Supporting Information for

The synthesis and characterization of carboxybetaine functionalized polysiloxanes for the preparation of anti-fouling surfaces

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S1. Synthesis of PDMS-g-CB

S1.1 Synthesis of hydrogen-containing polysiloxanes (PDMS-co-PHMS)

PDMS-co-PHMS was synthesized via ring-opening polymerization (ROP) of D_4, D^H_4 and MM with strong acid cation exchange resin as the catalyst. [1] The hydrogen content and molecular weights of PDMS-co-PHMS can be controlled by adjusting the mole ratio of reactants, and the following synthetic process takes $MD^H_8D_{24}M$ as an example.

Typically, a 250 mL three-necked flask was charged with D_4 (0.3 mol, 88.98 g), D^H_4 (0.1 mol, 24.05 g), MM (0.05 mol, 8.11 g) and 1.13 g Purolite® CT175 (1 wt%). The resulting mixture was stirred with a mechanical stirrer at 65 ºC under a nitrogen atmosphere for 12 h. To stop the polymerization, the mixture was cooled and the viscous solution was filtered through a glass filter under reduced pressure to remove the solid catalyst. Then the volatile oligomers were distilled off under reduced pressure (< 15 mmHg) at 150 ºC for 3 h. PDMS-co-PHMS containing different D^H units (D^H / ($D+D^H$) = 1/3 ~ 1/8) were prepared, which are colorless, viscous oils.

FT-IR (KBr, cm^{-1}): 2965, 2905 (-CH_3, ν); 2158(Si-H, ν); 1410 (-CH_3, δ_{as}); 1262 (-CH_3, δ_s); 1095, 1032 (Si-O-Si, ν); 912 (Si-H, δ); 802 (Si-C, δ_{as}); 690 (Si-C, δ_s).

^{1}H-NMR δ ($CDCl_3$, ppm): -Si(CH_3)$_2$-O-, 0.075 (DDD); 0.096 (DDD^H); 0.119 (D^HDD^H). -Si(CH_3)H-O-, 0.147 (DD^HD); 0.171 (D^HD^HD); 0.197 ($D^HD^HD^H$). -Si-*H*, 4.683 (DD^HD); 4.697 (D^HD^HD); 4.715 ($D^HD^HD^H$).

The molecular weight and hydrogen content of various PDMS-co-PHMS were measured with GPC and ^{1}H-NMR, as shown in **Table S1**.

Table S1. Molecular weights and hydrogen content of various PDMS-co-PHMS

Notation [a]	D^H / (D^H+D)	H% calc. [b]	M_n (×10^3 g/mol) calc. [b]	GPC (×10^3 g/mol)			H% NMR [c]
				M_n	M_w	PDI	
$MD^H_8D_{16}M$	1/3	0.438	1.83	1.79	3.24	1.81	0.418
$MD^H_8D_{24}M$	1/4	0.331	2.42	2.39	4.23	1.77	0.319
$MD^H_8D_{32}M$	1/5	0.266	3.01	2.83	4.82	1.70	0.256
$MD^H_4D_{20}M$	1/6	0.213	1.88	1.76	3.06	1.74	0.205
$MD^H_4D_{24}M$	1/7	0.184	2.18	2.10	3.75	1.79	0.181
$MD^H_4D_{28}M$	1/8	0.162	2.47	2.26	3.95	1.75	0.160

a. Reaction conditions: temperature: 65°C, reaction time: 12 h, catalyst dosage: 2.0%, stirring rate: 400 rpm.

b. Throretical H% and M_n were calculated from the designed molecular formula.

c. H% was determined by ^{1}H-NMR spectrum based on end group analysis.

S1.2 Synthesis of PDMS-g-DMAA via hydrosilylation

The hydrosilylation reaction of PDMS-co-PHMS with DMAA was carried out in the presence of Karstedt's catalyst. Typically, 10.20 g DMAA (0.12 mol) and 98 mg Karstedt's catalyst (20 ppm, 0.5 wt%) was weighted into a 250 mL round-bottom flask equipped with a dropping funnel, a reflux condenser and a gas inlet cock. After purging the flask with nitrogen, 24.18 g $MD^H_8D_{24}M$ (0.01 mol) were added via the dropping funnel under a continuous flow of nitrogen and the reaction allowed to proceed at 100 ºC. During the course of reaction, samples were removed periodically and monitored by FT-IR spectroscopy. The reaction was conducted until no Si-H absorption (2154 cm^{-1}) was detected by FT-IR spectroscopy. Then the resulting liquid was distilled under reduced pressure to remove the remaining DMAA to afford the final product.

