Chronic Myeloid Leukemia Drug Evaluation Using a Multisignal Amplified Photoelectrochemical Sensing Platform

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Supporting Information

ABSTRACT: Chronic myeloid leukemia (CML) is a malignant clone disease of hematopoietic stem cells. At present, the most effective therapy for CML is bone marrow transplantation, but this procedure is expensive, and it is often difficult to find appropriately matched bone marrow donors. As an alternative to marrow transplantation, a more effective anticancer drug should be developed to cure the disease; in addition, an effective system to evaluate the activity of the drug needs to be developed. Herein, we present a novel antileukemia drug evaluation method based on a multisignal amplified photoelectrochemical sensing platform that monitors the activity of caspase-3, a known marker of cell apoptosis. Manganese-doped Cds@ZnS core-shell nanoparticles (Mn:CdS@ZnS) were synthesized via a simple wet chemical method, which provided a stable photocurrent signal. A DEVD–biotin peptide and streptavidin-labeled alkaline phosphatase (SA-ALP) were immobilized successively at these nanoparticles through amide bonding and through specific interaction between biotin and streptavidin, respectively. The photocurrent of this sensing platform improved as the ALP hydrolyzed the substrate 2-phospho-l-ascorbic acid (AAP) to ascorbic acid (AA), a more efficient electron donor. The activity of caspase-3 was detected using this sensing platform, and thus, the efficacy of nilotinib for targeting K562 CML cells could be evaluated. The results indicate that nilotinib can effectively induce apoptosis of the K562 cells. This sensing platform exhibited sensitive, reproducible, and stable performance in studying the nilotinib-induced apoptosis of K562 CML cells, and the platform could be utilized to evaluate other anticancer drugs.

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by hyperproliferation of stem cells and subsequent differentiation into peripheral white blood cells.¹ There is no doubt that marrow transplantation is the most effective therapy to eradicate CML; however, only a minority of patients are eligible for transplant, which carries an appreciable risk of mortality or protracted morbidity.² Therefore, anticancer drugs are necessary to alleviate the development of CML and prolong life for the patients. Recently developed and effective drugs for the treatment of CML include imatinib mesylate,³ nilotinib,⁴ dasatinib,⁵ and interferon-α.⁶ Interferon-α, a general-use medicine, is widely used in the treatment of various diseases. Imatinib mesylate, nilotinib, and dasatinib, which are tyrosine kinase inhibitors, are CML-targeted medicines that can induce complete cytogenetic remission.⁷ Unfortunately, all the antileukemia drugs listed above may be associated with serious toxicity or gradually hampered by CML’s propensity to develop resistance to them.⁷ Thus, it is crucial to establish an effective method to evaluate the real-time efficacy of these drugs for monitoring the treatment of CML.

Caspase-3 is one of the most frequently activated cysteine proteases and is a well-established marker of cellular apoptosis, thus enabling it to be used to evaluate the therapeutic effects of drugs.⁸,⁹ Sensitive detection of caspase-3 activity is being investigated thoroughly to better evaluate drug resistance, make therapeutic choices, and monitor treatment.¹⁰ Conceivably, caspase-3 detection could also be an effective means to evaluate the effects of CML drugs and monitor the treatment of CML.¹¹ Traditional techniques for the detection of caspase-3 include flow cytometric-based analysis,¹² colorimetric/fluorometric assay,¹³ Western blotting,¹⁴ and electrochemical methods.¹⁵ Although promising, most of those methods require sophisticated instrumentation and a high degree of technical expertise and are time-consuming and labor-intensive. There-
fore, it is necessary to develop a highly sensitive, convenient, and low-cost approach for the detection of caspase-3 activity.

Photoelectrochemical sensing, which enjoys high sensitivity owing to different energy forms of excitation and detection, has aroused a great research interest in recent years.\textsuperscript{16−18} With advances in the fields of nanotechnology and biochemistry, photoelectrochemical techniques offer unique advantages of inherent simplicity, low cost, and sensing of various organic/inorganic molecules\textsuperscript{19} and biomolecules.\textsuperscript{20} The primary mechanism for photoelectrochemical sensing is based on specific recognition between a pair of biospecies, such as aptamers and cells\textsuperscript{21}, antigens and antibodies,\textsuperscript{22} biotin and avidin,\textsuperscript{23} or probe and target DNA.\textsuperscript{24} Usually these biomolecules are disease-related biomarkers, and thus, their detection is very important for early disease diagnosis and monitoring. However, to the best of our knowledge, there has been no report on the photoelectrochemical monitoring of caspase-3 activity to directly evaluate the medical value of anticancer drugs.

