

Electronic Supplementary Information (ESI)

Wireless Near-Infrared Electrical Stimulation of Neurite Outgrowth

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Experimental Section

Preparation of UCNPs. In a typical experiment, to a well-agitated transparent solution of ethylene glycol (EG) (25 mL) charged with NaCl (3.6 mmol), YCl_3 (1.56 mmol), YbCl_3 (0.4 mmol), ErCl_3 (0.4 mmol), and PAA (0.15 g) was added a stoichiometric amount of NH_4F in 18 mL of EG. The resulting mixture was agitated for another 15 min, then transferred to a 20 mL of Teflon-lined autoclave, and subsequently heated at 200 °C for 24 h. The obtained nanoparticles were collected by centrifugation, washed with ethanol and deionized water several times, and dried in an oven at 37 °C.¹

Preparation of PPy/UCNPs Films. ITO electrode surface was cleaned successively with acetone, ethanol, and water under sonication for 30 min, respectively. Subsequently, the surface was immersed in piranha solution for 5 seconds and washed with pure water. Then the surface of ITO was dispensed with UCNPs. 0.4 M pyrrole was added to the 10 mM of PBS solution containing and the PAA-UCNPs/ITO electrode was immersed into the solution for electropolymerization. The electropolymerization of pyrrole was carried out at a constant current of 0.00015 A for 600s, and the PPy films were thus formed. For comparison, PPy films were electropolymerized with similar ways.² According to the surface area of ITO, various PPy/UCNPs films with different area could be prepared as well.

Photocurrent Generation Measurement. Photocurrent measurement was carried out using the three-electrode system comprised of a platinum wire as the auxiliary electrode, an Ag/AgCl as the reference electrode and an indium tin oxide (ITO) glass as the working electrode. For the preparation of working electrode, PAA-UCNPs solutions were dropped on the ITO glass and dried at room temperature to form film (PAA-UCNPs/ITO), and the photocurrent on PPy/PAA-UCNPs/ITO, PPy/ITO and ITO were measured as well. The three electrodes were immersed into an electrolyte bath containing PBS (100 mM, pH 7.2) for photo-electrochemical measurements with a potential of 0.2 V. The working electrode was illuminated with a near infrared laser source (980 nm).³

Cell Culture. PC12 cells (rat pheochromocytoma, American Type Culture Collection) were cultured in DMEM (Gibco BRL) medium supplemented with 5% fetal bovine serum (FBS), 10% horse serum (HS) in a 5% CO₂ humidified environment at 37 °C. PC12 cells were seeded (10000 cells/cm²) and incubated for 24 h in serum-reduced media (1% HS and 0.5% FBS) Simultaneously, NGF (50 ng/mL) was added into the media to induce the differentiation of PC12 cells. Fresh NGF was added to the media every two days. During the NIR irradiation, the temperature was well controlled below 41°C during the NIR irradiation.

Measurement of Neurite Outgrowth of PC12 Cells. The level of neurite outgrowth was quantified by counting the number of PC12 cells and measuring the neurite length in three random images from triplicate experiments. The level of differentiation was determined as L0, L1, L2 and L3 according to the length of the neurites. L0 was defined as cells with no neurites, L1 was defined as cells with neurites whose length was shorter than the size of the cell body, L2 was defined as cells with neuritis whose length was between the original or twice the size of the cell body, and L3 was defined as cells with neurites whose length was longer than twice the size of the cell body. The differentiation efficiency was determined by dividing the number of neurite-bearing cells (L1, L2 and L3) by the total number of cells. In NIR irradiation stimulation experiment, we irradiated the 24-well plates with 980 nm laser for 5 min (2.5 W/cm²) after PC12 cells have grown for 24 hours on different interfaces. And after irradiation, fresh NGF was added to the media. For deep tissue irradiation, pork tissues (muscle tissue) with 0 mm (2.5 W/cm²), 2 mm (ca. 2.2 W/cm²), and 4 mm (ca. 1.9 W/cm²) thickness were placed on the top of 24 wells plate above the cell adhesive surface with a distance of 1 mm. The laser was irradiated from the top of the tissue.⁴

