Electronic Supporting Information

Biophotonic device based on conjugated polymer and macrophage-laden hydrogel for triggering immunotherapy

Zhiwen Yang,[abc] Qi Shen,[ab] Longjiang Xing,[c] Xuancheng Fu,[ab] Zhipeng Qiu,[c] Hongping Xiang,[d] Yiming Huang,[a] Fengting Lv,[a] Haotian Bai,*[a] Yanping Huo*[c] and Shu Wang*[ab]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China.

[b] College of Chemistry, University of Chinese Academy of Sciences, Beijing 100049, P. R. China.

[c] School of Chemical Engineering and Light Industry, Guangdong University of Technology. Guangzhou, 510006, P. R. China.

[d] School of Materials and Energy, Guangdong University of Technology. Guangzhou, 510006, P. R. China.

*Corresponding Authors. E-mail: baihaotian@iccas.ac.cn; yphuo@gdut.edu.cn and wangshu@iccas.ac.cn

Experimental

Materials

All chemical agents were purchased from commercial suppliers and used as received without further purification. Specifically, sodium alginate (from brown algae), gelatin (from porcine skin) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were obtained from Sigma Aldrich. 3-(4, 5- dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) was purchased from Xinjingke Biotechnology Co., Ltd (Beijing, China). Annexin V, FITC Apoptosis Detection Kit were purchased from Dojindo Laboratories (Shanghai, China). TNF alpha Mouse Uncoated ELISA Kit (88-7324-77), TGF beta-1 Mouse Uncoated ELISA Kit (88-8350-88), Hoechst 33342 (62242), Calcein AM (C3099) and PI (P1304MP) were purchased from Invitrogen (Beijing, China). Calcein Deep Red AM ester were purchased from Xi'an Biolite

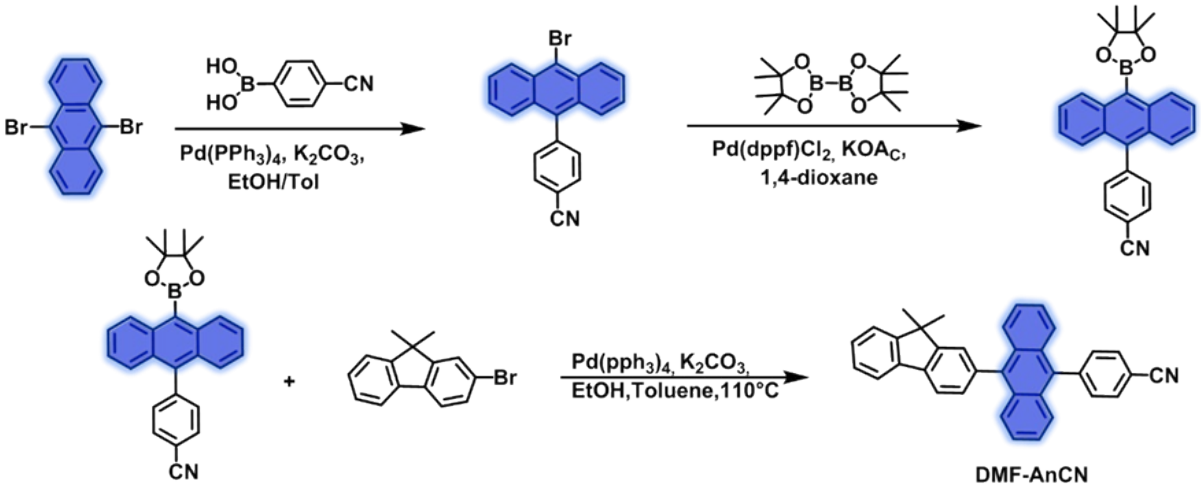
Biotech (Xi'an, China). iNOS Mouse ELISA Kit (ZK-M4929) were purchased from Ziker Biological Technology (Shenzhen, China). The primary antibodies TNF-α (ab183218), iNOS (ab178945) and second antibody Goat anti-rabbit IgG H&L Alexa Fluor 647 (ab150079) were purchased from Abcam. Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Materials (Hangzhou, China). Dulbecco's modified Eagle medium (DMEM) and Roswell Park Memorial Institute 1640 (RPMI 1640) were purchased from WISENT bioproducts (Nanjing, China). RAW 264.7, 4T1, B16 cells were purchased from Procell Life Science &Technology Company (Wuhan, China). 0.4 μm-sized transwell system was purchased from Corning. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, USA).

Measurements

UV-Vis absorption spectra were measured by JASCO V-730 Spectrophotometer. PL spectra were recorded using a Edinburgh FLS980 spectrometer and Hitachi F-4500 fluorometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AV400 spectrometers using DMSO-d_6 and $CDCl_3$ as solvent. MTT and ELISA analysis were analyzed on a microplate reader (BIO-TEK Synergy HT). Confocal laser scanning microscopy (CLSM) characterizations were performed by the confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan). The flow cytometric analysis was conducted by BD FACSCalibur™ flow cytometer. Rheological tests were performed on a DHR-1 rheometer (TA Instruments) equipped with a temperature controller. 3D printing experiments was performed on CPD1 printer (SUNP Biotech).

Synthesis of DMF-AnCN

DMF-AnCN were synthesized according to the procedure that we have been reported before [1]. H^{1} NMR (400 MHz, DMSO-d_6): δ(ppm) 8.19 – 8.08 (m, 3H), 8.01 – 7.95 (m, 1H), 7.69 (dddd, 6H), 7.55 – 7.36 (m, 9H), 1.53 (s, 6H). C^{13} NMR (400 MHz, $CDCl_3$): δ(ppm) 153.93, 153.93, 144.61, 144.61, 139.02, 140.20, 137.50, 137.50, 134.45, 134.45, 132.34, 132.34, 129.99, 129.46, 127.44, 127.16, 126.04, 125.68, 125.24, 122.75, 120.15, 119.92, 118.94, 111.63, 47.07, 27.25.