Herein, we describe a novel strategy in which we performed photoelectrochemical sensing of caspase-3 activity to evaluate the drug resistance of CML cells during nilotinib treatment (Scheme 1). Mn-doped CdS@ZnS core−shell nanoparticles (Mn:CdS@ZnS) were used as the photoactive material; these particles were synthesized through a novel wet chemical route.\textsuperscript{25,26} The Mn:CdS@ZnS nanoparticles generated higher and more stable photocurrent signals than did pure CdS nanoparticles, thus improving the sensitivity of the photoelectrochemical sensing scheme. The metal ion dopant, Mn\textsuperscript{2+}, effectively created an electronic state in the midgap region of CdS that prompted charge separation, inhibited recombination dynamics, and broadened the wavelength range of light absorption, thus increasing the photocurrent signal.\textsuperscript{25,27} The ZnS shell, which served as a passivation layer, effectively increased the photocurrent of CdS by preventing the formation of surface defects on CdS and by inhibiting electron recombination with the external redox couple.\textsuperscript{26,28,29} ZnS also increased the photocurrent signal due to its absorption of ultraviolet light. The Mn:CdS@ZnS core−shell nanoparticles were aminated with poly (ethylene imine) (PEI) at an ITO electrode, and then biotin−Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys (biotin−DEVD) peptides\textsuperscript{15,30} were immobilized at the modified electrode via strong amide bonding between PEI and the peptide. After cleavage by active caspase-3, the residual peptides were combined with streptavidin-labeled alkaline phosphatase (SA-ALP) via specific interaction between biotin and streptavidin. ALP hydrolyzed the substrate 2-phospho-L-ascorbic acid trisodium salt (AAP) to ascorbic acid (AA),\textsuperscript{31} thus creating a stronger electron donor and consequently resulting in an increased photocurrent signal. The intensity of the photocurrent was inversely proportional to the amount of active caspase-3 present in the system, and thus the efficacy of nilotinib could be evaluated through this multisignal amplified system.

Compared with the previous report,\textsuperscript{15} the photocurrent output could be detected without destroying the photoelectrochemical sensing platform, indicating that the platform might be retested only with the change of the substrate solution, whereas the electrochemical sensor had to be destroyed to obtain the Cd\textsuperscript{2+} and needed rebuilding for another test. In the research, Mn:CdS@ZnS nanoparticles were chosen as the photoelectric material without generating metal ions. The electrochemical sensor chose quantum dots as the electrochemical signal output and a stripping voltammetric method for detection, which would introduce heavy metal ions (Cd\textsuperscript{2+} and Hg\textsuperscript{2+}) and strong acid to induce some pollution. Alternatively, the electrochemical signals are easily disturbed by other metal ions and electrically active impurities, whereas the photoelectrochemical signals are more stable and easily reproducible because the increase in the signal output is related just to the generation of AA.

Finally, the ITO electrode is washed and modified much more easily and is less expensive than the GCE electrode to simplify the experimental process. Thus, the photoelectrochemical method has special advantages. Moreover, this photoelectrochemical sensing platform can be applied in the
evaluation of other anticancer drugs, and the photocurrent signals obtained are highly sensitive, reproducible, and stable. To our knowledge, these results are the first reported for photoelectrochemical sensing of caspase-3 for anticancer drug evaluation.

