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Paper-based electrochemical cyto-device for sensitive detection of cancer cells and in situ anticancer drug screening

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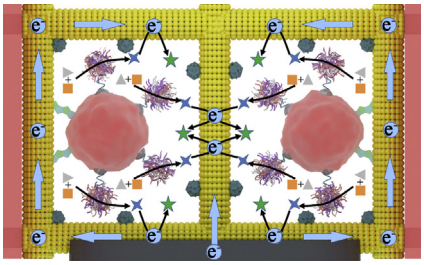
HIGHLIGHTS

- It is the first EC cancer cell detection in μ-PADs with high sensitivity.
- The 3D macroporous Au-PCE could inherently enhance the DPV response for cytosensing.
- Monitoring of multi-glycans expression is realized by in-electrode 3D cell culture.

GRAPHICAL ABSTRACT

In this work, a novel versatile microfluidic paper-based electrochemical cyto-device was fabricated for sensitive cancer cell detection and in situ screening of anticancer drugs in a multiplex manner based on in-electrode three-dimensional cell culture.



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ABSTRACT

In this work, using human acute promyelocytic leukemia cells (HL-60) as a model, a novel microfluidic paper-based electrochemical cyto-device (μ-PECD) was fabricated to demonstrate a facile, portable, and disposable approach for cancer cell detection and in situ screening of anticancer drugs in a high-throughput manner. In this μ-PECD, aptamers modified three-dimensional macroporous Au-paper electrode (Au-PE) was fabricated and employed as the working electrode for specific and efficient cancer cell capture as well as for sequential in-electrode 3D cell culture. This Au-PE showed enhanced capture capacity for cancer cells and good biocompatibility for preserving the activity of captured living cells. Sensitive cancer cell detection was achieved in this μ-PECD, which could respond down to four HL-60 cells in 10 μL volume with a wide linear calibration range from 5.0×10^2 to 7.5×10^7 cells mL^{-1} and exhibited good stability and reproducibility. Then, in situ anticancer drug screening was successfully implemented in this μ-PECD through monitoring of the apoptotic cancer cells after the in-electrode 3D cell culture with drug-containing culture medium, demonstrating its wide range of potential applications to facilitate effective clinical cancer diagnosis and treatment.

1. Introduction

Paper, one of the most important inventions in the human civilization and used for centuries, was endowed with transformative applications in 2007 in the innovative studies of Whitesides's group to construct microfluidic paper-based analytical

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devices (μ-PADs or lab-on-paper) [1] with the reduced dependence on expensive external instrumentation (e.g. no pumps are needed). The most obvious benefits of μ-PADs are the low-cost of paper and the highly developed infrastructure of the printing industry, making production of μ-PADs both economical and scalable. As an alternative to the first-generation lab-on-chip devices, the past six years have witnessed fast development and great research efforts in the field of μ-PADs [2–4] with focuses on developing novel fabrication techniques [5–8], inventing new functional concepts [9–12], and designing new prototype μ-PADs for analytical applications [13–17]. Moreover, lab-on-paper techniques represent promising and outstanding paradigms to portable and disposable devices for fluid manipulation, diagnostic testing, and environmental analysis, in which highly sensitive methods and complex functions must be combined with inexpensive, rapid, and simple fabrication and operation. Our group has also developed a series of analytical applications on μ-PADs for the sensitive detection of small molecules, proteins, and DNAs [18–21].

Recently, paper has been exploited as a novel platform for cytological or histological studies. The emerging paper-based cyto-devices [22–28] offer a promising potential to realize in situ, simple, miniaturized, and high-throughput cytological/histological researches in various area. As is well-known, cancer has been always the most pressing health concern, and until now, it remains the second leading cause of death worldwide [29,30]. It has been reported that early and accurate cancer diagnosis could provide an easier way to the effective and ultimately successful treatment of cancer [31]. Thus, sensitive and selective detection of cancer cells is important and fundamental to cancer diagnosis and therapy. Currently, various cyto-sensors, including chemiluminescent [32,33], fluorescent [34–38], electrochemical [39–44], electrochemiluminescence [45–49], and surface-enhanced Raman scattering [50] cyto-sensors, have been developed for cancer cell detection. Although these conventional approaches showed high sensitivity and selectivity for nondestructive detection of intact cancer cells, monitoring of cancer cells in μ-PADs would additionally provide a rapid, facile, inexpensive, portable, disposable, and high-throughput diagnostic tool to guide cancer treatment and therapy, especially in developing countries, resource-limited and remote regions.