FT-IR (KBr, cm^{-1}): 2963, 2903, 2814, 2764 (-CH_3, -CH_2-, -CH-, ν); 1462 (-CH_2-, δ); 1412 (-CH_3, δ_{as}); 1262 (-CH_3, δ_s); 1094, 1025 (Si-O-Si, ν); 801 (Si-C, δ_{as}); 689 (Si-C, δ_s).

^{1}H-NMR δ ($CDCl_3$, ppm): 0.05-0.09 (m, -Si-C*H*$_3$); 0.47, 0.49, 0.51 (t, -Si-C*H*$_2$); 0.90 (m, -Si-C*H*-); 0.97, 0.99 (d, -CH-C*H*$_3$); 1.46-1.54(m, Si-CH_2-C*H*$_2$-); 2.16 (m, -CH-C*H*$_2$ and –CH-CH_2-n(C*H*$_3$)$_2$); 2.17 (m, -C*H*$_2$-N(CH_3)$_2$); 2.21-2.28 (s, -N(C*H*$_3$)$_2$).

^{13}C-NMR δ ($CDCl_3$, ppm): 1.56-2.36 (-Si-*C*H$_3$); 12.31 (-Si-CH-*C*H$_3$); 15.55 (-Si-*C*H$_2$-); 20.45 (-Si-*C*H-); 21.66 (-Si-CH_2-*C*H$_2$-); 45.95 (-N(*C*H$_3$)$_2$); 61.92 (-Si-CH(CH_3)-*C*H$_2$-); 63.64 (-Si-CH_2-CH_2-*C*H$_2$-).

S1.3 Preparation of PDMS-g-CB via quaternization reaction

The tertiary amino functionalized polysiloxanes (PDMS-g-DMAA) were converted into zwitterionic carboxybetaine functionalized polysiloxanes by the quaternization reaction with sodium chloroacetate (SC). Typically, certain amount of PDMS-g-DMAA and SC (dissolved in distilled water) were mixed in a 1 / 1.2 molar ratio with an equal weight of dry isopropanol as the solvent. Then the mixtures were heated to 100 ºC for 24 h under nitrogen atmosphere. Afterwards, isopropanol and water were removed from the reaction vessel by reduced pressure distillation, and the residues were re-dissolved in a 50 / 50 (v / v) mixture of methanol and isopropanol. The insoluble impurities were separated by filtration, and the solvents were evaporated in vacuo. The residue solid was washed once again with ethanol, then re-precipitated with acetone, and the resulting product was dried in vacuum oven at 100 ºC for 3 h.

FT-IR (KBr, cm^{-1}): 2963, 2903, 2816, 2767 (-CH_3, -CH_2-, -CH-, ν); 1633 (C=O, ν); 1483, 1460 (-CH_2-, δ); 1395 (-CH_3, δ_{as}); 1262 (-CH_3, δ_s); 1090, 1026 (Si-O-Si, ν); 802 (Si-C, δ_{as}); 690 (Si-C, δ_s).

^{1}H-NMR δ (D_2O, ppm): 0.07-0.28 (m, -Si-C*H*$_3$); 0.67 (t, -Si-C*H*$_2$); 1.02 (m, -Si-C*H*-); 1.15 (d, -CH-C*H*$_3$); 1.82(m, Si-CH_2-C*H*$_2$-); 2.87-2.97 (m, -CH-CH2-N(C*H*$_3$)$_2$); 3.25(m, –CH-CH_2 -N(C*H*$_3$)$_2$); 3.38 (m, Si-CH-C*H*$_2$); 3.61 (t, Si-CH_2-C*H*$_2$-); 3.77, 3.89 (d, -C*H*$_2$-COO).

S2. Determination of critical micelle concentration (CMC) of PDMS-g-CB

The CMC of PDMS-g-CB was determined by two commonly used methods, namely fluorescence probes and electrical conductivity methods, as detailed in the following.