**EXPERIMENTAL SECTION**

**Materials and Reagents.** Cadmium chloride (CdCl₂·2.5H₂O) was purchased from Shanghai Jinshan Tingxin Chemical Reagent Co., Ltd. (China). Polyvinylpyrrolidone (PVP, K-30) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Thioglycollic acid (TGA), 2-mercaptoethanol (TME), and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. Zinc chloride (ZnCl₂) was purchased from Shanghai Chengdu Chemical Co., Ltd. (Shanghai, China). Manganese acetate tetrahydrate (Mn·(CH₃COO)₂·4H₂O or Mn(AC)₂·4H₂O, 99%) and sodium sulfide (Na₂S·9H₂O) were purchased from Nanjing Chemical Reagent Co., Ltd. (Shanghai, China). Thioglycolic acid (TGA), 2-phospho-L-ascorbic acid trisodium salt, 1-ethyl-3-(3-dimethylamino)propyl) carbodiimide (EDC), N-hydroxy succinimide (NHS), and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. Zinc chloride (ZnCl₂) was purchased from Zhenxin Chemical Reagent Co., Ltd. (Shanghai, China). Ethylene imine polymer (PEI, 99%, 10000) was purchased from Aladdin (China). Biotin–Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys (biotin–DEVD, 95%) was from Jili Biochemical Co., Ltd. (Shanghai, China). Western and IP cell lysis liquid (P0013) and streptavidin-labeled alkaline phosphatase (SA-ALP, 66 kDa) was obtained from Beyotime Institute of Biotechnology (China). 6-Mercapto-1-hexanol (MCH) was obtained from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Nilotinib was purchased from the Far Top Limited Co., Ltd. (Nanjing, Jiangsu, China). The Annexin V-key Flour488 Apoptosis/PL Detection Kit, Caspase-3 Colorimetric Assay Kit, and Cell Proliferation and Cell Toxicity Assay Kit were purchased from KeyGEN BioTECH Co., Ltd. (Nanjing, China). All other reagents were of analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore).

**Instruments.** Fourier transform infrared (FT-IR) spectra were obtained using a Bruker Vector 22 spectrometer in the frequency range of 4000–400 cm⁻¹. Electrochemical impedance spectroscopy (EIS) was performed on an Autolab potentiostat/galvanostat (PGSTAT 30, Eco Chemie B.V., Utrecht, Netherlands) with a three-electrode system in KCl solution (0.1 M) containing K₂[Fe(CN)₆]/K₃[Fe(CN)₆] (5 mM, 1:1) mixture as a redox probe and recorded at an open circuit potential of 192 mV with an amplitude of 5 mV over a frequency range of 0.01 Hz–100 kHz. Photoelectrochemical measurements were performed with a home-built photoelectrochemical system. A 500 W Xe lamp was used as the irradiation source. The photocurrent intensity was recorded on a CHI 660D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) with a three-electrode system. In the system, a modified ITO electrode with an effective area of 0.5 cm × 0.5 cm (type JH52, Beijing Zhongdingkey Technology Co. Ltd., Nanjing, China; ITO coating 30 ± 5 nm, sheet resistance ≤10 Ω square⁻¹) was used as the working electrode, a Pt wire was used as the counter electrode, and a Ag/AgCl electrode was used as the reference electrode. The substrate solution was 15 mM AAP in phosphate-buffered saline (PBS; 0.1 M, pH 8.5), which was deoxygenated by bubbling with nitrogen for 15 min before use in the photoelectrochemical system. The external voltage was 0 V.

**Preparation of Mn:CdS@ZnS Core–Shell Nanoparticles.** (1) Synthesis of Mn-Doped CdS Nanoparticles. Mn-doped CdS nanoparticles were prepared via a one-step synthetic route. First, CdCl₂·2.H₂O (0.114 g), PVP (0.2 g), and 10% w/w Mn(AC)₂·4H₂O was dispersed into 20 mL of twice-distilled water. After vigorous stirring, 0.1 g of TAA was added. The obtained solution was allowed to react for 2 h at 80 °C under vigorous stirring. Finally, the product was centrifuged at 8000 rpm three times, and the resulting Mn:CdS nanoparticle precipitate was dispersed into 30 mL of twice-distilled water.

(2) The Growth of a ZnS Shell on the Mn:CdS Surface. In a typical experiment, 20 μL of TGA and 600 μL of 10 mM ZnCl₂ was added to the obtained Mn:CdS solution. The pH of the mixture was adjusted to 11.5 with 1 M NaOH solution, and then 600 μL of 10 mM Na₂S was added. The resulting mixture was refluxed for 1 h at 60 °C in an inert N₂ atmosphere, then centrifuged at 8000 rpm three times. The resulting precipitate of Mn:CdS@ZnS core–shell nanoparticles was dispersed into twice-distilled water at a concentration of 2 mg/mL.

