Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

SUPPORTING INFORMATION

Organosilicon coordination enhanced the stability of black phosphorus at ambient conditions

Jianing Zhang,[a] Si Chen,[b] Yue Ma,[a] Dongyuan Wang,[a] Jian Zhang, [a] Yanding Wang, [a] Wenjun Li,[a] Zhiqiang Yu,[c] Han Zhang,*[b] Feng Yin,*[a] Zigang Li*[a]

[a]Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Shenzhen Graduate School of Peking University, Shenzhen, 518055, E-mail: lizg@pkusz.edu.cn, yinfeng@pkusz.edu.cn.

[b]SZU-NUS Collaborative Innovation Center for Optoelectronic Science and Technology and Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province College of Optoelectronic Engineering, Shenzhen University Shenzhen 518060 (P. R. China), E-mail: hzhang@szu.edu.cn.

[c]Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, PR China. E-mail: yuzq@smu.edu.cn

Content

Materials

Crystalline BP was purchased from a commercial supplier (Nanjing XF NANO). NMP (99.5%, anhydrous) was obtained from Aladdin Reagents. TMSCl, TBSCl and TMSOtf were obtained from Alfa-Aesar. DMEM, RPMI-1640 Medium, trypsin-EDTA, fetal bovine serum and penicillin/streptomycin were obtained from Gibco Life Technologies (AG, Switzerland). Human cervical cancer cells line, HeLa (CCL-2, American Type Culture Collection), was maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum. Human embryonic kidney cell line, 239T (CRL-3216, American Type Culture Collection), was maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (heat inactivated) (ATCC 30-2020), 2mM L-glutamine (ATCC 30-2214), 1% Penicillin/Streptomycin. Human mammary gland cancer cell line, T74D (HTB-133, American Type Culture Collection), was maintained in RPMI-1640 Medium, Catalog No. 30-2001, supplemented with 0.2 Units/ml bovine insulin and fetal bovine serum to a final concentration of 10%. Human liver cancer cell line, HepG2 (HB-8065, American Type Culture Collection), was maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum. Cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2.

Preparation of BP nanosheets

40mg of bulk black phosphorus power was added to 20 mL of N-methyl-2-pyrrolidone (NMP) in a 40 mL sealed conical tube and sonicated with a sonic tip for 4 h at a power of 1000 W. The dispersion was then sonicated in an ultrasonic bath continuously for another 10 h at a power of 300 W. The temperature of the sample solution was kept below 277 K using an ice bath. The resulting dispersion was centrifuged for 20 min at 3000 rpm, and the supernatant containing BP nanoplates was decanted gently. Then, the supernatant was centrifuged for 30 min at 14 000 rpm, and the precipitate was rinsed repeatedly with water and re-suspended in aqueous solution.

Synthesis of TMSCl@BP

An excessive amount of TMSCl was added to the 2 mg BP nanosheets solution in 5 mL NMP and the mixture was stirred in darkness under the protection of nitrogen for 18 h. The mixture was centrifuged at 3000 rpm for 30 s to remove the unmodified Bare BP then the supernatant containing TMSCl@BP was centrifuged at 12,800 rpm for 8 min for use in subsequent experiments.

Raman spectra

Raman spectra were performed on a Renishaw InVia Reflex Raman system (Renishawplc, Wotton-under-Edge, UK) with a grating spectrometer using a Peltier-cooled charge coupled device (CCD) detector, then coupled to a confocal microscope. The Renishaw WiRE 3.2 software was used to process the spectra. Raman scattering was excited using an argon ion laser (I = 514.5 nm).

Characterization of functionalized BP and bare BP nanosheets

The zeta potential of the functionalized BP and bare BP nanosheets were measured by a particle size analyzer system (90 Plus, Brookhaven Instruments). The UV-vis absorbance spectrum was recorded on a spectrophotometer (Shimadzu UV-2450). AFM measurements were performed with a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Samples for AFM images were diluted with deionized H_2O to yield a final concentration of 1×10^{-6} M. The sample (20 μl) was then dispersed on Si/SiO2 substrates, and dried in vacuum oven at 60 ˚C. Tapping mode was used to acquire the images under ambient conditions. XPS was conducted on the Thermo Fisher ESCALAB 250Xi XPS. FT-IR was conducted on the PerkinElmer Frontier™.

