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Supplementary Information

Synthesis of completely solvent-free biomedical waterborne polyurethane with excellent mechanical property retention and satisfactory water absorption

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EXPERIMENTAL SECTION

Materials

2, 2-dihydroxymethylpropionic acid (DMPA) and organic bismuth catalyst (Bi Cat.) were purchased from Shanghai Aladdin Biochemical Technology Co., LTD. Polycaprolactone diols (PCL1000) and polycarbonate diol (PCDL1000) (Mn=1000) were purchased from Shiru Chemical Industry Co., LTD. Isophorone diisocyanate (IPDI) was purchased from Costron Polymer (China) Co., LTD. 1,3-propylene glycol (PDO) was purchased from TCI (Shanghai) Chemical Industrial Development Co., LTD. Triethylamine (TEA), methylbenzene, isopropanol, dibutylamine, bromocresol green, phenolphthalein, concentrated hydrochloric acid (36%-37%) and sodium hydroxide were purchased from Chengdu Colon Chemical Co. LTD. Trifunctional polycaprolactone (CAPA3091) was purchased from Shenzhen Pastto Chemical Co. LTD. Polyethylene glycol (PEG1450)(Mn=1450) were purchased from Dow Chemical Company. Calcein-AM/PI live/death staining kit were obtained from Beijing Solarbio Science & Technology Co. Ltd. Methyl Thiazolyl Tetrazolium (MTT) kit was obtained from Shanghai Biyuntian Biotechnology Co. Ltd. All materials were used as received without further purification.

Preparation of MWPUs from CAPA3091

The whole reaction uses mechanical stirring, the size of the crescent shaped tetrafluoroethylene stirring rod is 30cm long, the blade is 9cm long and 1.5cm wide when unfolded. The reaction was carried out in a three-mouth flask with a capacity of 500ml.

Polycaprolactone polyol (PCL1000) was dehydrated at 110°C for 2 hours under vacuum.

Firstly, IPDI and catalyst were added to an oven-dried double-necked flask and stirred at 85°C and 300 rpm for 1 hour. Secondly, the 2,2-dihydroxymethylpropionic acid (DMPA) was added dropwise to the flask and stirred at 600 rpm for 1 hour at 85 °C.

Thirdly, the 1,3-propylene glycol (PDO) was added dropwise to the flask for 1 hour. Fourthly, the reactants were cooled to 50°C, and the mixture was neutralized with TEA and stirred for 15 minutes. Then isophorone diisocyanate (IPDI) was replenished to reduce the viscosity. Then, CAPA3091 was added in an ice bath and stirred at 1000 rpm for 15 minutes. Deionized water was added for emulsification and then stirred vigorously (2000 rpm) for 45 minutes. After, the emulsion was heated to 45°C and an additional catalyst was added to catalyze the microemulsion reaction for 3h. The whole reaction process was carried out by toluene-din-butylamine titration. After removal of bubbles by rotary evaporator, the MWPU with a solid content of 20 wt% were obtained. The R ratio in the prepolymer was varied from 1.3 to 1:4, 1.5, 1.6, 1.7, 1.8, which were coded MWPU1.3 MWPU1.4 MWPU1.5 MWPU1.6 MWPU1.7 as respectively.

The corresponding MWPU films were first obtained by pouring 30 mL of emulsion into a watch glass, dried at room temperature, and then dried in vacuo at 80 °C for 24 h to remove the residual water. The representative synthesis routine microemulsion waterborne polyurethane dispersions is shown in Scheme 1 and Scheme 2(a).

The control sample (CWPU) used to investigate the mechanism was emulsified without the addition of CAPA3091, and the rest of the operation was the same as above.

Freeze-dried cast porous scaffold

A serous emulsion of MWPUs was first dialyzed with a dialysis bag (Mw = 3500) to remove small and medium molecules. Then, the solid content of the emulsion was adjusted to 15% by adding deionized water. The 400 microlitre polyurethane emulsion was then injected into a 48-well plate using a pipette. The mold was first stored at 4 °C for 4 hours, then at -20 °C for 24 hours, and next placed in a freeze dryer until the water was completely sublimated. Finally, the mold was placed in a forced air blowing oven at 37 °C for 6 hours before being tested at 23 °C.