**Fabrication of the Photoelectrochemical Biosensor.** The photoelectrochemical biosensor was fabricated on an ITO electrode with an effective area of 0.5 cm × 0.5 cm. Briefly, the ITO electrodes were sonicated sequentially for 15 min each in acetone, NaOH (1 M) in an ethanol/water mixture solution (1:1, v/v), and pure water. The steps for fabricating the biosensor are outlined in Scheme 1. First, a solution of Mn:CdS@ZnS (25 μL) was dropped onto the ITO electrode and dried at room temperature. Then the Mn:CdS@ZnS/ITO electrodes were immersed into 1.0 wt % PEI aqueous solution for 60 min. The modified electrodes were rinsed with distilled water to remove excess PEI, and then they were activated by immersion in 1.0 mL aqueous solution containing 20 mg of EDC and 10 mg of NHS for 1 h at room temperature, followed by a thorough rinsing with PBS buffer (pH 7.4) to remove excess EDC and NHS. Ten microliters of 5 mM biotin–DEVD peptide was activated with 1.5 μL of 10 mM MCH in pH 5.2 acetate buffer for 1 h to prevent its terminal cysteine from forming disulfide bonds. Then, the activated biotin–DEVD was spread on the modified electrodes, and the electrodes were incubated for 12 h at 4 °C in 100% humidity. Subsequently, the electrodes were immersed into 1 mM MCH for 1 h to prevent the nonspecific adsorption of proteins in cell lysates. After being washed with PBS (pH 7.4), the electrodes were used as a photoelectrochemical biosensor and incubated with 100 μL of activated caspase-3 cell lysis solution for 1 h at 37 °C. After washing with PBS twice more, the prepared electrodes were incubated with 10 μL SA-ALP at 37 °C for 2 h. Before utilizing the electrodes for photocurrent measurements, they were rinsed with PBS to remove residual ALP.

**Detection of Caspase-3 Activity via the Photoelectrochemical Biosensing Platform.** Human leukemic K562 CML cells were chosen as model cells for these experiments, and nilotinib was used as the antileukemia drug to induce the apoptosis of the K562 cells. K562 CML cells were cultured with RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS, Sigma), 100 μg/mL streptomycin, and 100 μg/mL penicillin in an incubator (5% CO₂, 37 °C). At the logarithmic growth phase, 4 mL of K562 cells (5 × 10⁵ mL⁻¹) treated with 30 μM nilotinib in DMSO solution for 0, 4, 8, 12, 16, 20, 24, and 30 h, respectively, were

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collected, washed twice with PBS, and lysed in 200 μL of ice-cold Western and IP lysis solution for 30 min. After centrifugation at 13,000 rpm for 15 min at 4 °C, the supernatant containing active caspase-3 was stored at −80 °C. Using a similar procedure, the K562 cells were also treated with nilotinib at concentrations of 0, 5, 10, 20, 30, 40, and 50 μM in DMSO solution for 16 h, respectively.

Active caspase-3 specifically cleaved the biotin-DEVD peptide, and thus, the amount of uncleaved biotin-DEVD detected on the electrode was inversely proportional to the amount of active caspase-3 present in the system. In addition, the amount of SA-ALP attached to the modified electrode was proportional to the catalytic product AA, which resulted in photoelectrochemical signal variation. Thus, caspase-3 activity could be detected indirectly by observing variations in the photocurrent.

