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Rapid Identification and Quantification of Tumor Cells Using an Electrocatalytic Method Based on Gold Nanoparticles

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There is a high demand for simple, rapid, efficient, and user-friendly alternative methods for the detection of cells in general and, in particular, for the detection of cancer cells. A biosensor able to detect cells would be an all-in-one dream device for such applications. The successful integration of nanoparticles into cell detection assays could allow for the development of this novel class of cell sensors. Indeed, their application could well have a great future in diagnostics, as well as other fields. As an example of a novel biosensor, we report here an electrocatalytic device for the specific identification of tumor cells that quantifies gold nanoparticles (AuNPs) coupled with an electrotransducing platform/sensor. Proliferation and adherence of tumor cells are achieved on the electrotransducer/detector, which consists of a mass-produced screen-printed carbon electrode (SPCE). In situ identification/quantification of tumor cells is achieved with a detection limit of 4000 cells per 700 μL of suspension. This novel and selective cell-sensing device is based on the reaction of cell surface proteins with specific antibodies conjugated with AuNPs. Final detection requires only a couple of minutes, taking advantage of the catalytic properties of AuNPs on hydrogen evolution. The proposed detection method does not require the chemical agents used in most existing assays for the detection of AuNPs. It allows for the miniaturization of the system and is much cheaper than other expensive and sophisticated methods used for tumor cell detection. We envisage that this device could operate in a simple way as an immunosensor or DNA sensor. Moreover, it could be used, even by inexperienced staff, for the detection of protein molecules or DNA strands.

The development and application of biosensors is one of the leading sectors of state-of-the-art nanoscience and nanotechnology. Biosensors are under commercial development for numerous applications, including the detection of pathogens, the measurement of clinical parameters, the monitoring of environmental pollutants, and other industrial applications. The most effective way of providing biosensors to potential customers, especially those with limited budgets, might be to modify the technology so that it can run on everyday equipment, rather than on specialized apparatuses.[1] Enzymatic biosensors represent an already consolidated class of biosensors, with glucose biosensors among the most successful on the market. Nevertheless, research is still needed to find novel alternative strategies and materials, so that affinity biosensors—immunosensors and genosensors—could be used in more successful applications in everyday life.

Early detection of cancer is widely acknowledged as the crucial key for an early and successful treatment. The detection of tumor cells is an increasingly important issue that has received wide attention in recent years,[2−7] mainly for two reasons: (i) new methods are allowing the identification of metastatic tumor cells (for example, in peripheral blood), with special relevance both for the evolution of the disease and for the response of the patient to therapeutic treatments, and (ii) diagnosis based on cell detection is, thanks to the use of monoclonal antibodies,[8] more sensitive and specific than that based on traditional methods.

Detecting multiple biomarkers and circulating cells in human body fluids is a particularly crucial task for the diagnosis and prognosis of complex diseases, such as cancer and metabolic disorders.[9] An early and accurate diagnosis is the key to an effective and ultimately successful treatment of cancer, but it

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(1) Erickson, J. S.; Ligler, F. S. *Nature* **2008**, *456*, 178–179.
(2) Yucai, X.; Yin, T.; Wiegraebe, W.; He, X. C.; Miller, D.; Stark, D.; Perko, K.; Alexander, R.; Schwartz, J.; Grindley, J. C.; Park, J.; Haug, J. S.; Wunderlich, J. P.; Li, H.; Zhang, S.; Johnson, T.; Feldman, R. A.; Li, L. *Nature* **2009**, *457*, 97–101.
(3) Savage, P. A.; Vosseller, K.; Kang, C.; Larimore, K.; Riedel, E.; Wojnoonski, K.; Jungbluth, A. A.; Allison, J. P. *Science* **2008**, *319*, 215–220.
(4) Manganas, L. N.; Zhang, X.; Li, Y.; Hazel, R. D.; Smith, S. D.; Wagshul, M. E.; Henn, F.; Benveniste, H.; Djuri, P. M.; Enikolopov, G.; Maleti-Savati, M. *Science* **2007**, *318*, 980–985.
(5) Smith, J. E.; Medley, C. D.; Tang, Z.; Shangguan, D.; Lofton, C.; Tan, W. *Anal. Chem.* **2007**, *79*, 3075–3082.
(6) Cortez, C.; Tomaskovi-Crook, E.; Ohnston, A. P. R.; Scout, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *ACSNano* **2007**, *1*, 93–102.
(7) Medley, C. D.; Smith, J. E.; Tang, Z.; Wu, Y.; Bamrungsap, S.; Tan, W. *Anal. Chem.* **2008**, *80*, 1067–1072.
(8) Rector, I.; Rektorova, I.; Kubova, D. J. *Neurol. Sci.* **2006**, *248*, 185–191.
(9) Sidransky, D. *Nat. Rev. Cancer* **2002**, *2*, 210–219.

