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

Supporting information

Two-Photon Fluorescent Polydopamine Nanodots for CAR-T Cells Function Verification and Tumor Cells/Tissues Detection

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S1 DA color under different light

As Fig. S1 shown. It is no color of DA solution under bright filed, and there is no fluorescence under UV light. Combined with Fig. 1, it is clear that the color has changed in the synthesis process.

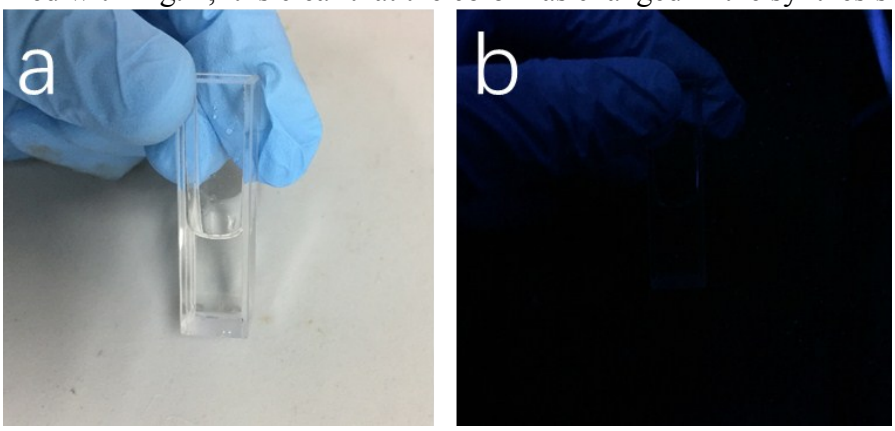

Fig. S1 Physical pictures of DA solution under bright filed and UV light. a, under bright filed; b, under UV light.

S2 Preparation process of PDA and OPDA nanodots

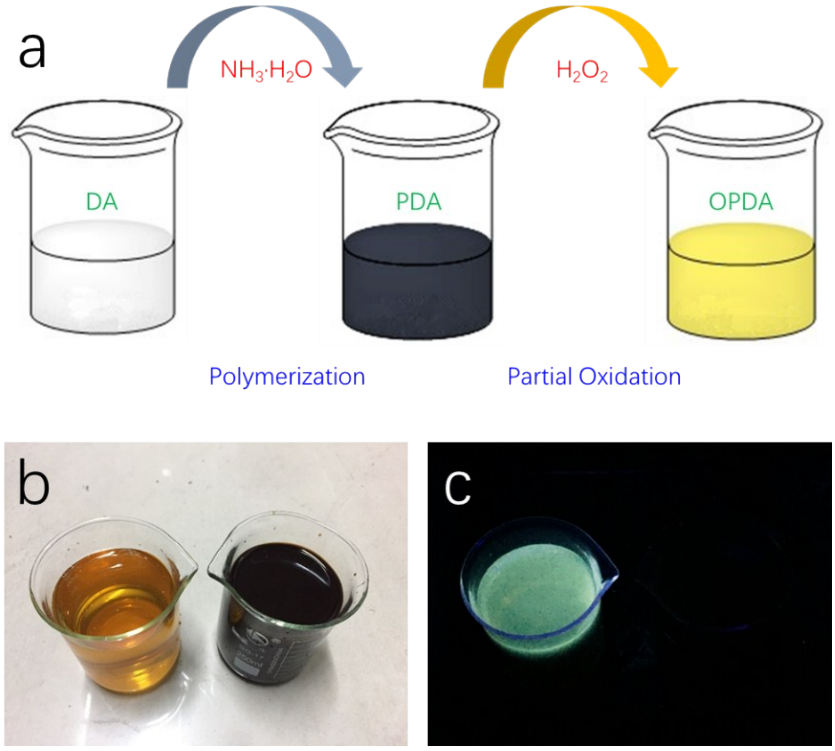

Fig. S2 a, Schematic of PDA and OPDA synthesis process, the beaker model is from ChemBioOffice 14.0.; b, digital picture of mass production PDA and OPDA under nature light; c, digital picture of mass production PDA and OPDA under UV light.

First, $NH_3 \cdot H_2O$ was added into DA solution. After 24 h, PDA was produced through polymerization reaction. Then, a certain H_2O_2 was added into PDA system. After 24h, OPDA nanodots was synthesized by partial oxidation. All the reactions were under room conditions, which provides much convenient advantage to mass production and application promotion.