■ RESULTS AND DISCUSSION

Characterization of Mn:CdS and Mn:CdS@ZnS. Scanning electron microscope (SEM) and transmission electron microscopy (TEM) images of Mn:CdS and Mn:CdS@ZnS nanoparticles are shown in Figure 1. The CdS nanoparticles were homogeneously sized and spherical (Figure 1A), and their average size slightly decreased after doping with Mn (Figure 1B). The Mn:CdS@ZnS particles were larger than the Mn:CdS particles as a result of the formation of the ZnS shell (Figure 1C). A TEM image of Mn:CdS (Figure 1D) indicates that the nanoparticles are composed of tens or hundreds of smaller nanoparticles. Energy-dispersive X-ray spectrometry (EDX) analysis and X-ray diffraction (XRD) patterns are shown in Figure 2. EDX analysis showed a weak elemental peak for Mn at ~580 eV with a ratio of ~0.8 at%, suggesting Mn was successfully doped into the CdS nanoparticles. An elemental peak for Zn at ~100 eV indicated the presence of the ZnS shell in Mn:CdS@ZnS nanoparticles. XRD patterns revealed the crystal structures of the nanoparticles. The XRD diffraction peaks from the nanoparticles matched well with those of bulk zinc-blended CdS, corresponding to the (100), (002), (101), (110), (103), and (112) planes; however, the diffraction peaks became weak and broad after Mn doping, indicating an increase in lattice defects. In contrast, the addition of the ZnS shell had little influence on the crystal structures of the CdS particles. The FT-IR spectra of Mn:CdS and Mn:CdS@ZnS are shown in Supporting Information (SI) Figure S1. The peaks at 1649 and 1637 cm⁻¹ were attributed to the stretching vibrations of amide bond from PVP, and the peaks at 1707 and 1468 cm⁻¹ were possibly attributed to carboxyl and C−H vibrations, respectively, indicating the successful synthesis of Mn:CdS@ZnS.33

Figure 1. SEM images of (A) CdS, (B) Mn:CdS, and (C) Mn:CdS@ZnS; (D) TEM image of Mn:CdS.

Figure 2. XRD patterns and energy-dispersive X-ray spectrometry (EDX) analysis of CdS, Mn:CdS and Mn:CdS@ZnS nanoparticles.
Photoelectrochemical Properties of Mn: CdS@ZnS Core–Shell Nanocrystals. CdS is a well-known narrow band gap semiconductor that is used in solar cells, since it can effectively absorb the visible range of solar energy and generate excitons composed of electron–hole pairs, as shown in Scheme 2. The electrons are transported to the conduction band while the holes remain in the valence band (I). Photocurrent is produced upon electron transfer from the conduction band to ITO (VI); however, electron recombination with holes (IV) or with the external redox couple (V) can also occur owing to the narrow band gap, and both of these mechanisms consequently decrease the photocurrent. The dopant Mn$^{2+}$ can produce shallow trap states in the midgap states to effectively capture and release charge carriers (electrons and holes; III), thus altering the charge separation and recombination dynamics to improve the efficiency of electronic transfer and to indirectly increase the photocurrent.27,34–36 Alternatively, electron transport between the valence band and dopant Mn$^{2+}$ trap states can extend the absorption profile to longer wavelengths (II), further improving the photoelectric properties of CdS.25,37,38

Figure 3A,B shows photocurrent measurements of Mn:CdS nanoparticles with different concentrations of Mn$^{2+}$. The CdS nanoparticles absorbed visible light to produce a photocurrent of ∼3 μA. With an increasing concentration of Mn$^{2+}$ precursor from 3 to 10% w/w, the Mn:CdS nanoparticles exhibited an obvious increase in photocurrent, achieving a maximum of ∼12 μA. The photocurrent decreased gradually as the concentration of Mn$^{2+}$ precursor was further increased above 10% w/w as a result of the creation of excess trap states induced by Mn doping, which led to inactivation of charge carriers. ZnS, a wide-band-gap semiconductor, can also make a limited contribution to the photocurrent. Even more importantly, ZnS acts as a passivation shell for the nanoparticles and in this capacity can significantly reduce the number of surface defect states, as well as confine charge carriers to the nanoparticle core due to the band offset potentials, thus avoiding loss of the charge carriers and reducing the light corrosion of CdS (V).28,39,40 The thickness of the ZnS shell greatly influenced the photoelectric properties of the nanoparticles, as shown in Figure 3C,D, for which various volumes of Zn$^{2+}$ (10 mM) were examined to optimize the photocurrent measurements for Mn:CdS@ZnS. The photocurrent increased with increasing Zn$^{2+}$, achieving a maximum of ∼25 μA at 600 μL of 10 mM Zn$^{2+}$, which is twice the value observed for Mn:CdS without a

**Scheme 2. Photophysical Processes of Mn:CdS@ZnS**

$^a$CB and VB are the conduction and valence bands, respectively. Process I represents the charge carrier transition between the valence band and conduction band of CdS; II represents the charge carrier transition between the valence band and Mn impurities; III represents the electron trapping and hole trapping by Mn impurities; IV and V represent electron recombination with holes and with the redox couple, respectively (recombination via pathway V is suppressed by the ZnS shell); and VI represents electron transfer from Mn:CdS to ITO.

