Interrogating Circulating Microsomes and Exosomes Using Metal Nanoparticles

*Yi-Ge Zhou, Reza M. Mohamadi, Mahla Poudineh, Leyla Kermanshah, Sharif Ahmed, Tina Saberi Safaei, Jessica Stojcic, Robert K. Nam, Edward H. Sargent, and Shana O. Kelley**

Prostate cancer (PCa) remains the second leading cause of cancer-related deaths in men.[1] According to the International Agency for Research on Cancer, there were more than 307 000 deaths from PCa worldwide in 2012 (accounting for 7% of male deaths). Although PCa is treatable at the early stages, it becomes castration resistant at the advanced stages and difficult to treat.[2,3] The level of prostate-specific antigen (PSA) is often used for screening of PCa and to monitor the progression of castration-resistant PCa;[4] however, PSA is not always reliable as a PCa biomarker due to its low specificity and the fact that benign conditions can also be associated with a PSA increase.[5] It is, therefore, necessary to develop novel biomarkers that are cancer specific and also detectable early in the course of PCa.

Exosomes (30–100 nm in diameter) and microsomes (100–1000 nm in diameter) are small vesicles secreted by most mammalian cells, and carry factors facilitating intercellular communication.[6] Since tumors are known to shed exosomes and microsomes that carry specific proteins, functional mRNAs, microRNAs, and DNAs, these tumor-secreted vesicles, especially exosomes, provide a potential biomarker resource for noninvasive diagnosis without the need to invasively access the tumor.[7,8] Using exosomes as markers is promising to overcome current technical challenges that still exist in cancer detection, such as the difficulty of early detection, the expense of invasive screening, low selectivity, and false-positive results. However, the detection of exosomes is challenging given the technical difficulties inherent in the isolation and molecular analysis of these nanoscale and molecularly diverse vesicles in clinical samples where billions of blood cells are present.[9]

The most commonly used isolation approaches are based on differential ultracentrifugation steps (often accompanied by multistep filterings).[10,11] These approaches are time consuming (>10 h) and require centrifugation up to 200 000 g.[12] Separation using affinity purification with specific antibodies (e.g., anti-CD63 or EpCAM) is another option although this method depends on the presence of target proteins.[13] Next-generation techniques have emerged based on this principle, such as affinity-binding beads,[14] microfluidic immunocapture[15–17] and alternating current electrohydrodynamic-induced nanoshearing for more efficient capturing.[18]

In addition to exosome isolation, the characterization of exosomes has been pursued intensely. Information on size, concentration, and morphology of exosomes can be obtained by optical and nonoptical methods such as electron microscopy, but these approaches cannot quantitate levels of these markers in patient samples. Conventional optical methods for cell analysis, such as dynamic light scattering and fluorescence microscopy, are not able to provide useful information due to their high detection limits.[19] Exosomal miRNAs, mRNAs and DNAs can be measured by PCR and agarose gel electrophoresis.[20,21] However, exosomes have to be lysed before the detection, which adds to the complexity of the testing. Exosomal proteins are typical markers for exosome detection, and are commonly detected by immunoaffinity-based approaches (e.g. enzyme-linked immunosorbent assay (ELISA) and western blot).[12] Unfortunately, these methods have shortcomings including poor sensitivity and highly manual workflows. Recently, surface plasmon resonance imaging was used for quantitative and label-free detection of tumor-derived exosomes, but this detection strategy relies on a customized instrument.[17]

Dr. Y.-G. Zhou, Dr. R. M. Mohamadi, Dr. S. Ahmed, Prof. S. O. Kelley
Leslie Dan Faculty of Pharmacy
University of Toronto
Toronto, ON M5S 3M2, Canada
E-mail: shana.kelley@utoronto.ca

M. Poudineh, T. Saberi Safaei, Prof. E. H. Sargent
Department of Electrical and Computer Engineering
University of Toronto
Toronto, ON M5S 3G4, Canada

L. Kermanshah, Prof. S. O. Kelley
Institute of Biomaterials and Biomedical Engineering
University of Toronto
Toronto, ON M5S 3M2, Canada

J. Stojcic, Prof. R. K. Nam
Division of Urology
Sunnybrook Research Institute
University of Toronto
Toronto, ON M4M 3M5, Canada

Prof. S. O. Kelley
Department of Biochemistry
University of Toronto
Toronto, ON M5S 3M2, Canada