Scheme S1. Synthetic routine of **DMF-AnCN**

Flexible OLED fabrication, encapsulation and characterization

Fabrication: PET-indium tin oxide (ITO) with the thickness of 0.175 mm were purchased from Zhuhai Kaivo Optoelectronic Technology Co. Ltd (ITO-M1015). The pre-coated ITO pattern were confirmed sheet resistance of 10-15 Ω per square and the transmittance of visible light is over 76%. Before fabrication, PET-ITO were cleaned for 5 minutes in isopropyl alcohol, detergent, deionized water, and isopropyl alcohol and then treated in a UV-cleaner for 30 min in sequence. Organic layers were deposited onto the ITO by high-vacuum (< 5×10^{-4} Pa) thermal evaporation. Deposition rates were controlled by independent quartz crystal oscillators, which were 1~2 Å s^{-1} for organic materials, 0.1 Å s^{-1} for LiF, and 5 Å s^{-1} for Al, respectively.

Encapsulation: A protecting adhesive PET layer (Shenzhen Mileqi Adhesive Tape Co. Ltd.) were attached onto ITO to cover all emission area 2×2 mm^2 ×4, followed by dropping 200 μL UV-curing encapsulation materials to cover protecting adhesive PET. The devices were then treated by a UV-flashlight with the power of 5 W for 2 min. All encapsulation process were conducted in a nitrogen-filled glove box (O_2 < 1 ppm; H_2O < 1 ppm). Encapsulation materials were prepared according to previous report [2].

Characterization: All the device characterization steps were carried out at room temperature under ambient laboratory conditions without encapsulation. EL spectra were taken by an optical analyzer, Photo Research PR745. Current density and luminance versus driving voltage characteristics were measured by Keithley 2400 and Photo Research PR745.

Hydrogel preparation

The hydrogels for 3D printing were prepared under aseptic conditions according to previous report. To start with, gelatin (4% w/v) was added to 80 μM PPV solution (or deionized water

for GA) at 37 ºC until it was completely dissolved. Alginate (1% w/v) was then added into the above system and the mixture was further shaken at 37 ºC until a uniform gel was formed. For M_0@PPV-GA and M_0-GA, RAW 264.7 cells were collected into centrifugal tubes for counting and final cell solutions with the density of 1×10^7 cells per milliliter were obtained. Centrifuge the above cell solutions and re-suspend the cells with the same volume of pre-gel at 37 ºC. The prepared pre-gels were kept under 4 ºC for 10 min before printing.

3D Printing

3D printing was conducted with a commercial 3D printer (CPD1, SUNP Biotech). To start with, the print platform was cooled to 10 ºC before printing, and 5 mL hydrogel was loaded into a BD syringe with a 220 μm nozzle followed by equipping on the printing arm with the temperature of 25 ºC. The 3D structures were printed and immediately cross-linked with 1 mL sterilized 2% w/v $CaCl_2$ solution for 5 min.

Cell culture

RAW264.7 cells were maintained in DMEM medium with 10% FBS. 4T1 and B16-F10 cells were maintained in RPMI 1640 medium with 10% FBS .Cells were cultured at 37 ºC in an atmosphere containing 5% CO_2。

Isothermal titration microcalorimetry (ITC) characterization

The stainless-steel sample cell was loaded with 0.6 mL of water or Gel/Alg solution. The PPV solution was inserted to the above solution dropwise with a Hamilton syringe. The system was stirred at constant rate of 60 rpm by a gold propeller. All the measurements were performed at 25.00 ± 0.01 ºC. To investigate the interactions between PPV and cells, cell solutions (in PBS, pH=7.4) or PBS were loaded in sample cell.

ESR measurements

PPV solutions with concentration of 100 μM was prepared and DMPO was sebsequently added, ensuring the final DMPO concentration of 100 mM. The resulting mixture was shaken vigorously and loaded into a quartz capillary tube before both ends were blocked by color clay. The spectrum for dark control was immediately collected while the capillary was irradiated for 15 min for light group. Another spectrum of DMPO control was collected using identical parameter settings.

Measurement of TNF-α, TGF-β and iNOS

The printed hydrogels were immersed in DMEM medium with 10% FBS for 4 h. 100 ng/ml LPS was added into medium to induce M_1 phenotype of LPS group. The control group and Device on group were treated with flexible OLED emission for 15 min. After 12 h, the culture medium was collected and the levels of various secreted cytokines were determined by ELISA, according to the manufacturer's instructions.

Rheological properties

Rheological measurements were taken with a DHR-1 rheometer (TA Instruments) with a 20 mm parallel-plate geometry. Temperature-scan tests were conducted at 1% strain and 10 rad/s. The hydrogel was kept at 4 ºC for 5 min, and then the temperature was elevated at a speed of 0.5 ºC/min from 4 ºC to 40 ºC. Shear-thinning test were measured by increasing the shear rate from 1 to 1500 s^{-1} at 25 ºC.

Immunofluorescence assay

The just printed cell gels were immersed in DMEM medium with 10% FBS (fetal bovine serum) for 4 h. 50 ng/ml LPS was added into medium to induce M_1 phenotype of M_1 group. The OLED-on group was treated with OLED emission for 15 min. After 12 h, a small part of cell gels was separated and immersed in 4% paraformaldehyde fix solution for 10 min, followed by staining process of TNF-α and iNOS according to manufacturer's protocol. Cell nuclei of M_1/blank groups were labelled by Hoechst 33342 before confocal analysis.

