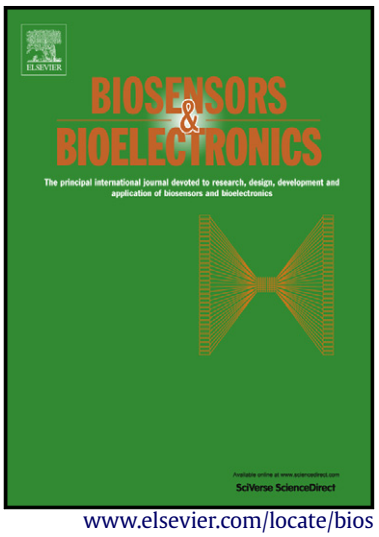

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Toward Therapeutic Effects Evaluation of Chronic Myeloid Leukemia Drug: Electrochemical Platform for Caspase-3 Activity Sensing

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Abstract: In recent decades, advanced therapies and novel scientific drug evaluation systems for chronic myeloid leukemia (CML) treatment are very urgent due to its increasing morbidity. The combination of dasatinib with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) was supposed to be effective for leukemia therapy. Taking full advantage of novel nano-biotechnology, we have developed a robust electrochemical cytosensing approach to profile the therapeutic effects of dasatinib and TRAIL by probing the activity of caspase-3 from apoptotic CML cells. The sensor was on a base of a glassy carbon electrode (GCE) modified with nano-materials composed of Au nanoparticles (Au NPs), poly(dimethyl diallyl ammonium chloride) (PDDA), and carbon nanotubes (CNTs). Then the platform immobilized the biotinylated DEVD-peptide (biotin-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys) via the strong bonding between Au NPs and the thiol group (Au-S bond). In particular, the sensor was then constructed with the environmentally friendly alkaline phosphatase (ALP) via the specific interaction between the biotin and streptavidin, and could retest detection indirectly for caspase-3 sensing by detecting the differential pulse voltammetry (DPV) signal of enzymatic catalysis product, ascorbic acid (AA). The results indicated that either dasatinib or TRAIL could successfully induce the apoptosis of CML cells, while the combination of dasatinib and TRAIL resulted in an improved therapeutic effect, suggesting a novel optimized strategy for CML therapy. This novel electrochemical sensing

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strategy exhibits attractive advantages of environmental benignity, simple performance, high stability, and may be readily expanded to evaluate other cancer therapeutic effects.

Key words: dasatinib; TRAIL; chronic myeloid leukemia; caspase-3; alkaline phosphatase ; electrochemical biosensor

1. Introduction

Chronic myeloid leukemia (CML) is one of the most common types of leukemia, which is induced by the active breakpoint cluster region (BCR)/Abelson (ABL) oncoprotein, a product of a reciprocal translocation of chromosome 9 and 22 and central to the pathogenesis of the CML (Drucker et al., 2001; Shah et al., 2007). While the morbidity of CML is gradually increasing in recent years, the matching rate of the bone marrow transplantation as the most effective treatment by far is still quite insufficient. Therefore developing an effective medication or treatment to control the progress of the disease is increasingly urgent. Dasatinib as a multikinase inhibitor targeting the BCR/ABL oncoprotein can inhibit the activity of the Adenosine Triphosphate (ATP)-binding site of BCR/ABL, which has been used as the frontline treatment for CML by inducing cell apoptosis and thus suppressing cancer cell proliferation (Gencer et al., 2011; Peng et al., 2004; Aguilera et al., 2009). However, the utilization of dasatinib is still hampered by dose-limiting pulmonary hypertension effects and the propensity of CML to develop resistance to a wide range of functionally unrelated anticancer agents (Hennigs et al., 2011; Sano et al., 2012). Alternatively, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a new member of the TNF superfamily, has been shown to kill tumor cells selectively by binding to their cognate death receptors, Death Receptor 4 (DR4) and Death Receptor 5 (DR5), with strong binding affinities, which stimulates rapid apoptosis in a variety of tumor cell lines (Lee et al., 2005; Zheng et al., 2013; Mitsiades et al., 2001; Spencer et al., 2009). Recently, Geng et al. reported that TRAIL exhibits synergism with several chemotherapeutic drugs in cancer cells by potentiating tumor cell apoptosis (Younes et al., 2003; Wang et al., 2008; Shankar et al., 2004; Geng et al., 2012). However, as far as we know, the combination of TRAIL and dasatinib has not

been introduced into the treatment of CML at present.