DOI: 10.1002/smll.201502365

www.MaterialsViews.com

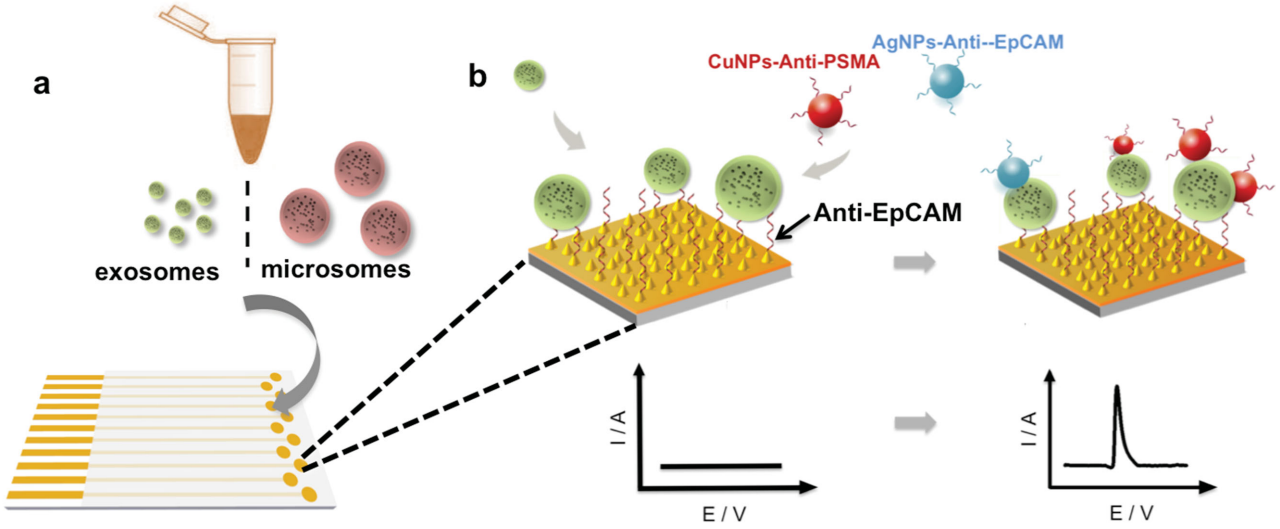

Figure 1. Schematic representation of the two-step isolation and analysis of exosomes and microsomes: a) capture step where vesicles are immobilized on aptamer-modified sensors, b) electrochemical detection of the captured exosomes/microsomes with Cu and Ag nanoparticles.

Electrochemical sensors have shown advantages such as fast response, simplicity, and low cost,[22] but have not been applied to the detection of exosomes and microsomes with clinically-relevant levels of sensitivity. Here, we describe a microfabricated chip with multiplexed gold sensors for the electrochemical analysis of the captured exosomes and microsomes. Electro-oxidation of metal nanoparticles (MNPs) is used to simultaneously report on the presence of specific surface markers on exosomes and microsomes. Silver nanoparticles (AgNPs) are used to report on EpCAM (epithelial cell adhesion molecule), a ubiquitously expressed epithelial cancer marker, while copper nanoparticles (CuNPs) are used to report on PSMA (prostate-specific membrane antigen), a biomarker enriched in exosomes and microsomes from PCa cells.[12,23] We used a simple centrifugation procedure that isolates exosomes and microsomes from cell culture supernatant or directly from prostate patient serum within 30 min with a maximum centrifugation of 16 100 g. Our results from the analysis of serum-derived exosomes and microsomes from PCa clinical samples show a significant increase in the levels of both EpCAM- and PSMA-containing vesicles over healthy controls, showing good selectivity and potential application of this technology in PCa monitoring. The present electrochemical assay exhibits a limit of detection (LOD) of 50 exosomes/sensor, showing higher sensitivity over recently reported approaches.[6,18,24]

To investigate the applicability of a nanoparticle-labeling strategy in the detection of exosomes or microsomes, we fabricated a sensor chip containing 11 individual circular gold electrodes that enables multiplexed readout (**Figure 1**a). Each electrode was electroplated with gold to form a layer of nanoflaked structure for more efficient capture of the vesicles (see Figure S1, Supporting Information for SEM image of the electrode after plating). As shown in Figure 1b, in order to specifically capture epithelial exosomes or microsomes, the surface of the nanostructured sensors was modified with thiolated anti-EpCAM aptamers. Epithelial exosomes or microsomes were then applied to the sensors for capture. For electrochemical readout, AgNPs and CuNPs (see Figure S2 for TEM characterization) were chosen as probes, because their oxidation potentials fall in the potential window of the gold electrodes and are well separated, allowing multi-marker detection. AgNPs and CuNPs were modified with thiol-conjugated anti-EpCAM aptamers and anti-PSMA aptamers, respectively, and the resulting MNP–aptamer conjugates were incubated with exosomes or microsomes captured by the chip to specifically recognize the corresponding surface markers. Linear sweep voltammetry (LSV) was then applied for the direct oxidation of AgNPs or CuNPs showing an electrochemical peak that suggests the presence and the amount of the surface markers.