Characterization

The infrared spectra of the dried WPU films were recorded on a Nicolet-560 (USA Thermoscientific) FTIR-ATR spectrometer ranging from 400 to 4000cm−1. The peak in 1600-1800cm^{-1} were separated by peakfit 4.0.

Samples were taken every half hour for the prepolymer, the emulsion was sampled after the emulsification, and the emulsion at 45 °C was sampled at half an hour interval. Acetone was used as solvent; the residual isocyanate content was determined by toluene-din-butylamine method.

Particle and morphologies were analyzed by a JEM-2100Plus field emission transmission electron microscope (TEM), operated at 200 kV and equipped with a LaB6 filament, and high-angle annular dark-field-scanning. When analyzing the particle morphologies, the samples were earlier stained with PTA by adding 2 wt % of PTA to the diluted latex. The mixture of diluted latex and PTA was sonicated for 30 min at a maximum power in a Bandelin Sonorex sonicator. A drop of this solution was spin-coated onto a carbon-coated cooper grid (300 mesh) at 1500 rpm for 3 min.

The particle size distribution and zeta potential of the MWPUs were determined by a Zetasizer Nano ZS90 (Malvern Instruments). The MWPUs were diluted with distilled water to around 0.01 wt % before conducting the tests.

The evaluation of emulsion stability was obtained by centrifuging (Lu Xiangyi TG16.5) at 3000rpm for 15min

The tensile testing was carried out on an electronic universal testing machine (Instron4302 Mode) with a crosshead speed of 300 mm/min. Rectangle sample films with a length of 15 mm and a width of 0.8mm were dried at 60 °C for 12 h before use. Wet mechanical properties were measured by soaking the strips in deionized water for 24h. At least three replicates of each resulting PU film were taken for the tests.

In the wet condition, the film rebound is stretched to 200% of the deformation at a speed of 50mm/min, and then recovered at the same speed to test. The cycle test ends after half an hour.

Rheological tests were carried out with a rheometer (TA, Discovery HR-3, US) with parallel plates (25 mm diameter, 0°) at 37 °C. In a typical experiment, the equilibrium swelled hydrogel was cut into a cylinder with the diameter of 25 mm and thickness of 1~2mm. The wet film is compacted with the parallel plates. The storage modulus (G') and loss modulus (G'') were recorded with constant strain of 0.1% while sweeping the frequency from 0.1 Hz to 100 Hz.

Dynamic mechanical analysis (DMA) of the PU films was performed on a TA Q800 dynamic mechanical analyzer, in the tensile mode at 1 Hz, using rectangular specimens of 6.46 mm *2mm *0.45 (length *width *thickness). The specimens were heated from −90 to 140 °C at a rate of 3°C min^{-1}. The glass transition temperatures (T_g) were determined by the peaks of the loss factor curves.

X-ray diffraction (XRD) was used to analyze the crystallization of the material. The MWPU films was tested using an XRD instrument (Rigaku Ultima IV) equipment performed at a voltage of 40 kV with a filament current of 40 mA. The scanning 2θ range was from 5°–70° with a scanning rate of 5 min^{-1}. The whole process takes place in a nitrogen atmosphere.

Differential scanning calorimetry (DSC) was carried out on a thermal analyzer (TA DSC250). All of the samples were weighed between 5 and 8mg. They were first cooled to −90 °C and isolated for 5min, and later heated to 260 °C at a rate of 10 °C/min. The midpoint temperature in heat capacity change of the second DSC scan is considered to be T_g.

The water contact angle of the resulting MWPU films was measured on a contact angle goniometer (DSA100, Germany) with the sessile drop method at room temperature. All the membranes were glued to the slide, three locations were tested for each membrane, and the results were averaged.

The transparency test was carried out by a UV3600 (Shimadzu, Japan) UV spectrophotometer with an integrating sphere accessory. The spectrum was collected in the 400 to 800 nm spectral range with a resolution of 0.5 nm. The membranes tested were of the same thickness and soaked for 24 hours.