Apoptosis, a highly regulated process, plays a crucial role in the aetiology of cancer with an intracellular process mediated partially by a series of aspartate-specific, cysteine-dependent proteases (Martinez et al., 2010; Zhang et al., 2011a). The effect to induce the apoptosis of tumor cells is one of the standards to evaluate the therapeutic effect of drugs. Caspase-3, which has been identified as a well-established cellular marker, is one of the most frequently activated cysteine protease in the early stage of apoptosis (Fadeel et al., 2000; Zhang et al., 2011b). By detecting the activity of caspase-3 during the treatment of tumor cells, the apoptosis of tumor cells and therapeutic effects of drugs can be objectively evaluated. Currently, the common methods used for caspase-3 activity screening, such as the western blot assay, flow cytometry and colorimetric/fluorometric assay (Ji et al., 2001; Belloc et al., 2000; Gurtu et al., 1997), are labor-intensive, time-consuming, and require highly technical expertise and precise instruments. In comparison to these methods, electrochemical approaches exhibit attractive advantages in terms of inherent simplicity, high sensitivity and low cost, and can be readily implemented into quantitative bioassays for clinical applications.

Herein we developed a novel nano-biotechnology-based electrochemical platform to profile the caspase-3 activity in drug-induced apoptotic cells by means of the combination of the alkaline phosphatase-based enzymatic reaction with cleavage of a biotinylated DEVD-peptide (biotin-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys). The sensor was constructed on the glassy carbon electrode (GCE) modified with nano-materials composed of AuNPs/PDDA/CNTs. The biotin-DEVD peptide was attached to the AuNPs/PDDA/CNTs/GCE electrode successfully by Au-S bond between the thiol group of the terminal Cys-residue and AuNPs surface. Then the sensor was immersed into the lysate, containing active casapase-3 obtained from the drug-induced apoptotic cells, to cleave a part of biotin-DEVD peptide effectively. The streptavidin-labeled alkaline phosphatase (SA-ALP) was modified on the sensor via the specific interaction between biotin of the peptide and streptavidin of SA-ALP. The ALP on the sensor could hydrolyze the substrate 2-phospho-l-ascorbic acid (AAP) to

yield ascorbic acid (AA) which was detected as a differential pulse voltammetry (DPV) signal. The peak current was proportional to the amount of ALP on the sensor, corresponding to uncleaved biotin-DEVD peptide, which in turn depended on the activity of caspase-3 in the cell lysates. In this way, the activity of caspase-3 could be indirectly evaluated. By measuring the caspase-3 activity, the therapeutic effect of dasatinib and TRAIL could also be successfully evaluated.

In the literatures, the detection of caspase-3 with electrochemical sensors needed high concentrated perchlorate acid ($HClO_4$) (Xiao et al., 2008), which was explosive easily with strong oxidizability, corrosivity and irritation; or CdTe quantum dots (Zhang et al. 2011b), which contained enviromentally unfriendly toxic heavy metal ions. In addition, these sensor structures were destroyed after giving the electrochemical signals, indicating that these sensors could not retest detection. In comparison, the electrochemical signals in our work came from the enzymatic reaction products without damaging the environmentally friendly sensor structure. The biosensor could retest detection with great reproducibility and strong stability. Using this electrochemical sensor, we explored the therapeutic effect of dasatinib and TRAIL to treat CML K562 cell lines by detecting the activity of caspase-3. The results indicated that either dasatinib or TRAIL could successfully activate caspase-3 and induce the apoptosis of K562 cells as expected, while the combination of TRAIL and dasatinib exhibited greater potency than either of them alone. Thus, the combination of TRAIL and dasatinib may provide a potential robust strategy for CML therapy, and show a significant meaning to the increasingly urgent CML treatment. To the best of our knowledge, this is the first work to use enzymatic reaction for electrochemical detection of caspase-3 activity, and meanwhile, the first attempt to study the effect of combining TRAIL with dasatinib for CML therapy.

<Scheme 1>

2. Experiment

2.1. Materials and Reagents

The biotinylated DEVD-peptide (biotin-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys) (1092.13 g/mol, 95.98% purity), was purchased from GL Biochem. Ltd. (Shanghai, China). Multi-walled CNTs (synthesized by means of chemical vapor deposition, >95% purity, 10−20 nm in diameter, 0.5−2.5 μm in length) were purchased from Chengdu Organic Chemical Co. Ltd. (Sichuan, China). Tris(hydroxymethyl)aminomethane (Tris) and dimethylsulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Dasatinib, TRAIL, AAP, Tris(2-carboxyethyl)phosphine (referred to herein as TCEP), PDDA (20wt%, MW = 200000 ~ 350000) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich. Western and IP cell lysis liquid (P0013) and streptavidin-labeled alkaline phosphatase (SA-ALP) were purchased from Beyotime Institude of Biotechnology (Jiangsu, China). A caspase-3 cell activity detection kit was purchased from Nanjing Keygen Biotech. Co. Ltd. (Jiangsu, China). The phosphate buffer solution (PBS, 0.01 M, pH 7.4) used in these experiments contained 136.7 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4 and 1.4 mM KH_2PO_4. All reagents used were of analytical purity grade.