The primary analytical technologies for cells on μ-PADs have relied on colorimetric methods [25,27,28] and fluorescent imaging techniques [22–24,26]. However, the semiquantitative “yes” or “no” answer of colorimetric methods is insufficient for early and accurate cancer diagnosis. Although fluorescent imaging techniques have a high detection sensitivity and rate, they are expensive, time-consuming, and requiring advanced instrumentation. Among the available detection methods on μ-PADs [2–4], electrochemical detection has attracted considerable interest which offers a considerable promise for cancer cell detection [51]. An additional advantage of electrochemical detection is simplicity of the instrumentation resulting in low electrical power requirements for in-field use [52]. Because of these features, its integration with paper-based cyto-device seems likely to be advantageous. To the best of our knowledge, no reports about electrochemical detection of cells in μ-PADs have been published. Therefore, in this work, a novel microfluidic paper-based electrochemical cyto-device (μ-PECD) with scalable and economical fabrication method was designed and fabricated to demonstrate the sensitive monitoring of cancer cells in μ-PADs by using fast-response differential pulse voltammetry (DPV) method. Human acute promyelocytic leukemia cells (HL-60), one of the most common fatal cancer cells that affects the blood and bone marrow [53], was used as a proof-of-concept cell line in this work to investigate the performance of this μ-PECD.

To enhance the sensitivity of this μ-PECD, the design and construction of biocompatible electrode interfaces that specifically target the cancer cells, competently maintain the normal activity of cells, and significantly facilitate the electron transfers between the electrochemical probes and electrode are of vital importance. Thus, in this work, Au-paper electrode [18,21,54] with three-dimensional (3D) incompact macroporous architecture, which is fabricated through the growth of a gold nanoparticle (AuNP) layer on the surfaces of cellulose fibers in the hydrophilic paper zone of paper electrode, was employed as the working electrode. In order to make this μ-PECD specific for HL-60 cell, cell-targeting aptamers, KH1C12, were chosen as molecular probes to functionalize this Au-paper electrode due to their salient properties, such as high affinity and specificity, ease of chemical modification, good stability, and low immunogenicity [55]. The electrochemical bioprobe used for cancer cell detection in this μ-PECD was the horseradish peroxidase labeled folic acid (HRP-FA). The HRP-FA bioprobe integrated both the specific recognition of folate receptor on the captured HL-60 cell surface by FA and amplification of electrochemical signal based on the HRP catalyzed oxidation of *o*-PD by H_2O_2.

Although the benefits of early cancer diagnosis were well documented, triggering of cancer cell apoptosis by anticancer drugs is another attractive chemotherapeutic strategy for leukemia [56]. Thus, to further expand the capacity of this μ-PECD, anticancer drug screening was successfully demonstrated in this μ-PECD through monitoring of drug-induced cancer cell apoptosis, which were obtained through in situ 3D culture of the captured cancer cells in Au-paper electrodes with drug-containing culture medium for a certain time. One important indicator of cell apoptosis is the translocation of the membrane phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane [57–59]. The principle of this proposed apoptosis monitoring in μ-PECD is that annexin-V can specifically recognize and bind to PS moieties in the presence of Ca^{2+}. Once PS molecules were externalized to the extracellular environment on the apoptotic cells, the interaction between HRP labeled annexin-V (HRP-annexin-V) bioprobe and PS became available. In addition, PS externalization cannot happen on the healthy or necrotic cells. Therefore, on the basis of the interaction between annexin-V and PS, the monitoring of cancer cell apoptosis in μ-PECD could be high specificity.

2. Experimental

2.1. Reagents and cells

All reagents were of analytical grade and directly used for the following experiments as supplied. Ultrapure water obtained from a Millipore water purification system (resistivity $\geq$ 18.2 MΩ cm) was used in all assays and solutions. Whatman chromatography paper #114 was purchased from GE Healthcare Worldwide and used with further adjustment of size (A4 size). Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, USA). HRP-annexin-V, HRP-FA, fluorescein isothiocyanate labeled FA (FITC-FA), and fluorescein isothiocyanate labeled annexin-V (FITC-annexin-V) were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Thiolated KH1C12 aptamer with high specificity for HL-60 was synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Tetrachloroauric acid (Au% > 48%), *o*-phenylenediamine (*o*-PD), cetyltrimethylammonium chloride, and H_2O_2 were obtained from Shanghai Chemical Reagent Co., Ltd. (China). Sylgard 184 polydimethylsiloxane (PDMS) base and curing agent were obtained from Dow Corning (Midland, MI). Sterile phosphate buffer saline (PBS, 0.01 M, pH 7.4) contained NaCl (136.7 mM), KCl (2.7 mM), Na_2HPO_4 (87.2 mM), and KH_2PO_4 (14.1 mM).