Exosomes and microsomes were isolated from VCaP cells (a cell-based model of human PCa) as a culture supernatant and from serum of PCa patients by a three-step centrifugation process. To validate our isolation procedure, we examined the morphology of the resulting exosomes (**Figure 2**a,d) and microsomes (Figure 2b,e) by transmission electron microscopy (TEM) characterization, which revealed that both vesicles were round in shape and uniformly distributed. The size distribution was quantitated (Figure 2c,f) by TEM analysis of 50 exosomes or microsomes, and shows that VCaP-derived exosomes and microsomes exhibit sizes ranging from 30 to 60 nm and 200 to 450 nm, respectively, while serum-derived exosomes and microsomes show sizes that are slightly larger. We also noticed from the TEM images the existence of vesicles with much smaller sizes among microsomes, likely exosomes that were not removed from the supernatant. No intact cells were detected.

To validate the idea of using extracellular vesicles (EVs) as a potential quantitative source of cancer markers and the direct MNP electrochemical strategy, we carried out the electrochemical analysis of VCaP-derived exosomes with differing concentrations. AgNPs–anti-EpCAM conjugates were used as markers to detect EpCAM, a transmembrane protein expressed exclusively in epithelial cells and epithelial-derived neoplasms and often used as a diagnostic marker for

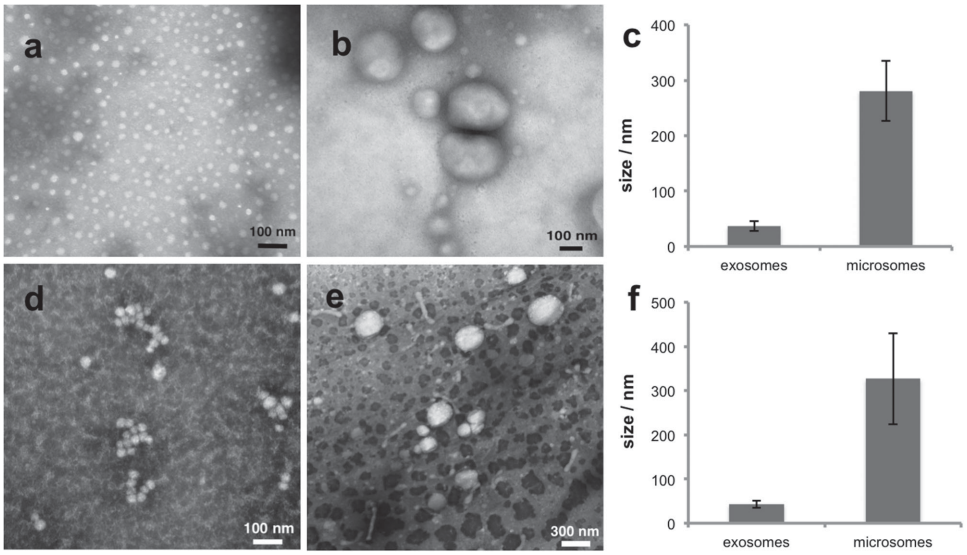

Figure 2. TEM characterization of a) exosomes and b) microsomes, and their size distributions c) from VCaP cell culture supernatant; d) exosomes and e) microsomes and their size distributions f) from serum of PCa patient.

cancers. CuNPs–anti-PSMA conjugates were used to detect PSMA, a PSA that is enriched in exosomes from PCa cells. In this experiment, VCaP cells were seeded at a density of 0, 2×10^4, 2×10^5 and 1×10^6 cells per well in a six-well plate. After 72 h of incubation, cell culture supernatant was collected as the source of exosomes and the control. As shown in **Figure 3**a, an oxidation peak at around 350 mV versus Ag/AgCl was detected, corresponding to the direct oxidation of AgNPs, which confirms that EpCAM is expressed on VCaP cell-derived exosomes. Similarly, Figure 3c shows an oxidation peak of CuNPs at around 600 mV, corresponding to the detection of PSMA on VCaP cell-derived exosomes. As shown in Figure 3b,d, the charges integrated from the oxidation peak of both AgNPs–anti-EpCAM and CuNPs–anti-PSMA increase as a function of the number of cells that produce exosomes, indicating that an increasing level of exosomes was secreted from higher number of cells.