Ion Release Assay. To assess the ionic release in an aqueous phase, the comparison of the different interfaces contained PAA-UCNPs with and without NIR irradiation was employed to study the ionic release effect. The irradiation sample had been irradiated with NIR for 120 minutes (2.5 W/cm²). Equal amounts of both samples experienced a 96 hours dialysis in deionized water, and RE ion concentrations in the dialysate were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Scanning Electron Microscopy (SEM) Study. In the SEM analysis, cells were seeded on a glass cover slip (10 mm in diameter). After treated with UCNPs, the differentiated PC12 cells were fixed with 2% glutaraldehyde and 2% paraformaldehyde in 0.01 M phosphate buffered saline (PBS) for 30 min at 4°C. Cells were dehydrated in an ascending series of ethanol (50%, 70%, 80%, 90%, and 100%). Finally, cells were immersed in HMDS (hexamethyldizilasane) and dried, and sputter coated with Au. Changes in cell morphology were monitored using SEM.

Intracellular Determination of ROS. The generation of reactive oxygen radicals was monitored using 2',7'-dichlorofluorescein diacetate (DCFH-DA), a nonfluorescent compound which reacts with intracellular free radicals and generating the fluorescent product dichloro-fluorescein (DCF). The DCF fluorescence intensity correlates with the amount of intracellular reactive oxygen radicals. To perform the experiment, 20 mM DCFH-DA solution was added to the PC12 cells and the mixture was incubated at 37 °C for 1h. The cells were then washed twice with PBS solution and finally the fluorescence intensity was monitored by flow cytometric analysis.

Apparatus and characterization. FT-IR characterization was carried out on a BRUKE Vertex 70 FT-IR spectrometer. The samples were thoroughly ground with exhaustively dried KBr. TEM images were recorded using a FEI TECNAI G² 20 high-resolution transmission electron microscope operating at 200 kV. SEM images were recorded using a HITACHI S-4500 instrument. The crystalline structures of the as-prepared samples were evaluated by X-ray diffraction (XRD) analysis on a Rigaku-Dmax 2500 diffractometer by using Cu_{Kα} radiation ($\lambda = 0.15405$ nm). The operation voltage and current were kept at 40 kV and 40 mA. UV-vis absorbance measurements were carried out on a JASCO V-550 UV-vis spectrophotometer, equipped with a Peltier temperature control accessory. For comparison, the UV-vis spectrum and upconversion fluorescence spectrum were normalized by the highest values of absorbance and fluorescence intensity, respectively.

Cell toxicity assays: PC12 cells were placed on 24-well plates equipped with PPy/PAA-UCNPs/ITO or equivalent area glass sheet in serum-reduced media (1% HS and 0.5% FBS). Simultaneously, NGF (50 ng/mL) was added into the media to induce

the differentiation of PC12 cells. Then the 24-well plates were exposed to 980 nm laser for 5 min (2.5 W/cm^2) after PC12 cells have grown for 24 hours. Fresh NGF was added to the media every two days. To validate cytotoxicity of the samples (with and without NIR), the PPy/PAA-UCNPs/ITO and equivalent area glass sheet were placed in another 24-well plates after incubating for 5 day. Then modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) diluted with serum-free media was added into the new 24-well plates. The absorbance values of formazan at 490 nm were demonstrated by an automatic plate read.

Immunofluorescent staining: After incubating for 6 day, cells were washed twice with ice-cold PBS, fixed with 4% paraformaldehyde, and *via* incubation with 0.5% Triton X-100. Cells were then blocked with PBS containing 5% BSA, incubated with a primary antibody Tuj1 and Nestin and then an appropriate FITC or 568 Dye-labeled goat secondary antibody. The nucleus was stained blue with DAPI. Cells were ultimately observed under a confocal laser scanning microscopy (CLSM).



Scheme S1 Schematic representation of the structure of the PPy/UCNPs photoelectrode.

