Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

A highly efficient and AIE-active theranostic agent from natural herbs

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Experimental procedures

Characterization of Berberine Chloride

Berberine Chloride was purchased from MERYER company and was purified by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6): 9.88 (s, 1H), 8.93 (s, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.0 (d, J = 8.0 Hz, 1H), 7.77 (s, 1H), 7.07 (s, 1H), 6.16 (s, 2H), 4.93 (t, J = 6.0 Hz, 2H), 4.08 (s, 3H), 4.06 (s, 3H), 3.2 (t, J = 6.0 Hz, 2H).

Bacterial imaging

A single colony of bacteria on solid culture medium (Luria broth for *E.coli* and *S. aureus*) was transferred to 10 ml of liquid culture medium and grown at 37 °C for 10 hr. The concentrations of bacteria were determined by measuring optical density at 600 nm (OD600), 10^9 colony forming unit (CFU) of bacteria was then transferred to 1.5 ml Eppendorf tubes, followed by 7,100 rpm for 3 min. After removal of supernatant, 500 mL BBR solution in PBS at certain concentration was added into the tube. After dispersion, the bacteria were then incubated at room temperature for certain time. To take confocal images, about 1 μL of stained bacteria solution was transferred to glass slide and covered by a piece of cover slip. The images were collected using 63 x objective. The excitation is 488 nm, the emission filter is 500-600 nm.

Bacterial killing study

10^8 CFU bacteria were dispersed in 1 mL PBS. After 20 min incubation with certain concentration of BBR, the solution was centrifuged at 7,100 rpm for 3 min, followed by removal of supernatant and PBS washing. Then the bacteria were dispersed again in PBS. The BBR treated bacteria solution were then exposed to white light for 30 min, while the control group was kept in the dark for the same period of time. The bacteria viability was then determined and quantified by the plate count method.

SEM studies

The concentration of *S. aureus* was diluted to an optical density at 600 nm (OD600) equals to 1. Afterwards, the bacteria were incubated with 5 μM BBR for 30 min and then illuminated in white light for 30 min. The bacteria solution was then treated with 5% glutaraldehyde at 4 ^{o}C overnight, followed by ethanol gradient dehydration for SEM studies. Bacteria without BBR-phototherapy were also imaged for comparison.

In vivo antibacterial test

16 of Wistar mice were divided into four groups: (1) control group without bacterial infection and treatment, (2) *S. aureus*-infected wounds without treatment, (3) *S. aureus*-infected wounds treated by BBR only, and (4) phototherapy group of BBR plus white light irradiation. The mice were anesthetized by intraperitoneal injection of 10% chloral hydrate. Then, two 1.5 × 1.5 cm^2 open excision wounds was cut on the both sides of the spine. 50 μL of *S. aureus* suspension (2×10^8 cfus) and 50 μL of BBR (4 μM) was inoculated over each wound and covered with sterile nonwoven fabrics. Then the wound were

irradiated by white light with a power density of 60 mW cm^{-2} for 30 min. On the 1st, 3rd and the 7th day after surgery, the entire wound with adjacent normal skin were harvested. The infectious tissues were separated and homogenized in normal saline. The homogenates were diluted 1000 times with normal saline. 20 μL of the bacteria solution was sprayed onto LB agar plate and subjected to culturing at 37 °C. After 24 h, the bacterial colonies on the plate were counted for analysis. The other tissues were fixed in 4% paraformaldehyde for the histological analysis.