S2.1 CMC determination by fluorescence probes method

The CMC of PDMS-g-CB was measured with pyrene as the fluorophore, which is the most commonly used hydrophobic probe in fluorescence spectroscopy. 10 mL pyrene/acetone soulution (6×10^{-4} mol / L) were injected into a series of 10 mL volumetric flasks, and the flasks were poured with nitrogen to remove acetone. Then certain amount of PDMS-g-CB were added into the flasks and diluted with de-ionized water (the concentration of PDMS-g-CB vary from 5×10^{-4} g / L to 20 g / L), followed by sonicating for 2 h. The solutions were equilibrated for 12 h at room temperature, and then steady-state fluorescence measurements were performed on a Hitachi F-4500 spectrophotometer with bandwidths of 5 nm and 2.5 nm for excitation and emission, respectively. The fluorescent spectra were measured between 350 nm and 480 nm with the excitation wavelength (λ_{ex}) of 334 nm.

Figure S1 shows the pyrene fluorescent intensity ratio (I_1 / I_3) as a function of various concentrations of PDMS-g-CB with different carboxybetaine grafting ratio (1/3 ~ 1/8).

S2.2 CMC determination by electrical conductivity method

As a novel zwitterionic polymer, PDMS-g-CB contains both cationic and anionic groups, thus the electrical conductivity method could be applied to measure the CMC value. A series of PDMS-g-CB were dissolved in de-ionized water to form aqueous solutions with different concentration (C, varied from 5×10^{-4} g / L to 20 g / L), and then the electrical conductivity (κ) were measured with a DDS-11A conductivity meter (Shanghai, China). The CMC values could be determined by the log C - κ curves.

Figure S2 show the conductivity plots for PDMS-g-CB with different carboxybetaine grafting ratio

