Mild Nanoencapsulation of Single Mammalian Cell by Surface Initiated Polymerization

Yan Zhang, [a] Jianing Li, [a] Qizhen Huang[a] and Faming Wang[a]

[a]*School of Public Health, Institute of Special Environmental Medicine, Nantong University, Nantong, 226019, China. E-mail: wangfaming1990@ntu.edu.cn*

General information

All reagents and solvents were purchased from commercial suppliers (Aladdin, Makclin Biochemical, Bidepharm, Meryerm, Energy Chemical, and J&K) and used without further purification. Penicillin, streptomycin, trypsin-EDTA, Dulbecco's Modified Eagle Medium, RPMI 1640 Medium and fetal bovine serum were purchased from Beijing Solarbio Science & Technology Co.,Ltd. 4-arm-PEG acrylate (10K) and HS-PEG-SH (3.4K), MAL-FITC, HS-PEG-FITC (1K) were purchased from Shanghai ToYongBio Tech. Inc. Proteinase K and Cell Counting Kit-8 was purchased from Shanghai Beyotime Biotech Inc. Rhodamine B modified Polystyrene nanoparticles (RB-PS, 50 nm), FITC-Dextran 20k, PEl coating Fe_3O_4 nanoparticles (30nm) were purchased from Xi'an QIYUE biology Co. The fluorescence images of cells were acquired using a NIKON ECLIPSE Ti2 and analyzed using ImageJ. Cell viability was measured by CCK-8 assay with a Tecan Infinite M Plex microplate reader.

Rheological experiment

Rheological characterization was performed on all hydrogel samples using Anton Paar MCR102 rheometer. The kinetics of the gelation process was characterized by time sweep oscillatory tests at 37 °C in 25 mm parallel-plate geometry. Oscillatory frequency and strain sweeps were conducted to find the linear viscoelastic region (LVR) of the formed hydrogels and to determine the time sweep oscillatory test conditions (frequency of 1 Hz and a strain of 1%). Each sample was dispensed on the preheated rheometer plate while in liquid state, the excess hydrogel was discarded. Each hydrogel sample was used for only one test. Each test was performed in triplicate, and the data represents the average of the three tests with corresponding standard deviation. The testing time to determine gelation time and modulus the humidified chamber was used for these experiments. The maximum statistical time is 100,000 seconds, and data is recorded every 10 s.

Cell culture

The human MDA-MB-231 (Catalog Number: SCSP-5043) and Jurkat T (Catalog Number: SCSP-513) cell line was acquired from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (CAS Institute), Shanghai, China. The MDA-MB-231 cells were cultured in DMEM medium supplemented with 10% FBS (Gemini, Woodland, USA) and 1% penicillin-streptomycin solution in a cell culture incubator. The Jurkat cells were cultured in RPMI 1640 medium. The cells were incubated at 37

°C under a humidified atmosphere of 5% CO_2. When the cells reached 80% confluency, they were washed twice with phosphate-buffered saline (PBS; pH 7.4). For experiments using detached cells, trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.25% trypsin, 380 mg/L EDTA) was added to the culture plate, which was then incubated at 37 °C for 3 min. After the cells were detached from the plate, DMEM was added for neutralization. The cells were collected by centrifugation (300 x g for 5 min) and washed twice with PBS. The suspended cells were collected by centrifugation (300 x g for 5 min) directly and washed twice with PBS.

TCEP treatment

The suspended cells at a density of 1-2 × 10^6 cells/ml were collected in 2-ml tubes by centrifugation at 300 x g and suspended in 1 ml of PBS containing 0.25, 0.5, 1, or 2 mM of TCEP. Then the samples were incubated at 37 °C for 20 min. After incubation, cells were collected by centrifugation (300 x g for 5 min) and washed twice with PBS. The attached cells were cultured in 6-well plate, and added in 1 ml of PBS containing 0.25, 0.5, 1, or 2 mM of TCEP. Then the samples were incubated at 37 °C for 20 min. After incubation, the cells were washed with PBS twice.

Maleimide-FITC Probe

The control cells or TCEP treated cells were washed with PBS twice. Then, Maleimide-FITC Probe (1 mg/ml) was diluted in PBS solution (final concentration: 3 μg/ml) and added to the cells, which were then incubated for 30 min in an incubator at 37 °C. During incubation, the tubes were tapped every 10 min. After 30 min, the cells were washed with PBS twice and analyzed by microscopy.

The PEG *in situ* encapsulation

The control cells or TCEP treated cells were collected by centrifugation (300 x g for 5 min) in 2 mL Tube, then the 4-arm-PEG acrylate (10K, 64 mg/mL) was prepared in PBS and added into the above cell palate (500 μL). Gently suspend the cell in the solution and incubation at 37 °C for 5 min. After that, the HS-PEG-SH (3.4K, 44 mg/mL) was prepared in PBS and added into the above mix solution (500 μL). The mixture was incubated for another 30 min at 37 °C. For the HS-PEG-FITC staining, HS-PEG-FITC was mixed together with HS-PEG-SH at a final concentration of 10 μg/mL and added into the cells incubated with 4-arm-PEG acrylate. The encapsulated cells were collected by centrifugation (300 x g for 5 min) directly and washed twice with PBS.

The cell viability test

The cell viability was quantitatively analyzed by the CCK-8 kit assay. 100 μL of cell suspension was seeded into 96-well plates. After incubating for certain time, 10 μL CCK8 was added to each well, and continue to incubate for 2 hours at 37 °C, the optical intensity in each well was measured at 450 nm using a microplate reader.

For the LBL protocol, we chose poly-lysine as polycations and alginate as polyanions, and to reduce the losses caused by the centrifugation process, we used adherent cells

for LBL encapsulation. Thus, MDA-MB-231 cells were seeded in 96 well plate at 10^5 cells/mL. After incubating at 10% FBS DMEM for 12h, the cells were washed twice by PBS. For the subsequent polyelectrolyte complexation, the cells were sequentially treated with 0.01% (w/v) poly-lysine for 1 min and 0.05% (w/v) alginate for 5 min. The process was repeated three times. Then, the cell viability was tested by CCK-8.
For the mineralization protocol, we chose metal (Fe^{3+}) and polyphenol tannic acid (TA) as the monomer, and to make the experimental conditions consistent, we also used adherent cells for the experiment. Thus, MDA-MB-231 cells were seeded in 96 well plate at 10^5 cells/mL. After incubating at 10% FBS DMEM for 12h, the cells were washed twice by PBS. For the subsequent mineralization, the DMEM of solution (0.05 mL) of TA (0.4 mg/mL) and the DMEM (0.05 mL) of $FeCl_3.6H_2O$ (0.1mg/mL) were added sequentially to the collected cells. The resulting suspension was mixed for 10 s and then washed with DMEM. The process was repeated three times. Then, the cell viability was tested by CCK-8.
For our method, to make the experimental conditions consistent, we also used adherent cells for the experiment. Thus, Thus, MDA-MB-231 cells were seeded in 96 well plate at 10^5 cells/mL. After incubating at 10% FBS DMEM for 12h, the cells were washed twice by PBS. The next encapsulation steps were same with the above descriptions.