Histological Examination

The collected tissues were fixed in 4% paraformaldehyde for the histological analysis, and then embedded in paraffin. The fixed tissues were cut into slices with a thickness of 4 mm. Next, H&E staining was carried out according to the standard protocols as described in the previous work.[1]

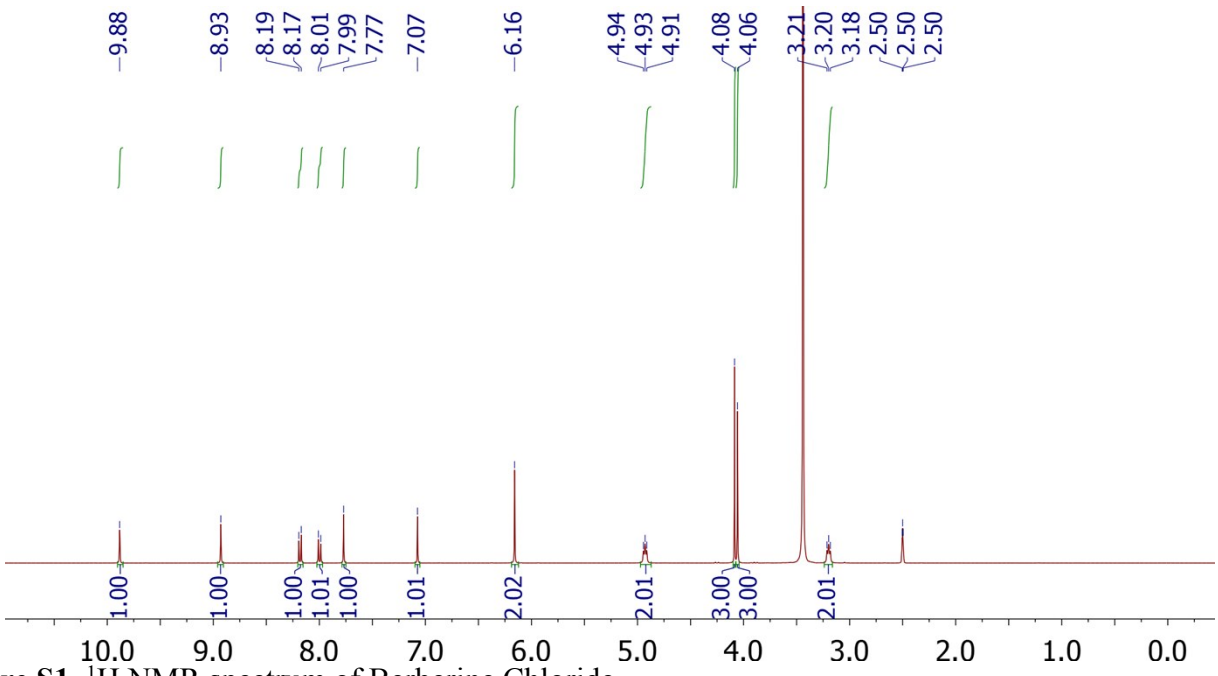

Figure S1. ^{1}H NMR spectrum of Berberine Chloride.

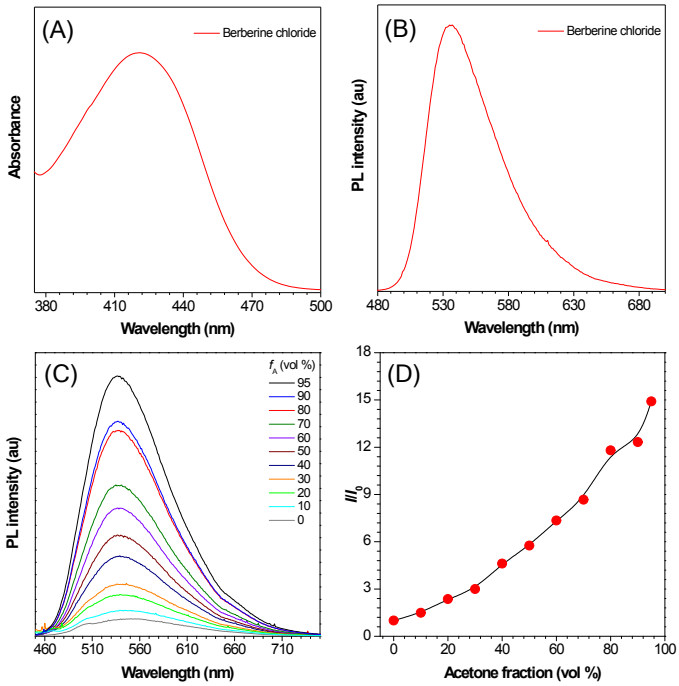

Figure S2. A) Absorption spectra of Berberine Chloride in the aqueous solution. B) PL spectrum of BBR in the solid state. C) PL spectra of BBR (10×10^{-5} M) in water/acetone mixtures with different acetone fractions (f_A); λ_{ex}: 410 nm. D) The plot of the emission maximum and the relative emission intensity (I/I_0) versus the composition of the mixture of BBR.