Cell viability test of PPV to 4T1 and B16-F10 cells

The 4T1 and B16 cells were seeded in 96 well plates at density of 5×10^3 cells per well for 12 h. PPV at varied concentrations were added into the cell culture medium separately. The cells were then incubated for 24 h. After removing the supernatant in the plates, MTT (0.5 mg/mL in fresh medium, 100 μL/well) was subsequently added to the plates followed by incubation at 37 ºC for 4 h. After co-incubation, the supernatant was removed and 100 ml DMSO was added to each well to dissolve the produced formazan. After shaking the plates for 60 s, absorbance values at 570 nm of each well were recorded by microplate reader.

Co-culture assay of PBM device and tumor cells

The 4T1 and B16-F10 cells were seeded into the lower chamber of 6-well transwell plates at density of 4×10^5 cells per well for 12 h. Simultaneously, printed hydrogels were immersed

in DMEM medium with 10% FBS for 4 h. 100 ng/ml LPS was subsequently added into the medium to induce M_1 phenotype of **LPS group**. The PBM device were activated for 15 min for **OLED-on** group. After 12 h, hydrogels were transferred to the upper chamber of the transwell plate for further 24 h co-culture. Finally, tumor cells were collected for flow cytometry analysis.

Macrophage Polarization under the Direct Treatment of PPV

Cells were seeded on confocal dishes at density of 1×10^5 cells per dish. M_1 group was treated with 100 ng/mL LPS for 12 hours. OLED-on group was treated with PPV (2 μM) and 15 minutes of irradiation of OLED, simultaneously. OLED-off group was treated with PPV (2 μM) only. After the treatments, PPV was removed and cells were thoroughly washed by PBS. After further incubation in the incubator for 12 hours, cells were fixed by 4% paraformaldehyde fix solution and stained for immunofluorescence assay according to manufacturer's protocol.

Statistics

Statistical analysis was performed in GraphPad Prism 6.

References

[1] L. Xing, Z.-L. Zhu, J. He, Z. Qiu, Z. Yang, D. Lin, W.-C. Chen, Q. Yang, S. Ji, Y. Huo and C.-S. Lee, *Chem. Eng. J.*, 2021, **421**, 127748.

[2] Z. Liu, P. Hong, Z. Y. Huang, T. Zhang, R. J. Xu, L. J. Chen, H. P. Xiang and X. X. Liu, *Chem. Eng. J.*, 2020, **387**,124142.

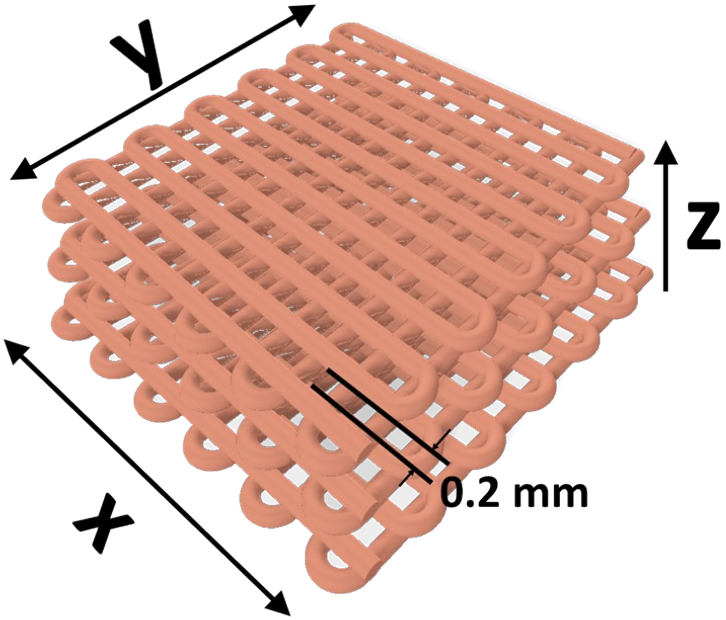


Fig. S1. Pattern of 3D printed hydrogel networks.

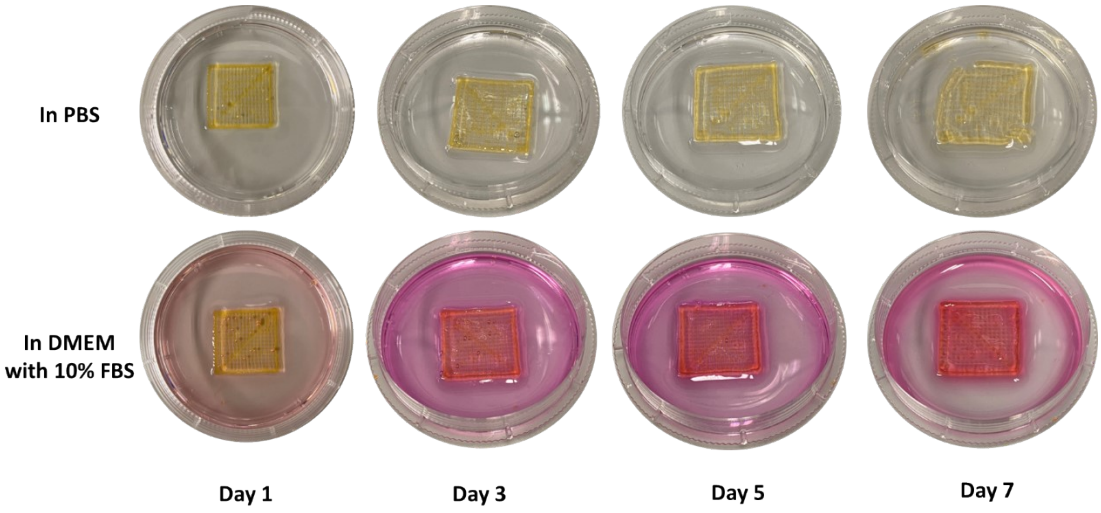


Fig. S2. Stability tests of **PPV-GA** in PBS and DMEM culture medium. The original dimension of printed structure (Day 1) is 20 mm × 20 mm.