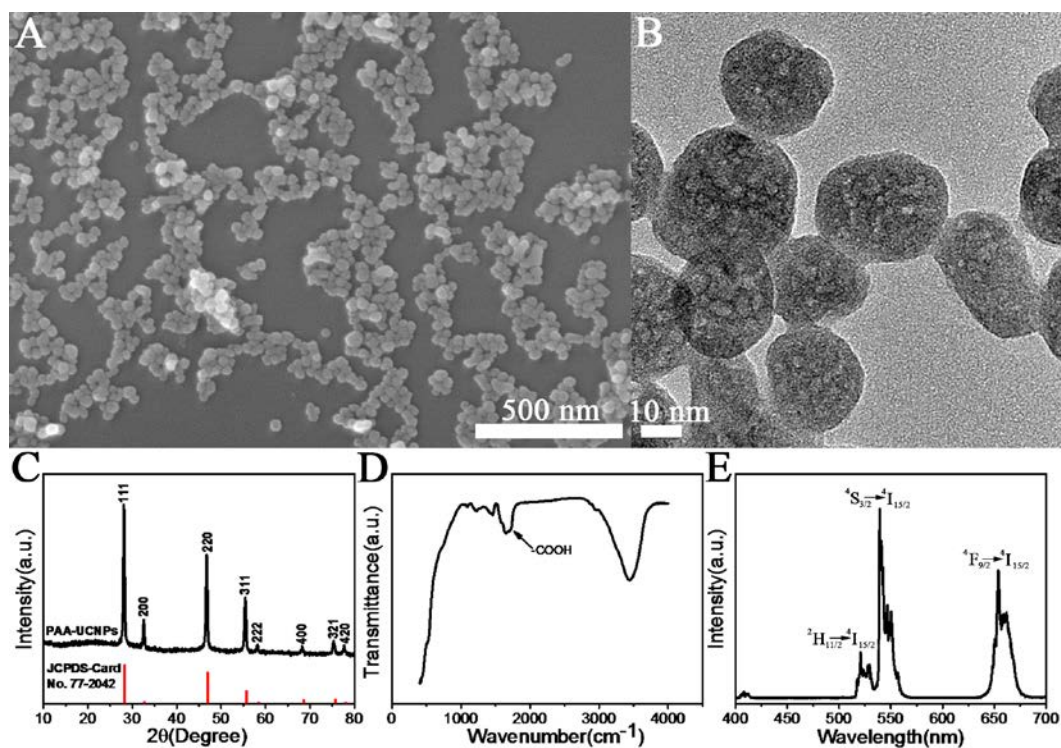


Fig. S1 (A) SEM image; (B) TEM image; (C) wide-angle XRD pattern; (D) FT-IR spectrum; (E) upconversion fluorescence spectrum of the UCNPs.



Fig. S2. Photographs of (A) UCNPs; (B) UCNPs/PPy; (C) PPy on the ITO (1cm × 1cm).

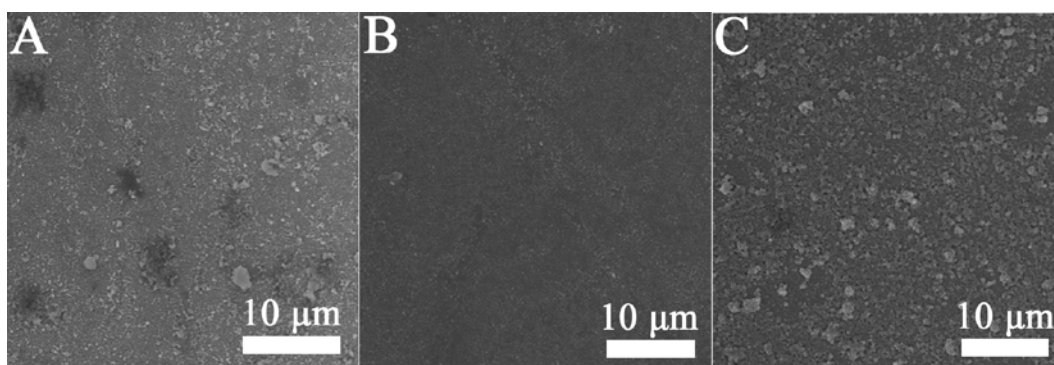


Fig. S3 SEM images of (A)UCNPs; (B) PPy; (C)UCNPs/PPy on the ITO.