S3 The stability of OPDA mixed with reductant

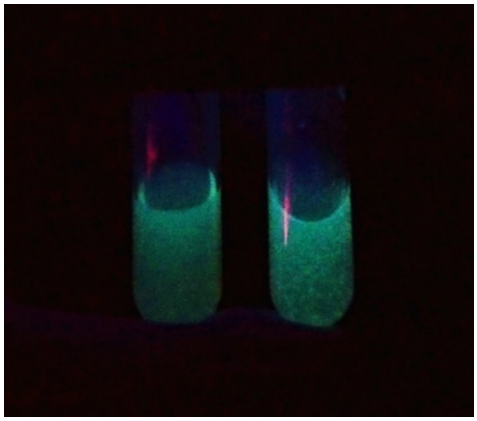

Fig. S3 The stability of OPDA mixed with reductant. Left, OPDA suspension; right, OPDA suspension mixed with $NaBH_4$ (10 mg/ml).

As Fig. S3 shown, OPDA nanodots cannot be reduced by reductant to confirm the stability of fluorescence.

S4 HRMS of PDA and OPDA nanodots

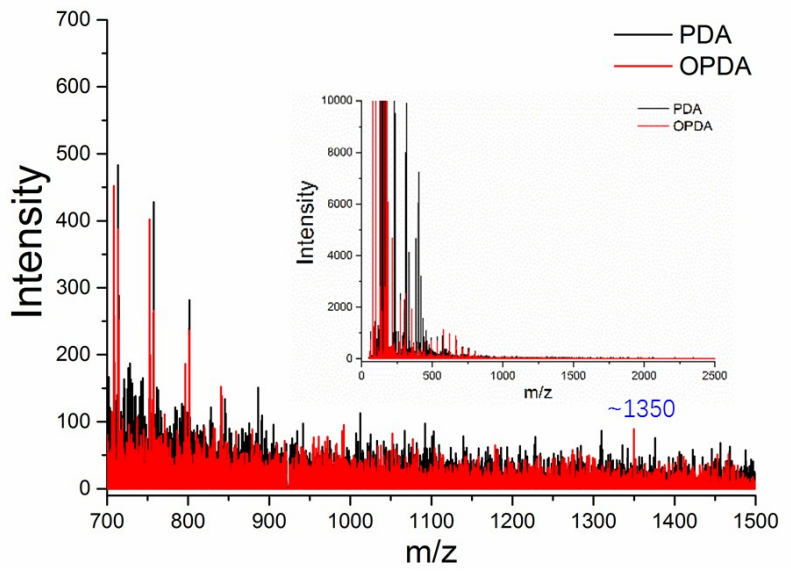

Fig. S4 HRMS of PDA and OPDA nanodots.

high resolution mass spectrometer (HRMS) spectra were measured to investigate the structure of PDA and OPDA. As Fig. S4 shown, the most reasonable molecular weight of PDA is about 1370, which means that the polymerization degree of PDA is about nine. OPDA has the similar molecular weight (~1350), and the polymerization degree is also about nine.

S5 The absorption spectrum of PDA

PDA nanodots can almost absorb all visible light following dark brown color (Fig. S5 and Fig. 1b).