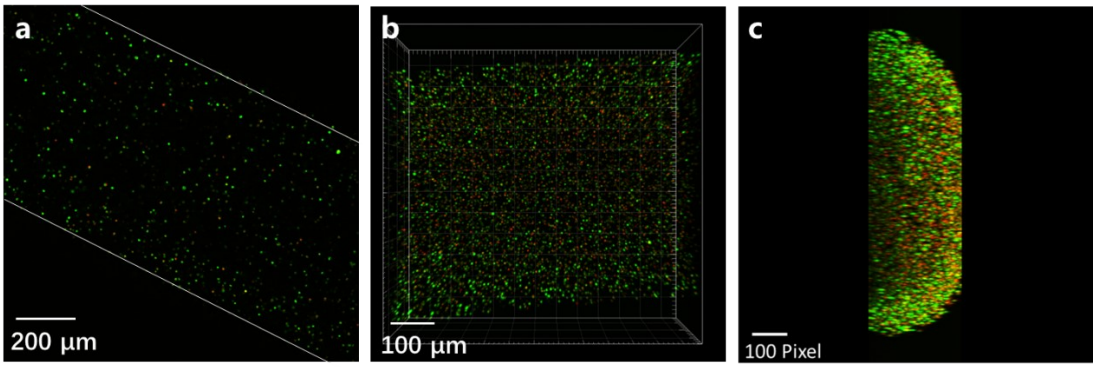


Fig. S3. (a) Live and dead cell stain of M_0@GA after 48-hour immersion in DMEM. Live cells were labeled by calcein-AM and dead cells were labeled by propidium iodide (PI). Front view (b) and (c) side view of 3D images of stained M_0@GA. The percentage of live and dead cells are 61% and 39%, respectively, determined by using image J for fluorescence intensity quantification.

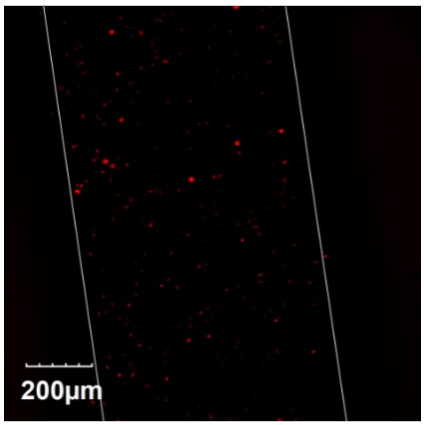


Fig. S4. Live cell stain of M_0@PPV-GA after 48-hour immersion in DMEM. Live cells were labeled by calcein-deep red.

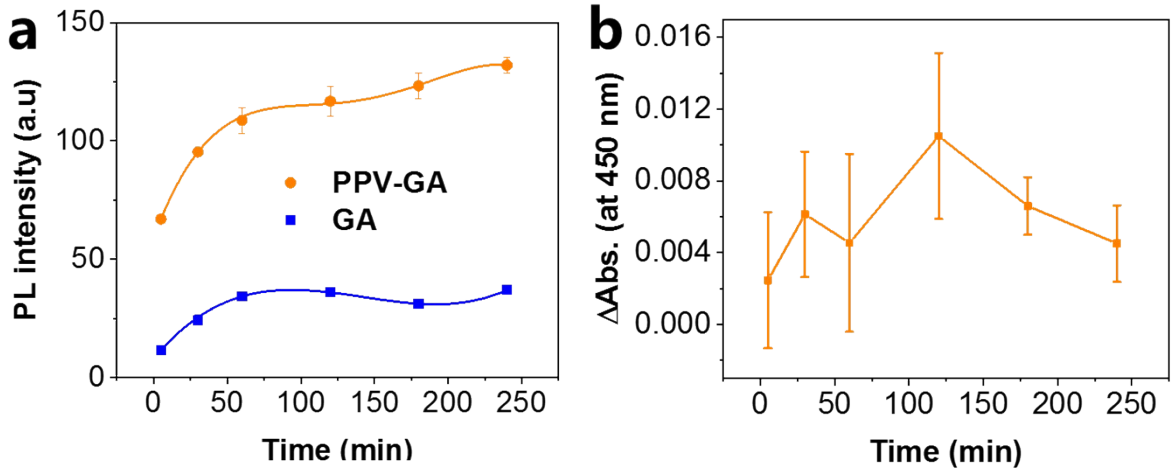


Fig. S5. (a) Diffusion curve of PPV from PPV-GA and GA within 4 h, determined by measuring the fluorescence intensity at 570 nm under excitation wavelength of 450 nm; (b) The corresponding ΔAbs. ($Abs_{PPV\text{-}GA}$ - Abs_{GA}) of each point in a), collected at 450 nm.