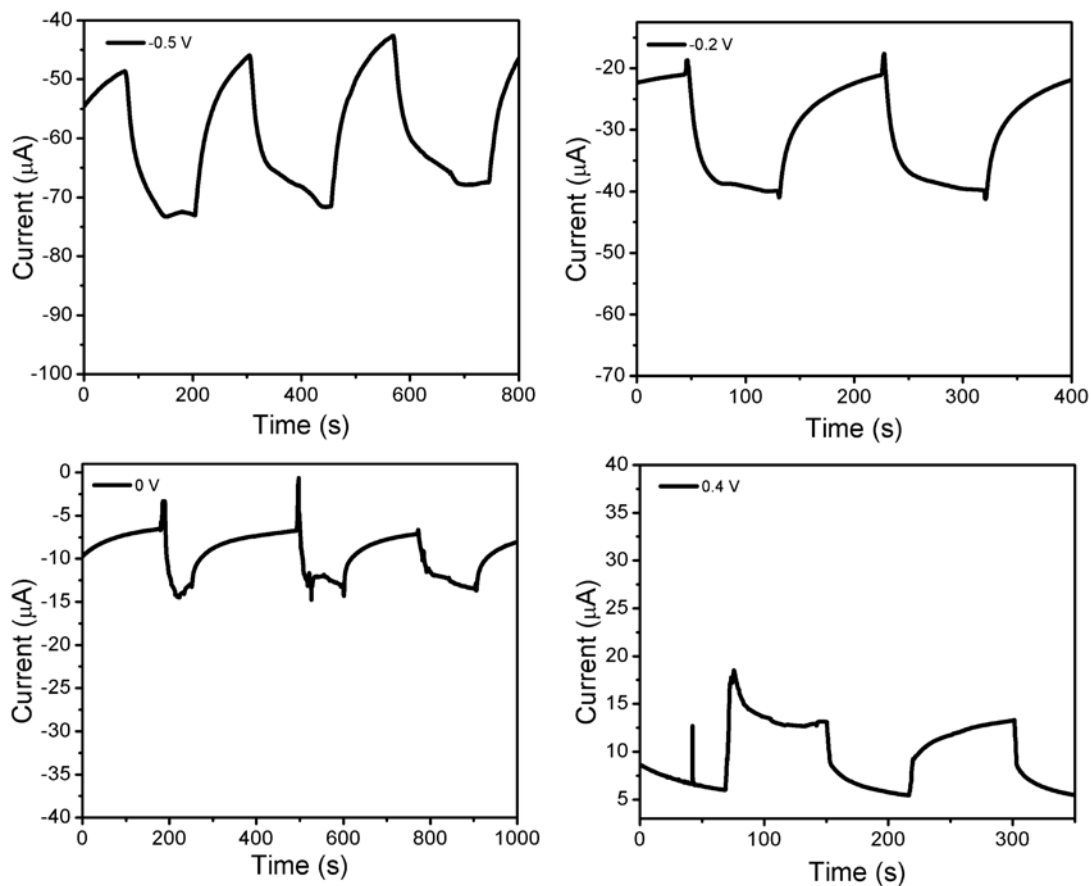


Fig. S4 Photocurrent of UCNPs/PPy at different potentials. All the potentials are vs. Ag/AgCl.

