Supplementary Material (ESI) for Lab on a Chip

Electrokinetic Gated Injection-based Microfluidic System for Quantitative Analysis of Hydrogen Peroxide in Individual HepG2 Cells– *Supplementary Information*

Xinyuan Zhang, Qingling Li, Zhenzhen Chen, Hongmin Li, Kehua Xu, Lisheng Zhang, Bo Tang*

1. Optimization of MCE

According to the voltage program in Table 1, three kinds of buffers (i.e., PBS, HEPES, and borate buffers) were tested. Considering the migration time and baseline stability, HEPES was selected. It was reported that the fluorescence intensity of DCF was pH-dependent[1] and its fluorescence increased with the increase of pH in the range of 7.0-8.2, considering the best physiological environment, pH 7.4 was adopted. The concentrations of HEPES buffer were examined ranging from 10-50 mM, as can be seen from Fig. S1a, the peak height increased with an increase in the buffer concentration until 30 mM, and then gradually decreased. Therefore, 30 mM HEPES was obtained.

Mannitol, with the hydroxyl groups on the opposite side of the chain, was often used as an small molecular additive.[2] The DCF signaling intensity was significantly affected by the concentrations of mannitol,[3] probably due to the formation of hydrogen-bonding interactions of the polyhydroxy additives with HEPES buffer and DCF so as to prevent free DCF molecules from adsorption onto the uncoated glass channels. A 10-50 mM mannitol was added to the buffer and the results were shown in Fig. S1b. 20 mM mannitol was employed because of less noise and higher peak height.

`

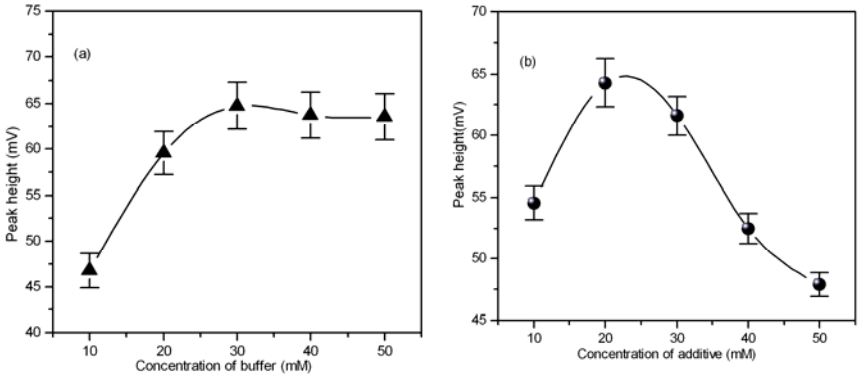
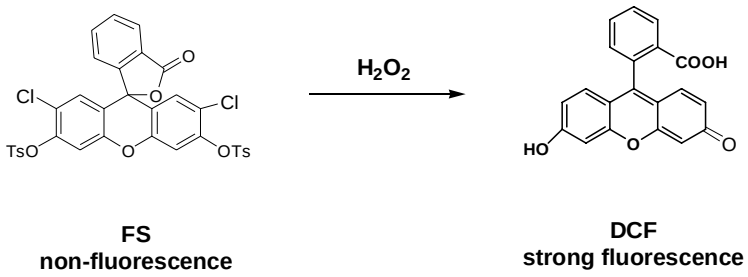


Fig. S1 (a) Impact of buffer concentration on peak height of DCF; (b) Effect of additive concentration on peak height of DCF.

2 Fluorescent probe

In order to interrogate the intracellular H_2O_2 at single-cell level, FS, a cell-permeable, sensitive and selective fluorescent indicator, was employed in this work. Upon treatment with H_2O_2, the probe would subsequently form the open, colored, and fluorescent product dichlorofluorescein (DCF), as shown in Scheme S1. The fluorescence spectra of FS and FS derivative were presented in Figure S2a. The mechanism was based on H_2O_2-mediated hydrolysis of sulfonates, thus allowing high selectivity for H_2O_2 over other ROS and reductants coexisted in biological systems as shown in Figure S2b. To further confirm that the other ROS and reductants did not interfere with the determination of H_2O_2 in single cells, the extracts of cells blank, cells depletion and FS-incubated cells were measured using the fluorescence spectrometer, the results were shown in Figure S2c. This demonstrated that these compounds did not interfere with the quantitative analysis of H_2O_2 in single cells.

Scheme S1. Chemical structure of FS and the reaction of FS with H_2O_2.



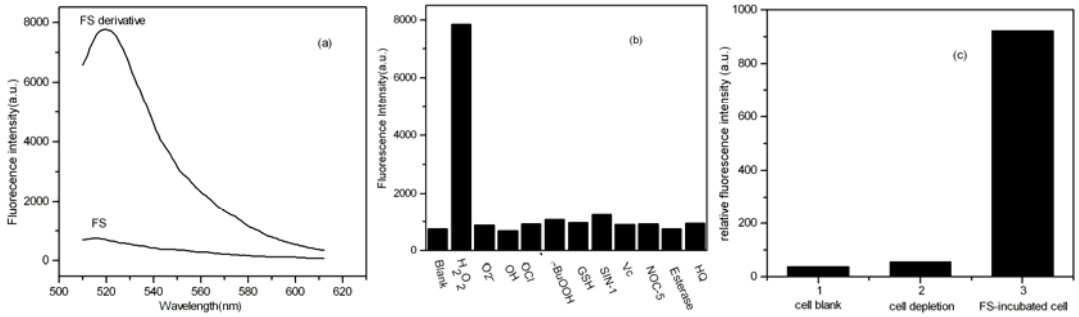


Figure S2 (a) Fluorescence emission spectra of 50 μM FS in the absence or presence of H_2O_2 (10 μM) in 0.10 M HEPES (pH 7.4) at 37°C for 40 min. H_2O_2 (10 μM) in utrapure water was freshly prepared before use. (b) Fluorescence responses of 50 μM FS in the presence of various ROS and reductants (10 μM for each) in the same conditions above; •OH was generated by reaction of Fe^{2+} with H_2O_2. (c) Fluorescence responses of the extracts of cells blank, cells depletion and FS-incubated cells in the same conditions above. Fluorescence spectra were obtained with FLS-920 Edingburgh Fluorescence Spectrometer (Edinburgh Analytical Instruments, Edinburgh, UK) with a Xenon lamp and 1.0cm quartz cells. The emission wavelengths were determined at an excitation wavelength of 493 nm and scanning the emission monochromator between 510 and 610 nm. The excitation slit and emission slit were both 1.5 nm.

3 The movie showing cell sampling, single cell loading and cytolysis

Movies showing cell sampling, single cell loading and cytolysis were obtained using CCD camera (DFC300FX, Leica, Germany) with a software (Leica, Application Suite) under a ×10 objective lenses of an inverted fluorescence microscope (DM-IL, Leica, Germany). In order to decrease the storage space, we just selected a typical segment from 14-24 s of one full video to show the single-cell analysis process (including cell sampling, single cell loading, cytolysis, etc.).

1. P. L. Desbene, C. J. Morin, A. M. D. Monvernay and R. S. Groult, *J. Chromatogr. A.*, 1995, **689**, 135–148.

2. F. T. Han, B. H. Huynh, Y. F. Ma and B. C. Lin, *Anal. Chem.* 1999, **71**, 2385–2389.

3. X. C. Gong, Q. L. Li, K. H. Xu, X. Liu, H. M. Li, Z. Z. Chen, L. L. Tong and B. Tang, *Electrophoresis*, 2009, **30**, 1983–1990.