DCFH-DA staining
MDA-MB-231 cells were seeded in 35mm confocal dishes at 1*10^5 cells/mL. After incubating at 10 % FBS DMEM overnight, the cells were washed twice by PBS. Then, the cells were divided into four groups. Control: the cells without any treatment; Oxidant group: the cells were treated by APS initial polymerization; UV group: the cells were treated by UV light initial polymerization; Our group: the cells were treated with our method. After different treatments, the cells were washed with PBS twice and incubating with DCFH-DA probe (10 μmol/L) for 30 min. After washing with PBS twice, the fluorescent images of DCFH-DA were collected by NIKON ECLIPSE Ti2 microscopy and analyzed using ImageJ.

γ-H2AX staining
MDA-MB-231 cells were seeded in 35mm confocal dishes at 1*10^5 cells/mL. After incubating at 10 % FBS DMEM overnight, the cells were washed twice by PBS. Then, the cells were divided into four groups. Control: the cells without any treatment; Oxidant group: the cells were treated by APS initial polymerization; UV group: the cells were treated by UV light initial polymerization; Our group: the cells were treated with our method. After different treatments, the cells were washed with PBS twice. Then, the DNA damage of the four groups were test by the standard protocol of Beyotime DNA Damage Detection Kit (γ-H2AX Immunofluorescence method, rabbit monoclonal antibody, red) and fluorescent images of γ-H2AX were collected by NIKON ECLIPSE Ti2 microscopy and analyzed using ImageJ.

Cell Young's modulus testing
Transferring the cell samples to the confocal microdishes and fixed onto the atomic

force microscope (Bruker, NanoWizard) on the AFM sample stage. The pyramid-shaped tip silicon nitride cantilever probe (BrukerMLCT-BI0) was selected for instrument calibration in the liquid phase. The stiffness was calibrated by determining the spring constant of the cantilever during thermal fluctuations at air temperature, with the spring constant range being 80 to 100 mN/m. The cantilever was positioned on the cell coating surface using a microscope, and the scanning height was set to 10 μm. The mechanical properties of the polymer coating on the cell surface were determined after indentation was made at a contact speed of 5 μm/s to reach the contact force. The force-indentation curve obtained was fitted to the Hertz model using the analysis software JPK Data Processing. The Poisson's ratio was set at 0.5, and the Young's modulus of the polymer coating was calculated. Gaussian distribution fitting was performed on the obtained data, and the results were obtained.

Pel-Fe_3O_4 nanoparticles (30nm) cytotoxic test

The cells were incubated with various concentrations of Fe_3O_4-PEI (10 ug/mL, 20 ug/mL, 40 ug/mL, 60 ug/mL, 80 ug/mL) for 24 h at 37 °C. Afterward, the culture is completed, gently remove the old medium, add 100 μL of DMEM medium containing 10% CCK8 to each well (v/v%), and continue to incubate for 2 hours at 37 °C, the optical intensity in each well was measured at 450 nm using a microplate reader.

The mechanical stresses experiment

Digesting the attached cells and resuspend them in 1mL of PBS buffer to obtain the cell suspension; Centrifugating at 300g for 4 minutes, then remove the supernatant and waste liquid. Add 10 μL of Hoechst 33342 and 990μL of PBS buffer and react in the dark at 25°C for 15 minutes; Centrifugating at 300g for 4 minutes and remove the supernatant waste liquid. Resuspend the cells in PBS buffer and wash them again to remove the residual staining solution. Then the cells were resuspended in 1mL PBS respectively to obtain cell suspensions with a cell concentration of 10^6 cells /mL Then the cells were centrifuged at high-speed centrifugation at 3000 x g for 10 minutes and repeated this process for 5 cycles. For each cycle of high-speed centrifugation of cell samples, transfer the samples to a 24-well plate, letting them stand in the dark for 15 minutes, and then use a 405 nm fluorescence channel to capture images using a Nikon inverted microscope (Nikon Ti2-U). The reserved cell numbers were counting by a python program via fluorescence images.

Selective permeability experiments

The native or encapsulated cells were suspended in 24-well plate. After that, 50 nm RB@PS or FITC-Dextran 20k were added. Then the cells were observed with a 405 nm, 488nm, 525 nm fluorescence channel using a Nikon inverted microscope (Nikon Ti2-U) and the images were captured.

For the proteinase K treatment experiment, the native or encapsulated cells were fixed by 4% PFA first for 30 min. After washing with PBS for 2 times, the cells were suspended in 24-well plate. After that, 2.5 μL Proteinase K (20 mg/ml) was mixed with 1 mL lysis buffer (50 mM KCl, 50 mM Tris-HCl, 2.5 mM EDTA, 0.45% NP-40) and

added into cells. Then, fluorescent images were collected by NIKON ECLIPSE Ti2 microscopy at different time points.

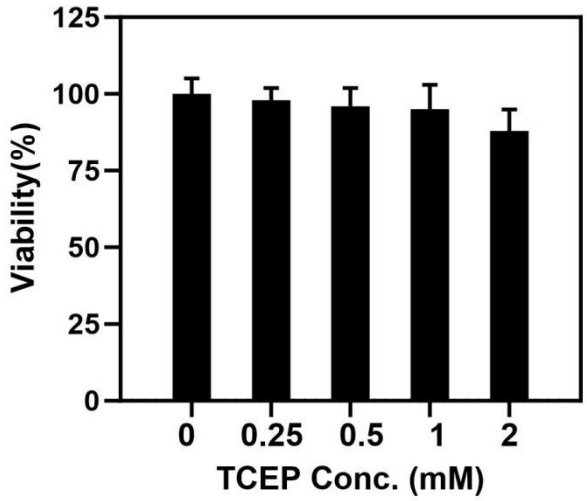

Figure S1. The cell viability of MDA-MB-231 cells after treatment with different TCEP concentration.