Scanning electron microscope

TMSCl@BP and BP nanosheets were centrifuged from NMP, dispersed in water and dropped onto Si/SiO2 substrates, and then all the samples were dried in vacuum oven at 60 ˚C, 30 min. The SEM images were obtained on the field-emission SEM (JEOL-7800M) at 5-10kV after gold coating for 120 s (EM-SCD500, Leica, Germany). The energy dispersive X-ray spectroscopy was conducted on the Oxford INCA 300 equipped on the SEM.

Hemolysis assay

The general procedure was showed here: Fresh ethylenediamine tetraacetic acid (EDTA)-stabilized rat whole blood sample was extracted from rat eyes. Typically, 5 mL of whole blood was added to 10 mL of calcium- and magnesium-free Dulbecco's phosphate buffered saline (PBS) and centrifuged at 500 g for 10 min to isolate RBCs from serum. This purification step was repeated three times, and then the washed RBCs were diluted to 50 mL in PBS. To test the hemolytic activity of TMSCl@BP, 0.1 mL of diluted RBC suspension (around 1 x 108 cells mL^{-1}) was added to 0.2 mL of TMSCl@BP and suspension solutions in PBS at different concentrations. The final concentration of TMSCl@BP ranges from 5 to 200 ppm. 0.1% Triton X-100 was used as the positive control. All the samples were placed on a rocking shaker in an incubator at 37°C for 2 h. After incubation, the samples were centrifuged at 12000 rpm for 5 min. The hemoglobin absorbance in the supernatant was measured at 540 nm, with 655 nm as a reference, using an iMark microplate reader (BioRad, Hercules, CA).

Cell viability test

HeLa Cell, T47D Cell and 293T cell were seeded into the 96-well plates at the density of 5×10^4 cells per milliliter and incubated in a humidified atmosphere of 5% CO_2 at 37 °C overnight. Then the cells were rinsed twice with PBS (pH 7.4) medium, and the medium was replaced with 100 μL of fresh DMEM medium containing different concentrations of BP or TMSCl@BP (0, 12.5, 25, 50, 100 and 200 ppm). Three multiple holes were set for every sample. The viability was measured using CCK-8 assay after cells had been cultured for 24h. The absorbance at 450 nm (OD 450 nm) was measured on a microplate reader (Bio-Rad ELx800, USA).

Photothermal Experiments

HeLa and HepG2 cells were seeded into the 48-well plates at the density of 2×10^5 cells per milliliter and cultured for 12 h, then the cells were rinsed twice with PBS (pH 7.4) medium, and 200 μL fresh

DMEM medium was added, with or without bare BP and TMSCl@BP (50 ppm). Three multiple holes were set for every sample. After incubation for 4 h, cells were treated with 808 nm light (2.0 W/cm2) for 10 min. The laser spot was moved constantly to fully cover the area of each well. The viability was measured using CCK-8 assay after cells had been cultured for 24 h. The absorbance at 450 nm (OD 450 nm) was measured on a microplate reader.