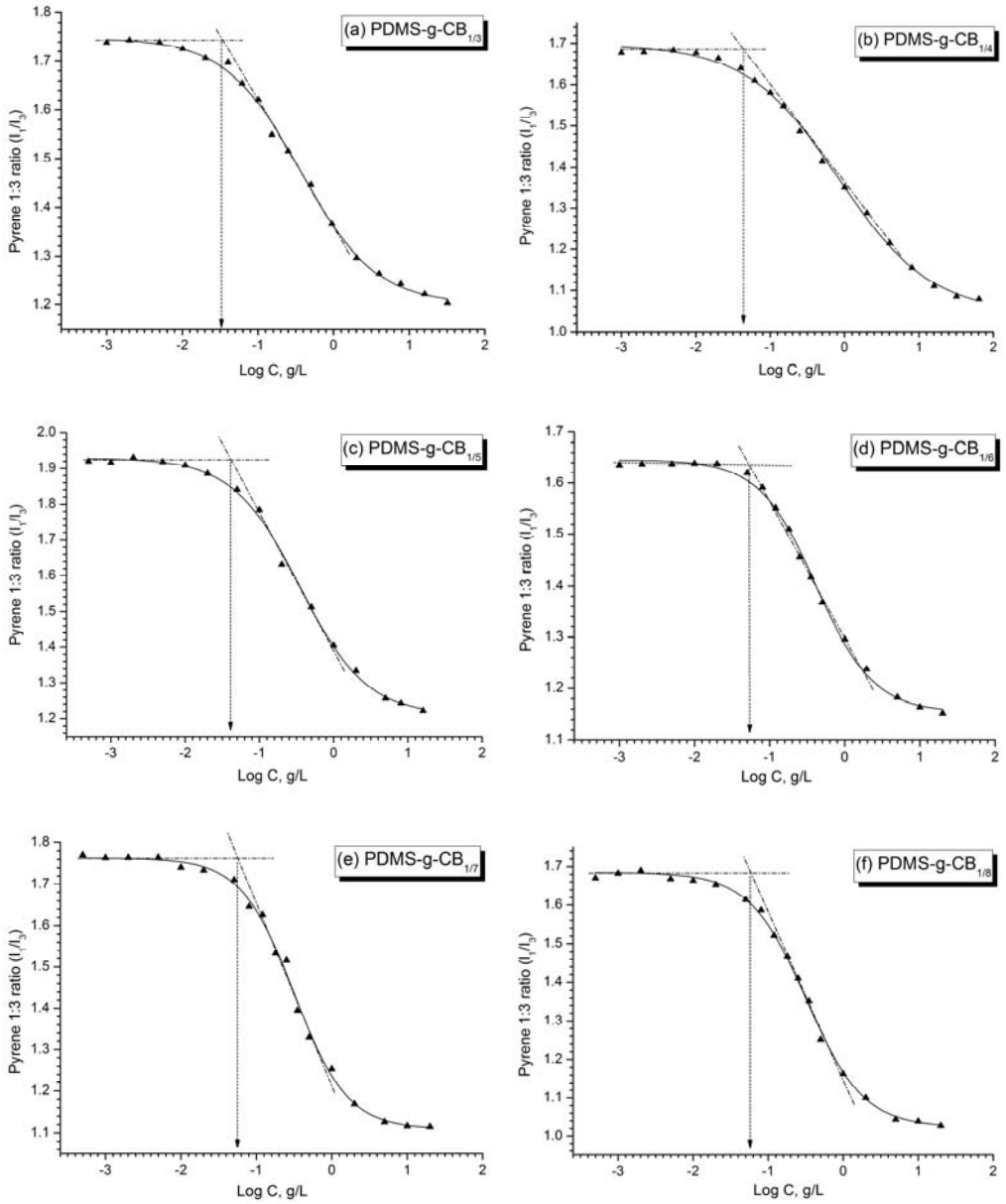

Figure S1. Plots of the intensity ratio I_1/I_3 of the vibrational bands in the pyrene fluorescence spectrum as a function of PDMS-g-CB concentration: (a) PDMS-g-$CB_{1/3}$, (b) PDMS-g-$CB_{1/4}$, (c) PDMS-g-$CB_{1/5}$, (d) PDMS-g-$CB_{1/6}$, (e) PDMS-g-$CB_{1/7}$, (f) PDMS-g-$CB_{1/8}$.