The water absorption of the resulting MWPU films was measured by this formula

$$Water\ absorption\ rate = \frac{M2 - M1}{M1}$$

Where M1 is the mass of the film before soaking in water, M2 is the weight after soaking in water for 24 hours. All films were of the same shape and three parallel samples were tested in each group.

The microscopic pore structure of the scaffolds was observed using a scanning electron microscope (Nova NanoSEM450) at 5 kV after gold coating.

The average size of the 3D porous scaffolds was collected from SEM images (n ≥ 6) and was semi-quantified by Image J. The porosity was calculated from the equation:

$$Porosity = 100\% * \frac{V\ scaffold * \rho\ MWPU - m\ scaffold}{V\ scaffold * \rho\ MWPU}$$

The compression properties of the scaffolds were evaluated using a commercial universal tensile tester (HZ-1004 Dongguan lixian instrument Scientific Co. Ltd). The mechanical properties of the scaffolds in the dry and wet state were tested. The compression speed was 10 mm min^{-1}, and a mechanical sensor of 1 kg was selected. All scaffolds were cylindrical in shape (10 mm in diameter and 5 mm in thickness). In the compression test, the compression process stopped when the maximum compression deformation reaches 70%. All mechanical performance tests were performed at 25 °C. All of the values were expressed as mean ± standard deviation (n ≥ 3).

Similarly, the scaffolds were soaked in water for 24h. The molding recovery rate of the scaffolds were calculated by the ratio of the height of the scaffolds before and after compression. And the swelling rate of the scaffolds were obtained by the ratio of the volume change before and after the stent was soaked in water.

The bio biocompatibility of the resulting MWPU scaffolds were measured by hemolysis test and total degradation solution toxicity test.

Using 10ml 1M NaOH solution to accelerated degradation 0.5g MWPU scaffolds until all solids disappeared, and 1M HCl solution was used to adjust pH of the degradation solution to 7.4. The 0.2μm filter cartridge was used for filtration and removal of bacteria. The filtrated degradation solution was diluted 100, 1000 and 10000 times by cell medium, respectively. 100μL of each degradation solution was added into 100μL medium containing L929 cells, and cultured at 37 °C for 3 days in a humid environment of 5% carbon dioxide. After culture to the expected time, the cell survival rate was verified by MTT method.

10ml of fresh human venous blood was taken and placed in a collection vessel containing anticoagulants. After centrifugation for three times, red blood cells were collected. Normal saline was added to form a 10% erythrocyte suspension, and the sample was diluted in half. The 5ml EP tubes were added with 1ml 5% erythrocyte suspension and 0.5g MWPU scaffolds, each sample was prepared for three duplicate samples. Using 500μL 0.9% normal saline and 500μL 4% erythrocyte suspension as the negative control and using 500μL 1%Tritan X-100 and 500μL red cell suspension as the positive control. All samples were store in oven at 37°C for 1h, then centrifuge at 4000rpm for 5min. Taking the supernatant 200μL in another 96-well plate and using enzyme marker to measure the absorbance of at 576nm. Erythrocyte hemolysis rate is calculated by the following formula.

$$Hemolysis\ rate\ （\%）=\frac{A\ sample-A\ negative\ control}{A\ positive\ control-A\ negative\ control}*100\%$$

Live/dead staining experiments were performed in 24-well plates (50 000 cells well^{-1}).

The L929 and RSC96 cells survival after co-culture with MWPU membrane (1cm*1cm) were observed by confocal laser scanning microscopy (LSCM, Nikon N-SIM) after utilizing a live/dead cell double-staining kit (calcein AM·PI) according to the instructions.

RESULT AND DISCUSSION

The stability of MWPU emulsion

When the R value reached 1.7, the distribution of the particle size changed from a single peak to a double peak because the IPDI content was significantly high, such that free IPDI in the water phase appeared, and the side reactions of IPDI with water caused chain extension to occur not only within the colloidal particles but also between the colloidal particles[1]. This result is consistent with the increasing proportion of urea carbonyl groups (Figs. S3(a-e)). The three sets of data exhibited similar trends in particle size variation. As summarized in Table S2, the particle size of polyurethane did not change significantly after one year of storage, and the distribution was slightly broadened.