SI figures

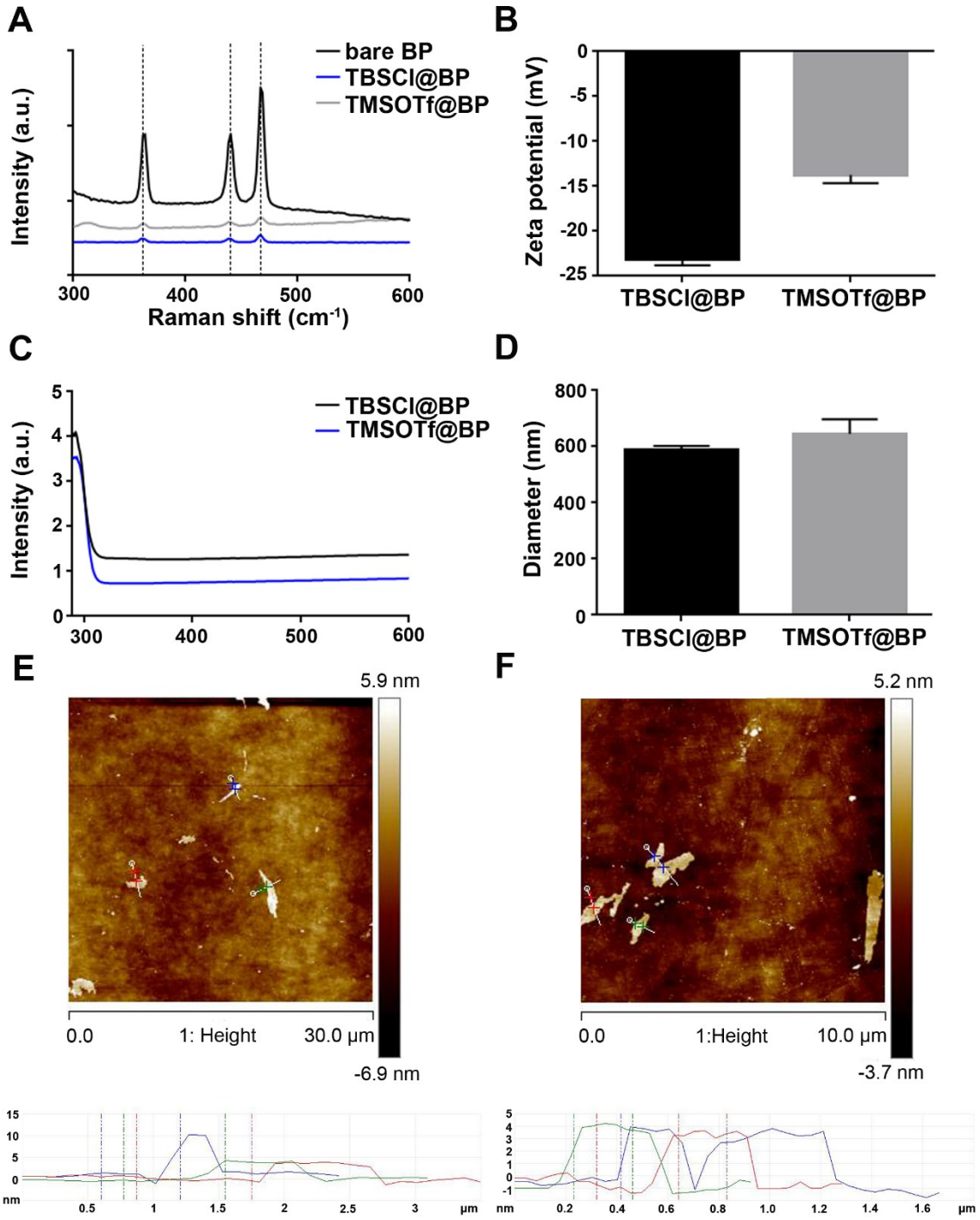

Figure S1 Characterization of TBSCl@BP and TMSOTf@BP. **A)** Raman spectra of TBSCl@BP and TMSOTf@BP. **B)** Average Zeta potential of TBSCl@BP and TMSOTf@BP. **C)** Absorption spectra of TBSCl@BP and TMSOTf@BP. **D)** hydrodynamic size of TBSCl@BP and TMSOTf@BP**.** AFM images of the **E)** TBSCl@BP and **F)** TMSOTf@BP**.**