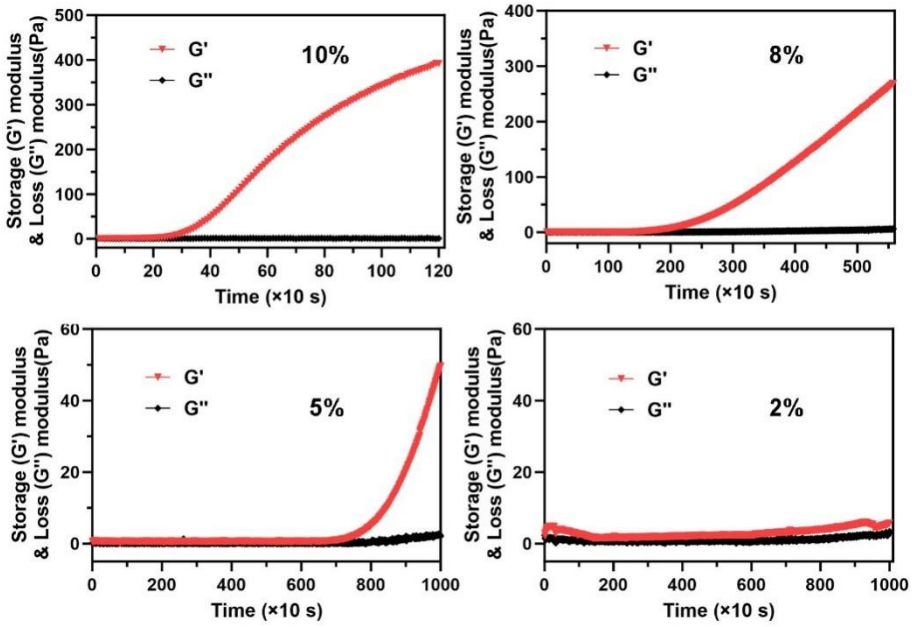

Figure S2. The Storage modulus and Loss modulus of different concentration monomer solutions (10%, 8%, 5%, 2%) tested by rotational rheometer.

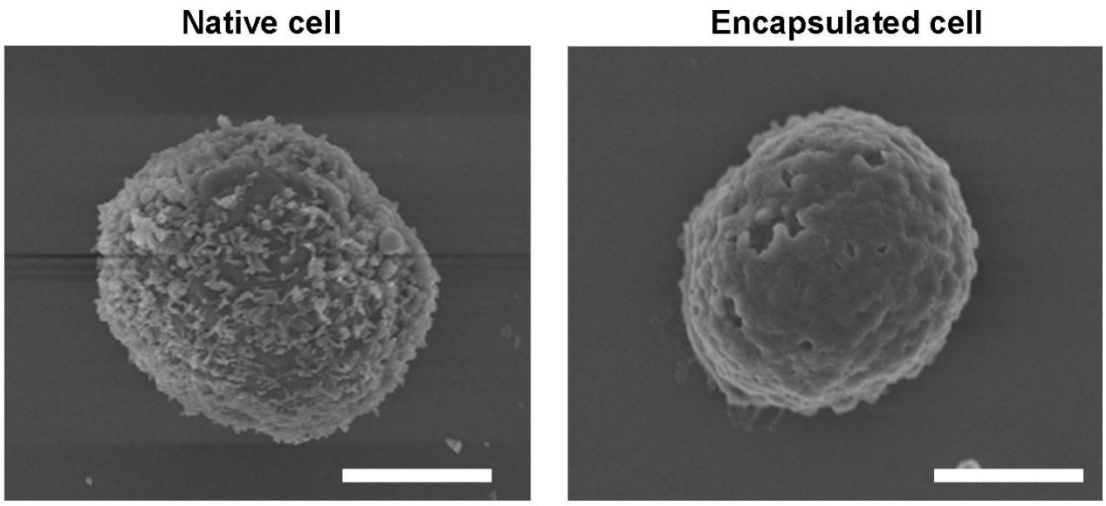

Figure S3. SEM micrographs of MDA-MB-231 cell before and after encapsulation. Scale bar: 10 µm.

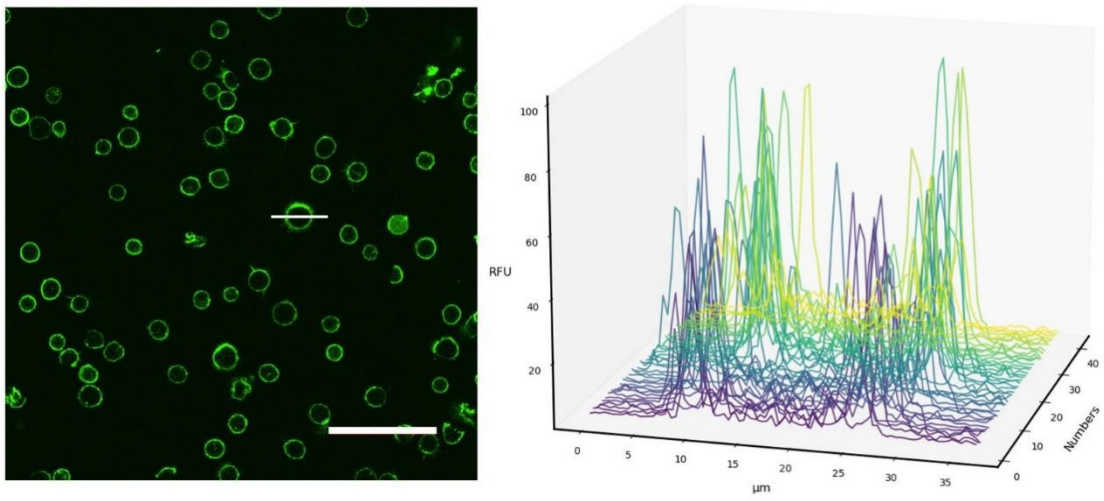

Figure S4. Confocal microscopy image of encapsulated cells and the relative fluorescence unit profile of individual encapsulated cells obtained using the ImageJ plot profile function. The plot confirms the uniform distribution of the polymeric shell around the cell. Scale bar: 100 µm.

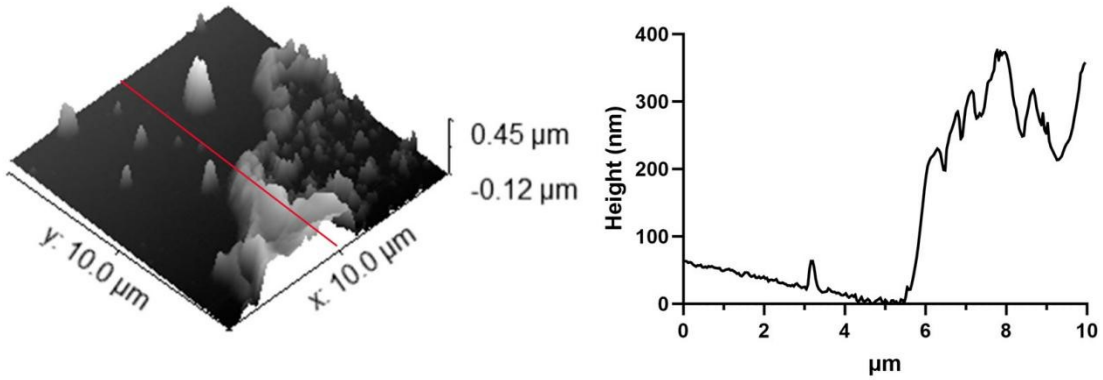

Figure S5. AFM height images and section view of a silicon wafer surface with grafted polymer encapsulation shell on surface. The mean height: 273.4 ± 54.8 nm.

