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Supporting Information

Water-soluble Highly Fluorescent Poly[poly(ethylene glycol) methyl ether methacrylate] for Cell Labeling

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Expermental

Materials and method: PEGMA, *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), CuBr (99%) and 2-bromoisobutyryl bromide (99%) were purchased from Sigma-Aldrich Chem. Co. All other reagents and solvents were purchased from either Sigma-Aldrich or Merck Chem. Co., and were used as received. *N*,*N*'-bis(2-[2-hydroxyethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide (PBI-OH) was prepared according to the method reported in the literature[1]. 3T3 fibroblasts, mouse macrophages (RAW 264.7) and KB cells were purchased from ATCC. ^{1}H NMR spectra were recorded on a Bruker DMX-300 with tetramethylsilane as the internal standard and $CDCl_3$ as the solvent. UV/Vis absorption spectra in the wavelength range of 200 to 900 nm were obtained from a Shimadzu UV-3101PC spectrophotometer. Fluorescence emission spectra in the wavelength range from 501 to 700 nm were measured on a Photon Technology International luminescence spectrometer. The fluorescence quantum yield was calculated using Rhodamine 6G in methanol as the fluorescence standard (Φ_f = 95%). The photostability of the PBI-P(PEGMA) aqueous solution (2.5 mg/mL) was tested by exposing the solution (3 mL in quartz cells) to natural light for 2 weeks. Gel permeation chromatography (GPC) was performed on an HP 1100 high pressure liquid chromatograph using poly(ethylene glycol) as the calibration standard, equipped with an HP 1047A refractive index detector and a Plgel MIXED-C 300-7.5 mm column (packed with 5 μm gel particles).

Synthesis of N,N'-bis{2-[2-[(2-bromo-2-methylpropanoyl)oxy]ethoxy] ethyl} perylene-3,4,9,10-tetracarboxylic acid bisimide (PBI-Br): PBI-OH (570 mg, 1 mmol) was suspended in dry CH_2Cl_2 (150 mL) and Et_3N (0.43 mL, 3 mmol). After cooling to 0 ^oC, 2-bromoisobutyryl bromide (0.69 g, 3 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise, with continuous stirring for 1 h. Chloroform can also be used as a solvent for this reaction. The reaction was kept stirring for another 24 h at room temperature. The resultant $(C_2H_5)_3N{\cdot}HBr$ and unreacted *N,N*'-bis(2-[2-hydroxyethoxy]ethyl)perylene-3,4,9,10-tetracarboxylic acid bisimide were removed by filtration. After removal of the solvent by rotary evaporation and purification by passing through a silica gel column using $CHCl_3$ as eluent, a dark red solid *N,N'*-bis{2-[2-[(2-bromo-2-methylpropanoyl)oxy]ethoxy] ethyl} perylene-3,4,9,10-tetracarboxylic acid bisimide (PBI-Br) was obtained. ^1H NMR ($CDCl_3$-*d*): 8.71 (4H, ArH), 8.65 (4H, ArH), 4.48 (4H, CH_2), 4.31 (4H, CH_2), 3.90 (4H, CH_2), 3.80 (4H, CH_2), 1.84 (12H, CH_3).

Synthesis of PBI-P(PEGMA): PBI-Br (100 mg, 0.12 mmol), CuBr (34.6 mg, 0.24 mmol), and PEGMA (2.0 g, 4.21 mmol) were first dissolved in 10 mL of THF. The solution was purged with argon for 20 min, and PMDETA (41.6 mg, 0.24mmol) was then added. After purging with argon for another 10 min, the flask was sealed and kept in a 45 °C oil bath for 2 h under stirring. The resulting mixture was diluted with THF at a reaction mixture/THF volume ratio of 1:3. The dilute solution was passed over a neutral alumina column to remove the catalyst. The resulting solution was concentrated using a rotary evaporator, precipitated by large amount of ethyl ether, and then dried under vacuum. This crude product was further purified by dialysis in water for 48 hours. The pure PBI-P(PEGMA) polymer was recovered after freeze-drying.

Cytotoxicity: The cytotoxicity of the PBI-P(PEGMA) polymer was evaluated by determining the viability of 3T3 fibroblasts, macrophages and KB cells after incubation in medum containing PBI-P(PEGMA) polymer at concentrations of 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL.

Control experiments were carried out using the complete growth culture medim without the polymer. Cell viability testing was carried out via the reduction of the MTT reagent (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide). The MTT assay was performed in a 96-well plate following the standard procedure with minor modifications. These cells were seeded at a density of 1×10^4 cells per well and incubated at 37 °C for 24 h before the medium was replaced with one containing the PBI-P(PEGMA) polymer. The cells were incubated at 37 °C for another 24 h in the medium containing the PBI-P(PEGMA) polymer. The culture medium in each well was then removed and 90 μL of media and 10μL MTT solution (5 mg/mL in PBS) were then added to each well. After 4 h of incubation at 37 °C, the medium was removed and the formazan crystals were dissolved with 100 μL of DMSO for 15 min. The optical absorbance was then measured at 560 nm on a microplate reader (Tecan GENios). The results were expressed as percentages relative to that obtained in the control experiments. The differences in the results obtained from PBI-P(PEGMA) polymer and the controls were analyzed statistically using the two-sample *t*-test. The differences observed between samples were considered significant for $P<0.05$.