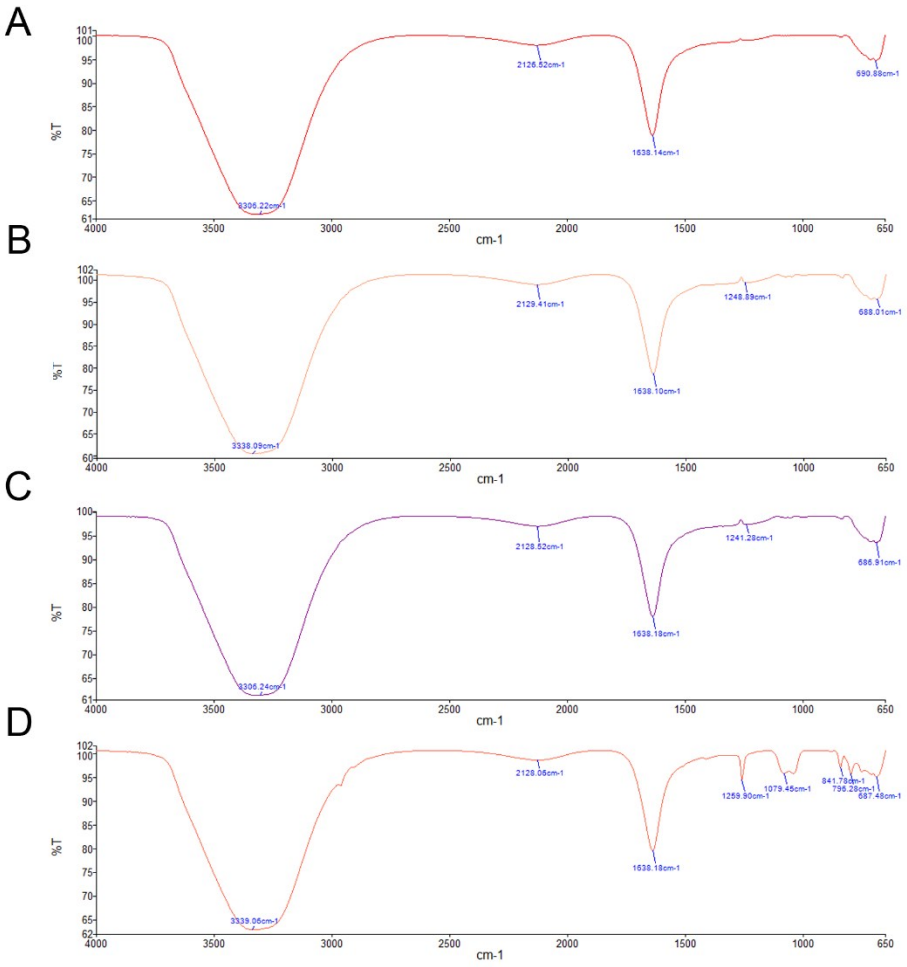

Figure S2 FT-IR sprctroscopy for **A)** bare BP, **B)** TBSCl@BP, **C)** TMSOTf@BP and **D)** TMSCl @BP.