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requires new sensitive methods for detection. Many current methods for the routine detection of tumor cells are time-consuming (e.g., immunohistochemistry), expensive, or require advanced instrumentation (e.g., flow cytometry[10]). Hence, alternative cost-effective methods that employ simple/user-friendly instrumentation and are able to provide adequate sensitivity and accuracy would be ideal for point-of-care diagnosis. Therefore, in recent years, there have been some attempts at cell analysis using optical-based biosensors.[11−14] In addition to optical biosensors, sensitive electrochemical DNA sensors,[15−20] immunosensors,[20−22] and other bioassays have all recently been developed by our group and others,[23] using nanoparticles (NPs) as labels and providing direct detection without prior chemical dissolution.[24,25]

Herein, we present the design and application of a new class of affinity biosensor. Based on a new electrotransducing platform, this is a novel cell sensor inspired by DNA sensors and immunosensors. This platform consists of a screen-printed carbon electrode (SPCE), coupled to a new nanoparticle-based electrocatalytic method that allows rapid and consecutive detection/identification of in situ cell proliferation. Detection is based on the reaction of cell surface proteins with specific antibodies conjugated to gold nanoparticles (AuNPs). Use of the catalytic properties of the AuNPs on hydrogen formation from hydrogen ions[26] makes it possible to quantify the nanoparticles and, in turn, to quantify the corresponding attached cancer cells. This catalytic effect has also been observed for platinum[26] and palladium[27] nanoparticles, but AuNPs are more suitable for biosensing purposes, because of their simple synthesis, narrow size distribution, biocompatibility, and easy bioconjugation. The catalytic current generated by the reduction of the hydrogen ions is chronoamperometrically recorded and related to the quantity of the cells of interest. The basis of the method is that the protons are catalytically reduced to hydrogen in the presence of AuNPs at an adequate potential in acidic medium. This approach, using the catalytic current resulting from the reduction of hydrogen ions, has been employed for the detection of both platinum(II)[28] and gold(I)[29] complexes used as labels in genosensor designs. However, to our knowledge, the present study is the first where the chronoamperometric method is used for AuNPs, with all the advantages that the latter bring to biosensing applications.

To evaluate the efficacy of this cell sensor as a probing tool for the detection of tumor cells, we studied whether the human tumor HMy2 cell line (HLA-DR class II positive B cell) could indeed be detected and whether another human tumor PC-3 cell line (HLA-DR class II negative prostate carcinoma) was not detected. Tumor cells can be grown on the surface of SPCEs, showing a similar morphology to those in other conventional cell growth substrates. Incubation of cells with AuNPs conjugated to antibodies was carried out in either a direct or indirect way. In the former, a commercial mouse monoclonal antibody (mAb) directed against DR molecules was conjugated to AuNPs, whereas in the indirect method, incubation with unconjugated anti-DR monoclonal antibody was followed by secondary antibodies attached to AuNPs. In both cases, the immunosensor was able to identify DR-positive tumor cells, but not the negative cells. As expected, the indirect method gave a higher signal, because of the amplification mediated by several secondary antibodies attached to the primary antibody.

We propose this cell sensor as a new class of biosensor device for the specific identification of cells in general and, more particularly, for the identification of tumor cells. The method does not require expensive equipment, it provides an adequate sensitivity and accuracy, and it would be an ideal alternative for point-of-care diagnosis in the future.

Given the increased use of various metallic nanoparticles, we envisage possible further applications of these smart nanobiocatalytic particles for other diagnostic purposes. The simultaneous detection of several kinds of cells (e.g., to perform blood tests, detect inflammatory or tumoral cells in biopsies or fluids) could be carried out, including multiplexed screening of cells, proteins, and even DNA.

MATERIALS AND METHODS

Chemicals and Equipment. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) and trisodium citrate were purchased from Sigma-Aldrich (Spain). Unless otherwise stated, all buffer reagents and other inorganic chemicals were supplied by Sigma, Aldrich, or Fluka (Spain). All chemicals were used as received, and all aqueous solutions were prepared in doubly distilled water. The phosphate buffer solution (PBS) consisted of 0.01 M phosphate buffered saline, 0.137 M NaCl, and 0.003 M KCl (pH 7.4). Analytical grade (Merck, Spain) HCl was used. The solutions were prepared with ultrapure water.

The human tumor cell lines used as targets in this study were HMy2, a tumoral B cell line that expresses surface HLA-DR molecules (class II of major histocompatibility complex), and PC-3, a tumoral prostate cell line that does not express the DR protein.