2.2. Apparatus

Scanning electron micrographs (SEM) and Energy-dispersive X-ray spectrometry (EDX) analysis were measured on an S-4800 scanning electron microscope. Electrochemical measurements were performed on a CHI 660c workstation (Chenhua Apparatus Corporation, Shanghai, China) with a conventional three-electrode system containing a saturated calomel electrode as the reference, a platinum wire as the auxiliary, and the modified GCE as the working electrode. Electrochemical impedance spectroscopy (EIS) and Cyclic voltammograms (CVs) were performed with an Autolab electrochemical analyzer (Eco. Chemie., The Netherlands). Supporting electrolyte: 0.1 M KCl aqueous solution containing 5 mM $Fe(CN)_6^{4/3-}$,

scan rate: 100 mV s^{-1}, frequency range: 0.01 Hz−100 kHz, amplitude: 50 mV. Colorimetric analysis of caspase-3 activity was detected at 405 nm using a Bio-Rad 680 microplate reader. Zeta Potential was tested on a Nano-z Zeta Potential Analyzer. UV-Vis absorption spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). Contact angle was examined by the OCA30 Video-optical Contact Angle measurement instrument (Dataphysics Instruments Gmb.).

2.3 Preparation of AuNPs/PDDA/CNTs nano-composites

CNTs were introduced with carboxylate functionalities by a mixture of nitric acid and sulfuric acid (Zhang et al., 2011b), and dispersed in ultrapure water to a concentration of ~5.0 mg/mL. Then, 2.0 mL carboxylated CNTs solution was dispersed into 8 mL 1.2 wt% PDDA aqueous solutions and sonicated for 60 min to obtain a homogeneous black suspension. After high-speed centrifugation (21, 000 rpm, 20 min) with ultrapure water for at least three times to remove the residual PDDA, the sediment was dispersed into ultrapure water with a concentration of ~1.0 mg/mL. Then, 1.0 mL PDDA-capped CNTs aqueous solution was dispersed into 5 mL AuNPs aqueous solution and sonicated for several minutes. The AuNPs used with an average size of 16 nm were prepared from $HAuCl_4$ aqueous solution and sodium citrate according to literature report (Zhang et al., 2008). The mixture was centrifuged and washed with ultrapure water at 9000 rpm for 10 min at least three times to obtain AuNPs/PDDA/CNTs nano-composites, which were dispersed in water with a concentration of ~1.5 mg/mL.

2.4 Assembly of Electrode Interface for Cytosensing

GCE (4 mm diameter) was polished to a mirror finish successfully (Zhang et al., 2010). After successive sonication in ethanol/deionized water (1:1, v/v), the electrode was rinsed with deionized water and dried at room temperature. Then 10 μL AuNPs/PDDA/CNTs were dropped on the pretreated GCE electrode and it was placed at room temperature to dry. 10 μL biotin-DEVD peptide (5 mM) was activated for 1 h with 1.5 μL TCEP (10 mM) in acetate buffer (pH 5.2) to destroy the disulfide bonds

of terminal cysteine. Then, the activated biotin-DEVD was spread on the modified electrode which was allowed to incubate for 12 h at 4 °C under 100% humidity. Subsequently, the electrode was immersed into 1 mM MCH for 1 h to remove nonspecifically adsorbed DEVD, and then was washed with PBS (pH 7.4) thoroughly for cytosensing. The modified electrode was immersed into 100 μL of cell lysates containing active caspase-3 for 1 h at 37 °C and washed twice with PBS, and then incubated with 10 μL ALP-SA solution (0.4 mg/mL) in PBS buffer (0.01 M pH 8.5) for 2 h at 37 °C to form biotin-streptavidin bioaffinity complexes. The biosensor was washed with PBS to remove any physically adsorbed bioconjugates before electrochemical measurements.

2.5 Electrochemical Detection

DPV was chosen as the detection method due to its high sensitivity, strong resolution and selectivity which can reduce the interference current, improve the sensitivity, separate the peak potentials and lower the detection limit. The substrate solution was 15 mM AAP solution in Tris-HCl buffer (50 mM, pH 8.5). While the sensor was immersed into the substrate solution, the ALP on the electrode hydrolyzed the substrate AAP to yield AA, which was detected as a DPV signal indirectly associated with caspase-3 activity with a conventional three-electrode system comprised of saturated calomel electrode (SCE) as reference, platinum wire as auxiliary electrode and the modified electrode as working electrode. DPV detection was carried out from −0.3 V to 0.3 V under N_2 atmosphere with a potential step of 4 mV, an amplitude of 50 mV, a pulse width of 0.05 s, a pulse period of 0.2 s and a quiet time of 8 s.

