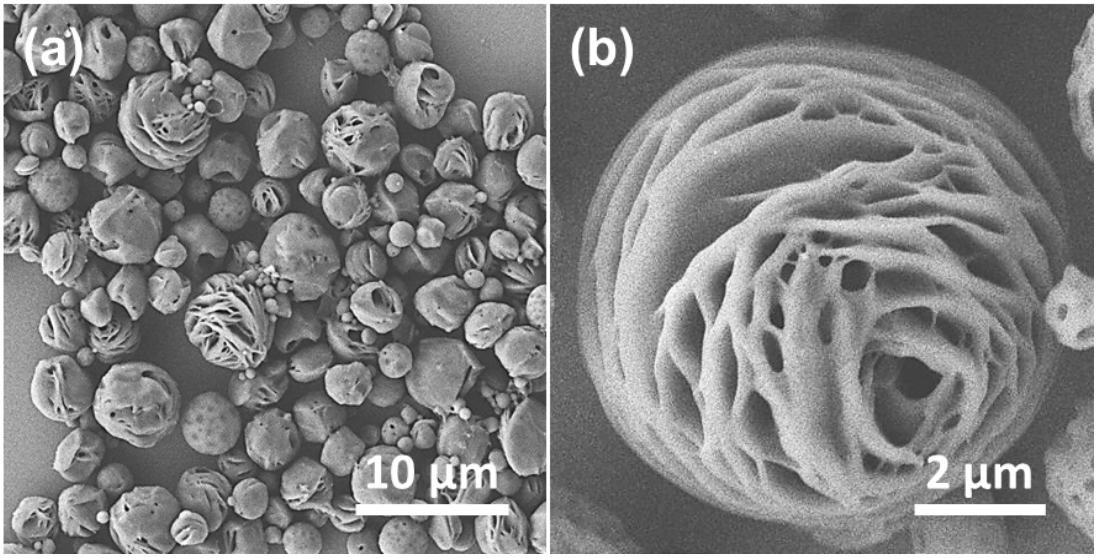

Fig. S9 SEM images of PCL plate-like microparticles generated at lower mixing temperature (4˚C).

References:

[1] R. Vogel, G. Willmott, D. Kozak, G. S. Roberts, W. Anderson, L. Groenewegen, B. Glossop, A. Barnett, A. Turner and M. Trau, *Anal. Chem.*, 2011, 83, 3499.

[2] A. Shahini, M. Yazdimamaghani, K. J. Walker, M. A. Eastman, H. Hatami-Marbini, B. J. Smith, J. L. Ricci, S. V. Madihally, D. Vashaee and L. Tayebi, *Int. J. Nanomed.*, 2014, 9, 167.

[3] A. Musyanovych, R. Rossmanith, C. Tontsch and K. Landfester, *Langmuir*, 2007, 23, 5367.