It is noteworthy that the intensity of the charge for the detection of CuNPs–anti-PSMA shows a seven-to-eightfold of increase over that of the detection of AgNPs–anti-EpCAM. There are three factors that may account for this observation. First, the oxidation of Cu is a two-electron transfer process while the oxidation of Ag is one-electron transfer. Second, the CuNPs are larger than the AgNPs, resulting in different loadings of metal bound to exosomes. Finally, flow cytometry analysis (Figure S3, Supporting Information) shows that the

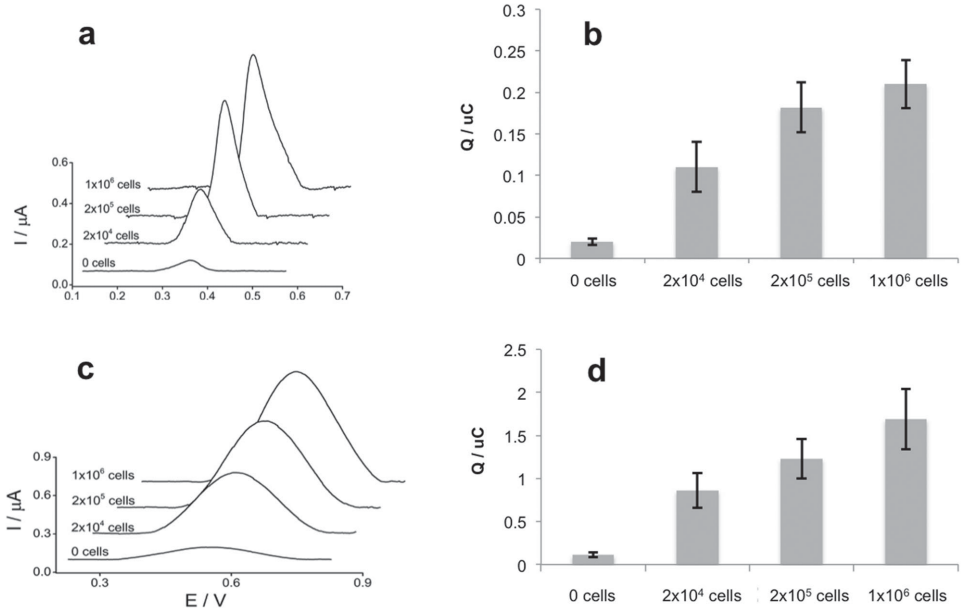

Figure 3. Evaluation of the electrochemical sensor performance for exosome detection. LSV profile showing the signal intensity of exosomes secreted from differing number of VCaP cells using a) AgNPs–anti-EpCAM and c) CuNPs–anti-PSMA as markers. Intensity of charges integrated from the oxidation peaks was shown in b) and d) corresponding to the level of EpCAM and PSMA expressed on exosomes.

www.MaterialsViews.com

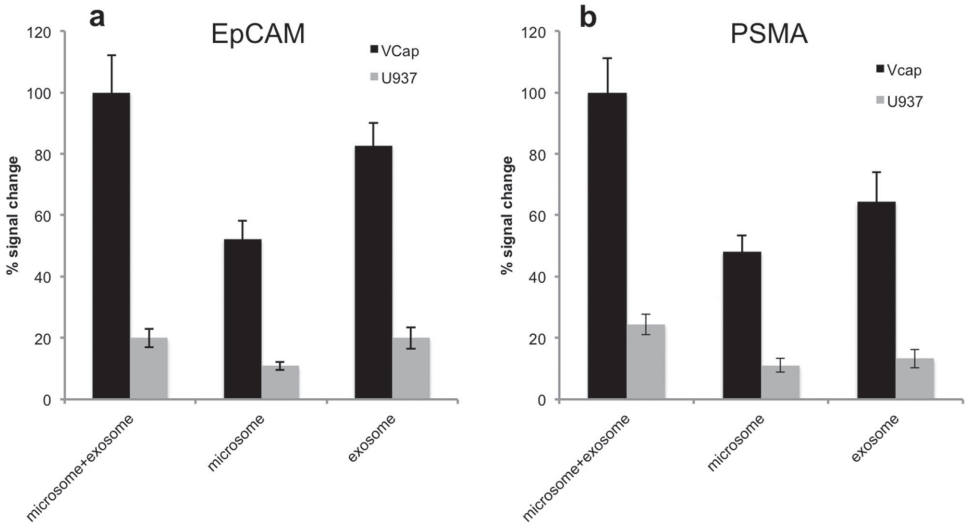

Figure 4. Electrochemical assay for the detection of fractions containing microsomes+exosomes, microsomes only, and exosomes only isolated from VCaP cell culture supernatant using a) AgNPs–anti-EpCAM and b) CuNPs–anti-PSMA as markers. Normalized intensity of charges integrated from the oxidation peaks was plotted to show exosomal and microsomal protein levels of EpCAM and PSMA.

level of PSMA expression is higher than EpCAM for both exosomes and microsomes. Nonetheless, these experiments demonstrate that the electrochemical approach allows rapid detection of exosomes. Control samples from blank samples (0 cells) did not exhibit statistically significant levels of signal, suggesting that the detection strategy is specific.