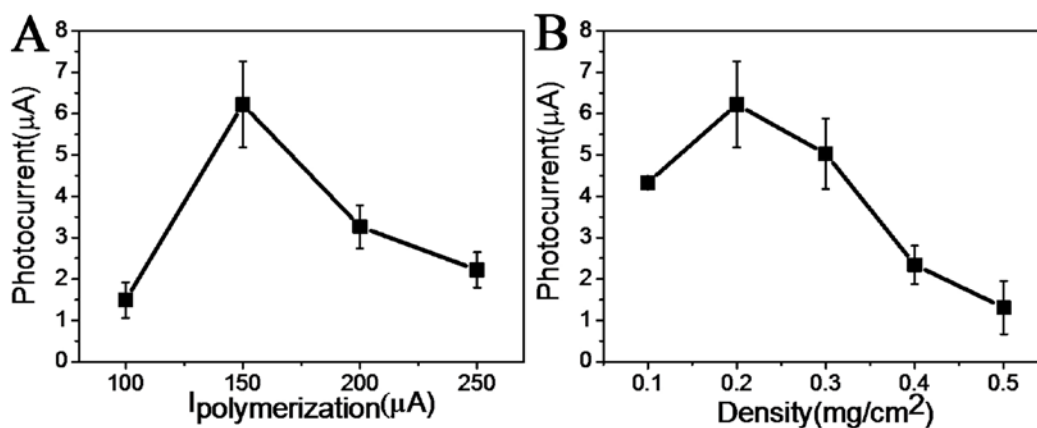


Fig. S5 (A) Optimization of the polymerization current when preparing PPy/UCNPs electrode (the density of UCNPs is 0.2 mg/cm²). (B) Optimization of the density of UCNPs when preparing PPy/UCNPs electrode (the polymerization current is 150 μA). The power density of laser at 980 nm is 5 W/cm².

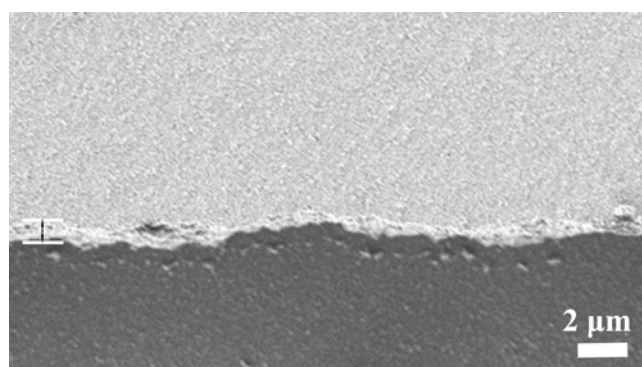


Fig. S6 The cross section SEM image of UCNPs/PPy (the density of UCNPs is 0.2 mg/cm²; the polymerization current is 150 μA.).

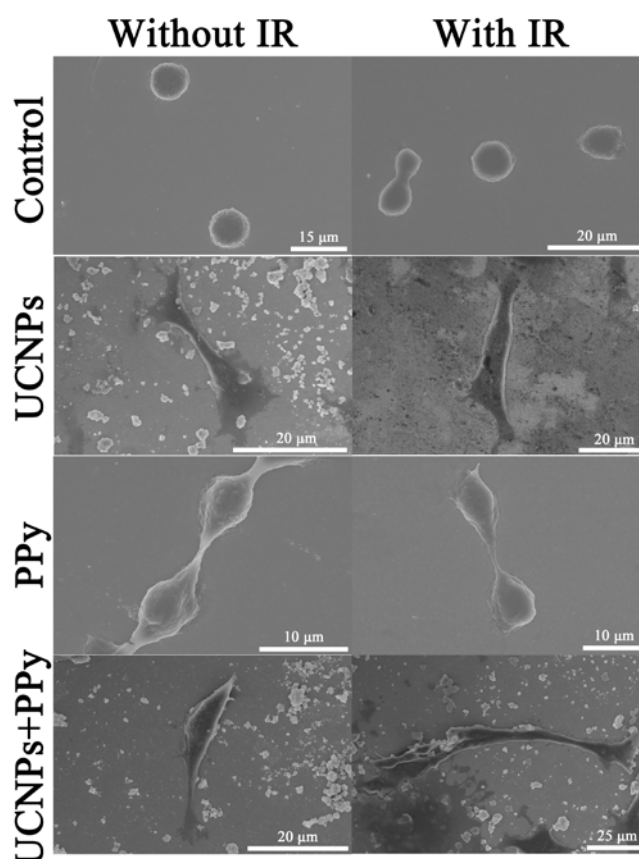


Fig. S7 SEM images of PC12 cells after 2 days on different interfaces with and without NIR irradiation.