FTIR-ATR spectrum analysis of MWPU films

The chemical structure of the MWPU films was determined by FTIR-ATR, and the data are shown in Fig. S2. In the FTIR-ATR spectra of all films, the absence of the isocyanate peak in the 2250cm^{-1}-2270 cm^{-1} region totally disappeared which indicates the completion of the urethane reaction. The broad peaks at 3365cm^{-1} are assigned to the -N-H stretching vibrations of the polymers. In addition, all peaks appeared from 1600cm^{-1} to 1800cm^{-1} correspond to the presence of five types of carbonyl peaks in the polymer matrix: one for the C=O stretching vibration of the urethane from 1706cm^{-1} and the other for the -C=O stretching vibration of the urea from 1639cm^{-1} respectively [2] [3].The small peak at 1460 cm^{-1} is assigned to the bending vibration of the -N-H bond. In FT-IR spectra of all MWPUs, a small broad band in the range 977cm^{-1}-1211 cm^{-1} corresponds to the presence of -O-C=O and -C-N bonds. The presence of all such bonds confirmed that the urethane linkage was successfully formed in the polymer.

Water contact angle of MWPU films

Fig. S5 shows the water contact angle data of MWPU films with different R values. It can be seen that the incorporation of IPDI decreased the water contact angles of MWPU films, indicating an improvement in surface hydrophilicity. There are two factors that affect the water contact angles of the films: the polarity group content and the crosslink density. A significant downward trend was observed for the PU films when the R value increased from 1.3 to 1.7. Specifically, the water contact angle of the MWPU1.3 film is 84.2°, while the water contact angle of the film decreases to 64.9° when the R value increases to 1.7.

This is due to the stronger formation of urethane and urea bonds. When the R-value increases, CAPA3091 as a flexible long-chain crosslinker, which can exposed more polar groups appear on the surface, and the surface is so hydrophilic that the contact angle becomes smaller[4, 5]. This result is well cross-checked with the Fig. S3.

Samples	PCL1000（g）	IPDI-1（g）	DMPA（g）	PDO（g）	IPDI-2（g）	CAPA3091（g）
MWPU1.3	16.52	13.53	1.83	0.221	1.044	10.43
MWPU1.4	16.52	13.53	1.83	0.221	2.171	10.43
MWPU1.5	16.52	13.53	1.83	0.221	3.286	10.43
MWPU1.6	16.52	13.53	1.83	0.221	4.445	10.43
MWPU1.7	16.52	13.53	1.83	0.221	5.534	10.43
CWPU	16.52	13.53	1.83	0.221	1.044	0

Table S1. Formulations for synthesizing MWPU and CWPU.