3. Results and discussion

3.1 Characterization of AuNPs/PDDA/CNTs and the electrochemical platform

A three-dimensional (3-D) architecture of AuNPs/PDDA/CNTs was fabricated as the base of the cytosensor with a layer-by-layer (LBL) approach. The AuNPs/PDDA/CNTs could improve the charge transfer rate and increase the effective

surface area of the electrode. Via Au-S bond between the thiol group of the terminal Cys residue and AuNPs surface, the biotin-DEVD peptide could be modified on the sensor successfully. Besides, the low toxicity and good hydrophilicity of AuNPs/PDDA/CNTs could increase the peptide loading, improve the biocompatibility, retain bioactivity, and result in enhanced sensitivity of the sensor. The stepwise preparation process of AuNPs/PDDA/CNTs was proved by scanning electron microscopy (SEM) and Zeta Potential (Figure S1). Before modification, the as-prepared carboxylated CNTs were mostly single tubes that were long and straight, with a diameter of ~10 nm (Figure S1A). Upon absorption of positively charged PDDA, the negative-charged CNTs became bundled, more curly and inseparable (Figure S1B). The AuNPs, which were negatively charged (Figure S1D), were electrostatically tethered to the positive-charged surface of the CNTs/PDDA, abundantly and uniformly (Figure S1C). Energy-dispersive X-ray spectrometry (EDX) analysis (Figure S2) and UV-vis absorption spectra (Figure S3) also revealed the successful bonding of AuNPs towards PDDA-modified CNTs. The hydrophilicity was analysed by contact angles in Figure S4. The excellent hydrophilicity could somewhat enhance the biocompatibility. The contact angle of bare GCE was ~68° while that of the GCE modified with AuNPs/PDDA/CNTs decreased to ~40°, indicating that the modified electrodes had good hydrophilicity, in favor of peptide loading. Besides, AuNPs/PDDA/CNTs have low toxicity, which would improve the biocompatibility, retain bioactivity, and result in enhanced sensitivity. (Shi et al., 2014).

<Fig. 1>

The formed 3-D architecture on the GCE provided an effective matrix for the immobilization of biotin-labeled DEVD-peptide via Au-S bonds, thus enabling the DEVD-peptide to remain stable and retain its bioactivity on the electrode surface. The DEVD-peptide-functionalized electrode was first immersed into the MCH aqueous solution to block the residual active sites, and then immersed into the apoptotic cell lysates containing active caspase-3, which selectively cleaved the N-terminus of DEVD-peptide. This cleavage event led to the biotin label loss. Subsequently, the remained biotin label was coupled with the SA-ALP signaling probe through

biotin-avidin interaction. The cyclic voltammetry spectra of the electrode at different modified stages showed the successful assembly of the electrochemical platform (Figure 1A). As expected, after modified with AuNPs/PDDA/CNTs, a significant increase of the peak current was observed due to the high conductivity of AuNPs/PDDA/CNTs. The peak current gradually decreased after the modification of biotin-DEVD peptide and MCH (the inert electron and mass-transfer blocking layers), and then increased again when the biotin-DEVD peptide was selectively cleaved by the active caspase-3. Caspase-3 here was obtained from cell apoptosis with 10 nM dasatinib for 10h. Finally, the recognition of SA-ALP with the rest biotin-DEVD peptide on the electrode, led to a slight decrease of the peak current. The EIS data of different electrode stages was consistent with the CV results (Figure 1B).

3.2 The optimization of detection conditions

ALP can generate an electroactive product AA by enzymatic hydrolysis of AAP substrate, which was chosen for the indirectly electrochemical evaluation of caspase-3 activity because enzyme catalytic reaction allows a long-term steady reaction rate for a prolonged time (Akanda et al., 2011) and keeps environmentally friendly. AAP was chosen as the enzymatic substrate for its high water-solubility, stability and low cost while AA could provide a detectable signal for DPV measurements (Preechaworapun et al., 2008; Moore et al., 2003). The DPV signal of AA oxidation was proportional to the amount of uncleaved biotin-DEVD on the electrode surface, which in turn depended on the activity of caspase-3 in the cell lysates. In this way, by measuring the electrochemical response of AA, the therapeutic effect of dasatinib and TRAIL can be simply evaluated. The DPV signals were examined when the sensors were built in different processes as shown in Figure S5 and Figure S7. When the ALP-modified electrode was put into the Tris-HCl buffer without AAP, no DPV peak was observed, while the DPV signal exhibited two peaks at around −0.6 V and 0 V (vs. SCE) into the AAP solution (Figure S5B), corresponding to the peak of the oxidation of AAP and AA separately (Figure S5A). It indicated that the enzymatic hydrolysis was successfully carried out. When the sensor was immersed into the active caspase-3

lysate (from K562 cells with 10 nM dasatinib for 10 h) and then modified with ALP, no DPV peak could be observed in the pure Tris-HCl buffer (Figure S7a) and in the Tris-HCl buffer containing the active casapase-3 lysate (Figure S7b) without AAP. It indicated that some hydroquinones in the lysate had no interference on DPV detection. When the sensor was not modified with ALP, the DPV signal only showed the peak at 0.6 V in the AAP solution (Figure S7c). By contrast, besides the peak of AAP oxidation, another peak current of ~40 μA at 0 V could be observed (d) when the sensor modified with ALP was immersed into the pure lysate without caspase-3, corresponding to the peak of AA oxidation. The peak current of AA oxidation was consistent with the sensor without cleavage (Figure S5). It indicated that the pure lysate without caspase-3 could not selectively cleave the N-terminus of DEVD-peptide.