In order to compare the levels of the specific protein markers displayed on microsomes and exosomes, the approach was applied to the three VCaP-derived fractions from our centrifugation procedure: i) microsomes + exosomes, ii) microsomes only and iii) exosomes only. A VCaP cell culture supernatant was collected from a flask after 1×10^6 VCaP cells were seeded for 72 h and the corresponding control sample was from U937 cell (a nonepithelial cell line) culture supernatant prepared with the same procedure. AgNPs–anti-EpCAM and CuNPs–anti-PSMA were used to detect EpCAM and PSMA, respectively. In order to better understand the level of EpCAM and PSMA on exosomes or microsomes in the vesicles obtained (exosomes + microsomes), we compared the normalized signal intensities (charge) of the three products in **Figure 4**. In Figure 4a, data are shown that indicates that the detection of EpCAM from the fraction containing microsomes + exosomes shows higher signals than those containing microsomes only or exosomes only, while the signal intensity of microsomes is slightly lower than exosomes. Figure 4b shows that the detection of PSMA follows a similar trend. This observation indicates that microsomes, like exosomes, express specific protein markers originated from the parental cells, which is in agreement with the previous studies.[25] Meanwhile, our flow cytometry analysis (Figure S3, Supporting Information) further confirmed this conclusion.

To demonstrate the ability to detect exosomes and microsomes in patient samples, the electrochemical assay was applied to compare the levels of EpCAM and PSMA expression on exosomes and microsomes from serum of 10 PCa patients and healthy individuals. Distinct profiles were observed as shown in **Figure 5** for patient samples

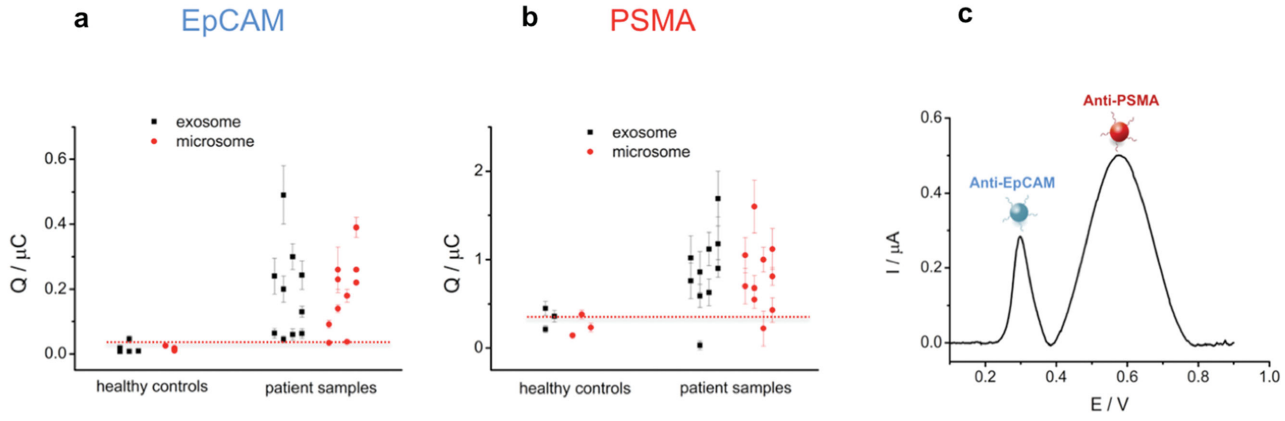

Figure 5. Electrochemical detection of exosomes (black squares) and microsomes (red circles) from serum of 10 prostate cancer patients and healthy individuals. AgNPs–anti-EpCAM and CuNPs–anti-PSMA were used as markers for the detection of a) EpCAM and b) PSMA, respectively. Intensity of charges from the oxidation peaks was plotted to show the exosomal protein levels of EpCAM and PSMA from patient samples and healthy controls. c) LSV detection of EpCAM and PSMA on exosomes from serum of prostate cancer patient.