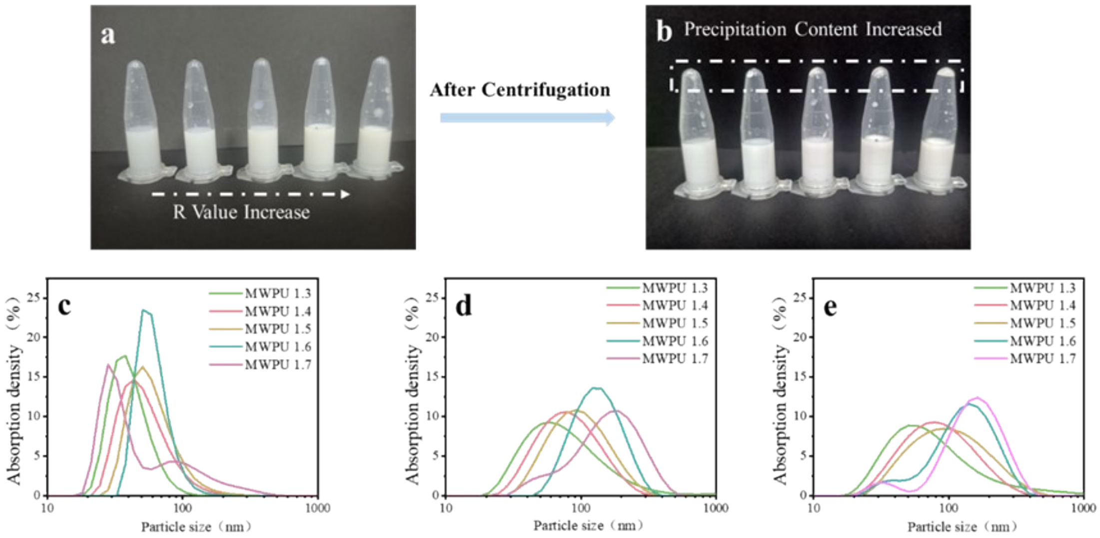

Fig. S1 Appearance of MWPUs obtained using varying R ratios before one year (a) before centrifugation and (b) after centrifugation. (c) Particle size distribution of MWPUs obtained using varying R ratios before one year (d) before centrifugation and (e) after centrifugation.

Sample	**Particle size one year ago （nm）**	**Zeta potential one year ago （mv）**	**Before centrifugal particle size （nm）**	**Before centrifugal Zeta potential （mv）**	**After centrifugal particle size （nm）**	**After centrifugal Zeta potential （mv）**
MWPU1.3	57.7	-39.3	64.87	-45.7	61.89	-51.8
MWPU1.4	79.5	-45.2	74.96	-46.6	73.23	-40.3
MWPU1.5	86.3	-54.8	91.78	-39.0	85.14	-42.0
MWPU1.6	110.6	-55.2	112.6	-46.5	108.3	-47.4
MWPU1.7	136.3	-60.1	128.1	-46.5	123.8	-54.3

Table S2. Properties of the resulting MWPU emulsions.