**Figure 3.** (A) Photocurrents of the Mn:CdS/ITO electrode with different concentrations of Mn(Ac)$_2$·4H$_2$O (mass ratio in contrast to CdCl$_2$·2.5H$_2$O); (B) relationship between the photocurrent and concentration of Mn(Ac)$_2$·4H$_2$O; (C) photocurrents of the Mn:CdS@ZnS/ITO electrode with different volumes of ZnCl$_2$; and (D) relationship between the photocurrent and the volume of ZnCl$_2$. Photocurrent spectra were recorded in PBS (0.1 M, pH 8.5) that contained 15 mM AA.
passivation shell. The photocurrent decreased with further increases in the volume of 10 mM Zn2+, possibly because the excessively thick ZnS shell interfered with the light absorption of CdS, and might have increased the diffusion resistance for electron transfer. The substrate solution also influenced the photocurrent. SI Figure S2 shows that the photocurrent of optimized Mn:CdS@ZnS nanoparticles was $\sim 25$ and $\sim 4 \mu A$ in 15 mM AA solution and 15 mM AAP solution, respectively. The phosphate group of AAP inhibits the particles’ capacity to capture holes, whereas AA can recombine with holes via oxidation of its enol group (Scheme 3); the produced hydrogen ions are rapidly consumed by the PBS buffer. Thus, utilization of AA can improve the photocurrent relative to that observed when AAP is used.41

**Scheme 3. Mechanisms of Hole Recombination with AA (top) and AAP (bottom)**

![Scheme 3](image)

**Fabrication of Photoelectrochemical Biosensor.** The fabrication process of the photoelectrochemical biosensor was monitored by means of EIS. EIS is a powerful tool for the study of electrode fabrication processes. Figure 4 displays the Nyquist plots of the impedance spectroscopy of the electrode throughout the modification process. The electron-transfer resistance ($R_{et}$) of the bare ITO electrode was $\sim 50 \Omega$ (curve a). After deposition of the Mn:CdS@ZnS nanoparticles, the $R_{et}$ of the modified electrode increased to $\sim 100 \Omega$ (curve b) as a result of blocking of electron transfer between the redox couple and the electrode. The $R_{et}$ increased slightly after the electrode was further modified with PEI and biotin–DEVD peptide (curves c and d).

The modified amount of peptide on the electrode was examined. In SI Figure S3, UV–vis absorbance values of peptides showed an approximate linear relationship with the logarithmic concentrations of peptides, and the maximum absorption peak presented a red shift trend with the increase in the peptide concentrations. The residual peptide that was washed and removed from the electrode after being diluted five times with ultrapure water showed a UV absorbance value of $\sim 0.6$, corresponding to a concentration of 0.5 mM. It indicated the residual peptide was $\sim 2.5$ mM while the modified-peptide on the electrode was the same as 2.5 mM. After the immobilization of MCH to block residual nonspecific binding sites, the $R_{et}$ increased markedly as a result of the poor conductivity of the passivated electrode (curve e); however, the subsequent cleavage of biotin–DEVD by active caspase-3 yielded an obvious decrease in the $R_{et}$ (curve f). Finally, the EIS showed a rebound (increase) in $R_{et}$ upon enzyme adhesion to the biosensor (curve g).

Figure 4 also shows the photoelectrochemical detection observed at various fabrication steps. In 15 mM AAP solution, the Mn:CdS@ZnS/ITO electrode yielded a photocurrent of $\sim 4 \mu A$ (curve a). In comparison, an obvious decrease in the photocurrent was observed after the electrode was modified except ALP, which was attributed to the creation of the electron transfer blocking layer (curve b). When the electrode was modified with ALP and immersed into 15 mM AAP solution, the photocurrent increased obviously (curve c), indicating that ALP catalyzed AAP to AA, which could improve the redox system, leading to an increase in the photocurrent.

