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Smartphone-Based Electrochemical Biosensors for Directly Detecting Serum-Derived Exosomes and Monitoring Their Secretion

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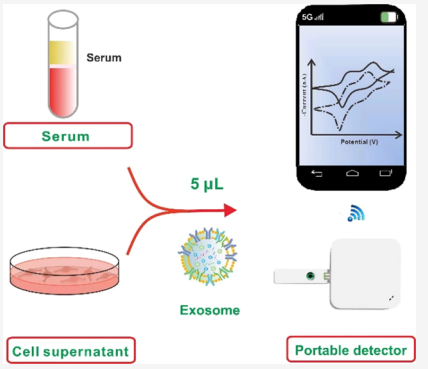
ABSTRACT: Exosomes are potential biomarkers, which play an important role in early diagnosis and prognosis prediction of cancer-related diseases. Nevertheless, direct quantification of exosomes in biological fluid, especially in point-of-care tests (POCTs), remains extremely challenging. Herein, we developed a sensitive and portable electrochemical biosensor in combination with smartphones for quantitative analysis of exosomes. The improved double-antibody sandwich method-based poly-enzyme signal amplification was adopted to detect exosomes. We could detect as low as 7.23 ng of CD63-positive exosomes in 5 μL of serum within 2 h. Importantly, we demonstrated that the biosensor worked well with microliter-level serum and cell culture supernatant. The biosensor holds great potential for the detection of CD-63-expressing exosomes in early diagnosis of prostate disease because CD63-positive exosomes were less detected from the prostate patient serum. Also, the biosensor was used to monitor the secretion of exosomes with the drug therapy, showing a close relationship between the secretion of exosomes and the concentration of cisplatin. The biosensing platform provides a novel way toward POCT for the diagnosis and prognosis prediction of prostate disease and other diseases via biomarker expression levels of exosomes.



INTRODUCTION

The intracellular multivesicular bodies first fuse with the cell membrane and are then released into the extracellular environment, and these small extracellular vesicles are defined as exosomes.[1−3] The exosomes have been discovered in diverse body fluids, such as plasma, saliva, lymph, ascites, semen, amniotic fluid, and cerebrospinal fluid.[4−10] They are believed to carry the transmembrane family proteins (CD63, CD81, and CD9)[11,12] and a cargo of lipids, proteins, DNA, mRNA, and microRNA from the parental cells and transfer this cargo to recipient cells.[3,13−15] Thus, exosomes can serve as extracellular messengers, raising the possibility of the enveloped exosome receptors in body fluids as novel noninvasive biomarkers for diseases.[16−19] However, exosome detection has been severely restricted by the technical difficulties in isolation and molecular analysis.

Differential ultracentrifugation, ultrafiltration, and density gradient separation are conventional methods to isolate and purify exosomes from cell culture supernatant and body fluids, which are time-consuming, tedious, and toilsome.[20−22] Affinity isolation with specific antibodies assembled on the beads was a high-cost method and limited because of the lack of specific protein, which also has low efficiency.[23,24] Hence, there is a growing demand for developing efficient and practical tools to detect and quantify exosomes originated from tumor to achieve diagnosis and prognosis prediction of cancer-related diseases. For exosome quantification, conventional methods such as enzyme-linked immunosorbent assay (ELISA) and western blots had low sensitivity and were laborious.[8,19,25] Recently, some new methods for exosome detection were developed, including microfluidic immune chips, surface plasmon resonance, frequency-locked microtoroid optical resonators, and so on.[26−48] Nevertheless, it is difficult to achieve point-of-care test (POCT) for these methods.

Currently, the medical care in many cases has to meet the requirements for the on-site test of patient care, which needs immediate acquisition of information on an individual's conditions, facilitating treatment decisions and further extensive testing in some specific environments. Electrochemical biosensors have held great potential in the analysis of exosomes.[31,49,50] They have played a significant role in the transition of medicine toward the development of point-of-care diagnostic devices.[31,49−52] These electrical devices are particularly useful for delivering the diagnostic information in a fast, simple, and effective fashion in connection to compact terminals such as mobile phones.[53−55]

Here, we report a novel portable electrochemical biosensor in combination with smartphones for sensitive electrochemical

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detection of exosomes. It took less than 2 h to achieve results of the quantitative analysis of exosome markers in serum and cell culture supernatant using screen-printed carbon electrodes (SPCEs) with fewer steps. The SPCE has been applied for mass production with very low cost and high reproducibility and has been adapted as a disposable biochip for wide use in the bioanalysis.[29,52,56] In our design, capture antibodies were immobilized on SPCEs to capture exosomes through the hydrophobic action of electrodes. The captured exosomes were then bound with multiple biotinylated detection antibodies because the exosomes could express many identical proteins and have little steric hindrance, followed by streptavidin-polyhorseradish peroxidase (SA-polyHRP)-based signal amplification. We could directly detect as low as 7.23 ng of exosomes in 5 μL of serum and cell culture supernatant without further pretreatment processes. For the CD63-positive exosomes, we found that CD63 on exosomes was less expressed in the case of serum from patients with prostate diseases. The CD63-positive exosome was also less expressed in human melanoma cells,[57] which is similar to our conclusion. The results demonstrated that the biosensor holds great potential to evaluate the expression change of CD63 and provides a new possibility for early diagnosis and prognosis monitoring of cancer. Also, the biosensor was used to monitor the secretion of the exosomes treated with drugs, demonstrating that the biosensor can be helpful for the drug screening for cancer-related diseases.