(10) Vermes, I.; Haanen, C.; Reutelingsperger, C. *J. Immunol. Methods* **2000**, *243*, 167–190.
(11) Fang, Y.; Ferrie, A. M.; Fontaine, N. H.; Yuen, P. K. *Anal. Chem.* **2005**, *77*, 5720–5725.
(12) Knauer, S. K.; Stauber, R. H. *Anal. Chem.* **2005**, *77*, 4815–4820.
(13) Skivesen, N.; Horvath, R.; Thinggaard, S.; Larsen, N. B.; Pedersen, H. C. *Biosens. Bioelectron.* **2007**, *22*, 1282–1288.
(14) Lin, B.; Li, P.; Cunninghamb, B. T. *Sens. Actuators B* **2006**, *114*, 559–564.
(15) Wang, J.; Liu, G.; Merkoçi, A. *J. Am. Chem. Soc.* **2003**, *125*, 3214–3215.
(16) Wang, J.; Polsky, R.; Merkoçi, A.; Turner, K. L. *Langmuir* **2003**, *19*, 989–991.
(17) Pumera, M.; Castañeda, M. T.; Pividori, M. I.; Eritja, R.; Merkoçi, A.; Alegret, S. *Langmuir* **2005**, *21*, 9625–9629.
(18) Castañeda, M. T.; Merkoçi, A.; Pumera, M.; Alegret, S. *Biosens. Bioelectron.* **2007**, *22*, 1961–1967.
(19) Marin, S.; Merkoçi, A. *Nanotechnology* **2009**, *20*, 055101.
(20) Ambrosi, A.; Merkoçi, A.; de la Escosura-Muñiz, A.; Castañeda, M. T. In *Biosensing Using Nanomaterials*; Merkoçi, A., Ed.; Wiley Interscience: New York, 2009; Chapter 6, pp 177−197.
(21) Ambrosi, A.; Castañeda, M. T.; Killard, A. J.; Smyth, M. R.; Alegret, S.; Merkoçi, A. *Anal. Chem.* **2007**, *79*, 5232–5240.
(22) de la Escosura-Muñiz, A.; Maltez-da Costa, M.; Merkoçi, A. *Biosens. Bioelectron.* **2009**, *24*, 2475–2482.
(23) de la Escosura-Muñiz, A.; Ambrosi, A.; Merkoçi, A. *Trends Anal. Chem.* **2008**, *27*, 568–584.
(24) Pumera, M.; Aldavert, M.; Mills, C.; Merkoçi, A.; Alegret, S. *Electrochim. Acta* **2005**, *50*, 3702–3707.
(25) Merkoçi, A.; Marcolino-Junior, L. H.; Marín, S.; Fatibello-Filho, O.; Alegret, S. *Nanotechnology* **2007**, *18*, 035502.
(26) Chikae, M.; Idegami, K.; Kerman, K.; Nagatani, N.; Ishikawa, M.; Takamura, Y.; Tamiya, E. *Electrochem. Commun.* **2006**, *8*, 1375–1380.
(27) Meier, J.; Schiøtz, J.; Liu, P.; Nørskov, J. K.; Stimming, U. *Chem. Phys. Lett.* **2004**, *390*, 440–444.
(28) Hernández-Santos, D.; González-García, M. B.; Costa-García, A. *Anal. Chem.* **2005**, *77*, 2868–2874.
(29) Díaz-González, M.; de la Escosura-Muñiz, A.; González-García, M. B.; Costa-García, A. *Biosens. Bioelectron.* **2008**, *23*, 1340–1346.

All cells were cultured in RPMI 1640 medium (Gibco, Life Technologies, Scotland) supplemented with 10% heat-inactivated fetal calf serum (FCS) (PAA, Austria), penicillin (100 U/mL), and glutamine (2 mM) (Gibco) at 37 °C in a humidified atmosphere containing 5% CO_2.

Measurement of HLA-DR expression was carried out with a commercial FITC-mouse antihuman HLA-DR mAb (Immunotech, France) and BH1, a human IgM mAb produced by the Immunology group of Vigo,[30−32] which recognizes human HLA-class II molecules on the surface of human monocytes, B lymphocytes, tumor B cell lines (HMy2, Raji, and Daudi), and tumor cells from patients suffering from hematological malignancies. As a control for positive recognition of both cell lines tested, the human mAb 32.4 was used, also produced by the Immunology group of Vigo. For the BH1 and 32.4 mAbs, FITC-conjugated rabbit antihuman IgM polyclonal antibodies (DakoCytomation, Spain) were used as secondary antibodies.

Electrochemical measurements were taken using an Ivium Potentiostat (The Netherlands) connected to a PC.

Cells were visualized under inverted and directed microscopes (Olympus IX50 and BX51, respectively, Olympus Optical, Japan), and photographs were taken with an Olympus DP71 camera.

A DEK248 semiautomatic screen-printing machine (DEK International, Switzerland) was used for the fabrication of the screen-printed carbon electrodes (SPCEs), as electrochemical transducers. The reagents used for this process were Autostat HT5 polyester sheet (McDermid Autotype, U.K.) and Electrodag 423SS carbon ink, Electrodag 6037SS silver/silver chloride ink, and Minico 7000 Blue insulating ink (Acheson Industries, The Netherlands).

The chambers used for cell incubation on the working area of the surface of the SPCEs were Lab-Tek eight-well chamber slides (Nunc, Thermo Fisher Scientific, Spain).

A JEOL JEM-2011 transmission electron microscope (JEOL Ltd., Japan) was used to characterize the gold nanoparticles.

Philips XL30 and JEOL JSM 6700F scanning electron microscopes were used to obtain images of cell growth on the surface of the sensor.

A Cytomics FC 500 Series Flow Cytometry Systems and CXP and MXP software (Beckman Coulter, Fullerton, CA) was used for the cell fluorescence measurements.