**Optimization of Detection Conditions.** To obtain the optimum experimental conditions, the concentrations of AAP and ALP, enzyme catalysis reaction time, and immersed time of caspase-3 were varied, and the resulting photocurrents were compared.

The concentration of AAP and ALP were key factors that affected the photocurrent. With increasing AAP and ALP concentrations (Figure S5A,B), the photocurrent increased rapidly until a plateau was observed at 15 mM AAP and 0.15 mg/mL ALP, respectively. After reaching this saturation concentration, the photocurrent stabilized. Thus, the optimum conditions were determined to be 15 mM AAP and 0.15 mg/mL ALP.

The enzyme catalysis reaction time was another key factor that affected the production of AA. Figure 5C shows photocurrent variations for different reaction time. An approximately linearly positive shift of photocurrent was
observed with increasing reaction time from 0 to 10 min; for longer time, the photocurrent exhibited a plateau. Therefore, 10 min was selected as the optimum reaction time.

As the immersion time in caspase-3 was increased, fewer residual peptides were modified with ALP. As a result, a smaller amount of AA was produced, leading to an observed decrease in photocurrent (Figure 5D). The photocurrent signal stabilized after soaking in caspase-3 for 60 min, which we determined to be the optimum immersion time. Actually, the photocurrent output presents an increasing trend after the ALP is modified on the platform, although the sensing platform indeed generates a negative signal because caspase-3 activity is higher. Thus, the final signal output is still accurate and stable.

The Detection of Caspase-3 Activation from K562 Cells Induced by Nilotinib. Nilotinib, which provides targeted therapy for CML by inducing growth arrest and

Figure 5. Relationship among photocurrent and concentration of AAP (A), concentration of ALP (B), enzymatic reaction time (C), and caspase-3 immersion time (D). All the measurements were carried out with external optimum conditions. The active caspase-3 was obtained from K562 cells induced by 30 μM nilotinib for 16 h.

Figure 6. Concentration dependence and time course of caspase-3 activation by nilotinib. (A) Photocurrent intensity of the biosensor cleaved by caspase-3, which was produced by K562 cells incubated with different concentrations of nilotinib: 0, 5, 10, 20, 30, 40, and 50 μM for 16 h. (B) Relationship between the photocurrent and concentration of nilotinib. (C) Photocurrent intensity of the biosensor cleaved by caspase-3, which was produced by K562 cells incubated with 30 μM nilotinib for different times: 0, 4, 8, 12, 16, 20, 24, and 30 h. (B) Relationship between the photocurrent and nilotinib incubation time. Error bars represent one standard deviation for three independent measurements.
apoptosis of CML cells, is poised to revolutionize the treatment of CML and dramatically increase long-term survival rates, especially for patients who exhibit resistance to imatinib. Thus, nilotinib was chosen as a model antileukemia drug to induce the apoptosis of K562 cells in our photoelectrochemical detection of caspase-3. Our developed sensor produced consistent photocurrent signals. Figure 6 shows the concentration dependence and time course of caspase-3 activation from K562 cells induced by nilotinib in DMSO solution. In the absence of nilotinib, the photocurrent was at its maximum observed value due to the minimum caspase-3 activity. The photocurrent decreased significantly, with an approximately linear negative shift as the nilotinib concentration was increased up to 30 μM; further increases in nilotinib concentration resulted in increases in the photocurrent (Figure 6A,B). In addition, the photocurrent decreased rapidly with increasing nilotinib incubation time, as expected, and reached a minimum plateau at 16 h incubation (Figure 6C,D). These results suggest that the optimum concentration and incubation time of nilotinib for K562 cells were 30 μM and 16 h. In summary, our proposed photoelectrochemical sensing platform effectively monitored caspase-3 activation during K562 cell apoptosis with great sensitivity and high stability.

**Summary**: Our developed sensor produced consistent photocurrent signals during K562 cell apoptosis, and the optimal concentrations were determined as 30 μM and 16 h for nilotinib. The sensor could effectively monitor caspase-3 activation with great sensitivity and high stability.

**Contrast experiments**. To verify the conclusion above, some experiments were performed, such as the colorimetric method for caspase-3 activity (Figure 7), the cytotoxicity experiment (Figure 8), and the flow cytometry experiment (Figure 9). The experimental process was operated as the related kit instructions.