EXPERIMENTAL SECTION

Reagents and Materials. Antibodies adopted in our study were as follows: purified mouse anti-human CD63 (556019, BD Pharmingen), biotinylated anti-human CD63 (353017, Bio Legend), and purified mouse anti-human CD81 (MAB4615, R&D). Casein, bovine serum albumin (BSA), *cis*-platinum, Tween 20, and DMSO were purchased from Sigma-Aldrich. The TMB (K-blue low-activity substrate, H_2O_2 included) was ordered from Neogen (Lansing, US). Streptavidin-PolyHRP40 (SA-polyHRP40) and streptavidin-PolyHRP80 (SA-polyHRP80) were bought from Fitzgerald Industries International (Acton, MA). A Pierce bicinchoninic acid (BCA) assay kit was ordered from Pierce Chemical, Rockford, IL, U.S.A. Heat-inactivated exosome-depleted fetal bovine serum (FBS) was ordered from System Biosciences. A human prostate cancer cell line (PC-3) was purchased from the Type Culture Collection of the Chinese Academy of Sciences. NANOpure water (>18.0 MΩ, Milli-Q) was used in all experiments. Human serum from prostate-diseased (n = 40) and healthy donors (n = 20) was from RuiJin Hospital (North shanghai, China) volunteers with agreement in this study. All human subjects gave written and informed consent before participation in the experiments. Serum was aliquoted and kept at −80 °C until being used. The serum was filtered through a 0.22 μm filter (Millipore) before electrochemical detection. The collected cell culture medium supernatant was first centrifuged at 2000g for 10 min at 4 °C to remove the large cell fragments. To thoroughly remove cellular debris and extracellular vesicle, the supernatant was filtered through a 0.22 μm filter before direct detection. The SPCE used was homemade, and the electrochemical detection was performed on a universal mobile and portable electrochemical detector. The SPCE and portable electrochemical detector could also be purchased from Zhejiang Nanosmart Biotechnology Co., Ltd.

Cell Culture and Exosome Isolation. First, PC-3 cells were cultured in F12K nutrient mix medium supplemented with 10% heat-inactivated FBS containing 2 mM L-glutamine and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin) at 37 °C under conditions of 5% CO_2. Then, cells were washed twice with 10 mM phosphate buffered saline (PBS) and cultured in exosome-depleted F12K nutrient mix medium supplemented with 10% heat-inactivated exosome-depleted FBS for 48 h. After incubating for 48 h, exosomes were isolated from cell culture supernatant with the standard ultracentrifugation protocol with modification. The cell culture media supernatant was collected and centrifuged at 2000g for 10 min at 4 °C. To thoroughly remove cellular debris, the supernatant was filtered through a 0.22 μm filter. The resulting supernatant was collected and centrifuged at 10,000g for 20 min. Then, the supernatant was centrifuged at 110,000g for 70 min, and the precipitate was washed in PBS solution. Then, the solution was ultracentrifuged at 100,000g for 70 min at 4 °C and was resuspended in PBS solution. The protein concentration of the exosomes was determined using a BCA protein assay kit according to the manufacturer's instructions.

Characterization of the Isolated Exosomes. *Transmission Electron Microscopy Imaging.* Exosomes were first diluted in PBS and then were placed on carbon-coated EM grids for fixing for 15 min with 2% glutaraldehyde (Sigma, Deisenhofer, Germany). 2% uranyl acetate was used to stain for 5 min. The imaging was performed under an electron microscope (FEI Company, Eindhoven, Netherlands) equipped with a digital camera Keen View (SIS, Germany).

Atomic Force Microscopy Imaging. Exosomes diluted in PBS were adsorbed to freshly cleaved mica sheets for 3 min and were rinsed with deionized water and air-dried. Atomic Force Microscope (Nano Scope V controller, Bruker Instruments) in the tapping mode was used.

Measurement of the Size Distribution by Nanoparticle Tracking Analysis. Nanoparticle tracking analysis (NTA) was carried out with a Nanosight system. Isolated exosomes were diluted with 10 mM PBS and were ultrasonicated before analysis.

Establishment of the Smartphone-Based Electrochemical Biosensor. First, the preparation of SPCE was carried out as follows: The SPCE was washed in Milli-Q water before use, and then, the SPCE was blow-dried with nitrogen. Then, 7 μL of 50 μg/mL purified mouse anti-human CD63 as capture antibodies diluted in carbonate buffer (pH 9.6) was dropped on the working electrode and incubated at 4 °C overnight. The electrode was then washed with 10 mM PBS. The modified SPCE could be stored at 4 °C until being used. In the optimization experiment, the prepared SPCE was then incubated for 1 h at 37 °C with 50 μL of 2% BSA and 1% casein in 10 mM PBS and was washed with 10 mM PBS. Washing steps used here and during the whole analysis were essential to reduce nonspecific binding, and lack of the washing steps could deteriorate sensitivity.

Exosome Detection Based on the Electrochemical Biosensor. SPCE was incubated for 45 min at 25 °C with a 5 μL drop of exosomes at different concentrations, followed by washing with 10 mM PBS. Next, a 6 μL drop of 6.25 μg/mL biotinylated anti-human CD63 antibodies was added to the working electrode. After incubation for 0.5 h at 25 °C, the biochip was washed as the above method. The SPCE was then incubated for 15 min at 25 °C with a 5 μL drop of SA-polyHRP40 diluted with 10 mM PBS containing 1% BSA and