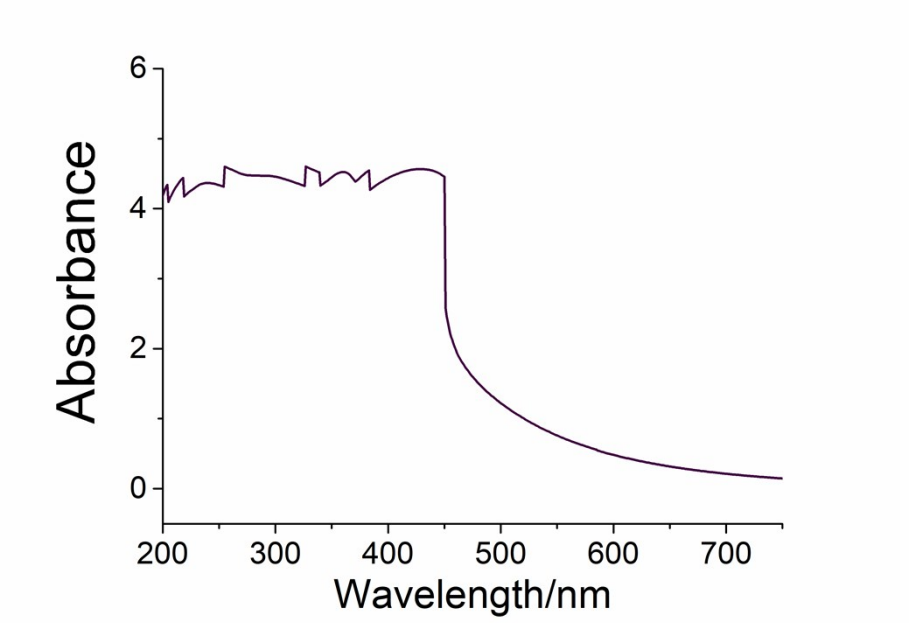

Fig. S5 The absorption spectrum of PDA nanodots

S6 The Log–Log curve

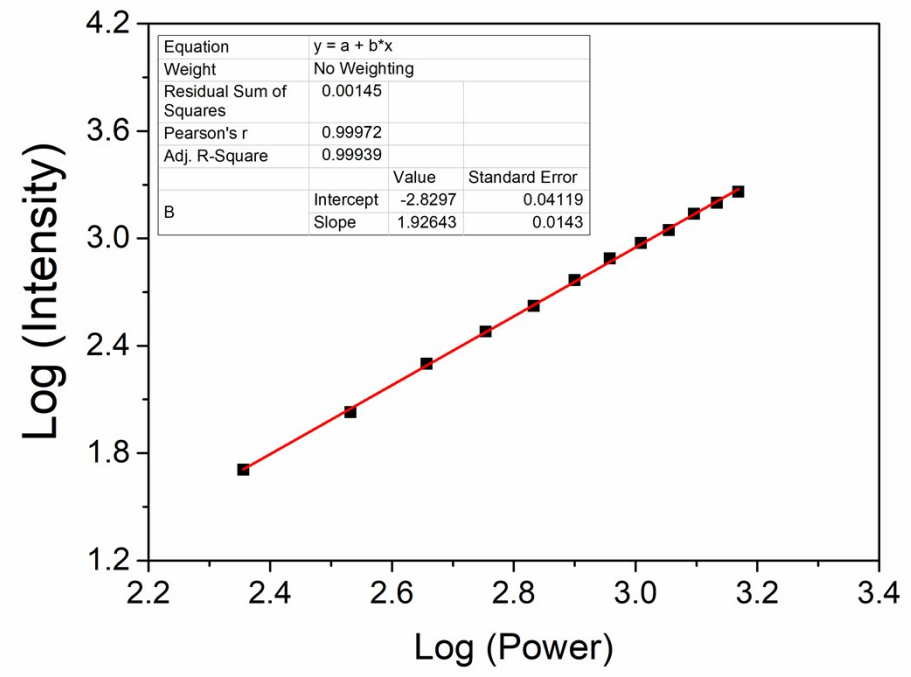

Fig. S6 The Log–Log curve of squared dependence of induced fluorescence signal and incident irradiance intensity.

The fluorescence intensity of OPDA nanodots under different power at 800 nm excitation was measured. The range of laser power is from 274 mW to 1475 mW. The Log–Log curve is shown as Fig. S6. The Log–Log curve is linear, and the slope is about 2. Therefore, the result can provide the solid evidence to confirm the two photon fluorescent property of OPDA nanodots.

S7 The scheme of one-photon and two-photon excited fluorescence process

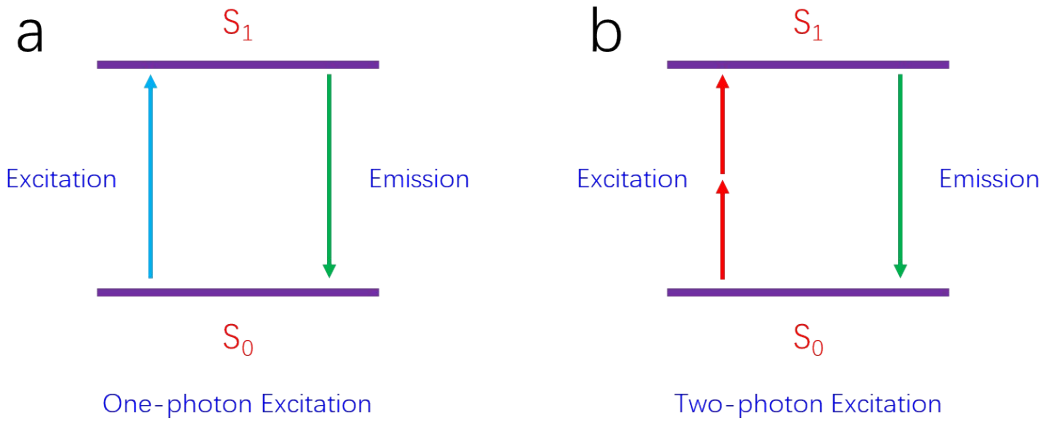

Fig. S7 The scheme of one-photon and two-photon excited fluorescence process. a, one-photon excited fluorescence process; b, Two-photon excited fluorescence process.