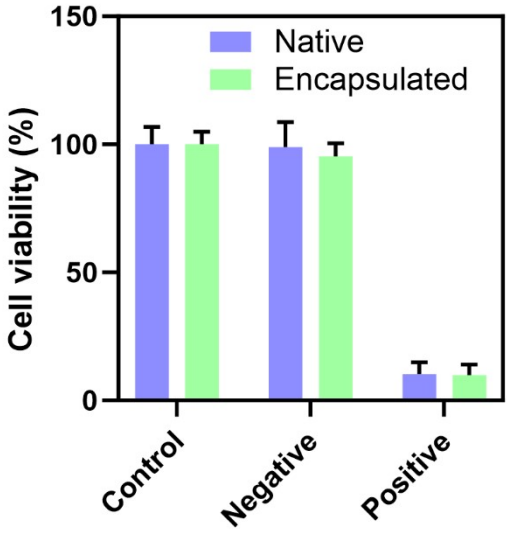

Figure S6. The cell viability of different treatments on native and encapsulated cells via CCK-8. Control: the cells treated with DMEM medium; negative group: the cells treated with 10% PBS added DMEM medium; positive group: the cells treated with 10% phenol added DMEM. Data were presented as mean ± standard deviation as indicated by error bars (n = 6).

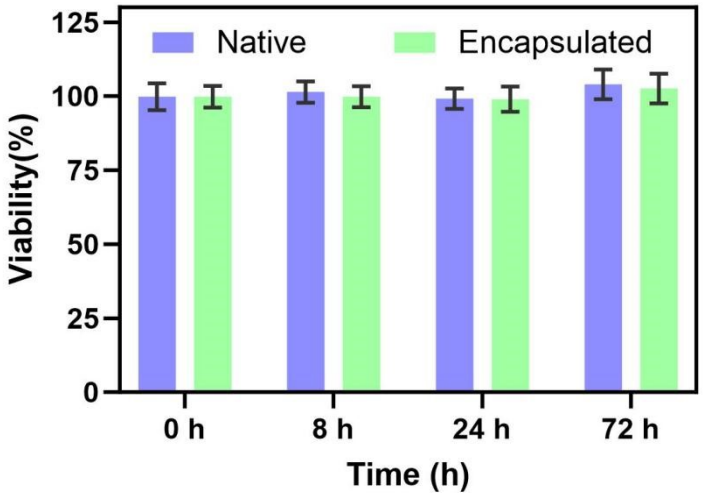

Figure S7. The cell viability of native and encapsulated MDA-MB-231 cells on different times after treatment. Data were presented as mean ± standard deviation as indicated by error bars (n = 6).

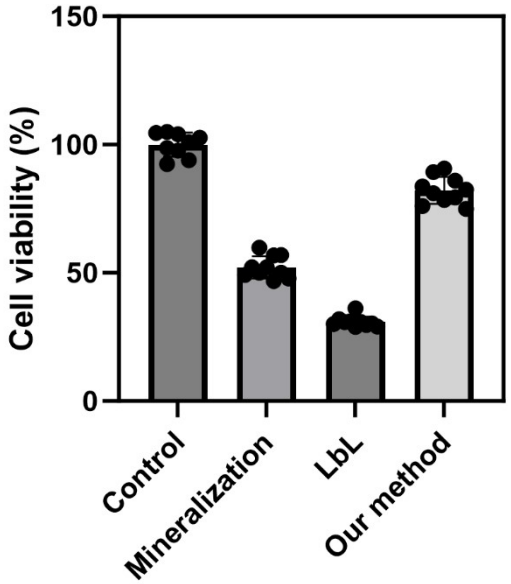

Figure S8. The cell viability of cells encapsulated by different strategies (control, mineralization, LbL and our method) tested by CCK-8. Data were presented as mean ± standard deviation as indicated by error bars (n = 10).

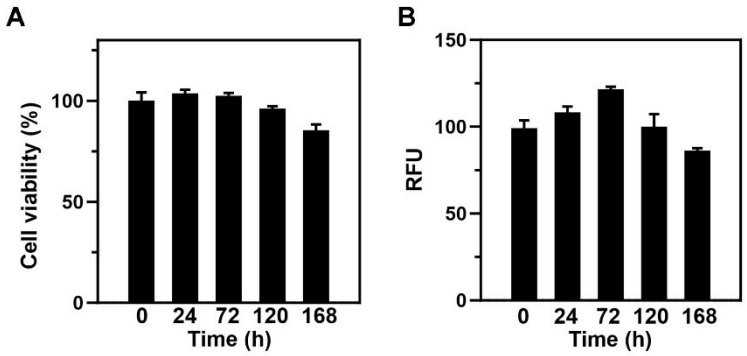

Figure S9. (A) The cell viability of encapsulated cell tested by CCK8 assay after culturing different times (0-168h). (B) The metabolic activity of encapsulated cell tested by Alamar blue assay after culturing different times (0-168h). Data were presented as mean ± standard deviation as indicated by error bars (n = 6).

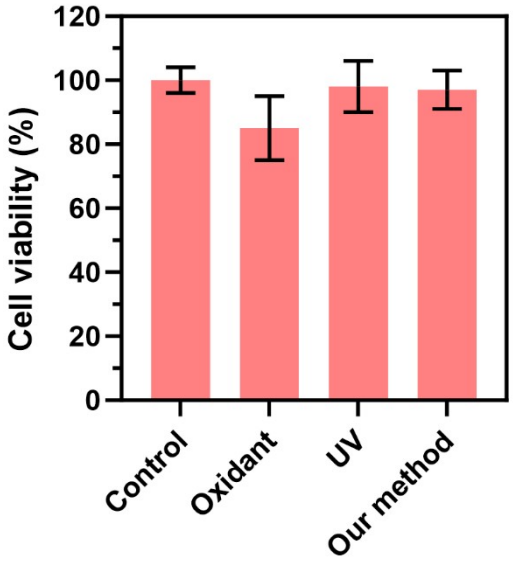

Figure S10. The cell viability of MDA-MB-231 cells in four groups (control, oxidant, UV and our method) tested by CCK-8 assay. Data were presented as mean ± standard deviation as indicated by error bars (n = 10).