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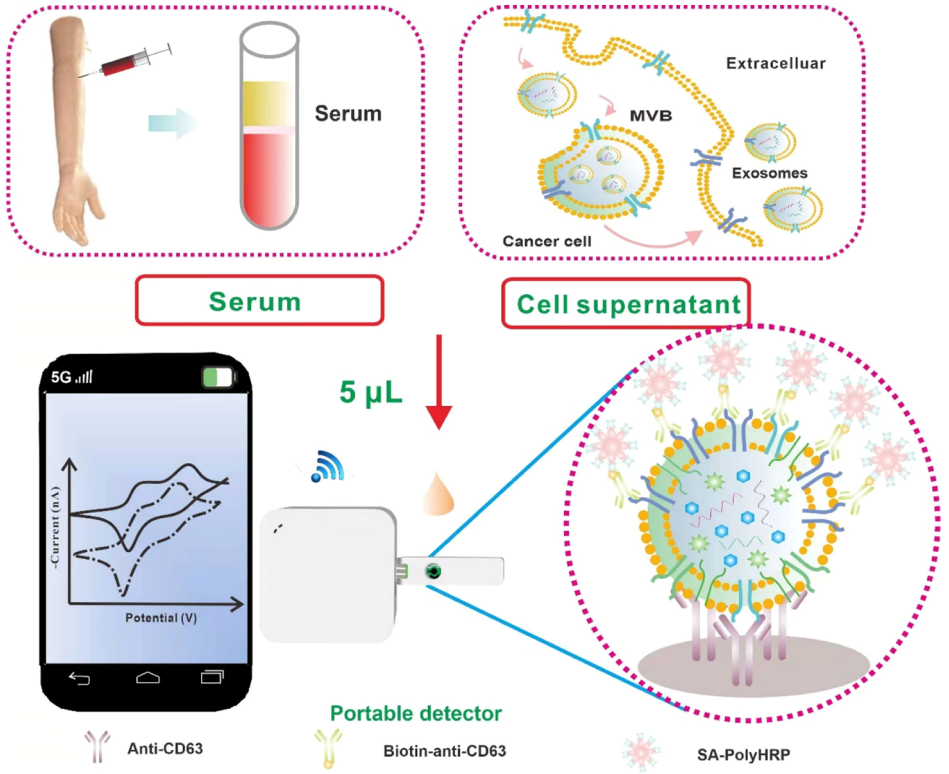
Scheme 1. Scheme of the Smartphone-Based Electrochemical Biosensor for Directly Detecting Serum-Derived Exosomes and Monitoring the Secretion of Exosomes



1% casein. After that, electrochemical detection was conducted in 50 μL of TMB substrate, using a homemade portable electrochemical detector and a universal smartphone equipped with a self-developed test app (Figure S1). Cyclic voltammetry (CV) was carried out at a scan rate of 100 mV/s, and the potential range was −0.3 to 0.6 V. The voltage of amperometry measurement was fixed at −50 mV. The electroreduction current was measured at 100 s after the HRP redox reaction reached a steady state. The detection of serum and cell culture supernatant was the same as above.

ELISA for Exosome Detection. Microtiter plates were coated with 50 μg/mL anti-human CD63 antibodies in carbonate buffer (pH 9.6) and incubated at 4 °C overnight. After washing with 0.01% Tween-20 in 10 mM PBS and blocking, purified exosomes were added and incubated for 2 h at 37 °C. After washing, the biotinylated anti-human CD63 antibody (5 μg/mL) was added and incubated for 1 h at 37 °C. After washing, the plate was incubated with SA-HRP diluted in blocking buffer for 0.5 h at 37 °C. After washing, TMB was added. The reaction was then stopped with 0.5 M H_2SO_4, and optical densities were recorded at 450 nm.

Cytotoxicity Assessments of Cisplatin. 10^5 cells were seeded in a 24-well plate and were incubated overnight for cell adherence. After washing with 10 mM PBS, cells were cultured with medium containing cisplatin at various concentrations. Cells without cisplatin were used as controls. After incubation for 3 days, the cell viability was determined from [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT assay (Sigma-Aldrich, Shanghai, China) and expressed as a percentage of $OD_{treatment}/OD_{control}$. All the experimental data of the cell survival rate were obtained from at least three independent experiments.

RESULTS AND DISCUSSION

Design of the Electrochemical Biosensor for Exosomes. Given the fact that a single exosome bears several surface-exposed copies of the CD63/CD81 protein,[58] our method adapted an electrochemically improved sandwich-type immunoassay configuration. We immobilized the antibody at the carbon interface of the screen-printed electrode through hydrophobic interaction overnight, and then, exosomes were captured using anti-human CD63/CD81. After sample incubation and exosome capture, biotin-modified mouse monoclonal antibodies for human CD63 (detection antibodies) were added to form a "sandwich"-type complex with exosomes, followed by the addition of SA-polyHRP to bind to biotin for signal output. For a single captured exosome, other copies of the CD63/CD81 proteins on the surface are exposed, and thus the captured exosome could allow the binding of a number of detection antibodies (relying on the number of CD63/CD81 proteins on each exosome), resulting in signal amplification (Scheme 1). Next, the binding of SA-polyHRP40 introduces further signal amplification because of its high catalytic activity as a poly-enzyme conjugate. The dual-stage signal amplification guarantees high-efficiency electrochemical signal output for the electrochemical biosensor, greatly improving its analytical performance.

The electrochemical measurements were performed on a smartphone-based device (Figure S1). To control the electrochemical biosensing reaction and process electrochemical signals, a smartphone was combined with a mini-electrochemical detector equipped with an electrode cartridge, and the android operating system could identify the portable EC detector through Wi-Fi or bluetooth and enabled fast readout (Scheme 1). The specific process of using smartphone for