The electrochemical transducers used for the in situ growth and detection of the cells were homemade screen-printed carbon electrodes (SPCEs), prepared as described in the Supporting Information.

Preparation and Modification of Gold Nanoparticles. The 20-nm gold nanoparticles (AuNPs) were synthesized by reducing tetrachloroauric acid with trisodium citrate, a method pioneered by Turkevich et al.[33] (see the experimental procedure for AuNP synthesis, transmission electron microscopy (TEM) images, and the Gaussian distribution of sizes in the Supporting Information).

The conjugation of AuNPs to FITC-mouse anti human DR mAb (αDR) and polyclonal rabbit antihuman IgM (αIgM) antibodies was performed according to the following procedure:[21] Two milliliters of AuNP suspension was mixed with 100 μL of 100 μg/mL solutions of each antibody and incubated at 25 °C for 20 min. Subsequently, a blocking step with 150 μL of 1 mg/mL BSA incubating at 25 °C for 20 min was undertaken. Finally, centrifugation was carried out at 14000 rpm for 20 min, and AuNP/αDR and AuNP/αIgM conjugates were reconstituted in PBS solution.

Effect of Gold Nanoparticles on Hydrogen Ion Reduction. Cyclic votammetric measurements were made to study the catalytic effect of gold nanoparticles on hydrogen ion reduction. A total of 25 μL of a 2 M HCl solution was mixed with 25 μL of a solution of AuNPs (different concentrations), the mixture was placed onto the surface of the electrodes, and cyclic voltammograms were recorded from +1.35 to −1.40 V at a scan rate of 50 mV/s. The background was recorded by placing 50 μL of a 1 M HCl solution onto the surface of the electrodes, following the same electrochemical procedure.

Qualitative analyses were carried out taking advantage of the chronoamperometric mode. Chronoamperograms were obtained by placing a mixture of 25 μL of 2 M HCl and 25 μL of the AuNP solution (different concentrations) onto the surface of the electrodes and, subsequently, holding the working electrode at a potential of +1.35 V for 1 min and then a negative potential of −1.00 V for 5 min. The cathodic current generated was recorded. The background (blank curve) was recorded by placing 50 μL of a 1 M HCl solution onto the electrode surface and following the same electrochemical protocol.

Similar measurements were taken for the AuNP/αDR and AuNP/αIgM conjugates.

Incubation of Cells. *Incubation of Cells in Flasks.* HMy2 and PC-3 cell lines were cultured in medium at 37 °C in a humidified atmosphere containing 5% CO_2 in tissue culture flasks (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ). The PC-3 cells were removed from the flasks using a cell scrapper, whereas the HMy2 cells were removed mechanically by mixing the flask, and the cells were counted in the presence of Trypan blue.

Incubation of Cells onto the Surface of Screen-Printed Carbon Electrodes (SPCEs). Only the working electrode surfaces were introduced into Lab-Tek eight-well chamber slides (Nunc, Thermo Fisher Scientific) after gasket removal and then fixed with mounting. (Supporting Information, Figure S1B,C). A fixed amount of cells was added into each well in a volume of 700 μL of culture medium and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2.

Detection/Quantification of Cells. *Immunological Reaction with Specific Antibodies.* (a) For the direct immunoassay, once cells had grown onto the electrode surface, they were washed with PBS, and 50 μL of AuNP/αDR was added onto the working electrode area and left there for 30 min at 37 °C.

(b) For the indirect immunoassay, cells were incubated with primary antibodies (either BH1 or 32.4 mAbs) for 30 min, washed with PBS, and then incubated with AuNP/αIgM for 30 min.

(30) Magadán, S.; Valladares, M.; Suarez, E.; Sanjuan, I.; Molina, A.; Ayling, C.; Davies, S.; Zou, X.; Williams, G. T.; Neuberger, M. S.; Brüggeman, M.; Gambón, F.; Díaz-Espada, F.; González-Fernández, Á. *Biotechniques* **2002**, *33*, 680–690.

(31) Suárez, E.; Magadán, S.; Sanjuán, I.; Valladares, M.; Molina, A.; Gambón, F.; Díaz-Espada, F.; González-Fernández, A. *Mol. Immunol.* **2006**, *43*, 1827–1835.

(32) Díaz, B.; Sanjuan, I.; Gambón, F.; Loureiro, C.; Magadán, S.; González-Fernández, A. *Cancer Immunol. Immunother.* **2009**, *58*, 351–360.

(33) Turkevich, J.; Stevenson, P.; Hillier, J. *Discuss. Faraday Soc.* **1951**, *11*, 55–75.