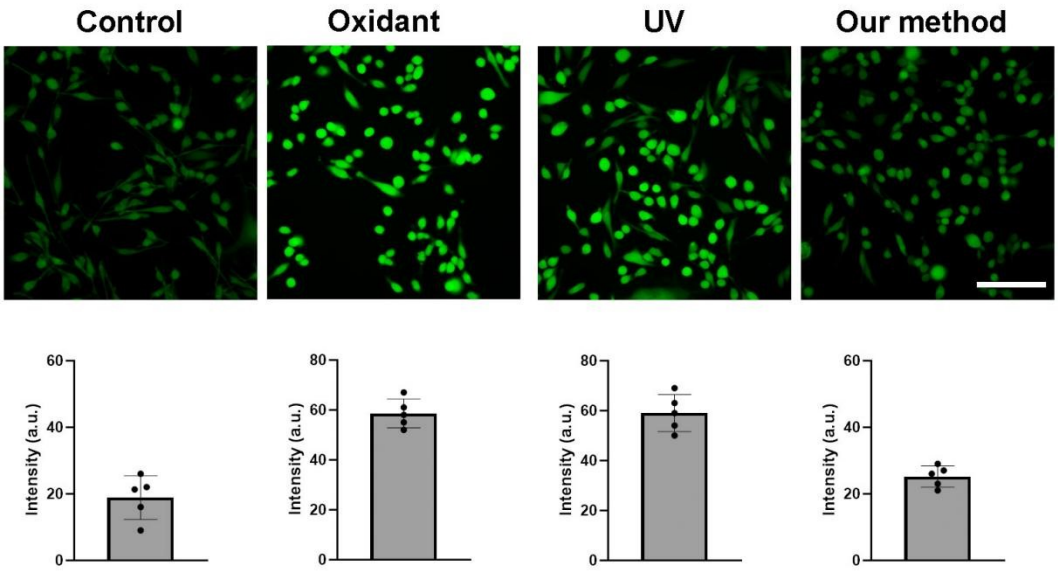

Figure S11. The fluorescent images of MDA-MB-231 cells in four groups (control, oxidant, UV and our method), as detected by the DCFH-DA probe. Scale bar: 100 μm**.**

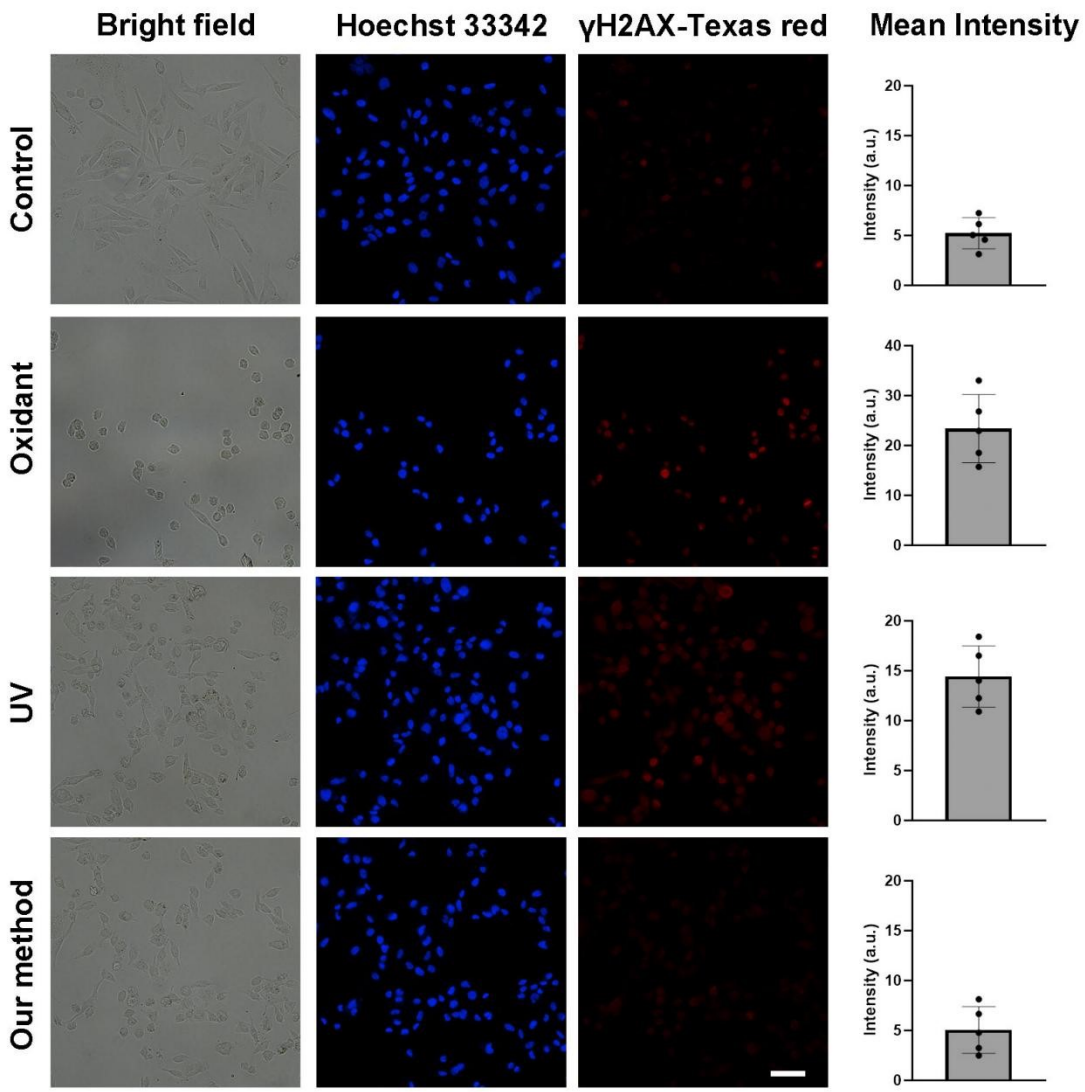

Figure S12. The γ-H2AX fluorescent images of MDA-MB-231 cells in four groups (control, oxidant, UV and our method). Scale bar: 100 μm.