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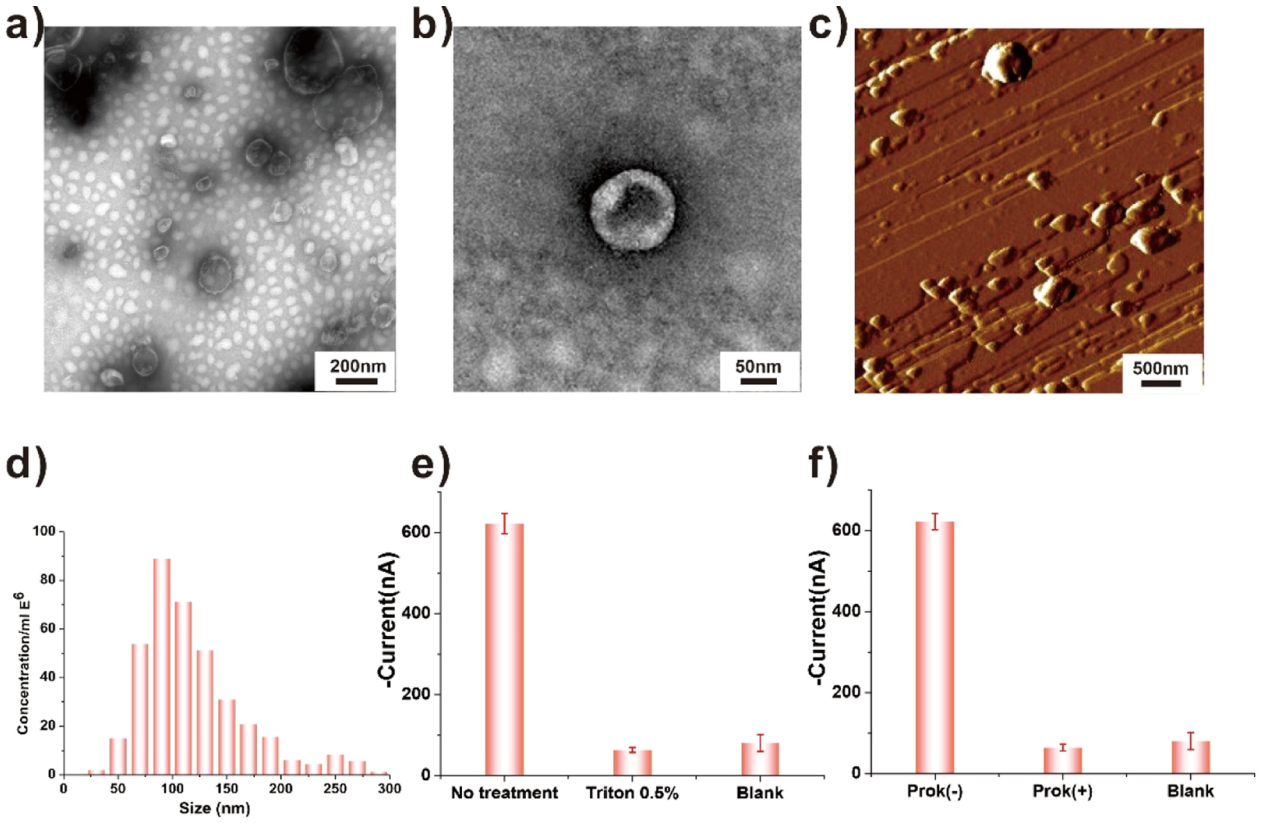

Figure 1. Characterization of exosomes and anti-CD63 recognition validation (a,b) TEM characterization of exosomes. (c) AFM amplitude image of exosomes. (d) Analysis of the size distribution of exosomes by the NTA. (e) Evaluation of electrochemical detection specificity against purified exosomes treated without or with 0.5% Triton X-100. (f) Evaluation of electrochemical detection specificity against purified exosomes treated with or without proteinase K. Exosomes were detected using CD63 antibodies. Exosomes were isolated and purified from PC-3 cell-cultured supernatant.

detection can be seen from the video screenshot in Figure S2. The disposable SPCE used here was a three-electrode system, which consists of a carbon working electrode (3 mm diameter), a carbon auxiliary electrode, and an Ag/AgCl reference electrode. The insulating layer was printed around the working area to form a reservoir of the electrochemical microcell. The SPCE could be inserted into the USB port of the portable EC detector and communicate with the mobile phone. SEM imaging results showed that the SPCE electrode had a rough surface with a larger surface area, which is more efficient for immobilization of capture antibodies, increasing significantly the capture ability of the biosensing electrode chip (see Figure S3 in Supporting Information for more detailed information) and improving the detection sensitivity of the electrochemical biosensor.

Validation of Exosome Isolation and Anti-CD63 Recognition. Exosomes used for the development of our biosensor were isolated from the prostate cancer cell line PC-3 by ultracentrifugation. TEM imaging was used to characterize the purified exosomes, showing particles with the classical cup-like morphology of exosomes. The cup shape might be formed during the drying process,[59] with a diameter of ~90 nm for most of particles (Figure 1a,b). AFM imaging (tapping mode) was used to characterize the exosomes, showing aggregation in bulging or irregular morphology (Figure 1c). The size distribution was characterized by NTA, showing that the diameter of particles ranged from 50 to 200 nm (see Figure 1d). Note, the NTA result showed that the size of the most of particles were 119 nm, larger than that characterized by TEM. This phenomenon might be caused due to the dehydration of exosomes during sample preparation process for TEM imaging. Because exosomes from different cell lines differ from each other, the exosome purified here was in the rational range. The morphological and physical characteristics determined using these methods corroborate with previous evidences.[32,60]

In our design, the capture antibody and the detection antibody for CD63 came from the same cell clone and can bind to the same epitope. In order to validate that only the intact exosome could be recognized by two antibodies and detected with the biosensor, the exosome was treated with Triton and proteinase K for disruption (Figure 1e,f). The biosensing results showed that the resulting current signal was almost the same as the background one while exosomes were disrupted by the treatment, indicating that the current signal came from the capture of intact exosomes rather than something else such as membrane fragments and protein complexes.