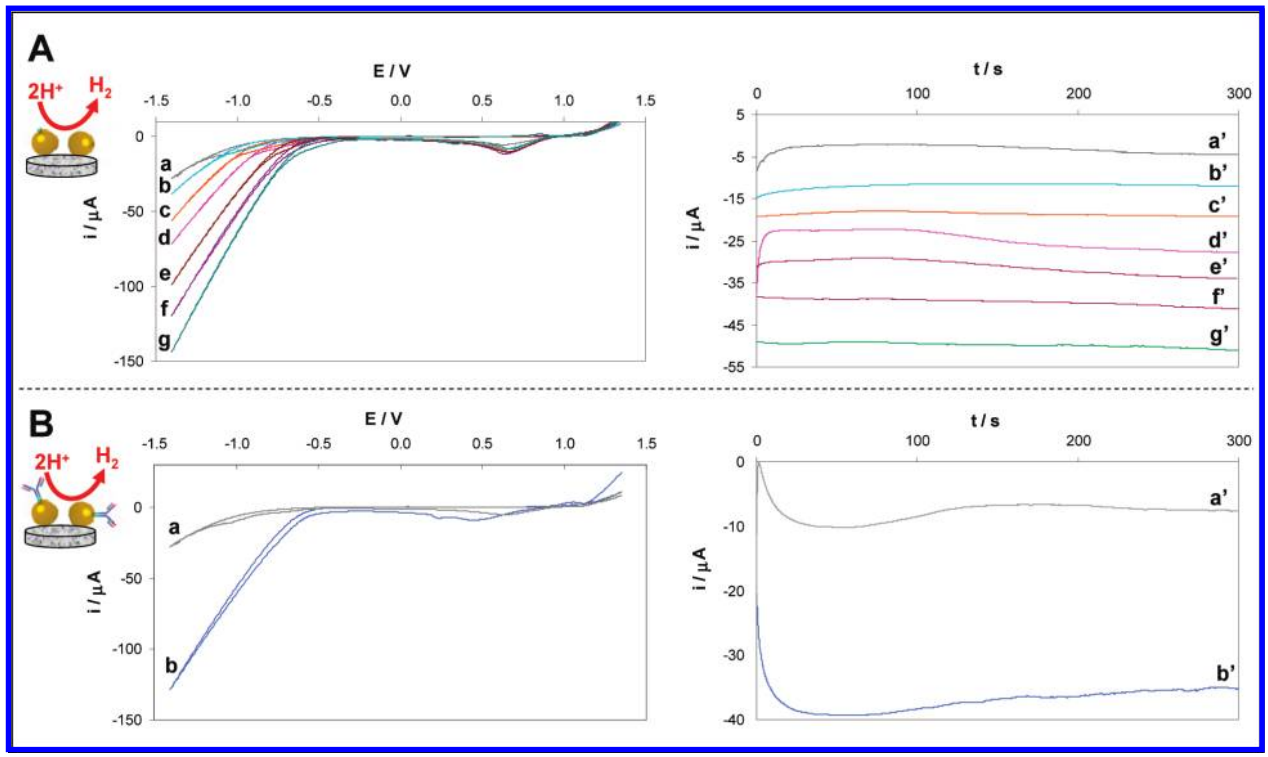


Figure 1. (A) Left: Cyclic voltammograms recorded from +1.35 to −1.40 V at a scan rate of 50 mV/s for a 1 M HCl solution (blank curve, a) and for increasing concentrations of AuNPs in 1 M HCl: (b) 0.96, (c) 4.8, (d) 24, (e) 120, (f) 600, and (g) 3000 pM. Right: Chronoamperograms recorded by applying a potential of −1.00 V for 5 min, using a 1 M HCl solution (blank curve, a′) and the same AuNP concentrations in 1 M HCl as detailed above (b′−g′). (B) Left: Cyclic voltammograms recorded under the same conditions as in A, left, for the blank (curve a) and for a solution of the conjugate AuNP/αDR (curve b). Right: Chronoamperograms recorded under the same conditions as in A, right, for the blank (a′) and for the conjugate AuNP/αDR (b′).

Analytical Signal Based on Hydrogen Evolution Catalyzed by AuNPs. Cells (after direct or indirect immunoassays) were washed with PBS solution to remove unbound AuNP/antibodies, and then 50 μL of 1 M HCl solution was applied to the SPCE surface. Subsequently, the electrode was held at a potential of +1.35 V for 1 min, and then a negative potential of −1.00 V was applied for 5 min. The cathodic current generated was recorded, and the value of the current at 200 s was chosen as the analytical signal.

Flow Cytometry. Flow cytometry analysis was carried out using 2×10^5 cells (HMy2 or PC-3) incubated at 4 °C for 40 min in the presence of commercial FITC-anti human DR mAb (αDR) or concentrated hybridoma supernatant containing BH1 and 32.4 mAbs. Cells were washed twice with PBS, and for indirect immunofluorescence, cells were stained with FITC-rabbit anti HuIgM (αIgM) Abs (Dako) at 4 °C for 30 min. The stained cells were washed five times with PBS, and the cellular fluorescence was measured using a flow cytometer.