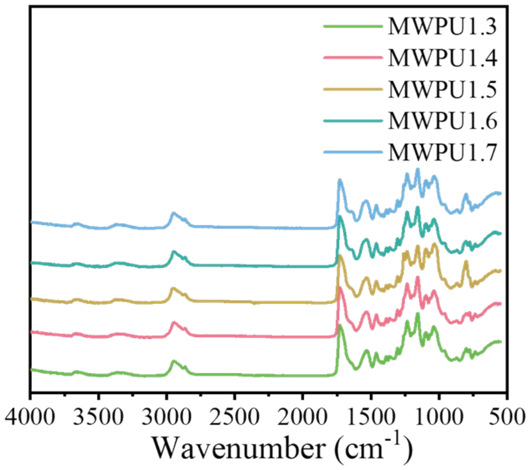

Fig. S2. Infrared spectrum of MWPU films

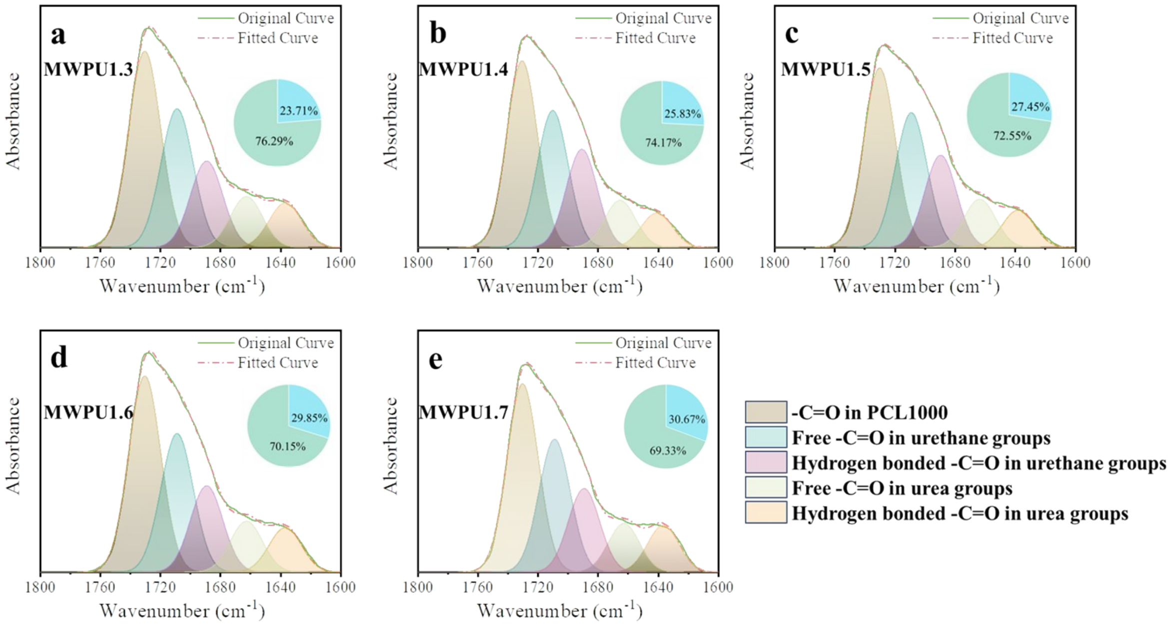

Fig. S3 (a-e) Schematic of the split peaks in the 1600–1800 cm^{-1} region (the pie chart represents the peak areas of the urea carbonyl (blue) and urethane carbonyl (green))

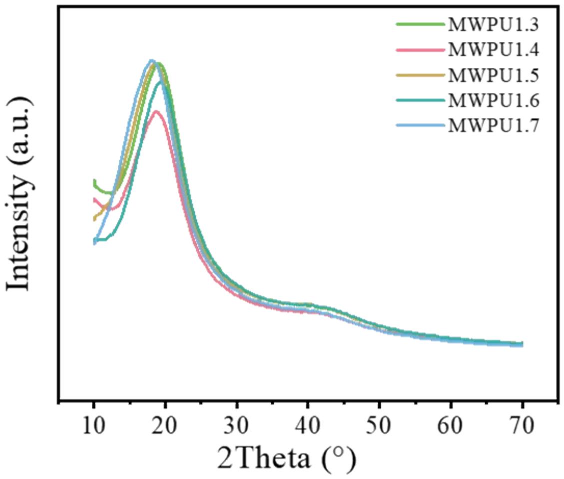

Fig. S4. XRD curve of MWPU films

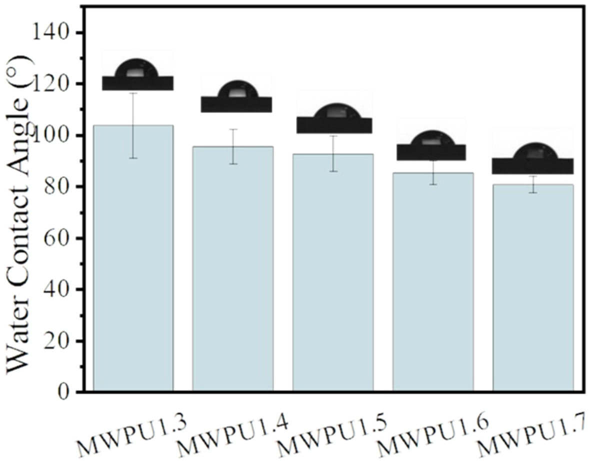

Fig. S5. Water contact angle of MWPU films.