<Fig. 2>

To optimize detection conditions, the dependence of the DPV peak currents under different experimental variables was investigated, including the concentrations of reaction substrate (AAP) and enzyme (ALP) (Figure 2) and time-dependent cleavage of biotin-DEVD by active caspase-3 (Figure 3). The results showed that the peak current of AA oxidation increased while increasing the concentration of ALP and AAP, and levelled off at 0.4 mg/mL of ALP and 15 mM AAP. In addition, the current decreased gradually with increasing immersion time from 0 to 120 min, and reached a minimum value after ~90 min. Thus, 0.4 mg/mL of ALP, 15 mM of AAP and 90 min of immersion time by active caspase-3 lysates were optimal conditions for electrochemical cytosensing.

<Fig. 3>

3.3 The detection of caspase-3 activation from K562 cells induced by dasatinib

<Fig. 4>

Time course of caspase-3 activation by dasatinib (in DMSO solution) is presented in Figure 4. In the absence of dasatinib, caspase-3 was not activated, leading to a

maximum current response. After the cells were incubated with 10 nM dasatinib, the current decreased gradually with increasing incubation time as expected, and reached a minimum after incubation for 12 h (Figures 4A and 4B). The dependence of dasatinib concentration (Emel et al., 2011) for caspase-3 activation was also detected (Figures 4C and 4D). While increasing concentration of dasatinib from 0 to 10 nM, the current response decreased gradually, and then increased from 10 to 50 nM, suggesting that the best concentration of dasatinib for the apoptosis of K562 cells was 10 nM. These results were basically consistent with the observation from a colorimetric method using a caspase-3 cellular activity assay kit (Figure S6). Thus, the proposed electrochemical cytosensor can be successfully used to monitor caspase-3 activity during cell apoptosis. And in addition, dasatinib is an effective inhibitor of cell proliferation and an apoptosis initiator for K562 cells.

3.4 The detection of caspase-3 activation from K562 cells induced by TRAIL (with dasatinib)

<Fig. 5>

TRAIL has shown promise for cancer therapy as it selectively induced apoptosis in tumor cells from different organs (Geng et al., 2012). Here, pure TRAIL was examined to induce apoptosis of K562 cells (Figure 5A and 5B). The peak current decreased gradually and reached a minimum value of ~18 μA at a TRAIL concentration of 500 ng/mL, which indicated that TRAIL could effectively induce the apoptosis of K562 cells. The combination of TRAIL with antitumor agents has also been reported to be a promising strategy for cancer therapy (Younes et al., 2003; Wang et al., 2008; Shankar et al., 2004; Geng et al., 2012). To find a suitable concentration of TRAIL combined with dasatinib for the apoptosis of K562 cells, we examined the apoptosis of the CML cells by combination of different concentrations of TRAIL with 10 nM dasatinib (DMSO solution) according to the optimum results above. As expected, the peak current reached a minimum of ~4 μA in a mixture of 10 nM dasatinib and 200 ng/mL TRAIL (Figures 5C and 5D), which was much lower than that obtained from 10 nM dasatinib or 500 ng/mL TRAIL alone. These results

indicated that the combination of dasatinib with TRAIL was believed to be a better strategy for CML therapy than either of them alone. The reduced dose of TRAIL to reach minimum peak current also suggested that dasatinib could improve sensitivity of the tumor cells to TRAIL by certain pathway.

3.5 Control experiments and the stability, reproducibility evaluation of the electrochemical platform

< Fig. 6>

Control experiments with normal cell lysates were also performed to verify the the effectiveness of the drugs (Figure 6A). The total liver cells from mice (Herein referred to as ML) and human liver cells (L-02) were chosen as the normal cells. Similar to the K562 cells, ML cells and L-02 cells were induced for apoptosis by 10 nM dasatinib, 500 ng/mL TRAIL, and the combination of 200 ng/mL TRAIL and 10 nM dasatinib for 10 h, respectively. The sensors were immersed into the obtained active caspase-3 lysates for 90 min before modification of SA-ALP. The electrochemical detection of AA showed that the peak currents of the sensors cleaved by active caspase-3 obtained from ML cells pretreated with dasatinib were almost consistent with those from ML cells without any drug treatment. While the peak current slightly decreased after the sensor was cleaved by active caspase-3 obtained from ML cells incubated with TRAIL or the combination drugs. Similarly, all the peak currents of AA oxidation with the sensor cleaved by caspase-3 from L-02 cells showed slight decrease after the cells were induced by these drugs. For K562 cells induced with these drugs, the peak current of AA oxidation decreased rapidly and greatly, with the minimum current of only ~4 μA for the combination drugs. The results indicated that dasatinib and TRAIL had slight effects on the apoptosis of both the ML cells and L-02 cells. However, dasatinib, TRAIL and the combination drugs could induce the apoptosis of K562 cells effectively. It suggested that dasatinib, TRAIL and their combinations may be potential for the treatment of CML.