RESULTS AND DISCUSSION

Catalytic Effect of AuNPs on Hydrogen Evolution. The catalytic properties of AuNPs, deposited onto SPCEs, are shown in Figure 1. Figure 1A (left) displays cyclic voltammograms (CVs) in 1 M HCl solutions recorded from +1.35 to −1.40 V, and Figure 1A (right) shows the corresponding chronoamperometric responses plotted at a fixed potential of −1.00 V. Curve a is the background CV that corresponds to the SPCE without AuNP, whereas curves b−g correspond to SPCEs with increasing concentrations of deposited AuNPs present in the HCl solution. The background CV (curve a) shows that the reduction of the medium's protons to hydrogen starts at approximately −0.60 V. In the presence of the AuNPs (curves b−g) on the surface of the electrode, the potential for hydrogen ion reduction shifts (by up to 500 mV, depending on the concentration of AuNPs) toward less negative potentials. Moreover, it can also be seen that, because of the catalytic effect of the AuNPs, a higher current is generated (up to 100 μA higher, as evaluated for the potential value of −1.40 V, depending on the concentration of AuNPs). The oxygen reduction on SPCE at very negative potentials (lower than −1.40 V) is not of great importance; therefore, the background signals are not affected.

The results obtained show that, if an adequate potential is fixed (i.e., −1.00 V), the intensity of the current recorded in chronoamperometric mode during the stage of hydrogen ion electroreduction (Figure 1A, right) can be related to the presence (curves b′−g′) or absence (curve a′) of AuNPs on the surface of the SPCE. A proportional increase of catalytic current was observed with corresponding increases in the concentration of AuNPs (from 3 nM (3000 pM) to 0.96 pM). The absolute value of the current generated at 200 s (response time of the sensor) was chosen as the analytical signal and used for quantification of the AuNPs.

The effect of other sources of hydrogen ions (e.g., HNO_3, H_2SO_4 at different concentrations ranging from 0.1 to 1 M) on the current intensity corresponding to the AuNPs was also studied (results not shown). The use of HNO_3 was avoided because of its corrosive effect on the transducer (screen-printed electrode based on carbon ink). Moreover, at the same

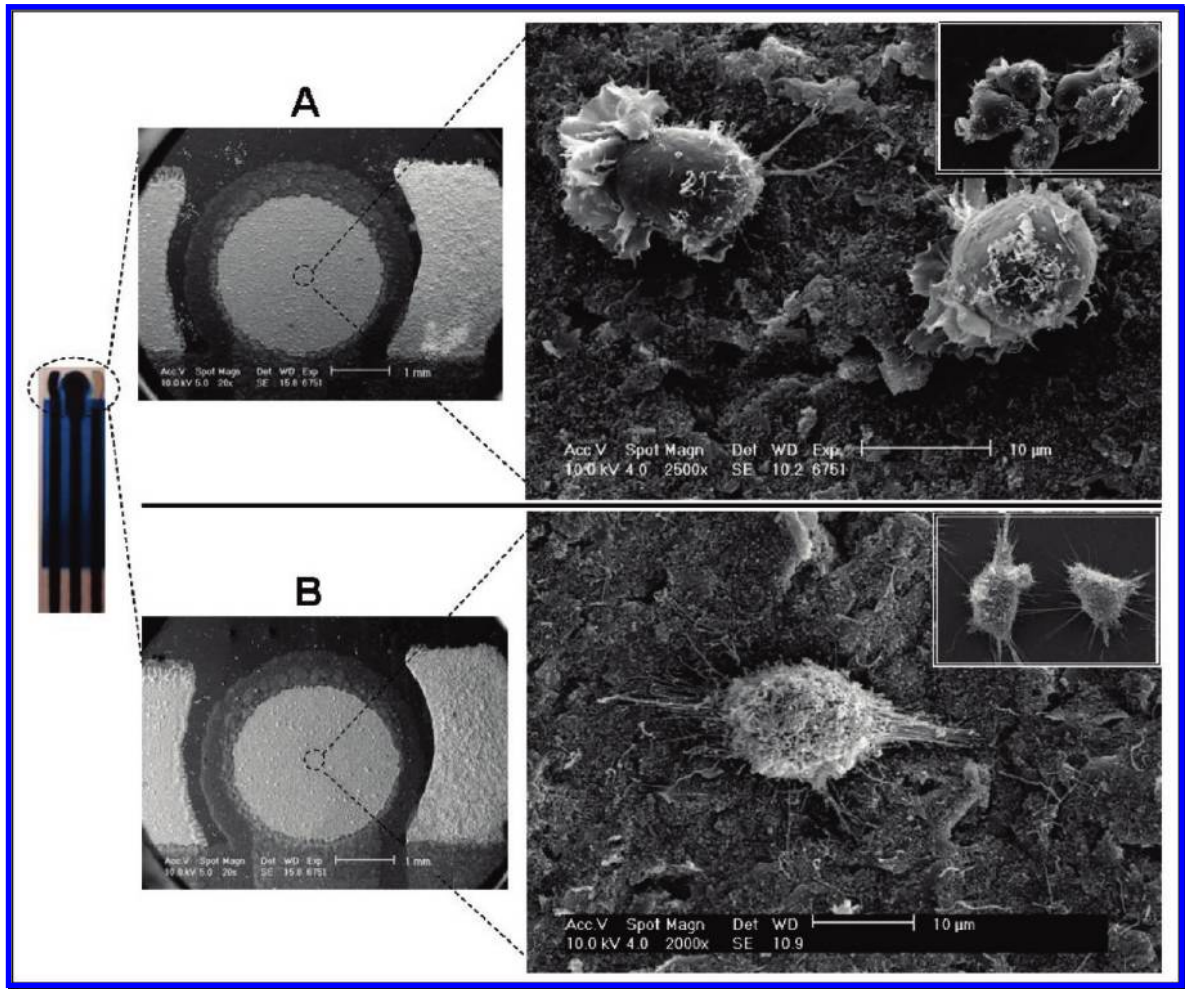


Figure 2. SEM images of the electrotransducer (SPCE) (left) with its three surfaces and details of the (A) HMy2 and (B) PC-3 cell lines on the carbon working electrode (right). Inset images correspond to cell growth on the plastic area of the SPCEs.