One-photon fluorescence process is that electron transferred to excited state (S_1) from ground state (S_0) by absorbing one photon, and then returned to S_0 by emitting fluorescence. However, for two-photon fluorescence, electron transferred to excited state (S_1) from ground state (S_0) by absorbing two photons rather than one photon, and then returned to S_0 by emitting fluorescence.

S8 The two photon fluorescence spectra of OPDA nanodots and fluorescein

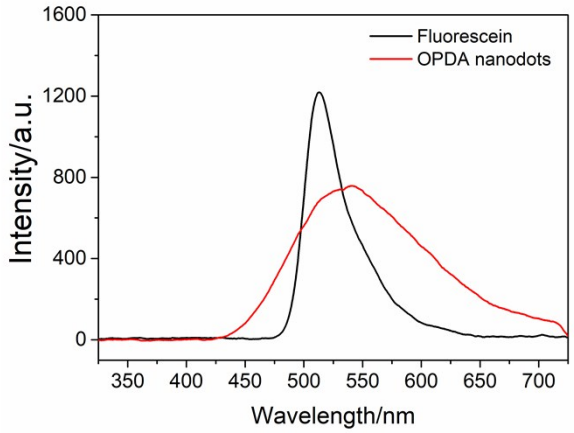

Fig. S8 The two photon fluorescence spectra of OPDA nanodots and fluorescein under 840 nm.

Fluorescein two-photon spectrum and OPDA nanodots two-photon spectrum under 840 nm excitation were provided for relative comparison. It is clear that the two-photon fluorescence integral area of OPDA nanodots under 1 mg/ml concentration is higher than fluorescein under 2 μM concentration. From the relative comparison, we can see that OPDA nanodots possess good two photon fluorescence property.

S9 The stability of OPDA fluorescence

OPDA was exposed on UV light to measure the stability of fluorescence property. After 14 d, there was little decrease in fluorescence intensity compared with the original one. Therefore, the stability of

OPDA is very high and can keep fluorescence for long time.

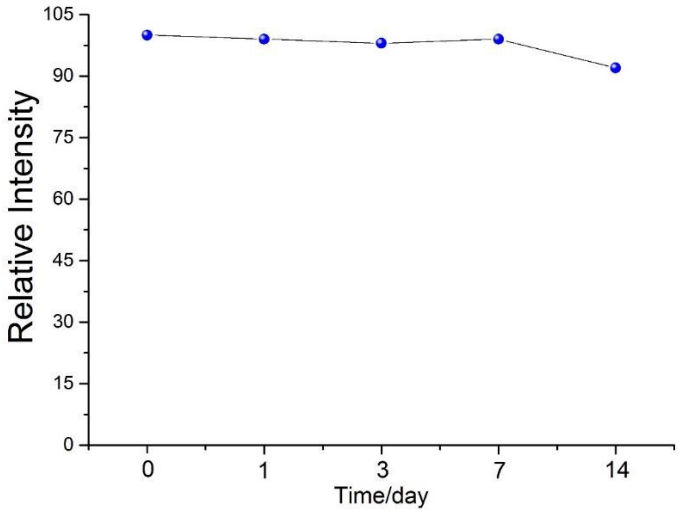

Fig. S9 The stability of OPDA fluorescence under UV light continuous irradiation. Power: 12 W; Distance: 15 cm.

S10 OPDA film fluorescence

ODPA film coated on glass can emit different color light from blue to green to red under different excitation light measured on common fluorescence microscope (Fig. S10). OPDA film shows blue color under excitation light from 330–385 nm. OPDA film shows green color under excitation light from 470-490 nm. OPDA film shows red color under excitation light from 510–550 nm;

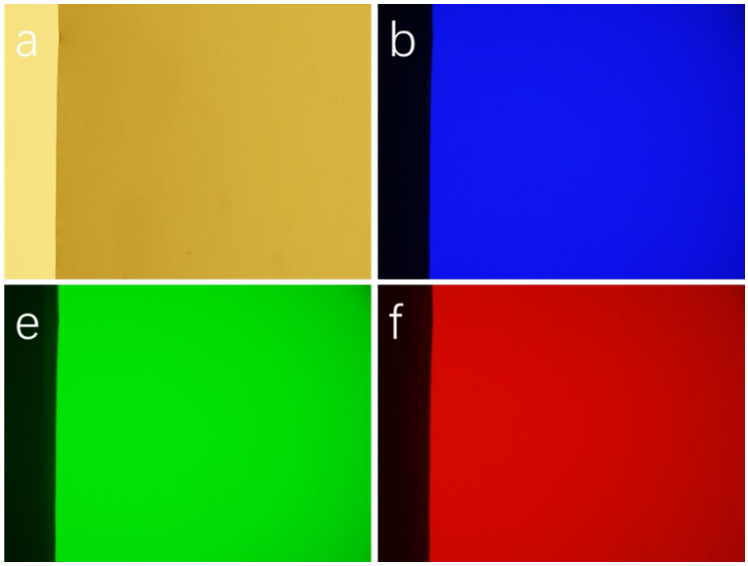

Fig. S10 OPDA film fluorescence under different excitation light. a, under bright filed; b, under excitation light from 330–385 nm; b, under excitation light from 470-490 nm; d, under excitation light from 510–550 nm.