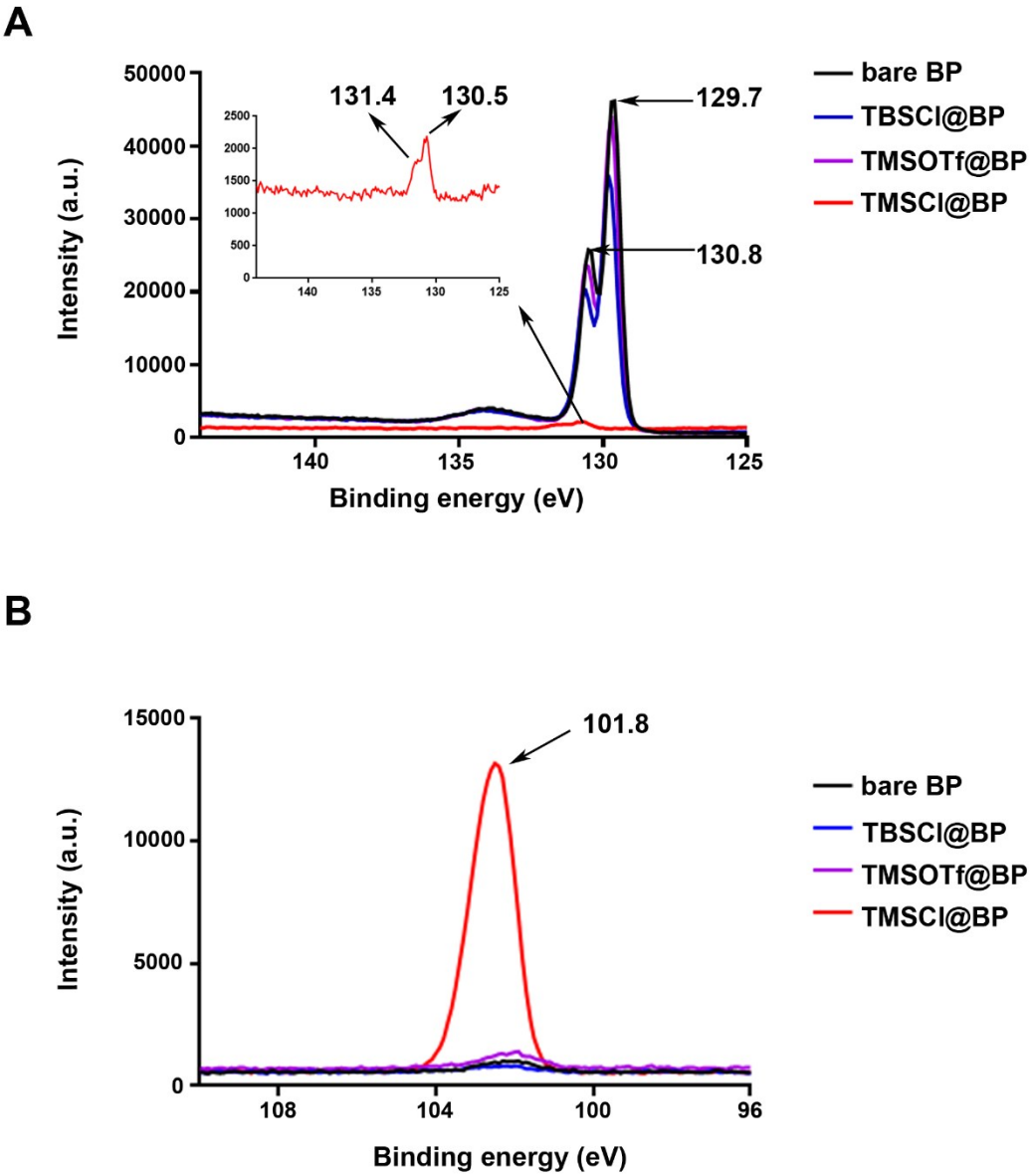

Figure S3 HR-XPS spectra of bare BP, TMSCl@BP, TBSCl@BP and TMSOTf@BP. **A)** HR-XPS spectra P2p; **B)** HR-XPS spectra of Si2p of bare BP, TMSCl@BP, TBSCl@BP and TMSOTf@BP. The samples are prepared by drop-casting the BP dispersion onto aluminium-foil substrates. Photoelectron spectroscopy studies were performed using a Thermo Fisher ESCALAB 250Xi surface analysis system equipped with a monochromatized Al anode X-ray source (X-ray photoelectron spectroscopy, XPS, hυ=1486.6 eV).