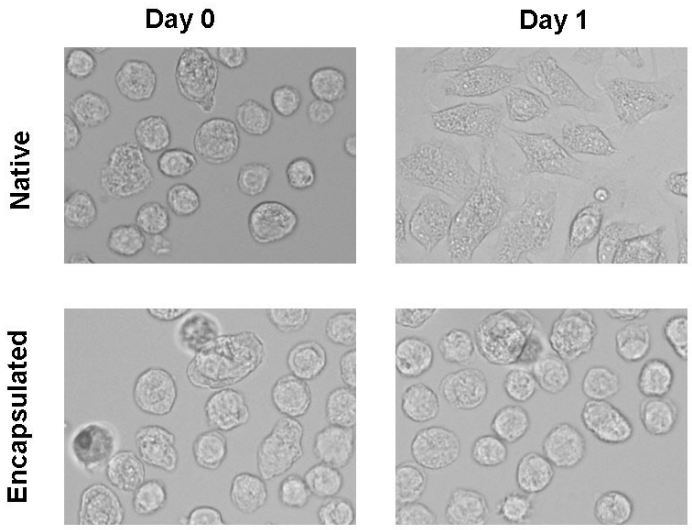

Figure S13. The adhesion ability of the native and encapsulated MDA-MB-231 cells was investigated by cell culture.

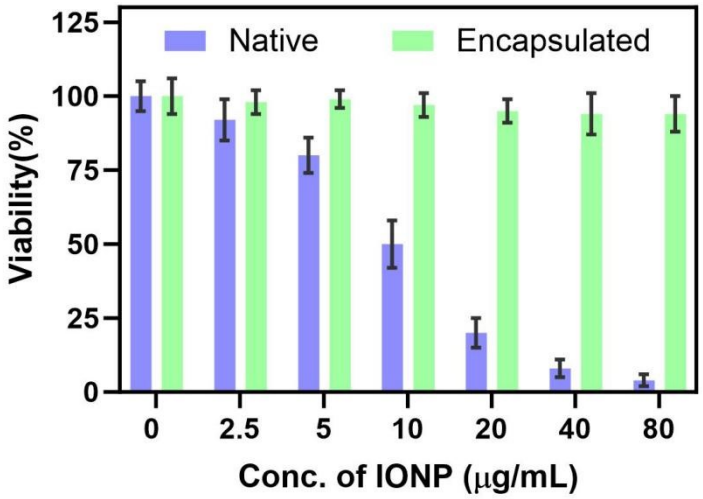

Figure S14. The cell viability of native and encapsulated MDA-MB-231 cells by CCK-8 assay after treating with different concentrations of polyethylenimine (PEI)-coated IONPs with a diameter of 30 nm.

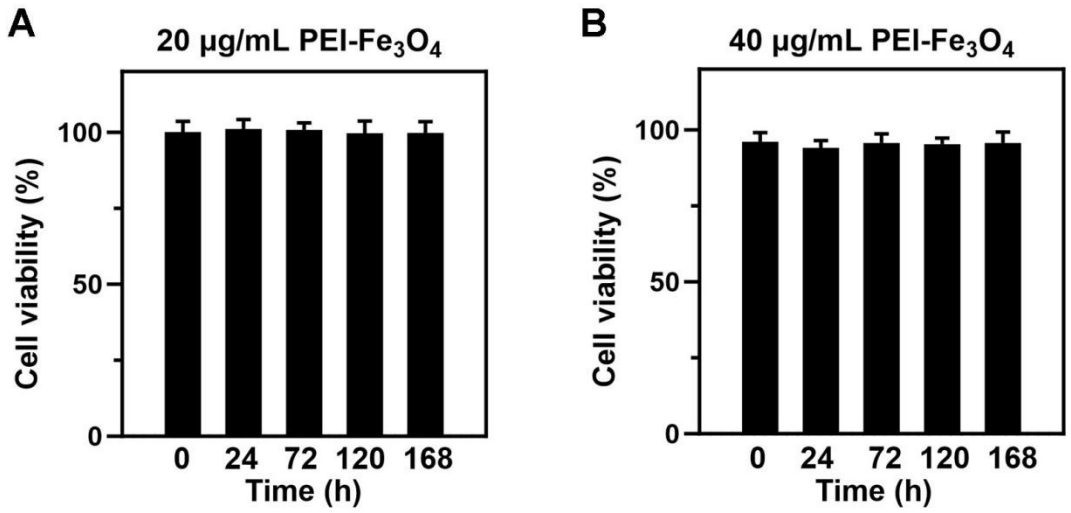

Figure S15. The cell viability of encapsulated MDA-MB-231 cells at different time (0-168h) post-encapsulation treated with 20 μg/mL PEI-Fe_3O_4 (A) and 40 μg/mL PEI-Fe_3O_4 (B), as tested by CCK-8 assay. Data were presented as mean ± standard deviation as indicated by error bars (n = 6).

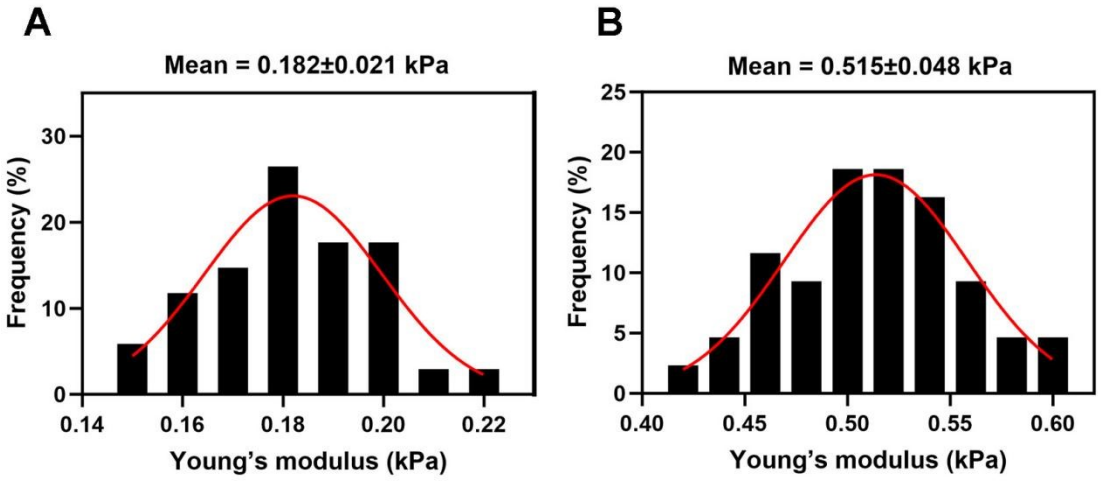

Figure S16. The Young's modulus of native cells (A) (with n=34) and encapsulated cells (B) (with n = 43) tested by AFM.