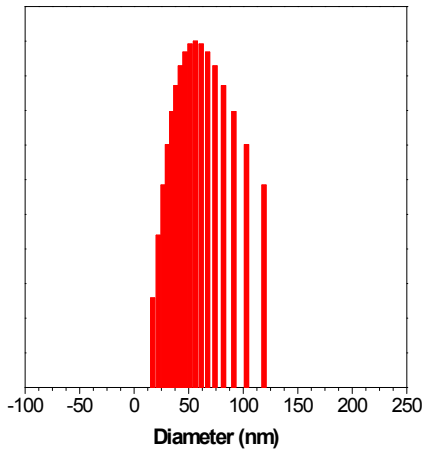

Figure S3. Particle size distribution of Berberine Chloride in the water/acetone mixture with a 95% acetone fraction. Concentration: 10 μM.

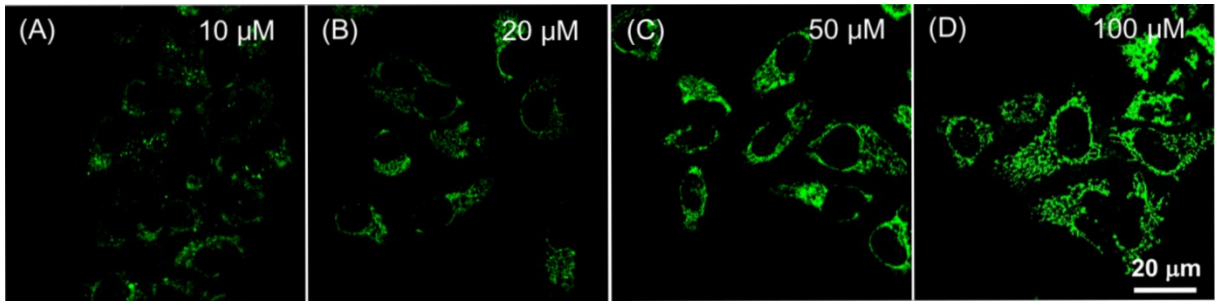

Figure S4. Confocal images of living HeLa cells after incubation with different concentrations (A) 10 μM, (B) 20 μM, (C) 50 μM, (D) 100 μM of BBR for 30 min. λ_{ex}: 488 nm. Scale bar = 20 μm.

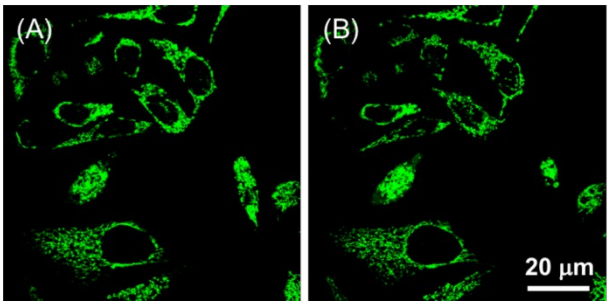

Figure S5. (A) Confocal images of HeLa cells stained with BBR, (B) Confocal images of HeLa cells which were kept in dark for 30 min after staining with BBR.