Optimization of the Electrochemical Biosensor. For the improvement of biosensor performance, it is vital to select antibodies and signaling probes. The right antibody would result in high-efficiency capture and binding of exosomes. Also, a right signaling probe would produce high-efficiency signaling amplification and a high signal-to-noise ratio. Because transmembrane superfamily proteins such as CD63 and CD81 are the most abundant protein markers found in exosomes isolated from any cell type virtually, the selection of the capture antibody was vital for the quantification of exosomes. Even though previous studies reported different

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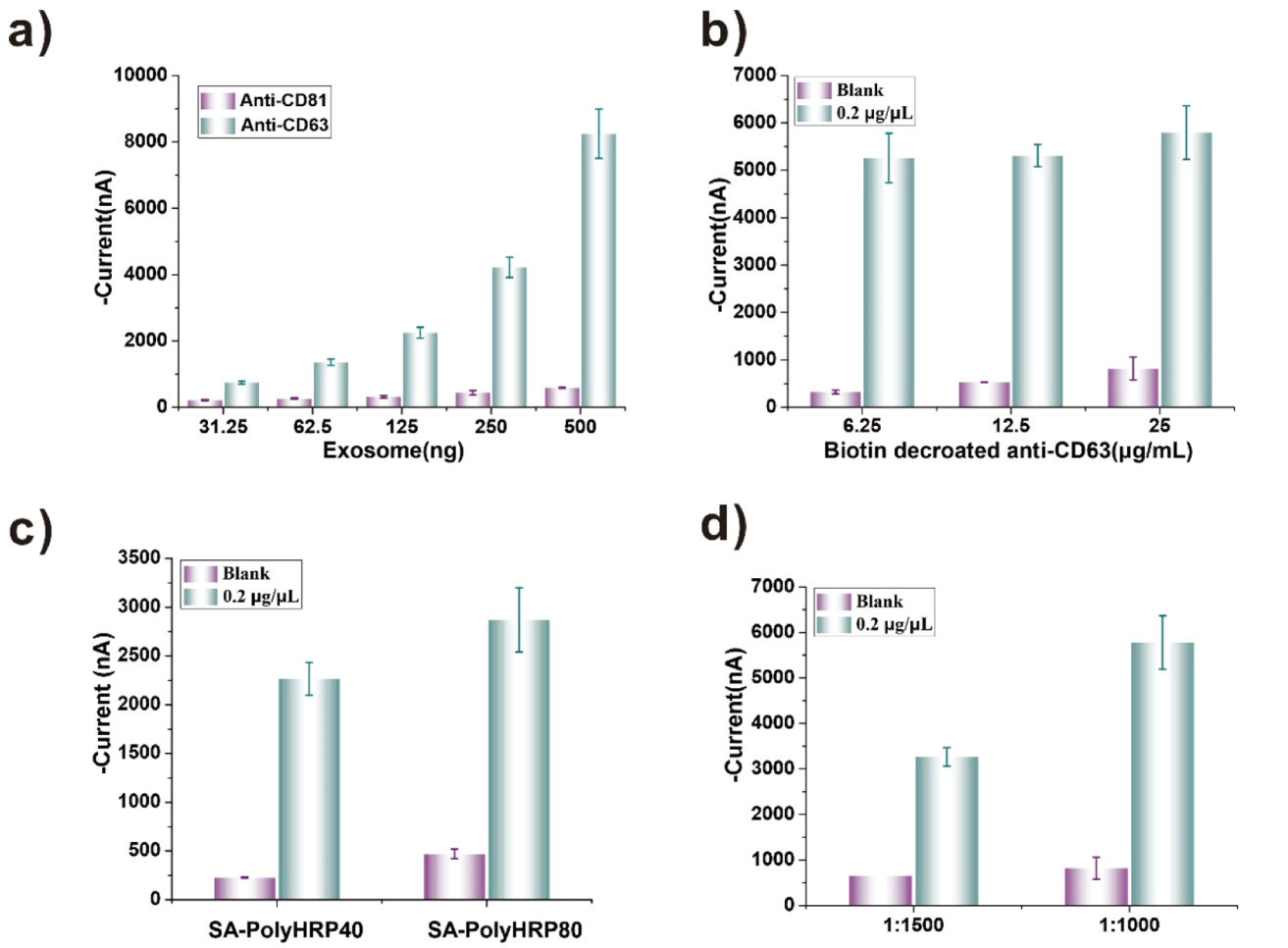

Figure 2. Optimization of the smartphone-based electrochemical biosensor (a) Comparison between the antibody CD63 and CD81 as capture antibodies. (b) Optimization of the concentration of the signal probe in detection without and with 1 μg of exosomes in 5 μL volume. (c) The comparison between the SA-polyHRP40 and SA-polyHRP80 in detection without and with 1 μg of exosomes in 5 μL volume. (d) Optimization of the concentration of SA-HRP40 in detection without and with 1 μg of exosomes in 5 μL volume.

markers to identify exosomes, CD63 and CD81 are commonly found at the plasma membrane.[61,62] Thus, we used electrodes modified with anti-CD63 and anti-CD81 to capture exosomes isolated from the supernatant of the PC-3 cell line. The biosensing results showed that the current signal of anti-CD63 immobilized electrodes was obviously higher than that of anti-CD81 (Figure 2a), probably because the expression of CD63 on exosomes is much higher than that of CD81. Then, we optimized the concentration of the detection antibody. We found that the background current values decreased significantly with the decreasing antibody concentration while the positive current signal values had minor changes. When 6.25 μg/mL antibody was used, the highest signal-to-noise ratio was obtained (Figure 2b). In our design, a SA-polyHRP conjugate was used to improve the sensitivity of the biosensor. After comparing the signal amplification efficiency between the detection effect of SA-polyHRP40 and SA-polyHRP80, we found that SA-polyHRP40 had a higher signal-to-noise ratio than SA-polyHRP80 (Figure 2c) because the latter caused much higher background signals. Also, we found that higher positive signals and low background signals could be obtained when the dilution rate of SA-polyHRP40 was 1:1000 (Figure 2d).