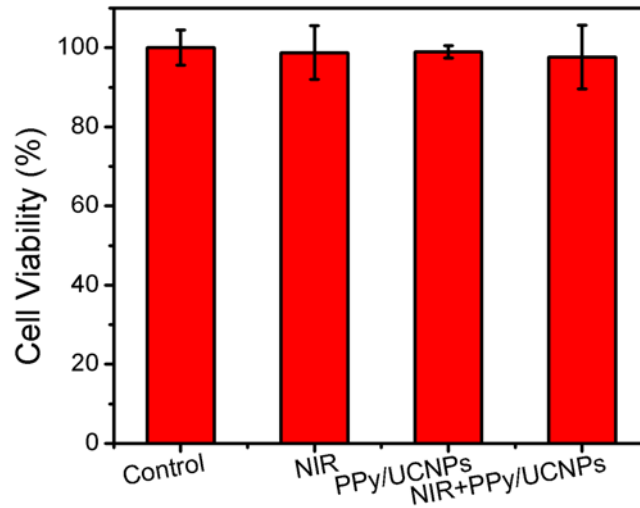


Fig. S8 Cytotoxicity studies of the UCNPs/PPy for PC12 cells evaluated by the MTT method. The NIR power here is 2.5 W/cm².

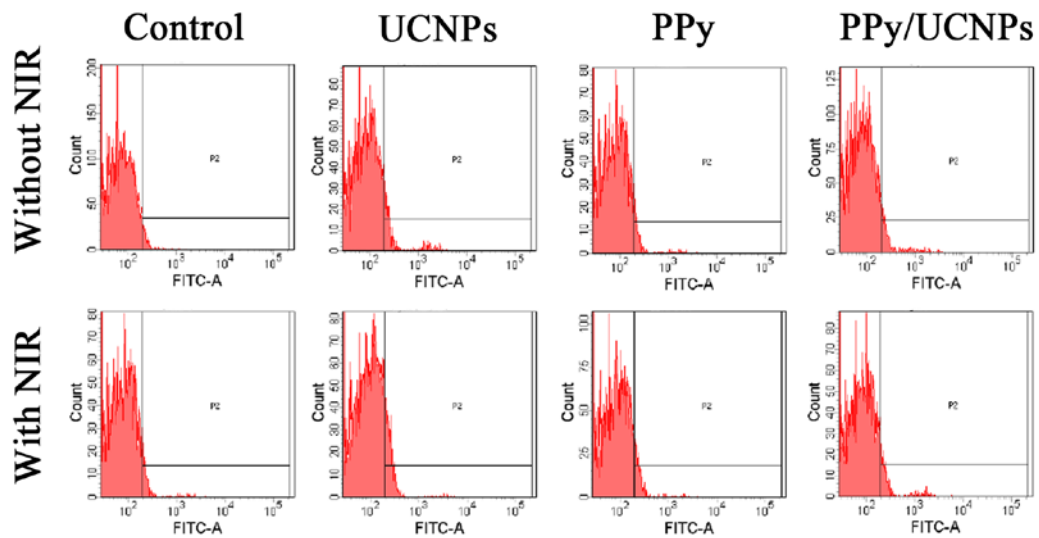


Fig. S9 Flow cytometry analysis to monitor the intracellular ROS of PC12 cells on different interfaces with and without NIR irradiation. The DCFH-DA was used as the fluorescence probe of ROS.