S11 The cytocompatibility of OPDA

As Fig. S11 shown, viability of Hela cells kept high after co-cultured with OPDA nanodots. There was only little decrease at 800 μg/ml concentration after 2d. However, the viability at 400 μg/ml concentration kept similar level with the control group. Even culture for 7d, cells treated with OPDA

still had high cell viability and typical cells morphology of Hela cells and MSCs (Fig. S12 and S13). Therefore, ODAP nanodots have high cytocompatibility.

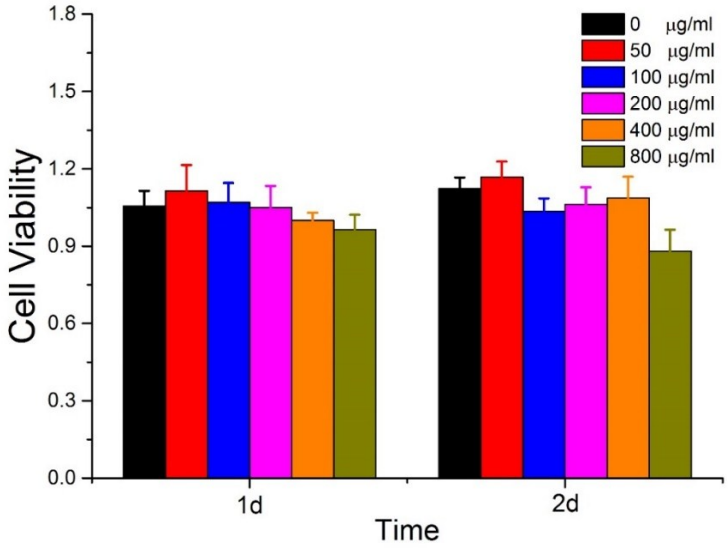

Fig. S11 Cell viability co-cultured with OPDA nanodots at different concentration.

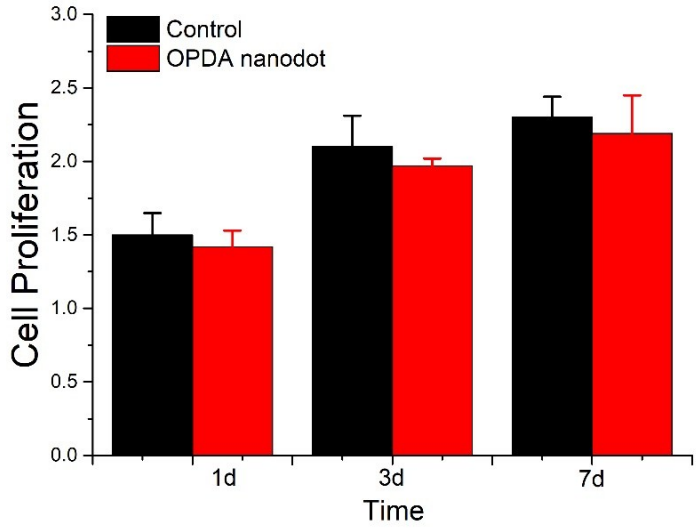

Fig. S12 Cell proliferation co-cultured with OPDA nanodots at 200 μg/ml concentration.

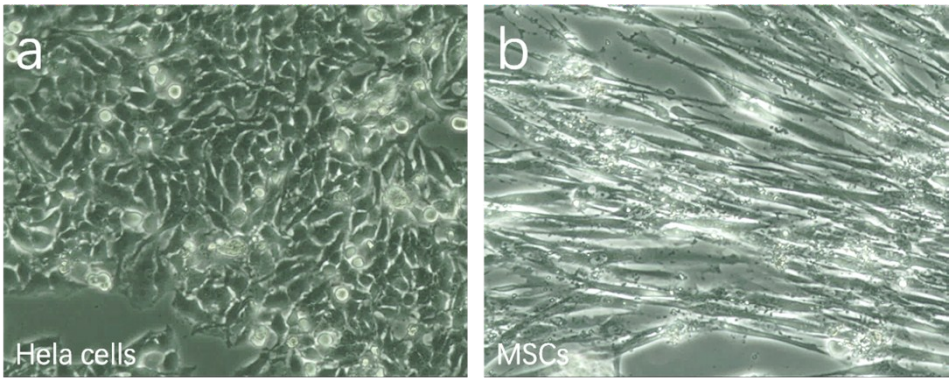

Fig. S13 Cell state co-cultured with OPDA nanodots at 200 μg/ml concentration after 7d under bright field.