Exosome Quantification with the Electrochemical Biosensor. To investigate the quantitative ability of the biosensor, different concentrations of isolated exosomes had been detected. As shown in Figure 3a, the typical HRP-based electrocatalytic process was observed in cyclic voltammograms. The reduction peak located at −50 mV apparently increased, resulting in a pair of asymmetric redox peaks that characterized the occurrence of electrocatalysis. Amperometry provided a more direct way to characterize such a HRP-catalyzed electrochemical process (Figure 3b). Upon the onset of the potential at −50 mV, we could observe a current (I) versus time (t) curve and the current reached a steady state within 100 s. The background current was as low as 219 nA, suggesting the low non-specific binding of SA-polyHRP. Significantly, when detecting 500 ng of exosomes, the current signal reached 8246 nA, while a very high signal-to-noise ratio of 37.6 was obtained. A good linear dose response was achieved in the range from 0 to 500 ng of exosomes. Regression equation: $Y = 218.28 + 16.62X$; R^2 value: 0.999, while only 5 μL of samples were used. The detection limit could be as low as 7.23 ng (background+3SD). The zoomed-in data points in lower ranges can be seen in Figure S4, and the LOD was calculated with background+3SD. The biosensor has a wider working range, compared with ELISA (Figure 3d). We found that SA-polyHRP40 had lower background signals than SA-polyHRP80 with a higher signal-to-noise ratio (Figure 2c) with the EC biosensor. In conventional ELISA reaction, the

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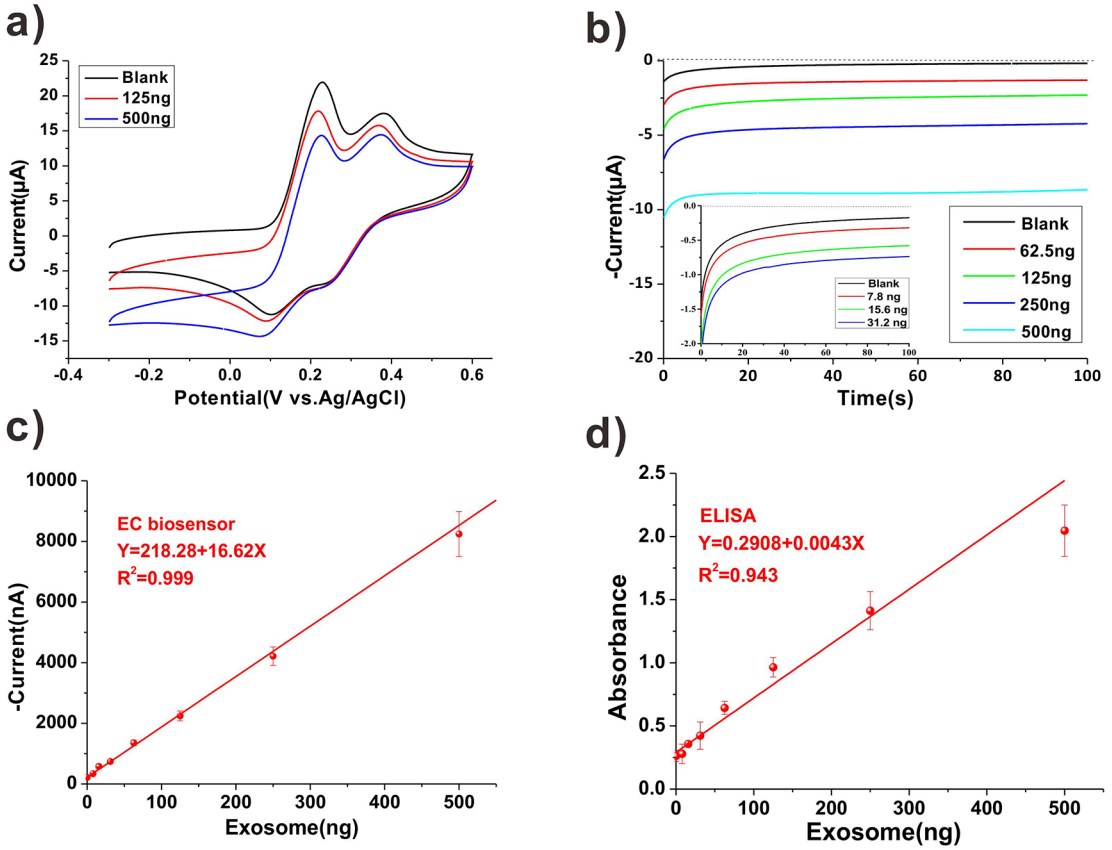

Figure 3. Exosome quantification analysis validated the quantitative ability of the smartphone-based electrochemical biosensor. (a) Cyclic voltammograms for the electrochemical biosensor in the absence and presence of exosomes. (b) Amperometry curves (i–t) for testing with a series of exosome concentrations. The potential was held at −50 mV. (c) Correlation curve for CD63-positive exosomes. (d) Correlation between ELISA measurements for CD63-positive exosomes in a dilution series. Data are representative of at least three experiments.

most commonly used reporter molecule is SA-HRP. If SA-polyHRP40 is used as a reporter in ELISA reaction, it may also cause relatively higher background signal noise, which would reduce the sensitivity of ELISA. Thus, we compared between the two assays with polyHRP40 and SA-HRP, respectively. An overall comparison showed that the biosensor was superior for the electrochemical detection of exosomes to conventional ELISA for the laboratory detection (Table 1).

Table 1. Comparison of the Electrochemical Biosensor and ELISA[a]

	EC biosensor	ELISA
sample volume	less than 10 μL	50–200 μL
dynamic range	3 logs	2 logs
sensitivity[b]	high (7.23 ng)	low (24.5 ng)

[a]ELISA. [b]Calculated by CD63/CD63 analysis (Blank+3 standard deviation).