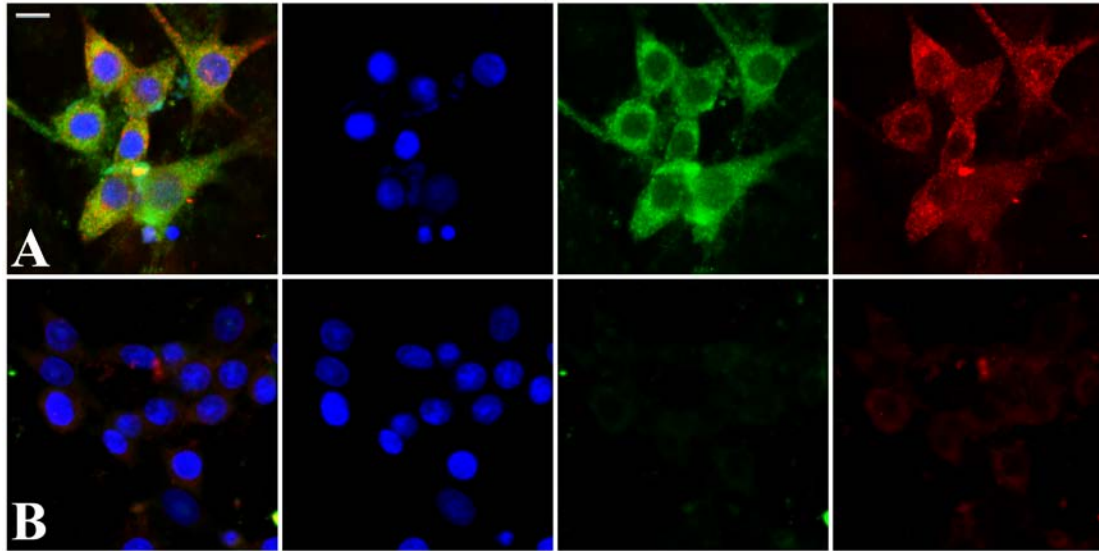


Fig. S10 (A) Immunofluorescence staining images of nestin (green) and Tuj-1 (red) in the differentiated PC12 cells on PPy/UCNPs photoelectrode. (B) Immunofluorescence staining images of nestin and Tuj-1 in the undifferentiated PC12 cells as a comparison. The nucleus was stained blue with DAPI. The scale bar is 10 μm .

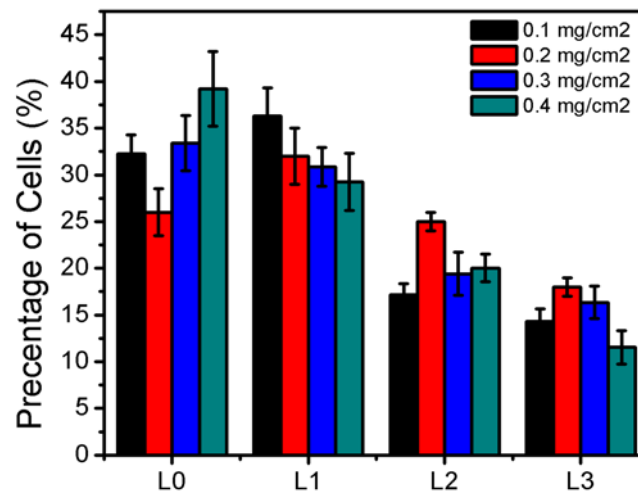


Fig. S11 Differentiation efficiency of PC12 cells on PPy/UCNPs photoelectrode with different amount of UCNPs irradiated by 980 nm NIR laser.

Because the amount of UCNPs on the PPy/UCNPs also affect their photocurrent generation. The degree of cell differentiation for PC12 is positive correlation with the photocurrent generated ability of PPy/UCNPs. Such positive correlation strongly supported that photogenerated interface electric field by PPy/UCNPs plays a key role in promoting neurite outgrowth.

Table S1. Ion release from the interfaces containing UCNPs with and without NIR irradiation.

Sample	Er[ppm]	Y[ppm]	Yb[ppm]
UCNPs	0.0314	0.5105	0.2628
UCNPs NIR	0.1148	1.480	0.7695
UCNPs IR/without NIR	3.66	2.90	2.93
UCNPs+PPy	0.0230	0.3278	0.1959
UCNPs+PPy NIR	0.0791	0.7378	0.3355
UCNPs+PPy IR/without NIR	3.43	2.25	1.71

References

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- 3 a) F. Pu, L. Wu, X. Ran, J. S. Ren and X. G. Qu, *Angew. Chem. Int. Ed.*, 2015, **54**, 892-896. b) F. Pu, L. Wu, E. G. Ju, X. Ran, J. S. Ren and X. G. Qu, *Adv. Funct. Mater.* 2014, **24**, 4549-4555.
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