concentration, H_2SO_4 gave lower sensitivity than was obtained for HCl.

The pretreatment at +1.35 V was also optimized. It was found that this previous oxidation is necessary for obtaining the best electrocatalytic effect in the further reduction step (hydrogen ion reduction at −1.00 V for 5 min). During the application of this oxidative potential, some of the AuNPs are transformed into Au(III) ions (released from the AuNP surface). These ions could also exert a catalytic effect on hydrogen evolution,[29] as could the significant number of AuNPs still remaining (results not shown) after the oxidation step.

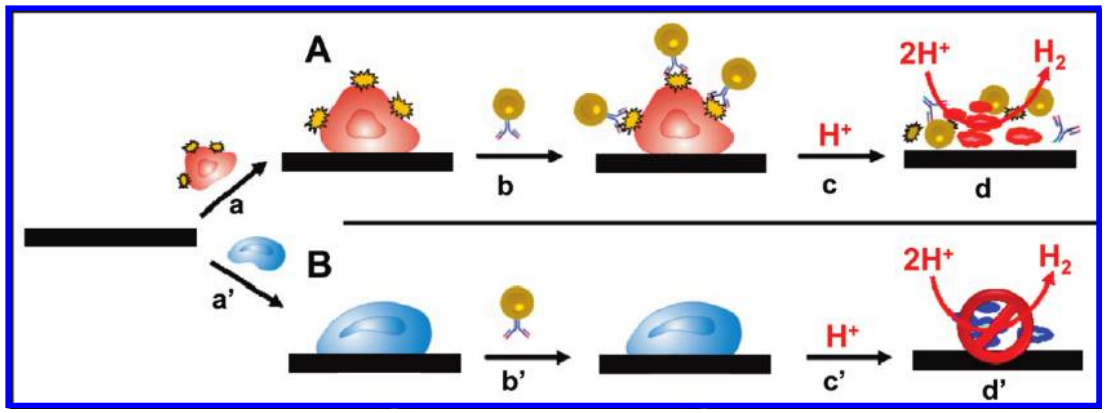
The behavior of AuNPs modified with antibodies was also evaluated for conditions similar to those used for the nonmodified AuNPs. A very similar response (see both CVs at Figure 1B, left, and corresponding chronoamperograms in Figure 1B, right) was observed for AuNPs modified with anti-DR mAb (AuNP/αDR).

Cell Growth on the Surface of SPCEs. After the evaluation of the SPCE to detect AuNPs using the catalytic mode, the next step was to study whether tumor cells can grow or attach themselves on top of the same electrodes. Two adherent human tumor cells (HMy2 and PC-3) that differ in the expression of surface HLA-DR molecules were used. HMy2 (a B-cell line) presents surface HLA-DR molecules, whereas PC-3 (a tumoral prostate cell line) is negative to this marker; they were used as target cells and "blank/control assay" cells, respectively. The growth of both cell lines on SPCEs was compared with that in flasks, the routine environment used in cell culture.

A total of 2×10^5 cells were placed onto the surface of the SPCEs through a small chamber with a window over the electrode area. Cell growth was allowed to take place on the surface of the working electrode. Figure 2 shows scanning electron microscopy (SEM) images of both cell lines attached to the working electrode of the SPCEs. Both types of cells were able to grow on the carbon surface, and most interestingly, they showed morphological features similar to those of cells growing on the plastic surface (inset images).

Identification of Cells Based on the Catalytic Detection of AuNPs. Scheme 1 shows the cell assay used to specifically identify tumoral cells starting from the SPCE electrotransducer. Both types of cell, HMy2 (Scheme 1A) and PC-3 (Scheme 1B), were initially introduced onto the surface of the SPCEs and allowed to grow (a,a′), prior to incubation with antibody-modified AuNPs (b,b′). Finally, analysis by electrocatalytic detection based on hydrogen ion reduction (d,d′) was carried out. Taking advantage of the catalytic properties of AuNPs on hydrogen evolution, antibodies conjugated with AuNPs were used to discriminate positive or negative cells for one specific marker. The presence of HLA-DR proteins on the surface of HMy2 cells was compared with PC-3 cells used as blanks. Two different antibodies were used for this purpose: a commercial anti-DR mAb

Scheme 1. (A) Tumoral Cell Line (HMy2) Expressing Surface HLA-DR Molecules Compared to a (B) Cell Line That Is Negative to This Marker (PC-3)[a]



[a] Cells were (a,a′) attached to the surface of the electrodes and (b,b′) incubated with AuNP/αDR, (c,c′) an acidic solution was added, and (d,d′) the hydrogen generation was electrochemically measured.