S12 Lambda experiment

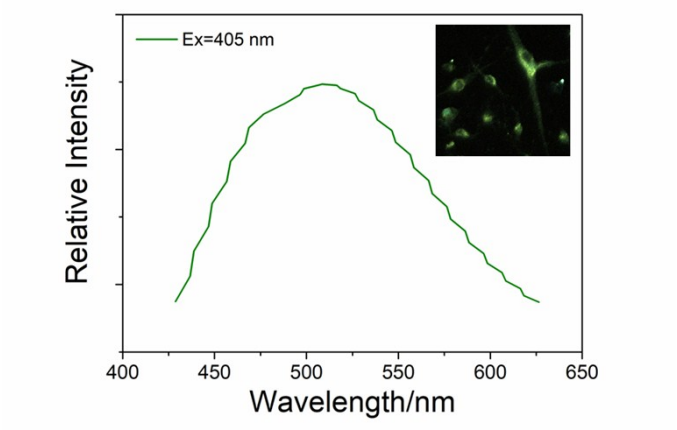

Fig. S14 Intracellular fluorescence spectrum of OPDA nanodots in situ under 405 nm excitation.

Lambda experiments have been performed, and result is shown in Fig. S14. The intracellular emission spectrum of OPDA nanodots is similar with extracellular result. Therefore, OPDA nanodots have good fluorescence stability, which provides the basis for long time living cells imaging.

S13 Cell imaging for suspension cells

After co-cultured with Raji cells, OPDA nanodots can image Rajic cells well, which confirms that OPDA can image suspension cells efficiently.

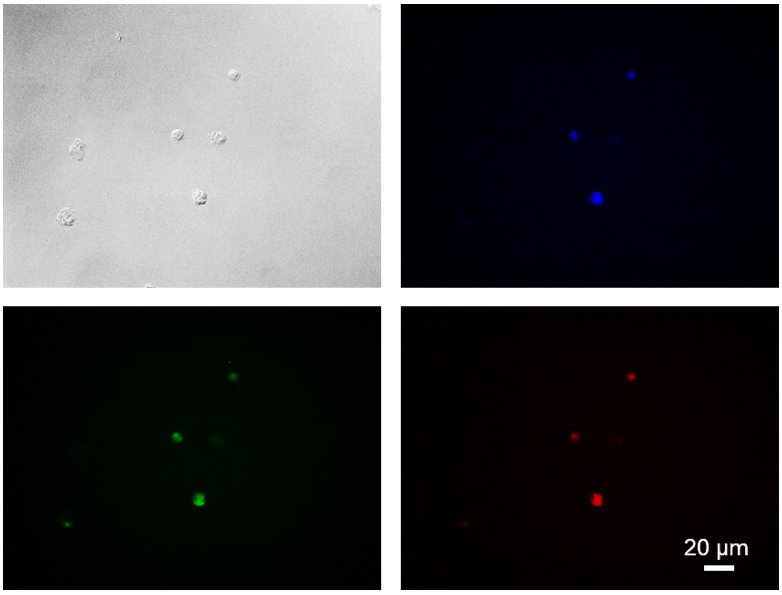

Fig. S15 Cell imaging for suspension cells of OPDA nanodots through different color.

S14 Emission spectrum of OPDA nanodots under 543 nm

It is clear that OPDA nanodots emit red color light under 543 nm wavelength excitation, which can be used to distinguish OPDA nanodots and EGFP.