The stability of the electrochemical platform was evaluated through the duplicate detections of DPV signals for caspase-3 activity (from K562 cells by 10 nM dasatinib for 10 h) with different cytosensors in the same condition. Figure 6B showed that the

DPV peaks basically remained constant, demonstrating its great stability. The reproducibility of the cytosensor was also appraised by detecting caspase-3 activated by 10 nM dasatinib with one sensor electrode for several times. Without rebuilding the structure, the sensor could retest detection on the premise of replacing the substrate solution only, because of the integrity of the structure during the test. Figure 6C showed that the DPV peak currents of duplicate detections with increased repetitions remained basically stable, with negligible decreasing trend, suggesting the stability and reproducibility of the cytosensor toward the detection of caspase-3. Based on this conclusion, the novel biosensor could retest detections successfully to ensure the accuracy of the results. The sensor could be stored for more than one week at 4 °C under 100% humidity before activated and could keep activity for at least 48 h at 37 °C under 100% humidity after activated (Figure S8).

4. Conclusions

In summary, we developed a novel electrochemical platform that combined the cleavage of DEVD-peptide by active caspase-3 with an ALP-based enzymatic catalytic reaction to enable sensitive detecting the activity of caspase-3 during cell apoptosis. This method allows the assessment of drug efficiency in activating caspase-3 at low concentrations within a short time as well as retest with high reaction rate, great reproducibility and strong stability, without damaging the sensor structure. Compared to other electrochemical sensors for caspase-3, this novel sensor was environmentally friendly and showed an excellent function for retest. With this electrochemical platform, the combination of dasatinib and TRAIL was suggested to have a certain potential as an antileukemic agent, and its therapeutic use may be of potential value for the treatment of CML. Sequentially, it is expected to be a significant tool to evaluate the drug efficacy, screen anticancer drugs, monitor the cancer treatment, and other promising applications in cancer research.

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Figure captions

Scheme 1. Fabrication of the nano-biotechnology-based electrochemical cytosensing platform

Figure 1. Cyclic voltammograms (CVs) (A) and Electrochemical Impedance Spectroscopy (EIS) (B) of stepwise modified electrodes: (a) bare GCE, (b) AuNPs/PDDA/GCE, (c) DEVD-peptide/Au-NPs/PDDA/GCE, (d) MCH/DEVD-peptide/AuNPs/PDDA/GCE, (e) MCH/DEVD-peptide/AuNPs/PDDA/GCE after cleavage by caspase-3, (f)

ALP-SA/MCH/DEVD-peptide/AuNPs/PDDA/GCE via cleavage by caspase-3. Caspase-3 here is obtained from cell apoptosis with 10 nM dasatinib for 10h.

Figure 2. (A) The concentration-dependence of DPV signals with ALP. (B) Relationship between the peak currents and the concentrations of ALP. (C) The concentration-dependence of DPV signal with AAP. The ALP on the cytosensor was set at 0.4 mg/mL. (D) Relationship between the peak currents and the concentrations of AAP solution.

Figure 3. (A) DPV measurements for caspase-3 activity after the cytosensor was treated with caspase-3 for different times. The caspase-3 activation was obtained from the K562 cells incubated with 10 nM dasatinib for 10 h. (B) Relationship between the peak currents and the immersed time.

Figure 4. Time course of caspase-3 activation and concentration-dependence of caspase-3 activation by dasatinib. (A) DPV measurements for caspase-3 activity after the K562 cells were incubated with dasatinib for different times (a to e): 0, 6, 8, 10 and 12 h. The concentration of dasatinib was 10 nM. (B) Plot for the dependence of peak currents on the incubation time of dasatinib. (C) DPV measurements for caspase-3 activity after the K562 cells were incubated with different concentrations of dasatinib (a to f): 0, 2, 5, 10, 20 and 50 nM. The K562 cells were incubated for 10 h. (D) Relationship between the peak currents and the concentrations of dasatinib. Error bars represent one standard deviation for three independent measurements.

Figure 5. Concentration-dependence of caspase-3 activation by TRAIL alone and by TRAIL combined with dasatinib. DPV measurements (A) and related peak currents (B) for caspase-3 activity from apoptotic K562 cells incubated with different concentrations of TRAIL (a to e): 0, 100, 200, 500 and 1000 ng/mL. The K562 cells were incubated for 10 h. DPV measurements (C) and related peak currents (D) for caspase-3 activity from apoptotic K562 cells incubated with TRAIL and dasatinib. The concentrations of TRAIL (a to e) were 0, 100, 200, 500 and 1000 ng/mL, while the concentration of dasatinib was the optimized 10 nM. The K562 cells were incubated for 10 h.

Figure 6. (A) DPV measurements for caspase-3 activity obtained from different cells with different drugs for 10 h. The cells are L-02 cells, ML cells and K562 cells, respectively. The drugs are pure DMSO (control), 10 nM of dasatinib in DMSO solution, 500 ng/mL of TRAIL, and the compounds containing 10 nM dasatinib and 200 ng/mL TRAIL, respectively. (B) DPV

measurements for caspase-3 activity by different cytosensors with the same condition. (C) DPV measurements for caspase-3 activity by the same cytosensor retested continuously for several times. Before detection, the cytobiosensors were immersed into the apoptotic cell lysates containing active caspase-3 obtained from the K562 cells incubated with 10 nM dasatinib for 10 h.