Direct Analysis of Clinical Serum Samples. To investigate the clinical feasibility of the biosensor, we detected exosomes in serum samples from clinical patients without purification. Our biosensor has the ability to block the nonspecific absorption so that serum exosomes could be captured and detected without purification. The SPCE used here not only has good conductivity and high loading capacity for capture antibodies but also allows the preferential adsorption of background proteins in the serum such as human serum albumin, which could play the role of blocking the electrode. Furthermore, a single captured exosome could bind to multiple biotinylated detection antibodies and SA-polyHRP (the conjugate of multiple HRP molecules), resulting in great amplification for positive signals. Thus, the electrochemical biosensor could achieve a good signal-to-noise ratio in the direct detection of serum samples. The level of CD63-positive exosomes in serum samples was compared between 40 prostate-diseased and 20 healthy individuals. Analyzing exosomes in human clinical samples showed that the current signals of exosomes were highly heterogeneous between patient and control samples and could conclusively differentiate patients from healthy individuals. The levels of CD63 expressed on exosomes were significantly lower in prostate patients ($P < 0.005$ for CD63; one-way ANOVA test) than in healthy groups (see Figure 4a). For prostate cancer, the increase in biomarkers such as survivin expressed on exosomes has been validated as reported,[63] which would cause a decrease in the commonly expressed protein such as CD63. Thus, the decrease in CD63-expressing exosomes could be a signal for the occurence of prostate disease. CD63 is usually used as a universal marker to capture and detect exosomes secreted by a variety of cancer cells. We selected a limited number of case samples to compare the CD63-positive exosomes between prostate-diseased and healthy donors. Analyses with receiver operating characteristic curves (ROC) determined an intrinsic specificity of 87.5% and a sensitivity of 85% for patients and the negatives (Figure 4b). These results demonstrated that the

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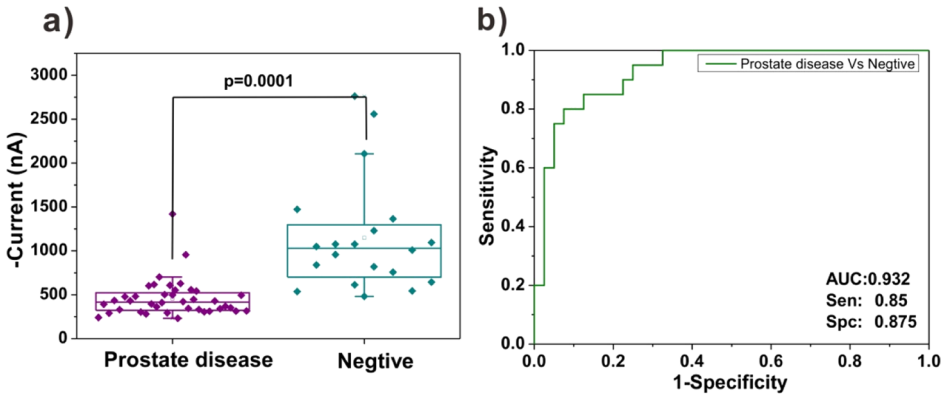

Figure 4. Electrochemical detection of exosomes validated the feasibility of direct analysis of serum from clinical patients. (a) Serum levels of CD63-positive exosomes in prostate patients and negative samples without extra purification. The panel shows a scatter plot for prostate diseases including cancer patients and benign prostate hyperplasia (n = 40) and healthy individuals (n = 20). The P-value was calculated by using one-way ANOVA test. (b) ROC curves between healthy donors and prostate disease patients assessing with CD63-positive exosomes.