and healthy controls for both EpCAM and PSMA surface markers. Variability in abundance was also observed across the patient group for both surface markers. We observed that a four- to fivefold increase on average for EpCAM expression was observed in exosomes from patient samples as compared to the healthy controls (Figure 5a), while an average three- to fourfold increase was observed for PSMA expression (Figure 5b). These data corroborate previous reports of significantly increased EpCAM and PSMA expressed on tumor-derived cells versus normal cells.[12,15] The increase may be a result of a higher level of exosomes shed by tumor cells than normal cells.[26] The detection of EpCAM and PSMA on microsomes follows a similar trend except that the levels of EpCAM and PSMA are slightly lower than those expressed on exosomes. It is also noted that PSMA, an ideal biomarker for the diagnosis of PCa, was detectable in nine out of ten individual patient samples derived exosomes and eight out of ten patient samples derived microsomes. As a complementary study, flow cytometry analysis was pursued as shown in Figure S3 (Supporting Information), where we observed that the intensity of EpCAM and PSMA expression on exosomes and microsomes from patient serum varied between individuals and that the level of PSMA was higher than that of EpCAM, which agrees with our electrochemical measurement.

We also demonstrated that the sensors were able to simultaneously detect two surface markers on exosomes from serum of prostate patients in a single electrochemical scan. In this experiment, a mixture of AgNPs–anti-EpCAM and CuNPs–anti-PSMA was applied to the sensor after exosomes were captured. As shown in Figure 5c, two peaks at around 300 and 600 mV were detected, corresponding to the electrochemical oxidation of Ag and Cu from AgNPs–anti-EpCAM and CuNPs–anti-PSMA, respectively. The two oxidation peaks were well separated from each other, enabling the detection of more than one type exosomal proteins at one time.

It is worth noting that in this study we have only tested one type of patients with localized PCa and the variations in the level of EpCAM and PSMA of secreted exosomes/microsomes could result from several factors such as age, tumor size, etc. As a future direction, different groups of patients with aggressive and nonaggressive diseases will be compared to study the correlation of EpCAM/PSMA levels and the aggressiveness/stage of cancer.

In this study, we present a multiplexed electrochemical sensor as a platform for the detection and characterization of exosomes microsomes by direct electro-oxidation of the labeled MNPs to recognize specific protein markers expressed on exosomes and microsomes. Compared to the conventional methods, our electrochemical strategy is fast, simple, cost-effective and requires small amount of sample (25 µL). Additionally, this approach is versatile, since the capture agent can be varied on the electrode and the recognition agent conjugated with MNPs can be adapted to detect different exosomes and microsomes. We demonstrate the profiling of surface markers associated with PCa directly from minimally invasive serum samples and show a significant increase in the levels of EpCAM and PSMA expressed on both exosomes and microsomes over healthy controls. Therefore, exosomes and microsomes can be used as potential tools in cancer diagnostics at an early stage, and the present electrochemical sensor provides a successful platform for protein marker detection from tumor-derived exosomes and microsomes.

Experimental Section

Chip and Sensor Fabrication: Chips with 11 working electrodes were fabricated and cleaned using the procedures previously described.[27,28] These electrodes were used as substrate for electrodeposition of nanostructured gold and capturing of cancer cells. The electrodeposition was performed at room temperature on an Epsilon potentiostat using a standard three-electrode system consisting of an Ag/AgCl reference, a Pt counter, and a 500 µm radius aperture Au working electrode. The plating condition was optimized and performed the plating at a gold concentration of 50×10^{-3} M under the plating potential of –200 mV for a duration of 120 s.

Isolation of Exosomes and Microsomes by Sequential Centrifugation: VCaP Cells and U937 cells were seeded at 1×10^6 cells in flasks in DMEM media (ATCC) with FBS depleted of exosomes and RPMI medium (Gibco), respectively. 72 h after seeding, the culture supernatant was collected for exosome isolation. The culture supernatant was centrifuged at 1000 g for 5 min for twice to eliminate cells and debris to obtain microsomes and exosomes. The third centrifugation was carried out at 16 100 g for 20 min. The resulting supernatant was collected as exosomes and the precipitate was microsomes and was suspended in PBS solution with 1% BSA for later use. Whole blood from PCa patients was directly used for isolation with the same procedure. The patient and healthy samples were from Sunnybrook Research Institute.

Conjugation of Metal Nanoparticles with Aptamers (Cu-Anti-PSMA): The conjugation of MNPs was carried out according to a literature protocol.[29,30] 10 µL of a solution containing an aptamer with a concentration of 100×10^{-6} M was mixed with 90 µL pH 9 PBS solution (60×10^{-3} M) containing 0.01 M TCEP to make a 10×10^{-6} M DNA solution. 200 µL MNPs was then added to the DNA solution. After the mixture reacted overnight on a vortex (AgNPs and anti-EpCAM mixture reacted on a shaking bed), 100 µL PBS (pH 7.4) solution was added to the mixture to obtain an ionic strength of 100×10^{-3} M and left for 6 h. 10 µL NaCl (3 M) was then added followed by another 2 h incubation. Another 4 µL NaCl (3 M) was added for a final 90 h incubation. Subsequently, the conjugates were centrifuged at 4000 rpm for 10 min to remove the excess. PBS solution containing 0.1% Tween and 1% BSA was then used to wash the precipitate before another centrifugation. The resulting MNP–aptamer conjugates were refrigerated for later use.