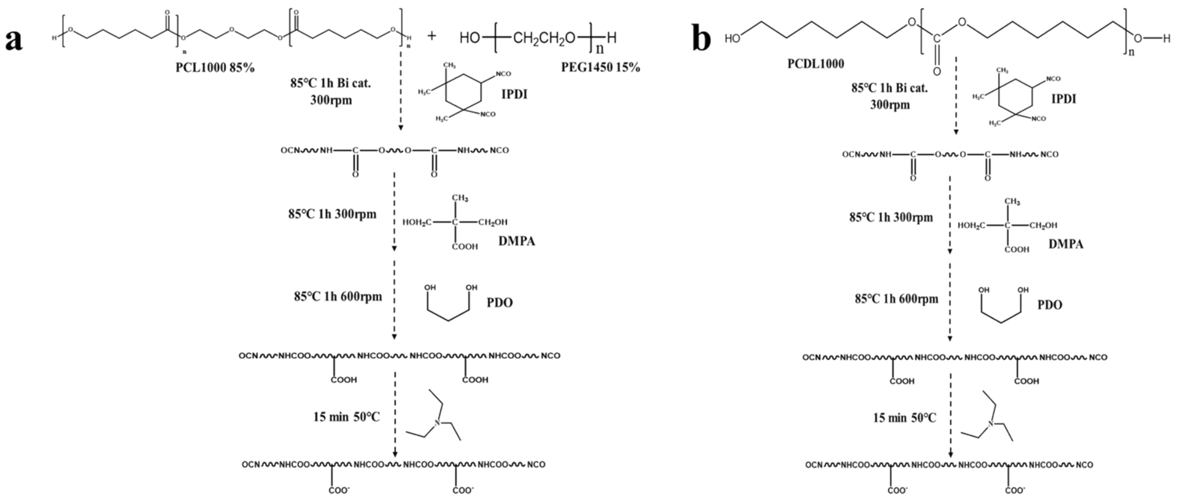

Fig. S6 The synthesis route of （a）EWPU （b）DWPU

Samples	PEG1450 (g)	PCL1000 (g)	IPDI-1（g）	DMPA（g）	PDO（g）	IPDI-2（g）	CAPA3091（g）
EWPU	2.61	14.42	13.53	1.83	0.221	4.445	10.432

Table S3 Synthetic formula of EWPU

Samples	PCDL1000（g）	IPDI-1（g）	DMPA（g）	PDO（g）	IPDI-2（g）	CAPA3091（g）
DWPU	16.52	13.53	1.83	0.221	3.286	10.432

Table S4 Synthetic formula of DWPU

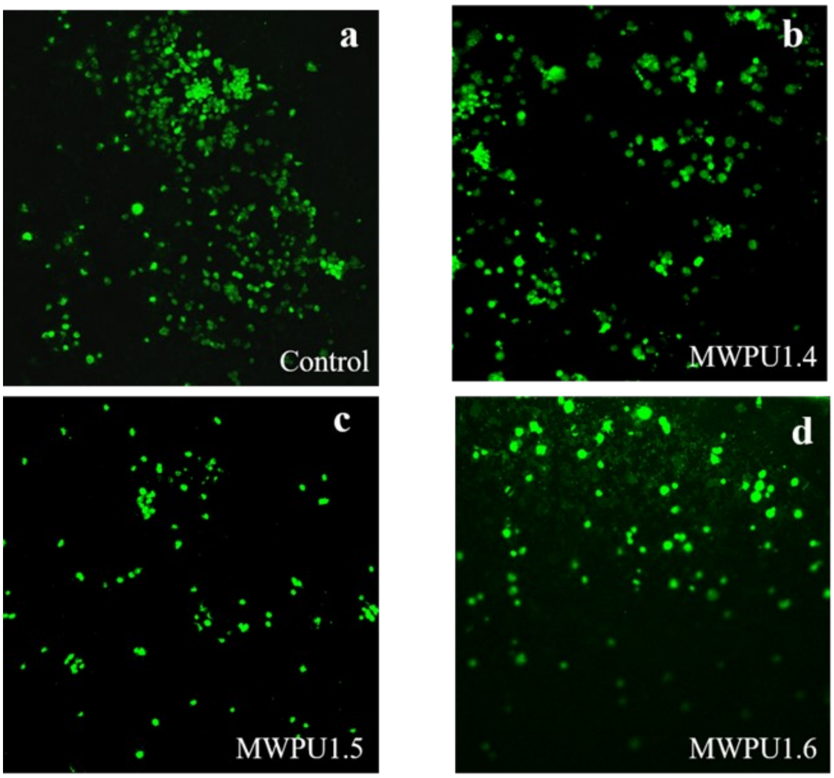

Fig. S7. Live-dead staining of RSC96 cells （3 days）

Reference paper

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