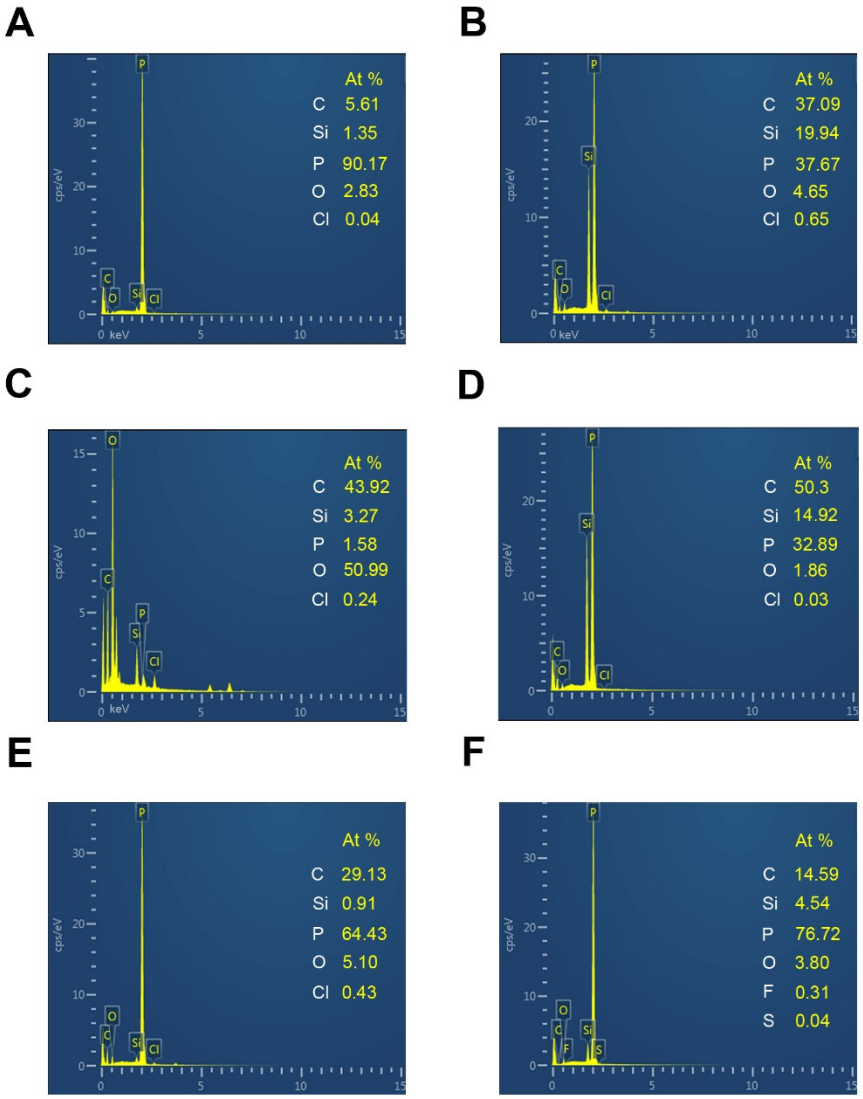

Figure S4 Energy dispersive X-ray spectroscopy (EDS) analysis of the fresh **A)** bare BP and **B)** TMSCl@BP and **C)** bare BP and **D)** TMSCl@BP after exposed in ambient condition for 3 days, and **E)** TBSCl@BP and **F)** TMSOTf@BP. The bare BP and TMSCl@BP, TBSCl@BP and TMSOTf@BP in NMP solution were centrifuged, dropped onto Si/SiO2 substrates and dried in vacuum oven at 160 ℃ for 3h. Then the energy dispersive X-ray spectroscopy was conducted on the Oxford INCA 300 equipped on the SEM.

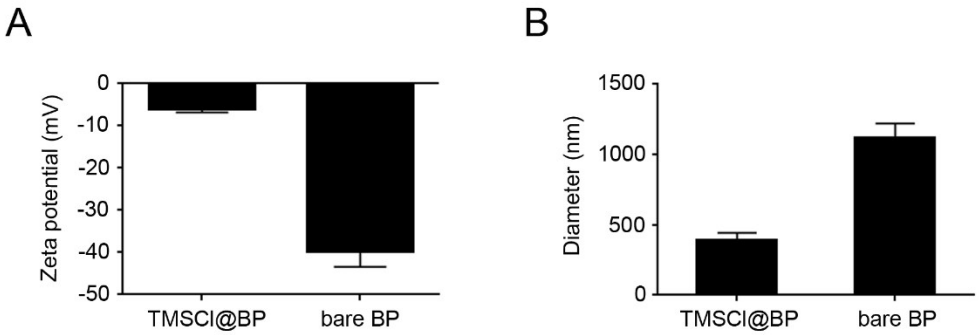

Figure S5 Characterization of bare BP and TMSCl@BP in PBS at pH 7.4. **A)** Zeta potential of bare BP and TMCl@BP. **B)** hydrodynamic size of bare BP and TMSCl@BP.