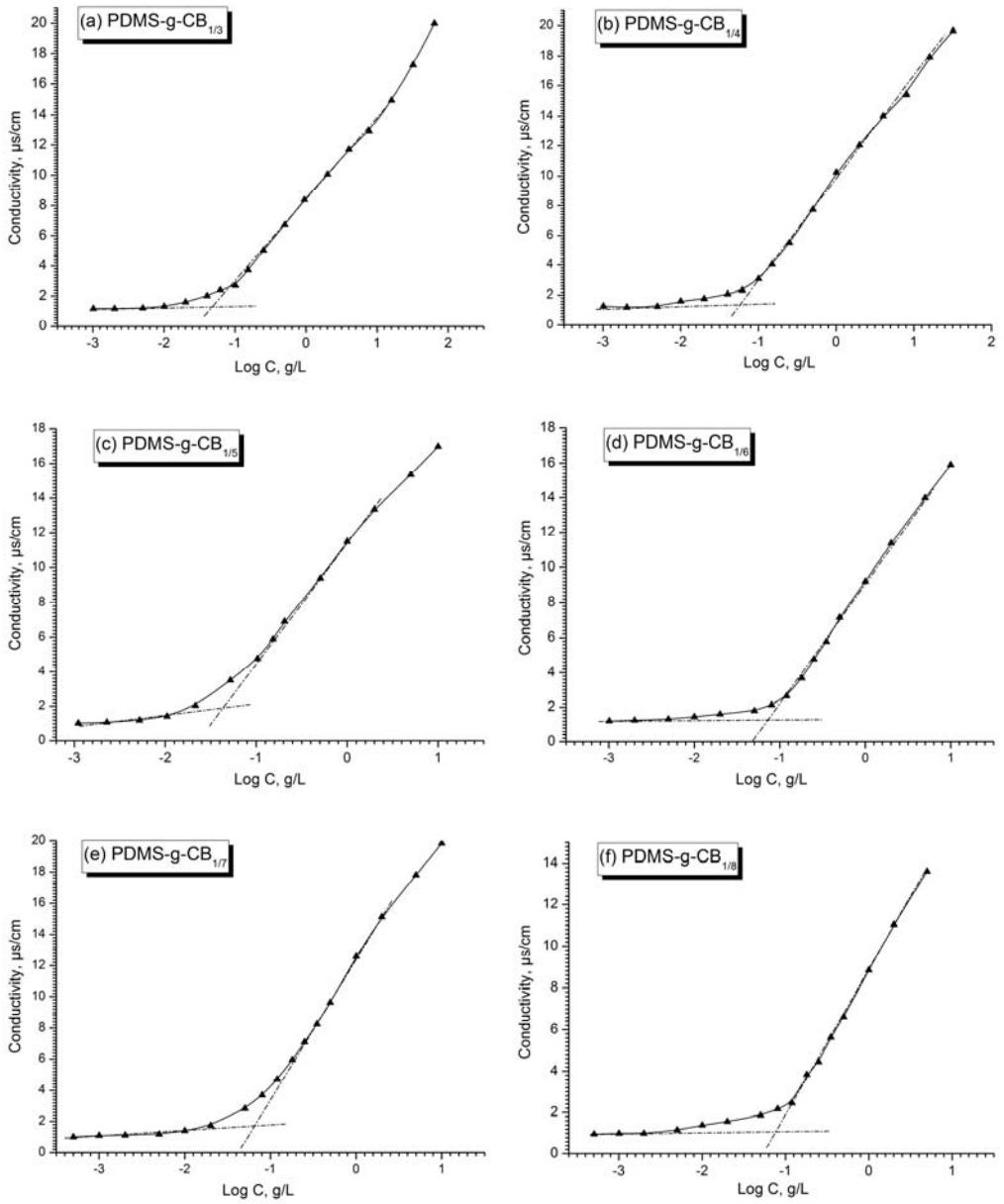

Figure S2. Plots of conductivity (κ) as a function of PDMS-g-CB concentration (log C): (a) PDMS-g-$CB_{1/3}$, (b) PDMS-g-$CB_{1/4}$, (c) PDMS-g-$CB_{1/5}$, (d) PDMS-g-$CB_{1/6}$, (e) PDMS-g-$CB_{1/7}$, (f) PDMS-g-$CB_{1/8}$.

S3. Skin irritation testing of PDMS-g-CB

Evaluation of the potential for PDMS-g-CB to cause skin irritation was conducted in rabbits *in vivo* according to ISO 10993-10: 2002, as detailed in the following.

Typically, the dorsal hair of three albino rabbits of the New Zealand strain (weight: 2.5 ± 0.2 kg) were shaven carefully with an electric clipper (area about 10 cm × 15 cm), and further treated with 4 % aqueous solution of barium sulfide for 24 h prior to testing. Subsequently, 50 wt% aqueous solution of PDMS-g-CB were smeared on the rabbits' hairless intact skin (as shown in **Figure S3**), 0.9 % physiological saline and gauze were used as the blank control and negative control, respectively. The testing sites were covered with sample and control for 6 h and then the drugs were washed with warm water. The appearance of each testing site at 1 h, 24 h, 48 h and 72 h after drugs removal were observed and recorded. Erythema and oedema were graded separately following the NAS procedures manual as shown in **Table S2**, and the irritation index was calculated and evaluated by the erythema and oedema grades (**Table S3**).

Table S2. Grading scale for skin irritation

Erythema and eschar formation	Grade	Edema formation	Grade
No erythema	0	No edema	0
Slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined)	2
Moderate to severe erythema	3	Moderate edema (raised about 1mm)	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1mm)	4

Table S3. The evaluation criterion of skin irritation

Irritation Index [a]	Intensity
0~0.4	no irritation
0.5~1.9	slight irritation
2~5.9	moderate irritation
6~8	severe irritation

a. Irritation Index = (Σ erythema grades of 24/48/72 h + Σ oedema grades of 24/48/72 h) /(3×number of rabbits).