Electrochemical Assays: 20 µL immobilization solution was applied to the chip and reacted at room temperature overnight. After washing the excess aptamer with PBS, 20 µL of 1×10^{-3} M MCH solution was added to the chip to block possible remaining active sites against nonspecific adsorption and allowed the reaction to proceed for 2.5 h. After another wash with pH 7.4 PBS, the chip was kept at room temperature in a humid environment for later use. The anti-EpCAM aptamer-modified chip was then incubated with exosomes or microsomes (25 µL) from both VCaP cell

supernatant and patient serum at room temperature for 1 h to capture exosomes and microsomes. After being washed with PBS, the exosomes or microsomes captured on the chip were exposed to the corresponding conjugates at room temperature for 1 h. Subsequently, the chip was treated using $BaNH_4$ for 10 min and thoroughly rinsed with PBS.

Electrochemical Measurements: For all electrochemical measurements, a conventional three-electrode setup was used with an Ag/AgCl reference and a Pt counter connected to an Epsilon potentiostat (BASi, West Lafyette, IN). For exosomes and microsomes detection, the chip was scanned in a solution containing pH 2 HNO_3 at a scan rate of 100 mV s^{-1} using LSV. The charge passed under the oxidation peak was calculated by integrating the peak area.

Transmission Electron Microscopy: The isolated exosomes or microsomes from VCaP cell culture supernatant or blood were stained with 2% phosphotungstic acid (PTA) with a concentration ratio of 4:1. The mixture was then loaded onto carbon-coated copper grids and left to dry at room temperature. TEM (Hitachi H-7000) was used to examine the morphology of exosomes and microsomes after the staining.

Flow Cytometry: First, exosomes and microsomes were isolated from VCaP samples at three different cell concentrations (10^4, 10^5, and 10^6 VCaP cells) and three patient samples. Then, each sample was diluted to final volume of 100 µL with PBS. Samples were incubated with 3 µL of APC-conjugated EpCAM and PSMA antibodies for 60 min. After incubation, 300 µL of PBS was added to the samples. At last step, samples were subjected to measurement by flow cytometry. In each sample, an unstained control was used to detect background staining. The results were later analyzed using FlowJo software (FlowJo LLC, Ashland, OR).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors acknowledge the Canadian Institute for Health Research for their generous support of this work through an Emerging Team Grant, the Ontario Research Fund for a Research Excellence Grant, the Canadian Cancer Society Research Institute for an Innovation Grant, and the Natural Sciences and Engineering Council of Canada. The authors thank the ECTI facility at University of Toronto for their cleanroom facilities.