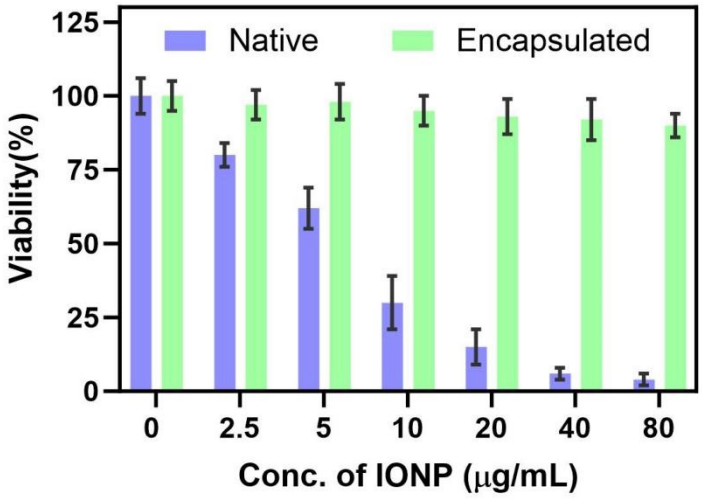

Figure S17. The cell viability of native and encapsulated Jurkat cells by CCK-8 assay after treating with different concentrations of PEI-IONPs with a diameter of 30 nm.

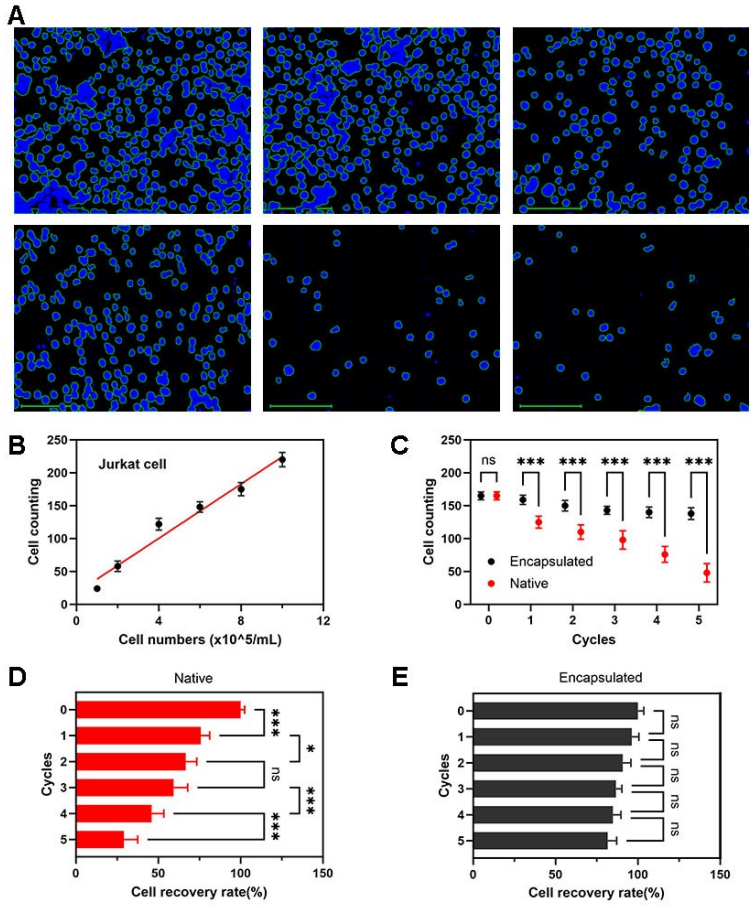

Figure S18. (A) The images of cells under different Jurkat cell numbers. (B) The calibration curve of cell counting and cell numbers. (C) The counting of the naked cell population (red) and encapsulated cell population (black) with different centrifuge cycles. The bar chart of native cells (C) and encapsulated cells (D) between recovery rate and centrifuge cycles. All data were means ± SD; n = 10. Statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001) was determined by the Student's t-test.

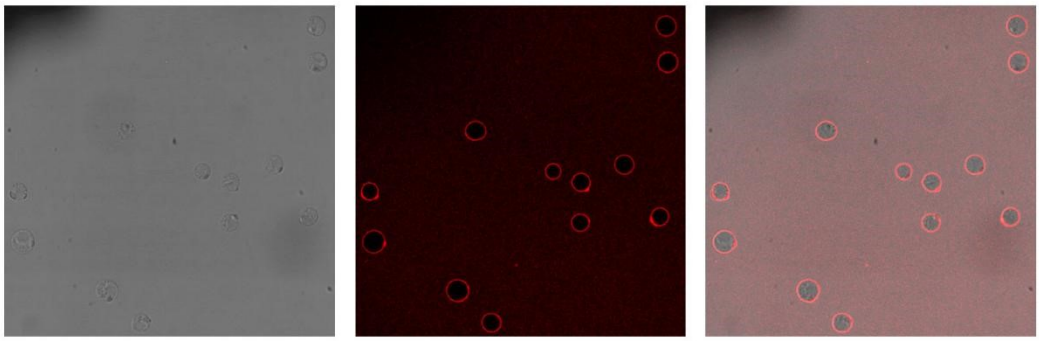

Figure S19. The images of the encapsulated cells incubated with 50 nm rhodamine B-modified polystyrene nanoparticles.