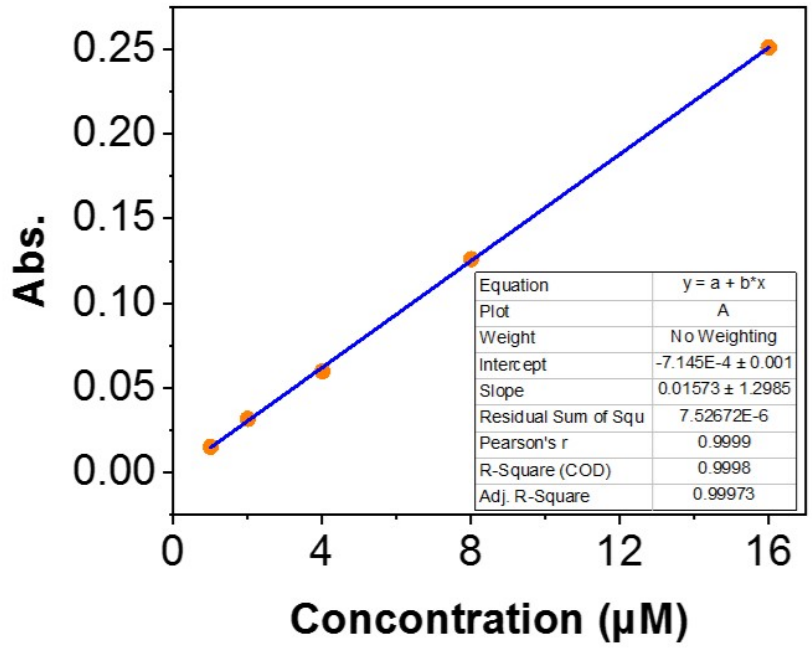


Fig. S6. Standard curve of the absorbance of PPV against concentrations at 450 nm.

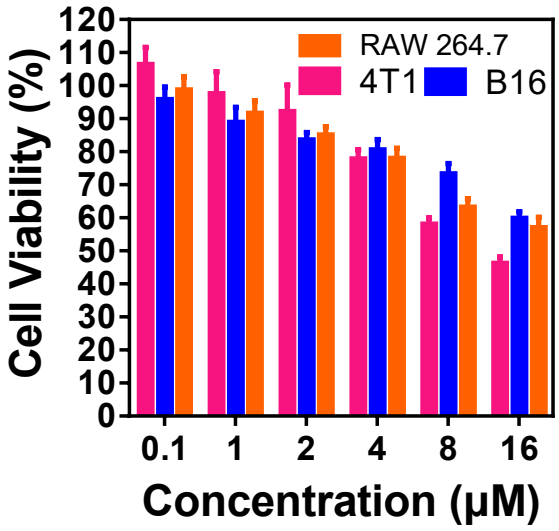
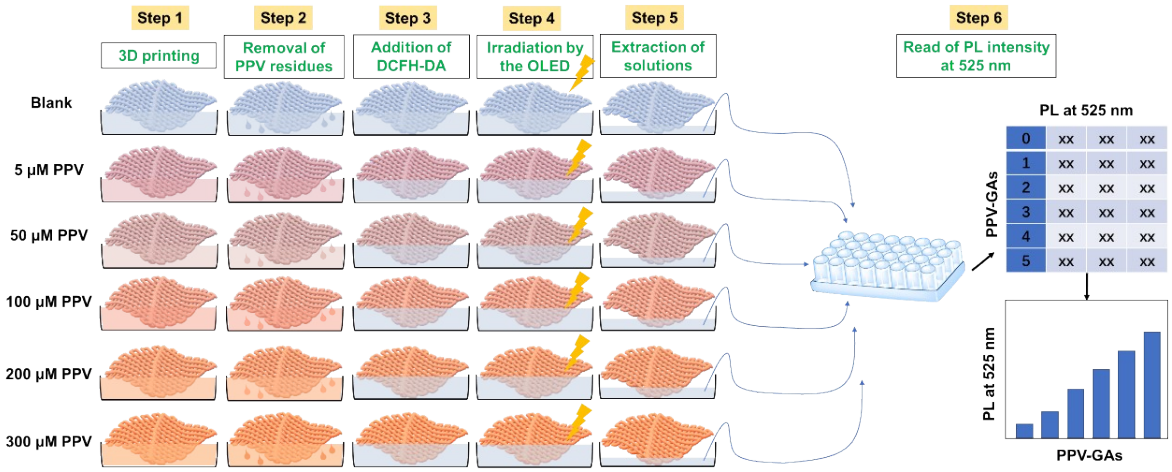


Fig. S7. Cell viability of macrophage (RAW 264.7), 4T1 and B16 cells, after the treatment of PPV with different concentrations.



Scheme S2: Schematical illustration of the experimental process of ROS generation ability test of PPV-GAs. PPV-GAs were 3D printed and immediately immersed into DMEM to dissolve free PPV. Afterwards, PPV-GAs went through a series of washing process using deionized water to thoroughly remove the residues of PPV and DMEM. The resultant PPV-GAs were immersed into the activated DCFH-DA stock solutions (1 M), followed by the irradiation of the OLEDs for 15 minutes. Then, the solutions were extracted and re-added into a 96-well culture plate to measure the PL intensity at 525 nm by using a microplate reader.