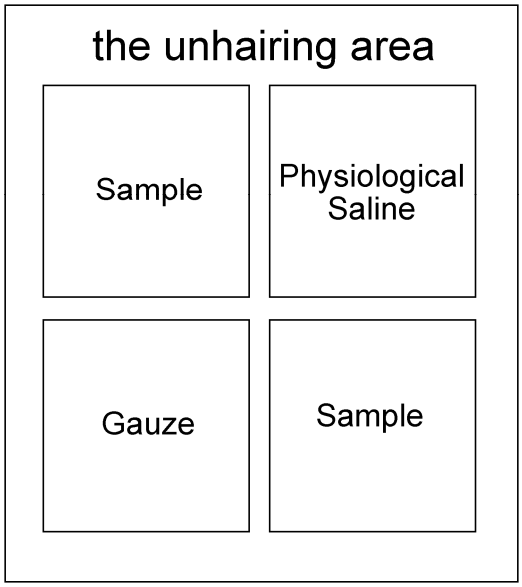

Figure S3. The schematic diagram of experimental area of skin irritation.

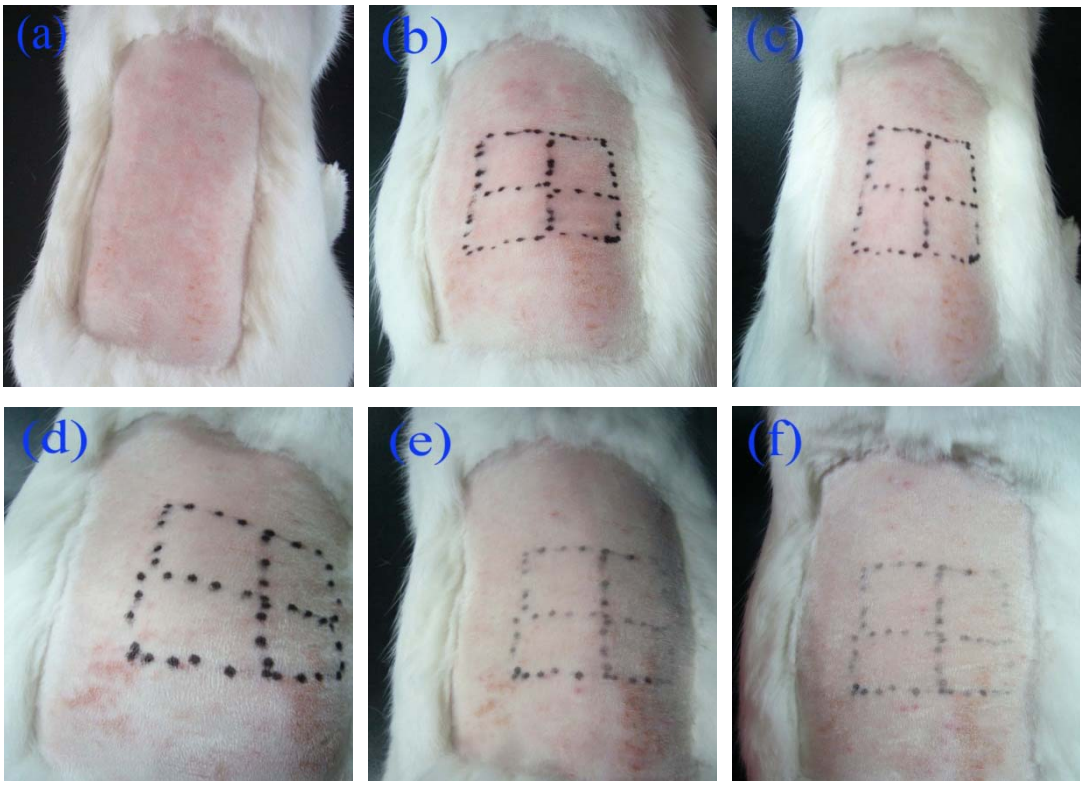

Figure S4. Images of skin irritation test at different stages: (a) after shaving, (b) dressing for 6h, (c) 1 h; (d) 24 h; (e) 48 h; (f) 72 h after removal of drugs.

S4. Acute oral toxicity testing of PDMS-g-CB

In order to assess the toxicity of PDMS-g-CB, the acute oral toxicity (LD_{50} as the index) was determined using NIH mice (22 ~ 25g), according to the OECD Acute Oral Toxicity Test Guideline 425 procedure (OECD TG 425), as detailed in the following.

