Electrochemical Identification of Leukemia Cells from Clinical Samples with Tetrathiafulvalene Probe at ITO Electrode

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Experimental section:

Synthesis of TTF-(COONBu\textsubscript{4})\textsubscript{2}

TTF-(COONBu\textsubscript{4})\textsubscript{2} was prepared as previously reported \textsuperscript{1}. The synthetic procedures was shown in Figure S1. Briefly, freshly distilled (OEt)\textsubscript{3}P was added to the mixture of precursors (compound 1 and 2) under a N\textsubscript{2} atmosphere. The mixture was reacted at 110 °C for 1 h and then cooled to room temperature. The suspension was precipitated by methanol for 4 hour, then the precipitate (compound 3) was filtrated, washed with methanol and chromatographed. TTF-(COONBu\textsubscript{4})\textsubscript{2} was prepared from compound 3 reacted with NBu\textsubscript{4}OH. Then the compound was purified and characterized as reported in Ref 1.

\[
\begin{align*}
\text{H}_3\text{COOC} & \quad \text{S} \quad \text{O} \quad \text{S} \quad \text{S} \\
\text{H}_3\text{COOC} & \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{S} \\
\text{(EtO)}\text{P} & \quad \text{COOCH}_3 \quad \text{COOCH}_3 \\
\text{NBu}_4\text{OH} & \quad \text{COONBu}_4 \quad \text{COONBu}_4 \\
\text{TTF-(COONBu}_4\text{)}_2 & \\
\end{align*}
\]

*Figure S1. Synthetic procedures of TTF-(COONBu\textsubscript{4})\textsubscript{2}.

Isolation of leucocytes from whole blood

This study was approved by Ethics Committee of Southeast University and adhered to Declaration of Helsinki. Leucocytes were isolated from whole blood as described early\textsuperscript{2}. The anticoagulated blood was diluted with an equal volume of PBS. Lymphocyte separation medium (Sinopharm Chemical Reagent Co., Ltd., China) was added to the blood solution and centrifuged for 15 min at 2000 rpm, 20 °C. Top layer containing the plasma was drew off. Lymphocyte cell layer was cautiously transferred into a new tube and washed by PBS (pH 7.2) twice. The morphologies of leucocytes was assessed by microscope (IX51, Olympus, Japan) and the concentrations were set to 3.0 \times 10^4 cells per milliliter.

Electrochemical identification of leukemia cells with TTF probe
ITO conductive glass (square resistance ≤ 40 Ω/cm²) was purchased from CSG Holding Co., Ltd. (Shenzhen, China) and was cut into strips of 3 cm×0.5 cm. ITO electrodes was ultrasonically washed for 5 min with acetone, ethanol and distilled water (18.2 MΩ/cm, purified by Milli-Q water purification system (Millipore, USA)) in sequence and dried with nitrogen gas.

All electrochemical experiments were performed on CHI 660B electrochemical workstation (CH Instruments Inc., USA) with an ITO electrode as the working electrode (WE), a platinum wire as the counter electrode (CE) and a silver wire as a quasi-reference electrode (RE). The place of electrodes were regulated by the XYZ positioning adjustment and illustrated with the video camera in a contact angle analyzer (CAM 2000, KSV Instruments, Finland).

As shown in Fig. 2, a drop of electrolyte was overspread the conducting surface of ITO electrode (0.25 cm², 0.5 cm×0.5 cm) and then the CE and RE were immersed within the droplet. The DPV was carried out with a 4 mV potential increment and a 0.1 s pulse period corresponding to an effective scan rate of 40 mV s⁻¹. We have assessed the effects of cell concentrations and incubation time for the analysis of relevant electrochemical probe with cells. Accordingly, TTF probe was added to cell suspensions and incubated for 5 min at room temperature. Differential pulse voltammetry (DPV) of the suspension solution were carried out in the droplet electrochemical system without any further separation.

Data processing and analysis

The potential shifts (ΔEP) and current changes (Ip/Ip0) of TTF derivative after incubated with cells were recorded and analyzed for the rapid identification of leukemia cells from clinical samples. The ΔEP was calculated as:

\[ ΔE_p = E_p - E_{p0} \]

Where \( E_p \) is the peak potentials of TTF probe with leucocytes from clinical samples and \( E_{p0} \) is the peak potentials of TTF probe.

ΔEP instead of the peak potential was employed in further analysis to avoid the slight drift in reference potential of the quasi-reference electrode. We then used ΔEP,1 and ΔEP,2 as the dependent variables and as the independent variable in significance tests with IBM SPSS statistics. \( P < 0.05 \) was accepted as significant difference.

Ultraviolet-visible spectroscopic study of interaction between TTF probe and leukemia cells/leucocytes

The interaction of TTF probe with leukemia cells or leucocytes was investigated with ultraviolet-visible spectroscopy. The leucocytes were isolated and the concentrations were set to 3.0 × 10⁴ cells per milliliter. TTF probe was added into the leucocytes solution and incubated for 5 min, and then the spectroscopies were collected on BioMate 3S UV-Visible spectrophotometer (Thermo Scientific, USA).

The UV-visible spectra of TTF probe, leukemia cells and leucocytes were presented in Figure S2. The peak A at around 278 nm can be assigned to the tyrosine and tryptophan residues in proteins. As shown in Table S1, the adsorption behaviors of leukemia cells and leucocytes were changed when incubated with TTF probe. Compared to the cells itself, the peak A performed a 3 nm red shift for leucocytes and a 3 nm blue shift for leukemia cells after incubated with TTF probes. There were about 9 nm shift between leukemia cells and leucocytes when interacted with TTF probe. Peak B at about 326 nm was due to the TTF core and almost unchanged after incubated with cells.
Figure S2. Ultraviolet-visible spectroscopic study of interaction between TTF probe and leukemia cells/leucocytes. Concentrations of TTF probe was $5.0 \times 10^{-5}$ M.

Table S1. Peaks of leukemia cells/leucocytes after incubated with TTF probe.

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak A</th>
<th>Peak B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>278</td>
<td>/</td>
</tr>
<tr>
<td>Leucocytes with TTF</td>
<td>281</td>
<td>326</td>
</tr>
<tr>
<td>Leukemia cells</td>
<td>275</td>
<td>/</td>
</tr>
<tr>
<td>Leukemia cells with TTF</td>
<td>272</td>
<td>327</td>
</tr>
<tr>
<td>TTF</td>
<td>/</td>
<td>326</td>
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References