> It is the first attempt to study the effect of combining TRAIL with dasatinib for the therapy of CML.

> The TRAIL combined with dasatinib was proven to be a more effective potential antileukemic agent than either of them alone.

> It is the first work to use enzymatic reaction for detection of caspase-3 activity, enhancing the specifity of the sensor.

> The environmentally friendly electrochemical platform could be used for retest without strong acid or heavy metal ions.

Scheme 1

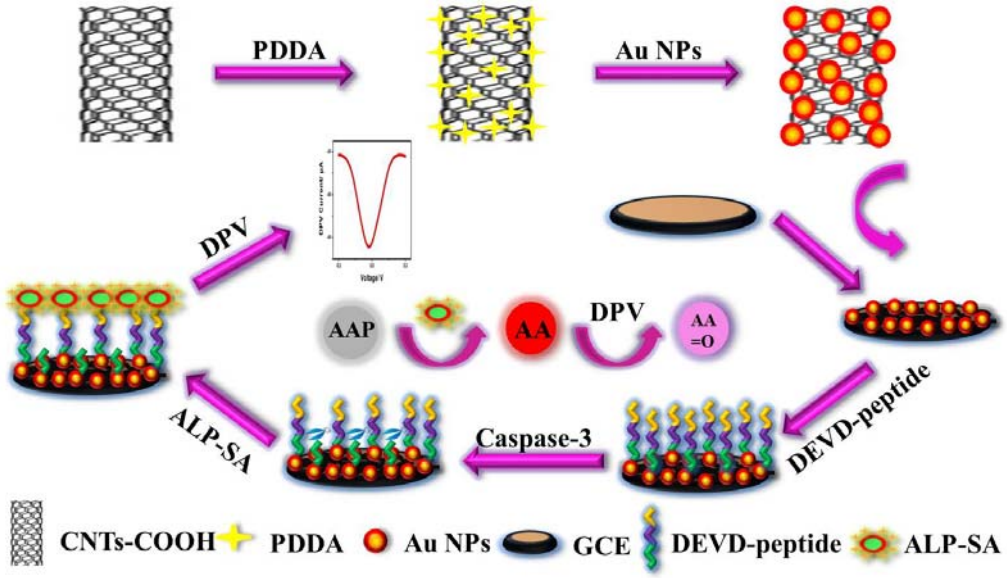

Figure 1

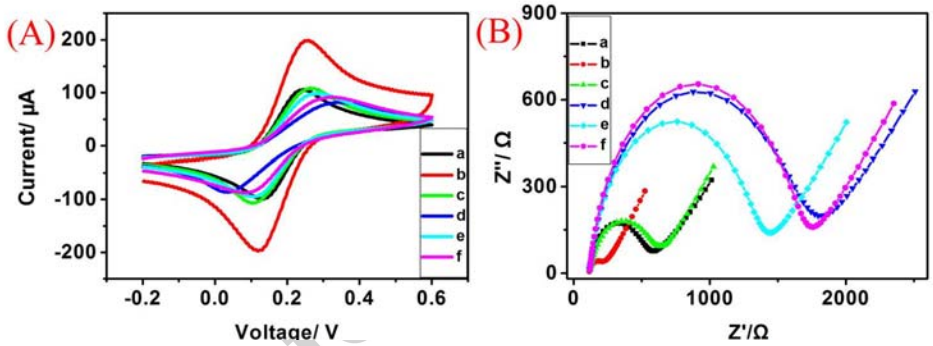

Figure 2

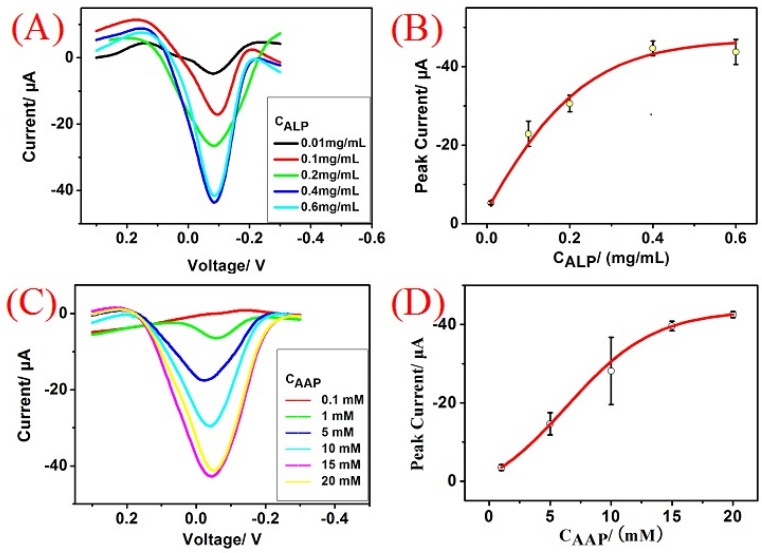

Figure 3

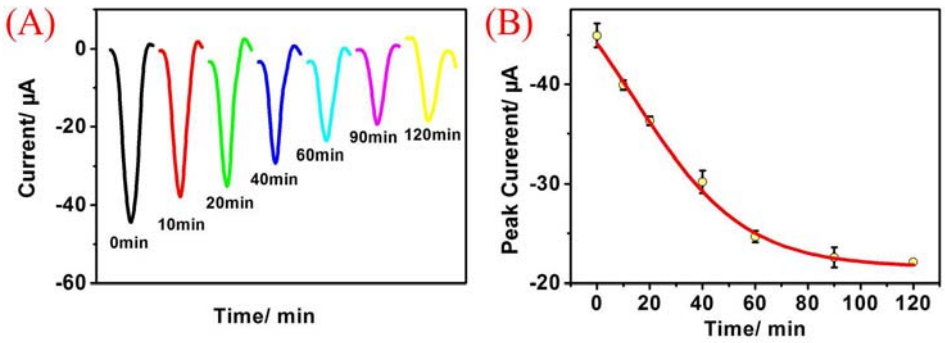

Figure 4

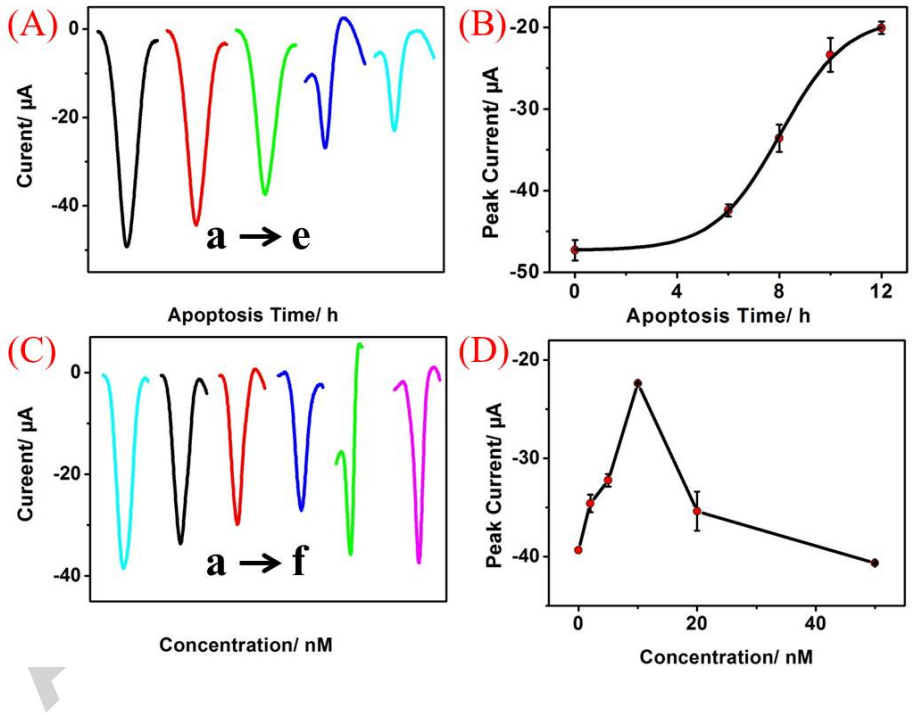

Figure 5

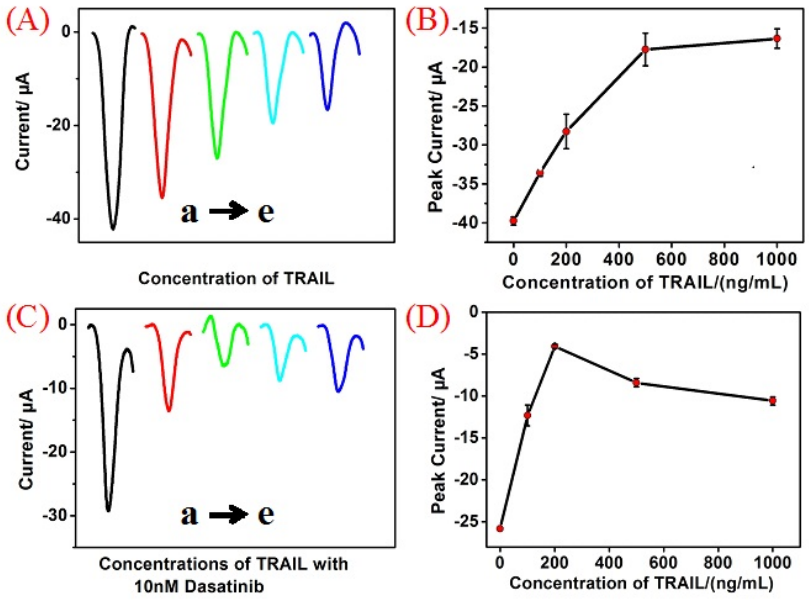

Figure 6