NIH mice were randomly divided into two groups (n = 8, male and female in equal): control group (identified as group A, observed for 7 days) and experimental group (identified as group B, observed for 14 days), and they were acclimatized for 7 days prior to dosing. Before the experiment, mice were fasted overnight. Then PDMS-g-$CB_{1/3}$ solution (300 mg / mL) was given to the experimental mice with a syringe through gastrointestinal tract at a dose of 5000 mg / kg, the control group mice were injected with 0.9 % steriled physiological saline as control. The symptom and mortality of group A was observed and recorded for 7 days, and group B for 14 days. The histopathological effects of PDMS-g-$CB_{1/3}$ on tissues and organs, such as heart, liver, spleen, kidney and lung, were studied by histological method (tissue sections), and those tissue sections were observed by light microscopy after HE coloration, as shown in **Figure S5**.

Table S4. The classification criteria of acute oral toxicity [a]

Classification	LD_{50} (mg/kg)
Extremely Toxic	≤5
Highly Toxic	5-50
Moderately Toxic	50-500
Slightly Toxic	500-5000
Practically Non-toxic	5000-15000
Relatively HarmLess	≥15000

a. According to the OECD Acute Oral Toxicity Test Guideline 425 procedure (OECD TG 425)

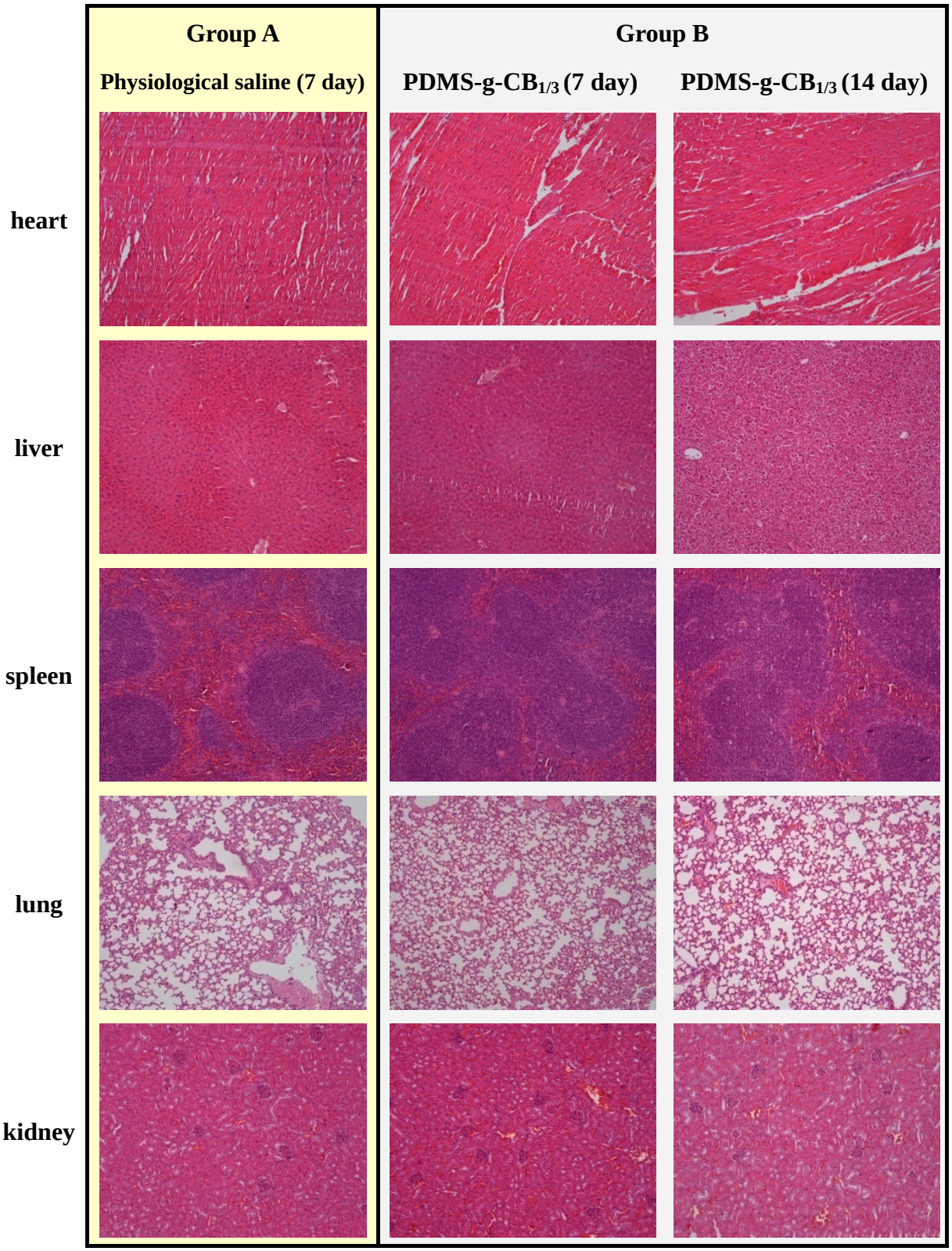

Figure S5. The images of tissue slice of physiological saline group (7 days) and the experimental group (7 days and 14 days).

S5. Haemolysis assay of PDMS-g-CB

Haemolysis assay was performed with fresh rabbit blood obtained from a New Zealand white rabbit, according to ISO 10993-4: 2002, as detailed in the following.