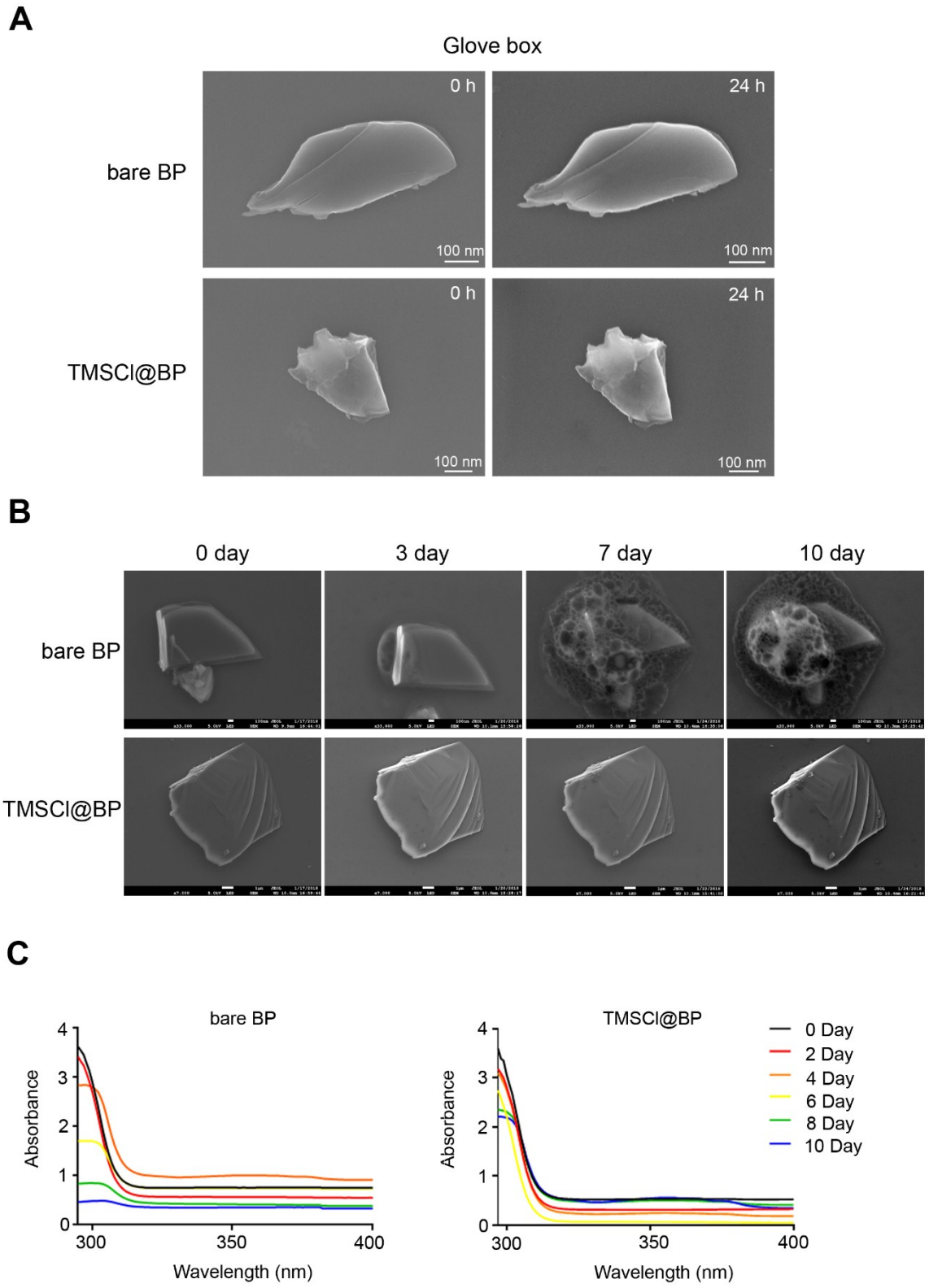

Figure S6 Surface morphology and the optical absorbance at different time point of bare BP and TMSCl@BP sheets in glovebox ambient conditions. **A)** SEM images of bare BP and TMSCl@BP exposed in glovebox for different time durations. **B)** SEM images of bare BP and TMSCl@BP exposed in air for different time durations. **C)** The optical absorbance of bare BP (left) and TMSCl@BP (right) at different time point.