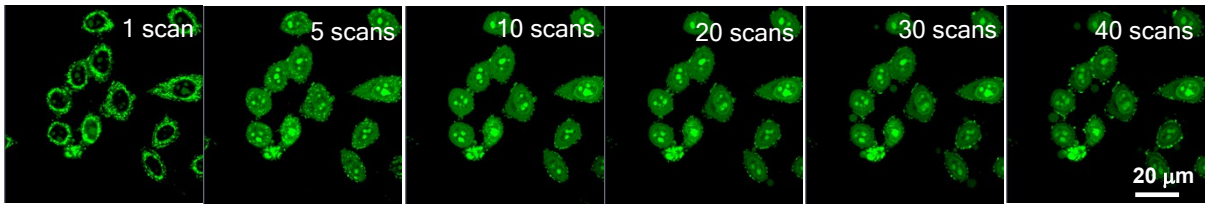

Figure S6. Light-induced staining transition of BBR in HepG2 cells. Confocal images of HepG2 cells stained with BBR upon increasing the number of scans of laser irradiation. λ_{ex}: 488 nm. Scanning rate: 22.4 s per frame. Scale bar = 20 μm.

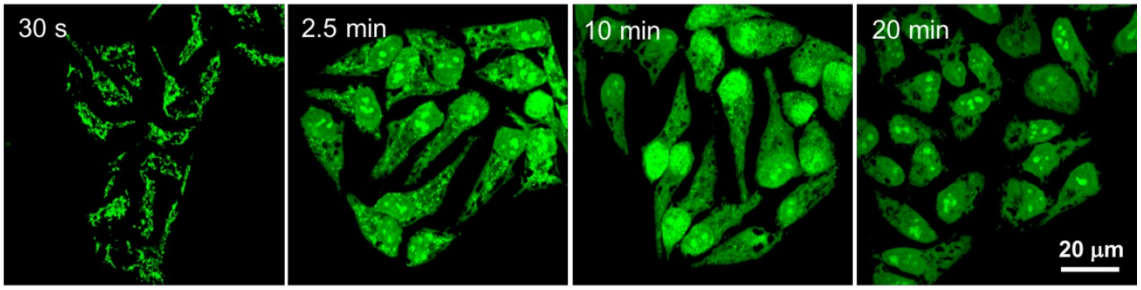

Figure S7. Confocal images of HeLa cells stained with BBR upon different durations of white light irradiation. Scale bar = 20 μm.

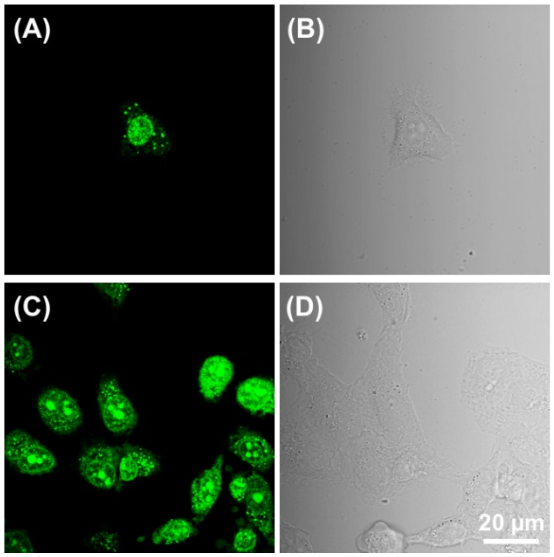

Figure S8. (A) Confocal images of CCCP (20 μM) treated HeLa cells stained with BBR. (B) Bright field. (C) Confocal images of hydrogen peroxide (10 μM) treated HeLa cells stained with BBR. (D) Bright field. Scale bar = 20 μm.