The blood was collected and certain amount of heparinized sodium was added. Then the red blood cells (RBC) were collected by centrifugation at 3000 rpm for 10 min, and washed with physiologic saline buffer for three times. Afterwards, diluted RBC suspension was prepared by mixing 1 drop of centrifuged RBC with 2 mL PBS. The sterilized b-PDMS films (Φ 7 mm × 1 mm) were equilibrated in 2 mL physiologic saline at 37 ºC for 30 min before 2 mL diluted RBC suspension was added. In comparison, 2 mL diluted RBC suspension mixed with 2 mL distilled water was taken as positive control, and 2 mL diluted RBC suspension mixed with 2 mL physiologic saline was taken as negative control. All the sample tubes were incubated at (37 ± 1) ºC for 60 min. Then the optical density (OD) of the clear supernatant fluids, which were obtained by centrifugation at 3000 rpm for 5 min, was measured with a UV2300 ultraviolet-visible spectrophotometer at 540 nm. The haemolysis ratio (HR) was calculated as follows:

$$HR(\%) = \frac{OD_s - OD_{nc}}{OD_{pc} - OD_{nc}} \times 100\% \quad (1)$$

Where OD_s, OD_{pc}, OD_{nc} were the OD_{540} value of b-PDMS sample, positive control (distilled water) and negative control (physiologic saline), respectively.

S6. Antibacterial activities of PDMS-g-CB

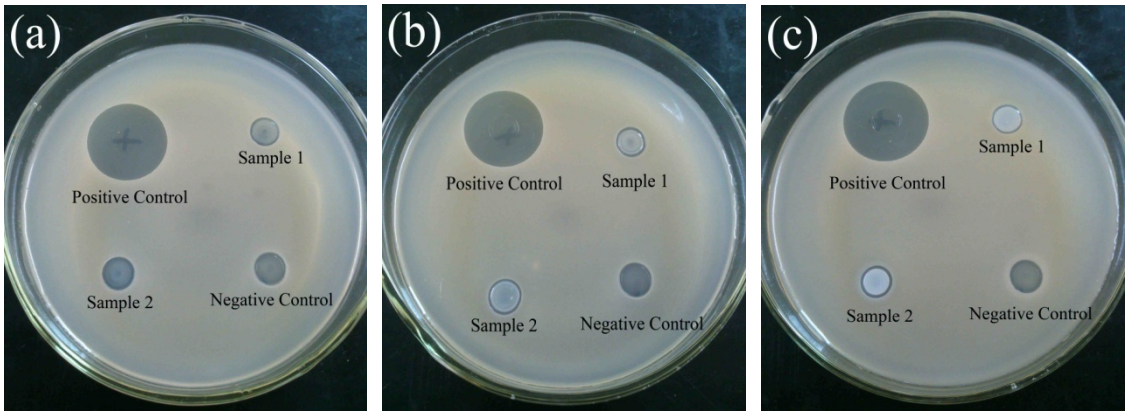

Figure S6. Antibacterial activities against E. coli with different concentration of PDMS-g-$CB_{1/3}$: (a) 15 mg/mL, (b) 30 mg/mL, (c) 60 mg/mL.

Reference

[1] L. Cheng, Q. Liu, L. Yang, Y. Lin, A. Zhang. *Polym Mat Sci Engi*, 2014, **30**, 25. (In Chinese)