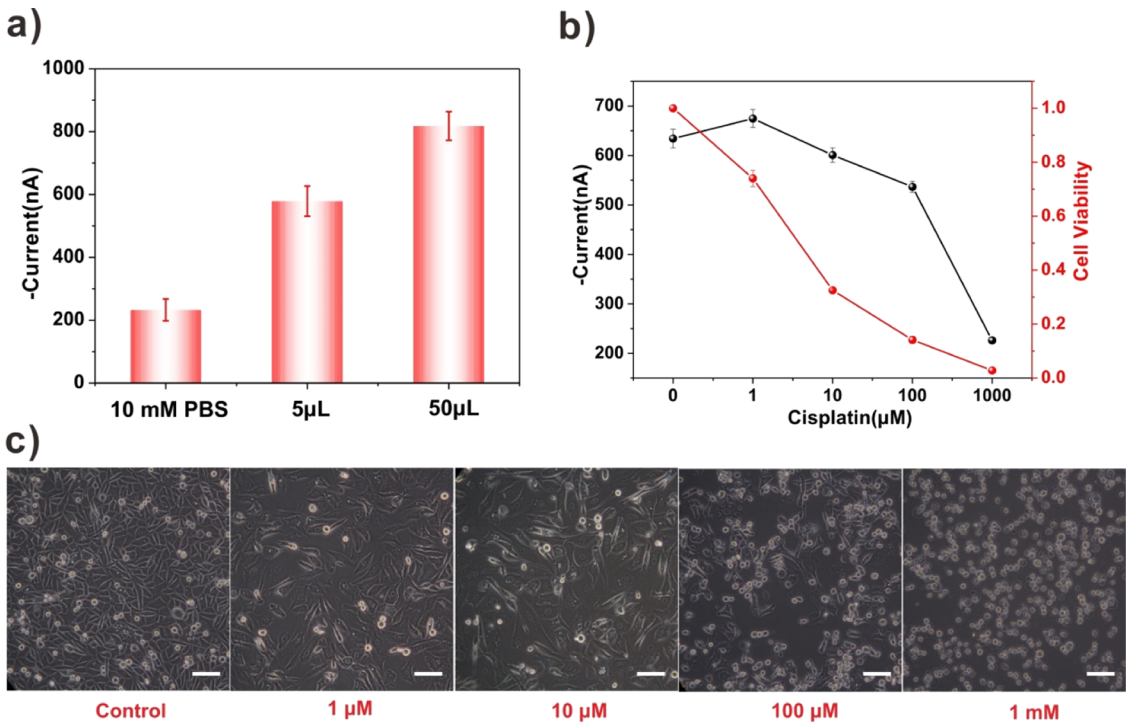

Figure 5. Electrochemical detection of exosomes validated monitoring the regulation of exosome secretion by cisplatin. (a) Electrochemical detection of exosomes in the cell culture supernatant without purification after incubation for 3 days with the CD63-positive detection system. (b) PC-3 prostate cancer cells were treated with increasing doses of cisplatin for 3 days, and dose response of PC-3 cells when treated with cisplatin with MTT. (c) Morphology of PC-3 cells treated with different concentrations of cisplatin for 3 days. The scale bar is 100 μm.

biosensor could detect circulating exosomes in serum samples without the need of an additional purification process. Especially, as low as 5 μL of serum would be enough for the detection. Therefore, it can be a powerful tool for early diagnosis of diseases through exosomes. Importantly, these results showed that the biosensor held a great promise for rapid disease diagnosis through exosomes toward POCT from biofluid samples.

Monitoring the Regulation of Exosome Secretion. Exosomes can not only be the biomarker for the diagnosis of cancer diseases but also be important indicators for the prognosis prediction of cancer and other diseases. Having demonstrated that the biosensor could be applied in direct detection of exosomes, we tried to verify that it was also a useful tool for monitoring the change in exosome levels modulated by drugs. It has been reported that cisplatin is a broad-spectrum anticancer drug and can be used as a first-line treatment for the adjuvant treatment of many cancers.[64,65] Although it was known that cisplatin could affect exosome release under non-cytotoxic doses, there has been few quantitative studies on the magnitude of the exosome excretion, which is very important for the drug usage of patients.[64,66] Here, for a quantitative study to assess the effect of the anti-cancer drug, we used the developed biosensor to detect exosome secretion changes for PC-3 cells treated with cisplatin, the first FDA-approved platinum compound for cancer treatment. First, we challenged the biosensor for the direct detection of exosomes in a cell culture supernatant

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without purification. Figure S4 shows that the CD63-positive exosomes were secreted by the PC-3 cell line after culturing for 3 days and could be obviously detected with the electrochemical biosensor. Also, no significant current difference was found between the FBS and the exosome-depleted FBS, indicating that the exosome detection was not interfered by intrinsic exosomes in FBS added to cell culture medium. Then, exosomes in the cell culture supernatant were directly detected after the PC-3 cell's incubation for 3 days. Only 5 μL of culture medium was needed for the detection, and the signal-to-noise ratio was 2.5 (see Figure 5a). Interestingly, under the drug treatment for PC-3 cells, exosomes in the cell culture supernatant slightly increased first and then decreased with the increase in cisplatin concentration, indicating the change in drug efficacy. With 1 μM cisplatin, the secretion of CD63-positive exosomes had been reinforced to some extent, which is consistent in lung cancer A549 cells.[64] The continuous decline of current signals indicated the continuous decrease of secreted exosomes, revealing the death of more and more PC-3 cells. The results were also verified by a cytotoxicity test. Figure 5b shows that the viability of cells had decreased as the concentration of the drug increased, demonstrating that the activity of the cells strongly depended on the duration of different concentrations of cisplatin. Also, cell imaging further confirmed a concentration-dependent mechanism for cisplatin-induced cytotoxicity (Figure 5c), implying that cisplatin could regulate the excretion of exosomes at specific concentration. The above results demonstrated that the biosensor could become an available tool to monitor the efficacy of chemical therapy for cancers.

CONCLUSIONS

In summary, we developed a rapid, sensitive, and portable biosensor in combination with smartphones for the quantification of exosomes. Exosomes in serum samples and the cell culture supernatant could be directly detected with the biosensor without jumbled isolation and purification processes, allowing the potential of POCT-based diagnosis and prognosis prediction of diseases through the biomarkers on exosome. Notably, the biosensor could facilitate the early diagnosis of various diseases, in which the expression of exosomes may change at their early stage. As shown in the example of prostate diseases, we found that the expression of CD63 in the exosomes of patients with prostate disease decreased in comparison with healthy donors. Because CD63 is a universal but not specific marker, we can infer that the increase in some specific markers on the exosomes of prostate disease leads to the decrease in the universal CD63 expression. Therefore, the decrease in CD63-positive exosomes is also an indicator of prostate disease. Importantly, different antibodies can be modified on the biosensing surface in the same way to capture different kinds of exosomes, making it possible to detect specific biomarkers on exosomes of other diseases. Being a novel biosensing platform for the on-site rapid detection of circulating exosomes, the biosensor could become a potential tool for assisting POCT diagnosis of diseases, helping to identify novel biomarkers and providing support for anticancer drug screening.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c04910.

Photograph of the smartphone-based instrument, specific process of using smartphone for detection, SEM of the working electrode, histogram showing the LOD of exosome assay with the EC biosensor and ELISA, and comparison of the FBS and the exosome-depleted FBS with the CD63 detection system (PDF)

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S.S. and J.S. conceived the study. J.S. designed and performed the experiments. J.S., S.C., X.D., and S.S. discussed the results. J.S., S.C., Y.D., Z.Z., X.J., X.D., and S.S. wrote and refined the article. All authors have read and approved the final article.

Notes

The authors declare no competing financial interest.

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