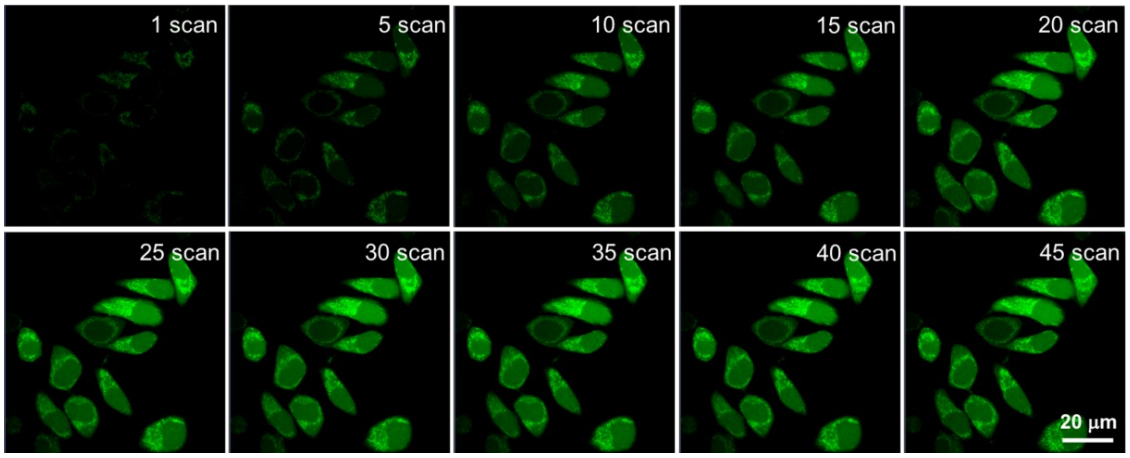

Figure S9. Confocal images of HeLa cells co-stained with BBR and H2DCF-DA (1 μM) at di□erent time under continuous white light illumination. λ_{ex}: 488 nm, 1% laser power, Scale bar = 20 μm.

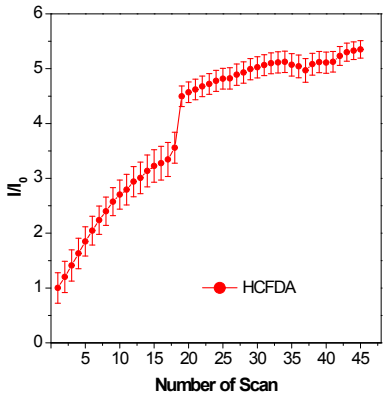

Figure S10. Statistical analysis of fluorescence intensity data in Figure S7.

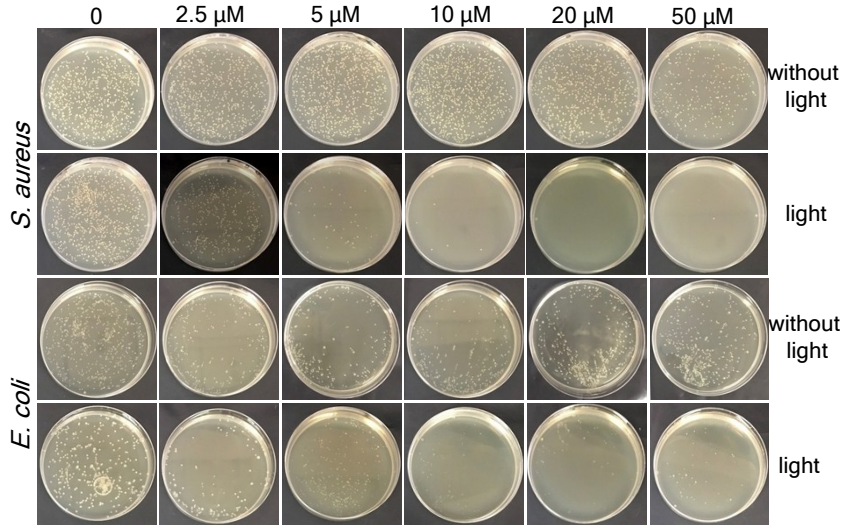

Figure S11. Plates of (the first and second rows) *S. aureus* and (the third and fourth rows) *E. coli* (the first and third rows) without and (the second and fourth rows) with light irradiation (60 mW/cm^2) for 30 min after incubated with BBR.

Reference:

1. Y. Zhu, C. Xu, N. Zhang, B. Yu and F.-J. Xu, *Adv. Funct. Mater.*, 2018, **28**, 1706709.