Identification of Type of Membrane Injury and Cell Death using Whole Cells-Based Proton-Sensitive Field-Effect Transistor Systems

Yuki Imaizumi, Tatsuro Goda, Akira Matsumoto and Yuji Miyahara

Institute of Biomaterals and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan

*Corresponding authors:
Tel: +81 3 5280 8097
Fax: +81 3 5280 8135
E-mail: goda.bsr@tmd.ac.jp (TG), miyahara.bsr@tmd.ac.jp (YM)
**Hemolysis assay**

Plasma components and buffy coat in defibrinated fresh sheep blood (Nippon Bio-test Laboratories, Tokyo, Japan) were removed by centrifugation at $1500 \times g$ for 5 min. The isolated erythrocytes were washed three times with isotonic Tris-buffered saline ($1 \times$ TBS, pH 7.4). Four volumes of washed and packed erythrocytes was diluted with three volumes of TBS to produce a 57% hematocrit solution. Then, 75 µL of TBS containing a desired concentration of chemical compound was added to 175 µL of the erythrocyte solution (final hematocrit: 40%), followed by incubation on ice for 20 min, after which the supernatant was isolated by centrifugation at $1500 \times g$ for 5 min at 4°C. The amount of hemoglobin was determined from the absorbance at 543 nm ($A_{543}$) using a microplate reader (infiniteM200, Tecan, Mannedorf, Switzerland). The erythrocytes with water (100% hemolysis) and TBS (0% hemolysis) served as positive (p.c.) and negative (n.c.) controls. The degree of hemolysis was calculated as

$$[\text{Hemolysis}] = (A_{543} - A_{543,\text{n.c.}})/(A_{543,\text{p.c.}} - A_{543,\text{n.c.}})$$
**Fig. S1.** A picture of the whole system including the sensor and well.

**Fig. S2.** Schematic illustrations explaining the mechanism for transient potential peaks during the exchange of NH$_4$Cl in the extracellular space.
**Fig. S3.** Scattered plots between the hemolysis (20 min) and LDH (15 min) assays. † and ‡ represent the hemolysis−/LDH+ and hemolysis+/LDH+ regimes, respectively. Data points identify the two signals at set concentrations with the mean ± SD (n = 3). LDH signals were normalized by those obtained at 1.0 mg mL$^{-1}$ TW20 for 15 min. Colours in symbol show chemical species. Dashed lines represent the thresholds. Correlation coefficient: $r$. 
Fig. S4. Scattered plots between the ISFET assay and the calcein release assay. *, †, and ‡ represent the ISFET+/calcein−, ISFET−/calcein+, and ISFET+/calcein+ regimes, respectively. The plots show the mean ± SD (n=3). r: correlation coefficient.
Fig. S5. Scattered plots between the ISFET and Live cell assays. *, †, and ‡ represent the ISFET+/live−, ISFET−/live+, and ISFET+/live+ regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.
Fig. S6. A scattered plot between the hemolysis (20 min) and WST-8 (6 h) assays. † and ‡ represent the hemolysis−/ WST-8+ and hemolysis+/ WST-8+ regimes, respectively. The plots show the mean ± SD (n = 3). $r$: correlation coefficient
Fig. S7. Correlation diagrams. (a–c) Scattered plots that compare the ISFET assay with the WST-8 assay for 12 h (a), 24 h (b), and 48 h (c). *, †, and ‡ represent the ISFET⁺/WST-8⁻, ISFET⁻/WST-8⁺, and ISFET⁺/WST-8⁺ regimes, respectively. (d–f) Scattered plots that compare the hemolysis assay with the WST-8 assay for 12 h (d), 24 h (e), and 48 h (f). † and ‡ represent the hemolysis⁻/WST-8⁺, and hemolysis⁺/WST-8⁺ regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.
**Fig. S8.** Correlation diagrams. (a–c) Scattered plots that compare the ISFET assay with the live assay for 12 h (a), 24 h (b), and 48 h (c). *, †, and ‡ represent the ISFET⁺/live⁻, ISFET⁻/live⁺, and ISFET⁺/live⁺ regimes, respectively. (d–f) Correlation diagrams that compare the hemolysis assay with the live assay for 12 h (d), 24 h (e), and 48 h (f). † and ‡ represent the hemolysis⁻/live⁺, and hemolysis⁺/live⁺ regimes, respectively. The plots show the mean ± SD (n = 3). r: correlation coefficient.
Fig. S9. Correlation diagrams. (a–c) Scattered plots that compare the following assays: the LDH assay and the calcein release assay (a), the LDH assay and the live assay (b), the calcein release assay and the WST-8 assay (c), and the calcein release assay and the live assay (d). The plots show the mean ± SD (n = 3). r: correlation coefficient.