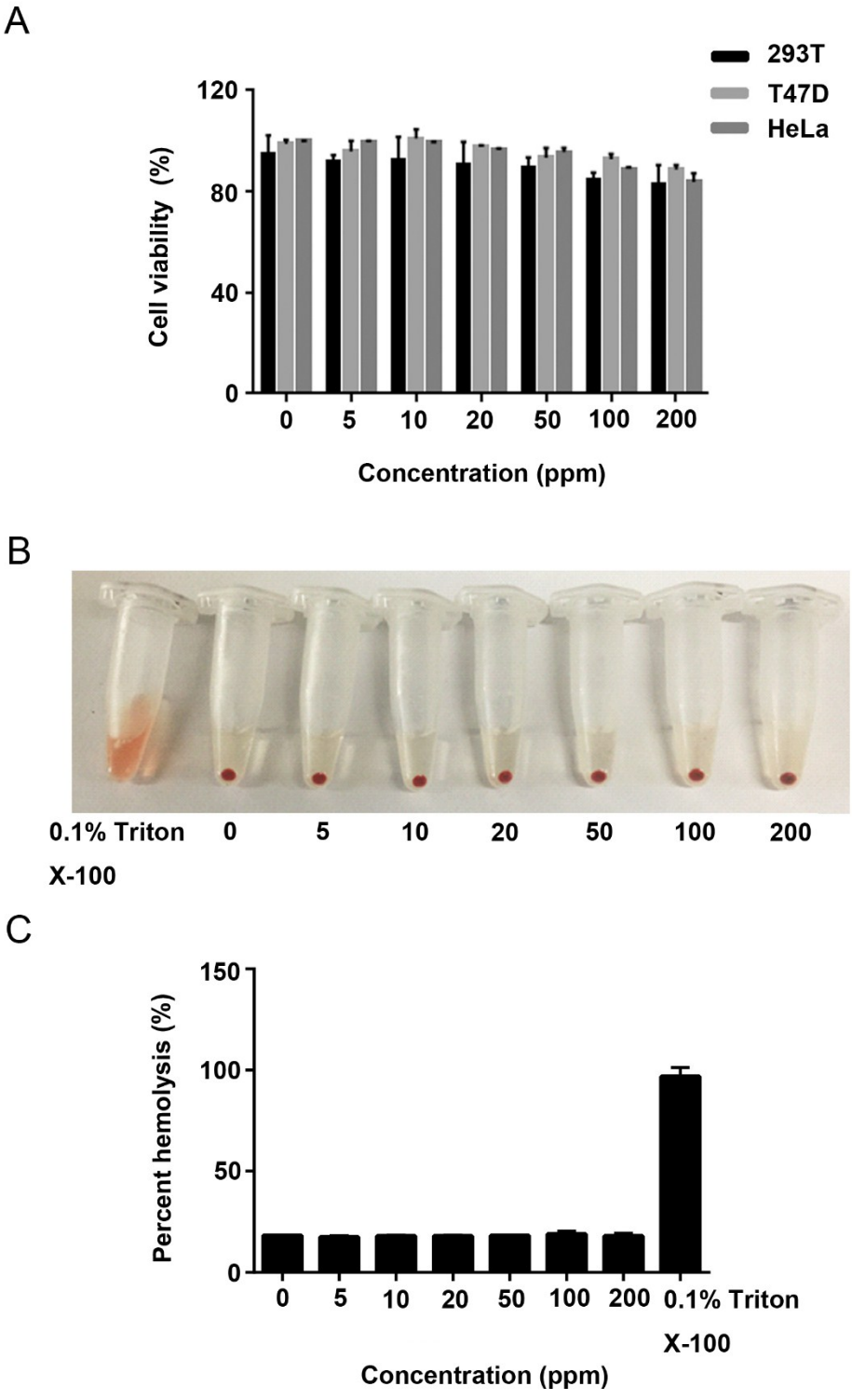

Figure S7 Cytotoxicity and hemolysis assays of TMSCl@BP. **A)** relative cell viability of 293T, HeLa and T47D cells after incubation with various concentrations (5, 10, 20, 50, 100 and 200 ppm) of TMSCl@BP for 24h. **B)** and **C)** Hemolysis assays of TMSCl@BP.