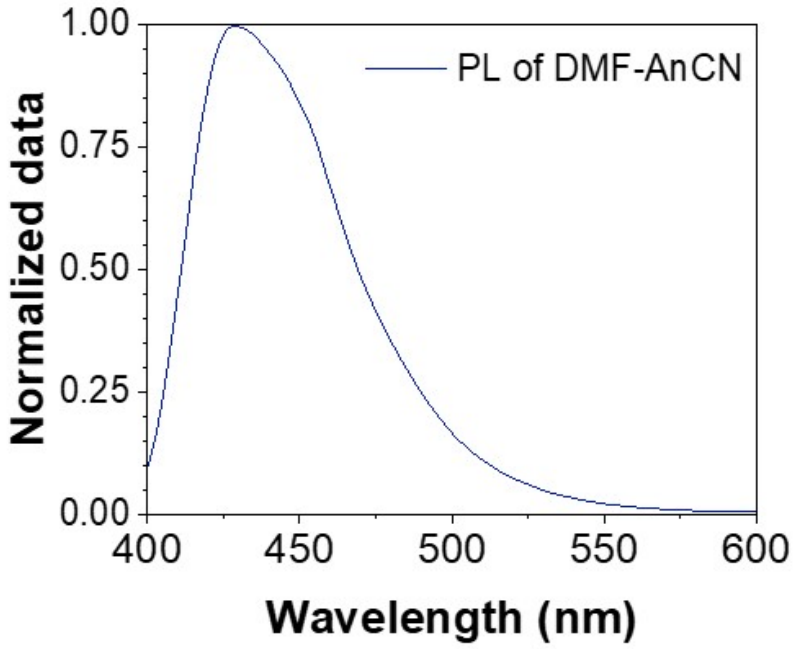
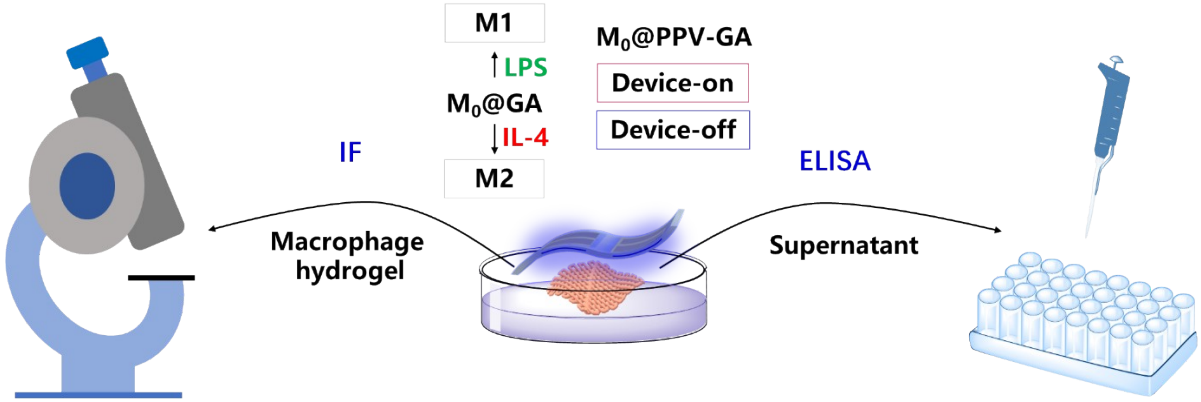


Fig. S8. PL spectra of DMF-AnCN



Scheme S3. Experimental scheme of immunofluorescence assay and ELISA analysis

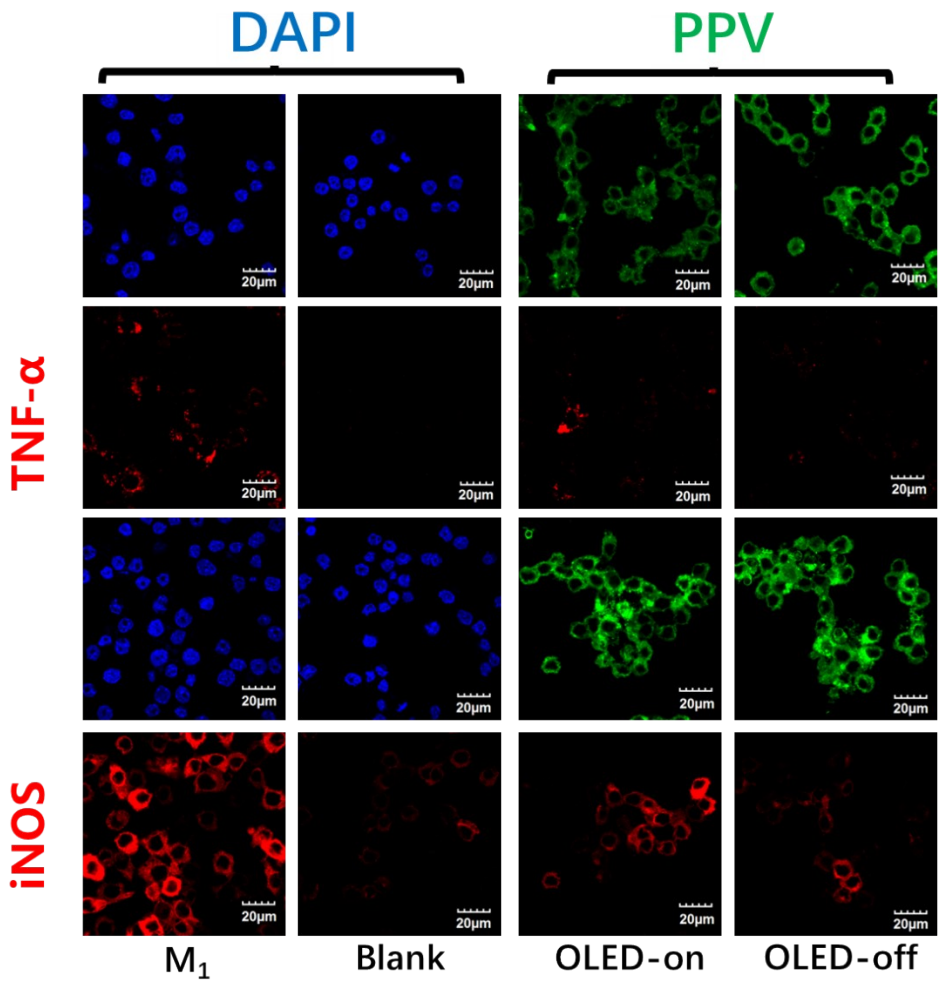
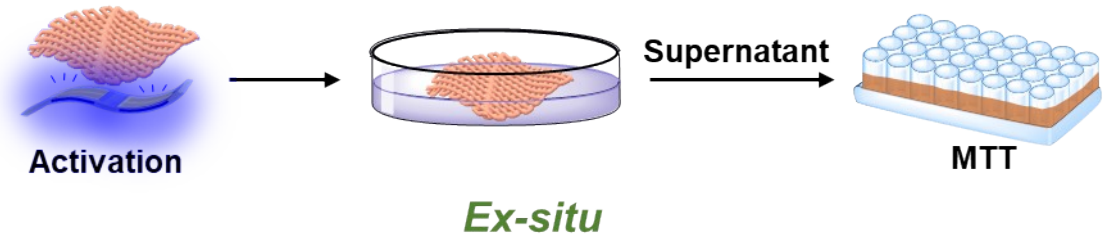