Confocal imaging: For confocal imaging experments, 3T3 fibroblasts, macrophages and KB cells were seeded on cover slips 24 h prior to labeling. The medium was then aspirated and replaced with a medium containing the PBI-P(PEGMA) polymer at a concentration of 0.5 mg/mL. The cells were cultured for another 24 h, and then washed with phosphate buffered saline (PBS; pH =7.4) extensively to completely remove loosely attached polymer in the medium. The cells were then fixed in 4% formaldehyde in PBS for 15 min. Following fixation, the cells were observed under a confocal laser scanning microscope with a FITC filter (Zeiss LSM 510 Meta, Germany). The control experiments were carried out with the same procedure as above except without the PBI-P(PEGMA) polymer.

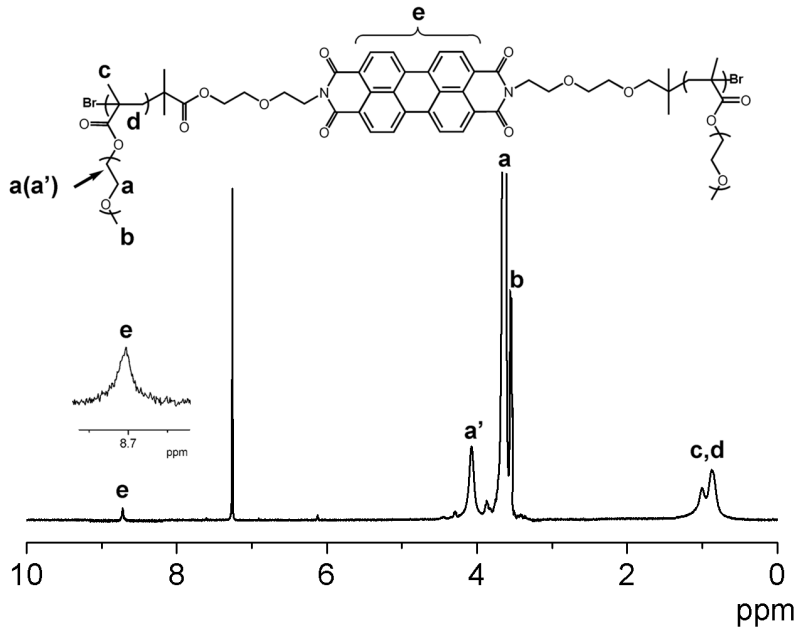

Fig. S1 ^{1}H NMR spectrum of PBI-P(PEGMA) polymer (in $CDCl_3$).

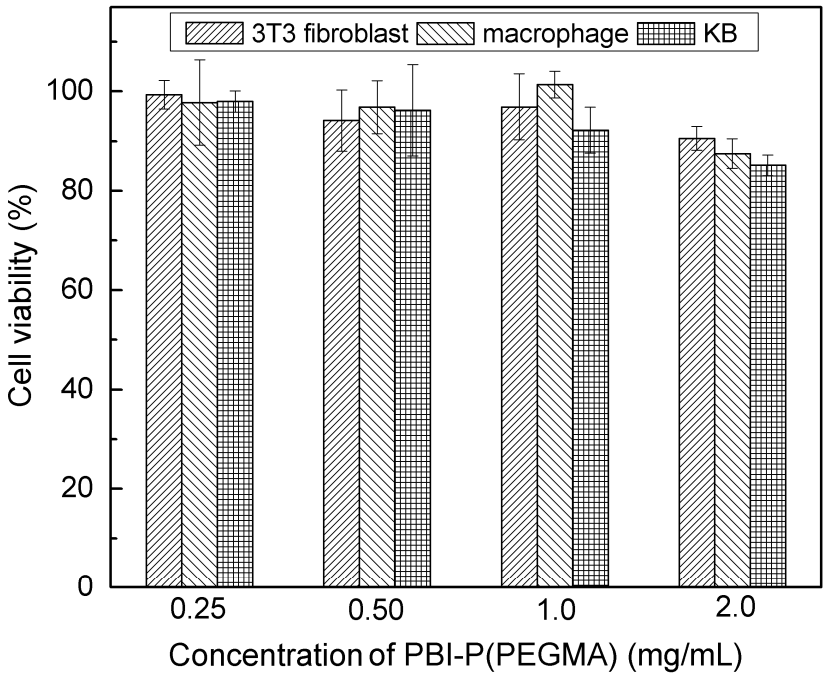

Fig. S2 Viability of 3T3 fibroblasts, macrophages and KB cells after incubation with PBI-P(PEGMA) polymer for 24 h.

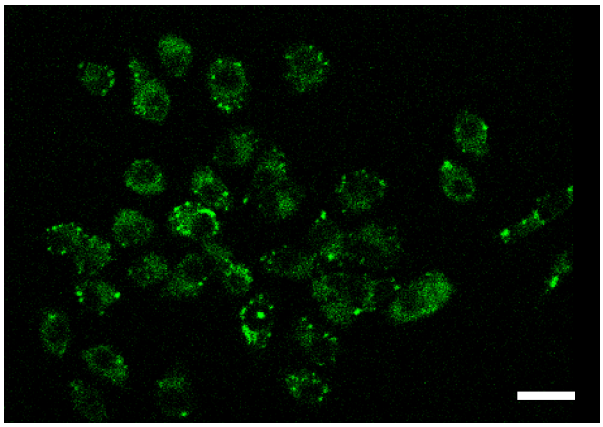

Fig. S3 Confocal laser scanning microscopy image of KB cells after culturing in medium containing 0.5 mg/mL PBI-P(PEGMA) polymer for 2h. Scale bar = 50 μm.

Reference

1. A. V. Ustinov, V. V. Dubnyakova, V. A. Korshun, *Tetrahedron,* 2008, **64**, 1467.