The caspase-3 activity was detected with a caspase-3 colorimetric assay kit. The K562 cells were first induced by different concentrations of nilotinib for 16 h, and the obtained active caspase-3 was investigated via the colorimetric method after combination with the chromophores. In Figure 7A, the absorption intensity of active caspase-3 with the chromophores increased with the increasing concentration of nilotinib until 30 μM and then decreased rapidly. Similarly, the induction time of 30 μM nilotinib for K562 cells was examined. Figure 7B showed that the absorption intensity of active caspase-3 was proportional to the induction time before 16 h and then fell back again, generally corresponding to the result of photoelectrochemical method above. It indicated that the photoelectrochemical sensing platform could successfully monitor the caspase-3 activity during the period of drug treatment.

The cytotoxicity experiment was performed to screen the suitable drug concentration. In Figure 8, the UV absorption intensity of K562 cells without drug or with only DMSO increased rapidly over time, indicating the significant cell division and proliferation. After the cells were induced by nilotinib, the absorption intensity decreased obviously with the increasing concentration of nilotinib and reached a large span between 30 and 40 μM with absorption intensity below 0.5 only for 8 h. It indicated that excessive drug concentration would introduce a strong toxicity that might induce cell death without active caspase-3. Thus, the caspase-3 activity decreased after 30 μM, as shown in Figure 6A,B. Alternatively, after induction time for 24 h, the cells’ activity and quantity reached a minimum level over 30 μM nilotinib. To obtain optimal treatment effect and avoid excessive toxicity, which might induce drug resistance and kill normal cells, the suitable drug concentration was chosen as 30 μM.

The cell condition after drug treatment was examined via flow cytometry experiment. Figure 9 showed that the normal cells without drug treatment exhibited slight apoptosis, and a small amount of DMSO had little effect on the cell proliferation. After treatment with 30 μM nilotinib for 16 h, the cells during the later period of apoptosis increased rapidly from ~7% to ~15%, and the cells during the early stage of apoptosis presented a significant increasing trend from ~3% to ~15%, to generate more active caspase-3 with the decrease in the photoelectrochemical signal output. It indicated that nilotinib could effectively induce cell apoptosis and inhibit cell proliferation without obvious cell death. The concentration of 30 μM is suitable for the treatment of K562 CML cells.

**CONCLUSIONS**

In summary, we developed a novel photoelectrochemical sensing platform to detect caspase-3 activity and thus evaluate anticancer drug effects by utilizing a DEVD-peptide specifically cleaved by active caspase-3 and an ALP-based enzymatic

Figure 7. Detection of caspase-3 activity via the caspase-3 colorimetric kit: (A) concentration dependence for caspase-3 activity from K562 cells induced by different concentrations of nilotinib for 16 h; (B) time course for caspase-3 activity induced by 30 μM nilotinib for different times.

Figure 8. Cytotoxicity experiment via a colorimetric method. Before detection, the K562 cells were incubated with different concentration of nilotinib for 8, 16, and 24 h, respectively.
reaction. The biosensor was fabricated using Mn:CdS@ZnS core–shell nanoparticles to obtain multisignal amplified photoelectric signals, allowing us to obtain more sensitive and stable signals. Using the sensor, we have successfully evaluated the anticancer drug nilotinib, which was demonstrated to be promising for the treatment of CML. This sensing platform may also be applied in other research areas, such as the screening of anticancer drugs and monitoring of cancer treatment.

**ASSOCIATED CONTENT**

* Supporting Information
  Additional figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure 9. Flow cytometry experiment of K562 cells before and after nilotinib treatment: the scatter distribution diagrams of K562 cells without drug treatment (A), with slight DMSO (B), and with 30 μM nilotinib in DMSO solution (C); and the flow cytometry analysis of apoptosis for K562 cells without drug treatment (D), with slight DMSO (E), and with 30 μM nilotinib in DMSO solution (F). Q1 refers to dead cells (black dots); Q2 refers to the later period of apoptotic cells (red dots); Q3 represents the normal cells (blue dots) and Q4 as the early period of apoptotic cells (green dots).