Fig. S9. CLSM images of macrophage stained with iNOS and TNF-α. The cells were counterstained with Hochest 33342 (blue) for M_1 and blank group.



Scheme S4. Schematic illustration of ex-situ simulation of anti-tumor activity of PBM device

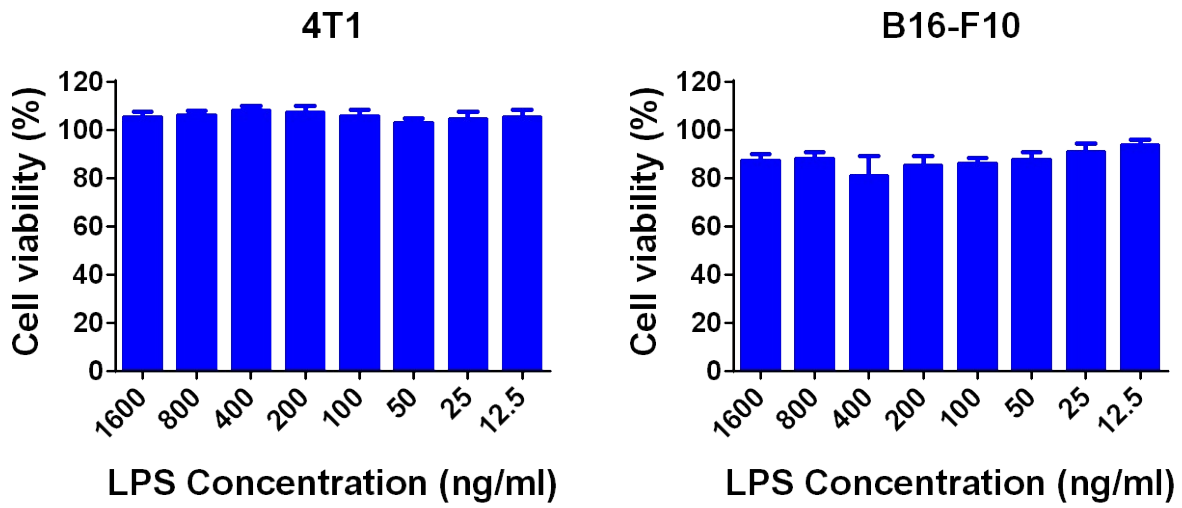


Fig. S10. The cell viability of 4T1 cells and B16-F10 cells in the presence of different concentrations of LPS.

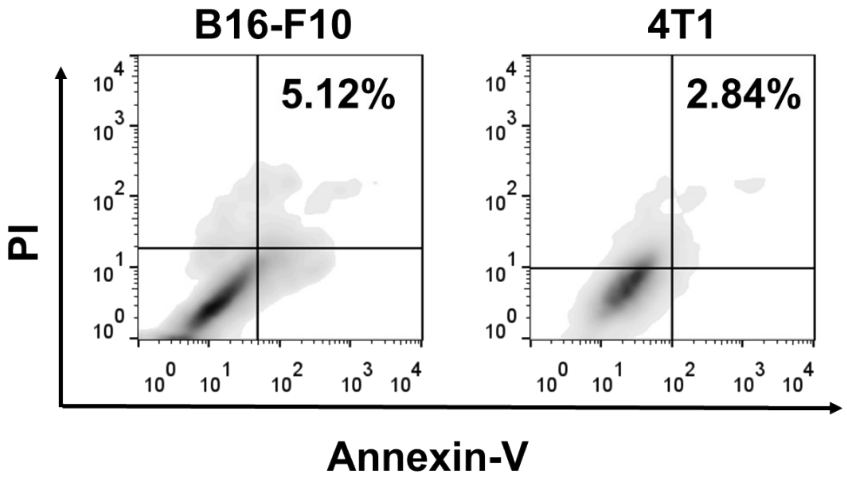


Fig. S11. Flow cytometry analysis of the apoptotic cells after the treatment of PPV and hydrogel vehicles (PPV-GA).