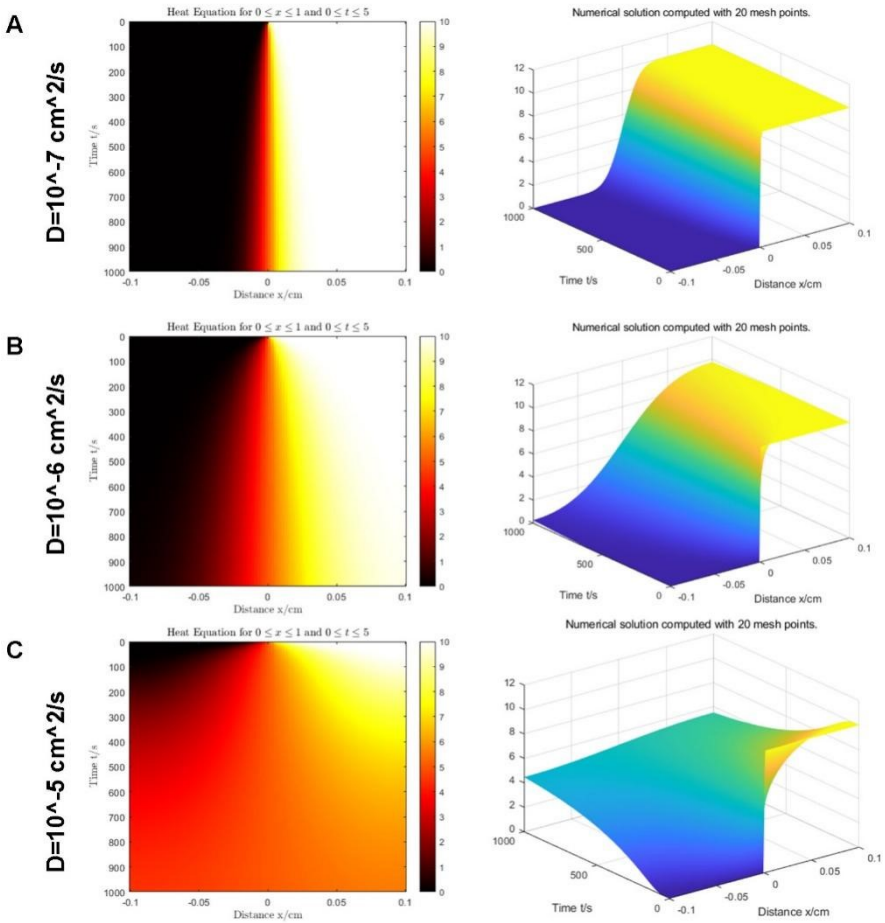

Figure S20. Our models predicted that molecules diffusion with the 3D diffusion curve and corresponding heat map at diffusion coefficients (D = 10^{-5} to 10^{-7} cm²/s).

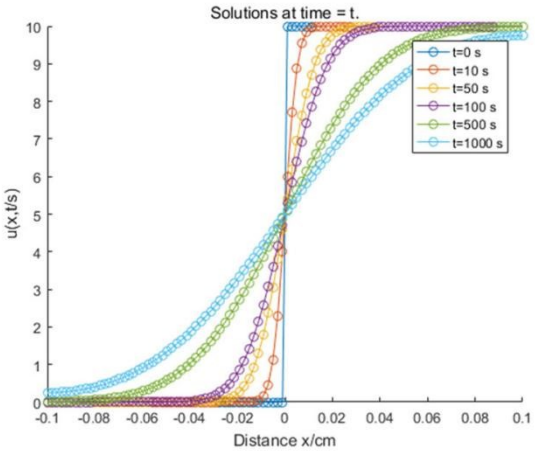

Figure S21. The diffusion curve of FITC-Dextran 20k (D= $8.7*10^{-7}$) at different times.

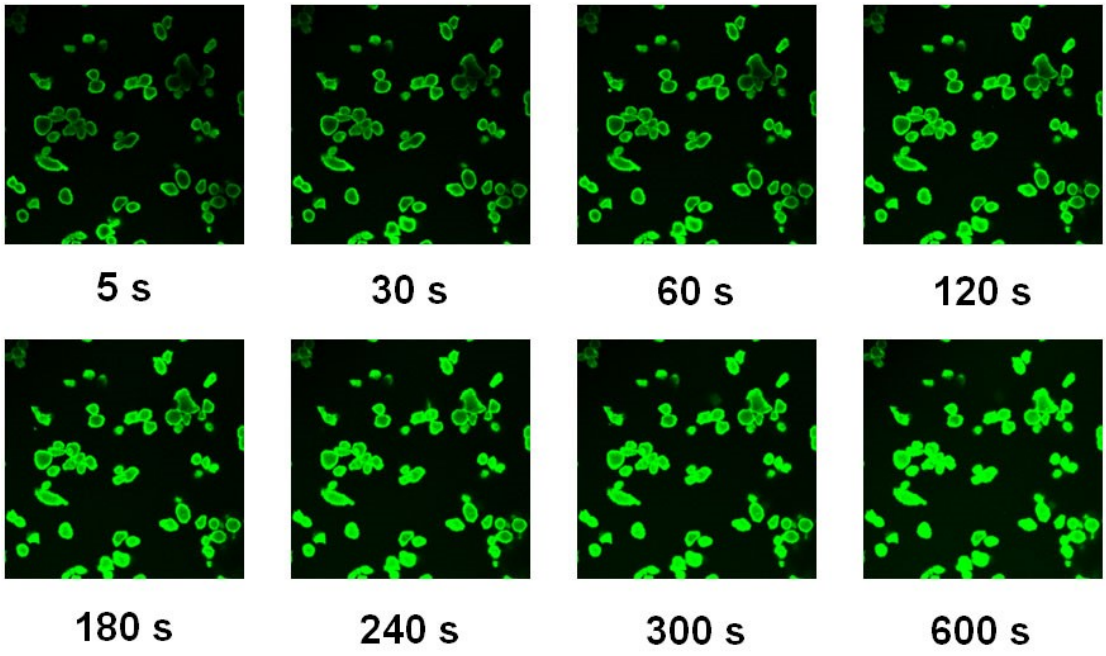

Figure S22. The diffusion images of FITC-Dextran (20k) into MDA-MB-231 cells at different times.

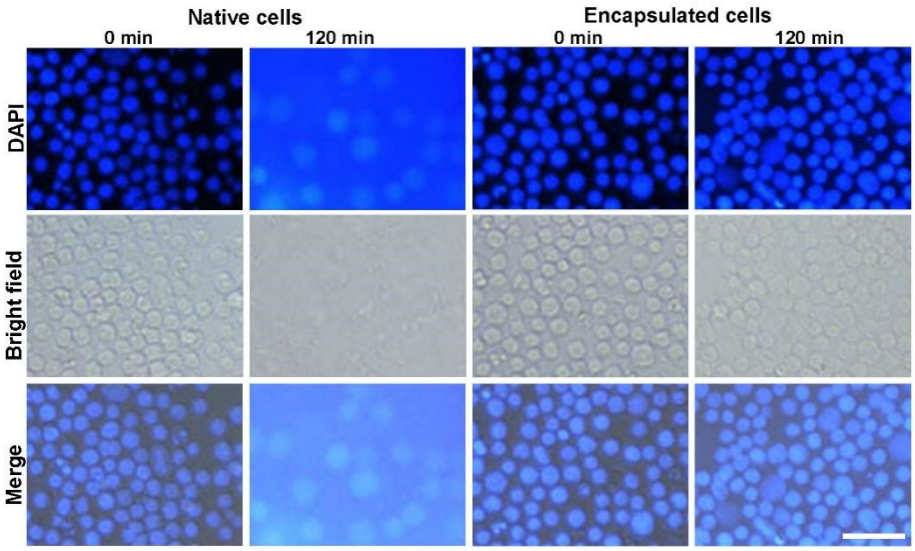

Figure S23. The fluorescent images of native and encapsulated MDA-MB-231 cells treated with a proteinase K lysis solution post-fixation for 0 min and 120 min. Scale bar: 100 μm.