[1] R. Siegel, D. Naishadham, A. Jemal, *Ca-Cancer J. Clin.* **2013**, *63*, 11.
[2] C. Palmberg, P. Koivisto, T. Visakorpi, T. L. Tammela, *Eur. Urol.* **1999**, *36*, 191.
[3] D. R. Berthold, G. R. Pond, F. Soban, R. de Wit, M. Eisenberger, I. F. Tannock, *J. Clin. Oncol.* **2008**, *26*, 242.
[4] M. S. Cookson, B. J. Roth, P. Dahm, C. Engstrom, S. J. Freedland, M. Hussain, D. W. Lin, W. T. Lowrance, M. H. Murad, W. K. Oh, D. F. Penson, A. S. Kibel, *J. Urol.* **2013**, *190*, 429.
[5] F. Tarhan, A. Orçun, I. Küçükercan, N. Camursoy, U. Kuyumcuoğlu, *Urology* **2005**, *66*, 1234.
[6] M. R. Speicher, K. Pantel, *Nat. Biotechnol.* **2014**, *32*, 441.
[7] D. Duijvesz, T. Luider, C. H. Bangma, G. Jenster, *Eur. Urol.* **2011**, *59*, 823.
[8] S. Khan, J. M. S. Jutzy, M. M. A. Valenzuela, D. Turay, J. R. Aspe, A. Ashok, S. Mirshahidi, D. Mercola, M. B. Lilly, N. R. Wall, *PLoS One* **2012**, *7*, e46737.
[9] A. Liga, A. D. B. Vliegenthart, W. Oosthuyzen, J. W. Dear, M. Kersaudy-Kerhoas, *Lab Chip* **2015**, *15*, 2388.
[10] K. Agarwal, M. Saji, S. M. Lazaroff, A. F. Palmer, M. D. Ringel, M. E. Paulaitis, *Langmuir* **2015**, *31*, 5440.
[11] L. Grasso, R. Wyss, L. Weidenauer, A. Thampi, D. Demurtas, M. Prudent, N. Lion, H. Vogel, *Anal. Bioanal. Chem.* **2015**, *407*, 5425.
[12] T. Liu, D. E. Mendes, C. E. Berkman, *Int. J. Oncol.* **2014**, *44*, 918.
[13] B. J. Tauro, D. W. Greening, R. A. Mathias, H. Ji, S. Mathivanan, A. M. Scott, R. J. Simpson, *Methods* **2012**, *56*, 293.
[14] K. Mizutani, R. Terazawa, K. Kameyama, T. Kato, K. Horie, T. Tsuchiya, K. Seike, H. Ehara, Y. Fujita, K. Kawakami, M. Ito, T. Deguchi, *Anticancer Res.* **2014**, *34*, 3419.
[15] S. S. Kanwar, C. J. Dunlay, D. M. Simeone, S. Nagrath, *Lab Chip* **2014**, *14*, 1891.
[16] C. Chen, J. Skog, C.-H. Hsu, R. T. Lessard, L. Balaj, T. Wurdinger, B. S. Carter, X. O. Breakefield, M. Toner, D. Irimia, *Lab Chip* **2010**, *10*, 505.
[17] H. Im, H. Shao, Y. I. Park, V. M. Peterson, C. M. Castro, R. Weissleder, H. Lee, *Nat. Biotechnol.* **2014**, *32*, 490.
[18] R. Vaidyanathan, M. Naghibosadat, S. Rauf, D. Korbie, L. G. Carrascosa, M. J. Shiddiky, M. Trau, *Anal. Chem.* **2014**, *86*, 11125.
[19] E. van der Pol, A. G. Hoekstra, A. Sturk, C. Otto, T. G. van Leeuwen, R. Nieuwland, *J. Thromb. Haemostasis* **2010**, *8*, 2596.
[20] S. Kruger, Z. Y. A. Elmageed, D. H. Hawke, P. M. Wörner, D. A. Jansen, A. B. Abdel-Mageed, E. U. Alt, R. Izadpanah, *BMC Cancer* **2014**, *14*, 44.
[21] B. K. Thakur, H. Zhang, A. Becker, I. Matei, Y. Huang, B. Costa-Silva, Y. Zheng, A. Hoshino, H. Brazier, J. Xiang, C. Williams, R. Rodriguez-Barrueco, J. M. Silva, W. Zhang, S. Hearn, O. Elemento, N. Paknejad, K. Manova-Todorova, K. Welte, J. Bromberg, H. Peinado, D. Lyden, *Cell Res.* **2014**, *24*, 766.
[22] S. O. Kelley, C. A. Mirkin, D. R. Walt, R. F. Ismagilov, M. Toner, E. H. Sargent, *Nat. Nanotechnol.* **2014**, *9*, 969.
[23] A.-K. Rupp, C. Rupp, S. Keller, J. C. Brase, R. Ehehalt, M. Fogel, G. Moldenhauer, F. Marmé, H. Sültmann, P. Altevogt, *Gynecol. Oncol.* **2011**, *122*, 437.
[24] M. Moscovici, A. Bhimji, S.O. Kelley, *Lab Chip* **2013**, *13*, 940.
[25] R. S. Israeli, C. T. Powell, J. G. Corr, W. R. Fair, W. D. W. Heston, *Cancer Res.* **1994**, *54*, 1807.
[26] A. J. Abusamra, Z. Zhong, X. Zheng, M. Li, T. E. Ichim, J. L. Chin, W. P. Min, *Blood Cells, Mol., Dis.* **2005**, *35*, 169.
[27] Y. Wan, Y.-G. Zhou, M. Poudineh, T. S. Safaei, R. M. Mohamadi, E. H. Sargent, S. O. Kelley, *Angew. Chem., Int. Ed.* **2014**, *53*, 13145.
[28] D. W. Kimmel, G. LeBlanc, M. Meschievitz, D. E. Cliffel, *Anal. Chem.* **2012**, *84*, 685.
[29] X. Chen, Y. Wang, Y. Zhang, Z. Chen, Y. Liu, Z. Li, J. Li, *Anal. Chem.* **2014**, *86*, 4278.
[30] M. M. Costa, A. de La Escosura-Muñiz, C. Nogués, L. Barrios, E. Ibáñez, A. Merkoçi, *Small* **2012**, *8*, 3605.

Received: August 6, 2015
Revised: November 9, 2015
Published online: December 28, 2015