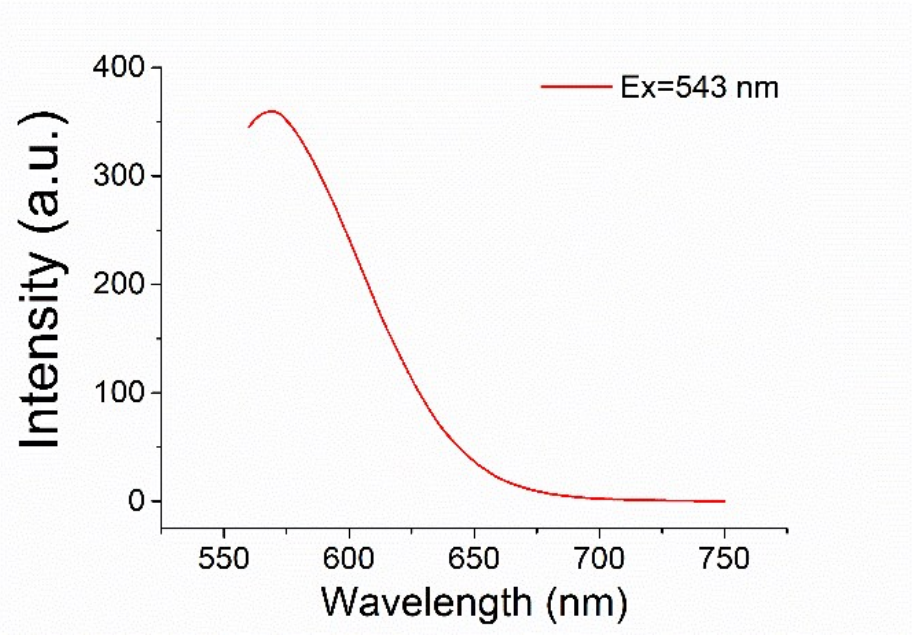

Fig. S16 Emission spectrum of OPDA nanodots under 543 nm.

S15 The response of PDA to H_2O_2 under different conditions

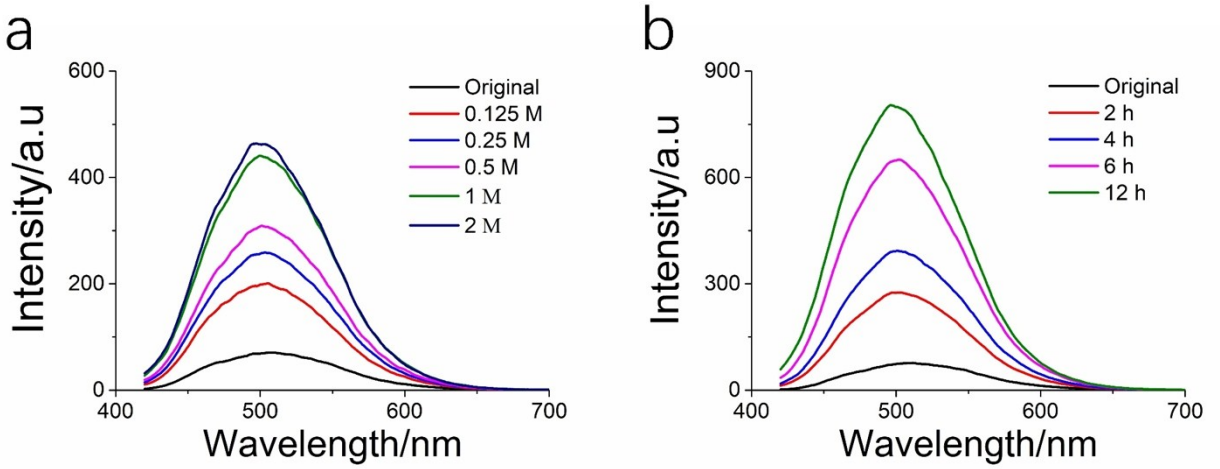

Fig. S17 The response of PDA to H_2O_2 under different conditions under 37℃. a, the response of PDA to H_2O_2 volume, reaction time, 4 h; b, the response of PDA to H_2O_2 under different reaction time, reaction concentration of H_2O_2, 0.5M.

To assess the effect of H_2O_2 on conversion of PDA to OPDA, fluorescent intensity of PDA solution was measured under different H_2O_2 concentration, different temperature and different time. With increase of the H_2O_2 concentration, the fluorescence intensity became stronger after reaction for 4 h (Fig. S17a). The fluorescence intensity changed slightly when the volume was over 1 M. The effect of time on fluorescence intensity was studied. After reaction for 2h, the fluorescent intensity had an obviously increase. When time was over 6 h, the increase rate became slow (Fig. S17b).