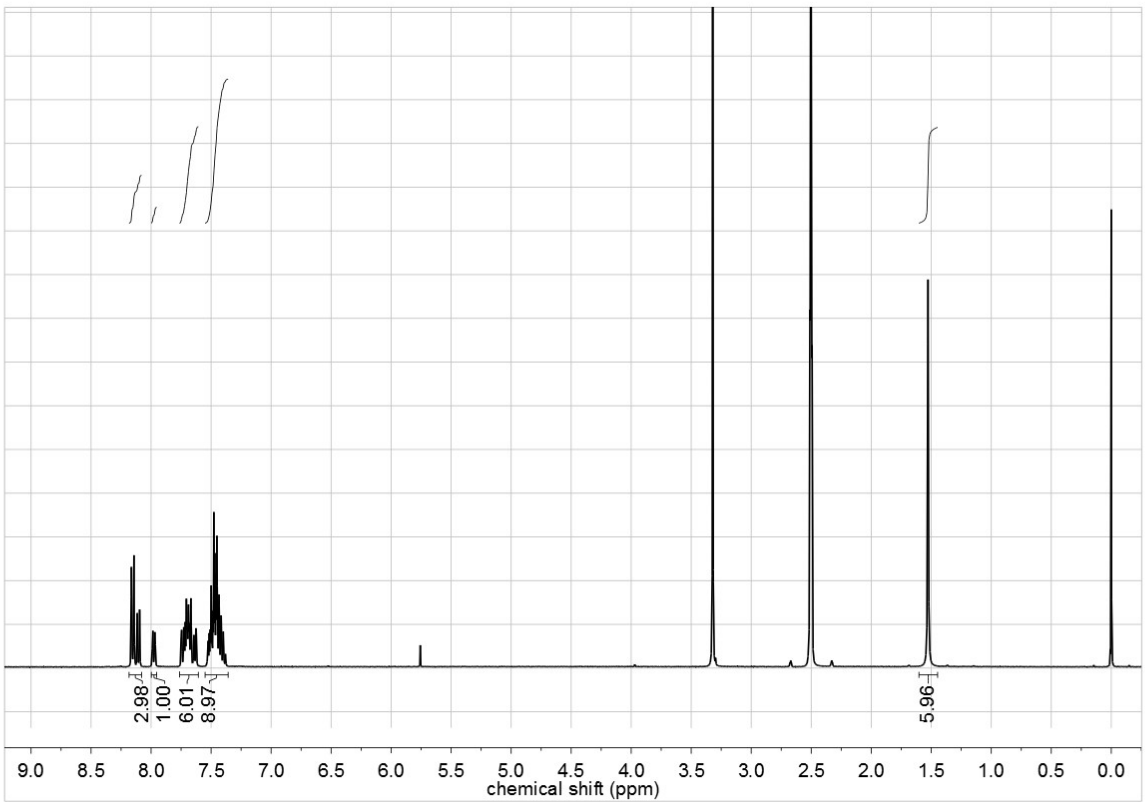


Fig. S12. ^{1}H NMR spectra of DMF-AnCN

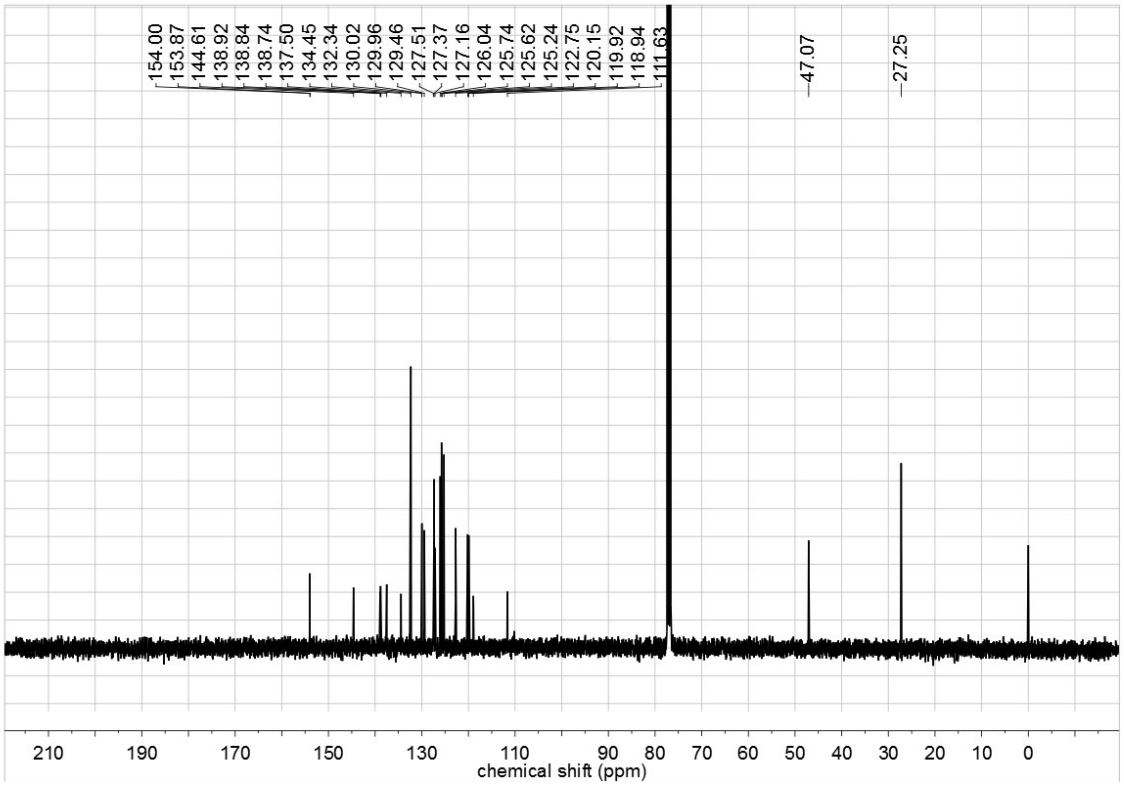


Fig. **S13.** ^{13}C NMR spectra of DMF-AnCN