S16 Physical pictures of PDA and PDA treated with H_2O_2 under 37℃ for 4h

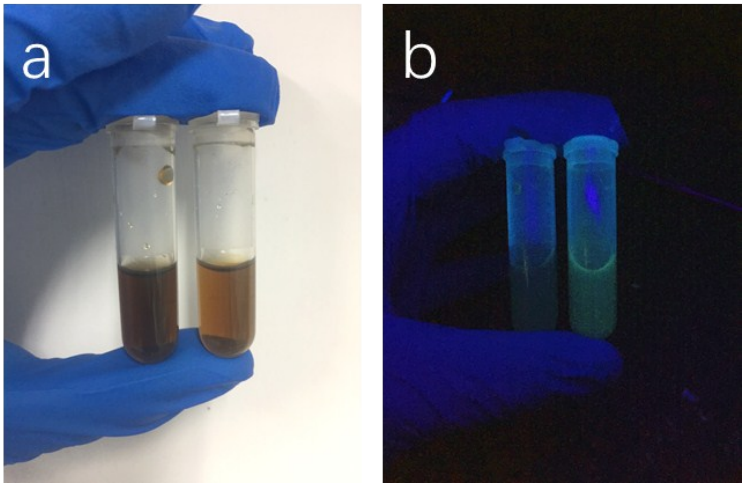

Fig. S18 Physical pictures of PDA and PDA treated with H_2O_2 under 37℃ for 4h. a, under bright field; b, under UV light. H_2O_2 concentration, 0.25M.

Physical pictures of PDA and PDA treated with H_2O_2 under 37℃ for 4h under bright field and UV light is shown in Fig. S15, and there was an obvious difference of fluorescence intensity between PDA and PDA treated with H_2O_2. After treated with H_2O_2, the color of PDA has become yellow from dark which means OPDA formation (Fig. S18a). The fluorescence intensity became very strong, which is consistent with the result of emission spectra (Fig. S18b).

S17 The ROS concentration measurement in cancer cells and normal cells

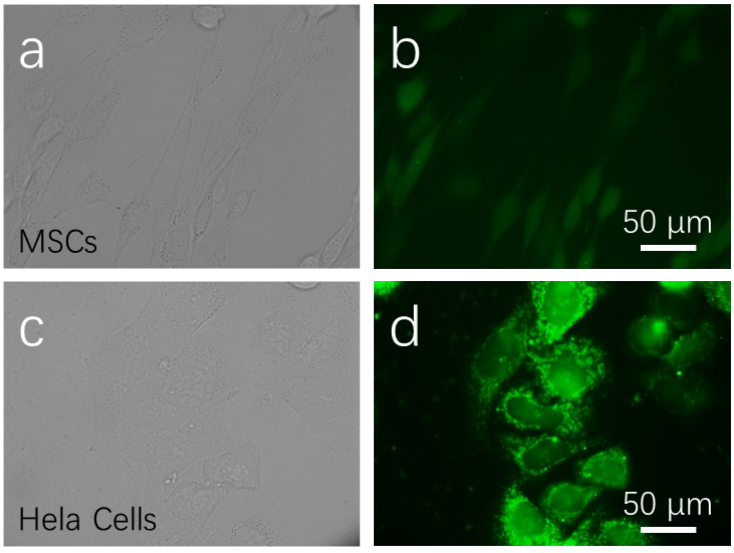

Fig. S19 The ROS concentration in cancer cells and normal cells. a and b, ROS concentration on MSCs cells; c and d, ROS concentration on Hela cells. a and c, images under bright field; b and d, images under 488 nm excitation.

To confirm the ROS concentration in cancer cells and normal cells, reactive oxygen species assay kit measurement was performed. There was much ROS concentration in cancer cells compared with normal cells (Fig. S19). The ROS concentration different provides the basis to obtain different fluorescence intensity to identify cancer/normal cells.

S18 The relative fluorescence intensity in different tissues

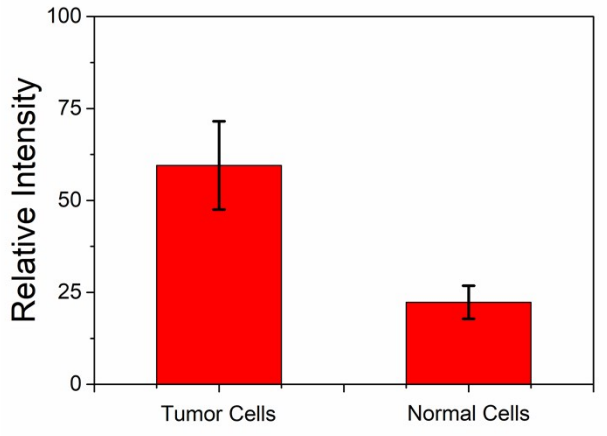

Fig. S20 The relative fluorescence intensity in tumor cells and normal cells measured by ImageJ.

The relative fluorescence intensity was measured by ImageJ. The mean value of normal and cancerous cells of muscle tissue and oral squamous carcinoma tissue is shown in Fig. S20. It is clear that there is higher fluorescence intensity in oral squamous carcinoma tissue (~60) than muscle tissue (~22).