(αDR) and a homemade BH1 mAb, both able to recognize HLA-DR class II molecules. For the commercial sample, αDR antibodies were directly labeled with AuNPs, whereas for the homemade BH1 antibody, a second step was necessary using AuNPs conjugated to secondary antibodies (αIgM). Another homemade antibody (32.4 mAb) that recognizes both types of cells was used as a positive control.

After addition of 50 μL of a 1 M HCl solution, when a negative potential of −1.00 V was applied, the hydrogen ions of the medium were reduced to hydrogen, and this reduction was catalyzed by the AuNPs attached through the immunological reaction. The current produced was measured. The electrochemical response in the presence of AuNP/αDR antibodies was positive in HMy2 (DR-positive cells), but not, as expected, in PC-3 (DR-negative cells) (Supporting Information, Figure S3A). This response was greatly increased by the use of secondary antibodies, as was observed for the BH1 mAb followed by AuNP/αIgM. For the control antibody (32.4 mAb), the electrochemical signals suggested that the PC-3 cells grew on the SPCEs and that recognition took place to a higher extent for these cells than for the HMy2 cell line.

These results concur with those for both cell lines in the immunofluorescence analysis by flow cytometry (Supporting Information, Figure S3B). The figure shows that, whereas HMy2 cells are positive to both the commercial αDR and the BH1 mAbs, PC-3 cells are negative to these antibodies. The same result was found for the positive control undertaken with the antibody 32.4, which recognizes both types of cells by immunofluorescence but has a higher intensity of recognition for PC-3 cells.

Effect of the Number of Cells on the Electrocatalytical Signal. To minimize the analysis time, the commercial αDR mAb was chosen for the quantification studies, even though the BH1 mAb had a higher response. Different quantities of HMy2 cells, ranging from 10000 to 400000, were incubated on the SPCEs and, subsequently, recognized by the AuNP/αDR. Figure 3A shows the effect of the number of cells on the electrocatalytical signal. An increase can be seen in the value of the analytical signal obtained that is correlated to the amount of HMy2 cells cultured. Although, because of the scale, no major differences can be appreciated in Figure 3A, a difference of around 170 nA was observed between control cells (blank) and 10000 cells. The inset curve shows that there is a very good linear relationship between

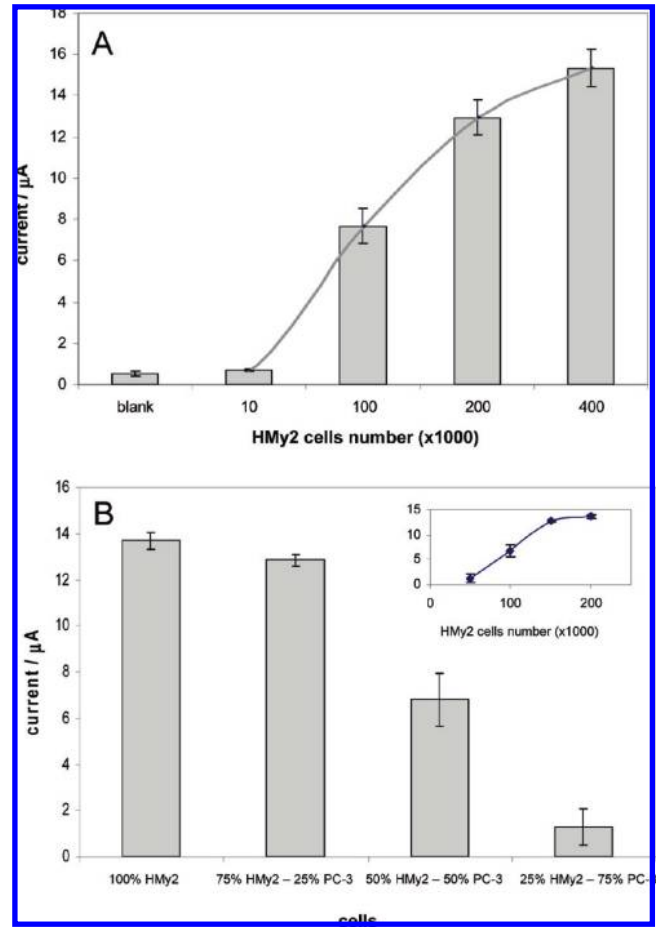


Figure 3. (A) Effect of the number of HMy2 cells on the electrocatalytical signals, after incubation with AuNP/αDR. (B) Electrocatalytical signals obtained with HMy2 cells, after incubation with AuNP/αDR in the presence of PC-3 cells at different HMy2/PC-3 ratios (the first bar, "100% HMy2", corresponds to 200000 HMy2 cells, whereas the last bar "25% HMy2−75% PC-3" corresponds to 50000 HMy2 cells and 150000 PC-3 cells).

the two parameters in the range of 10000−200000 cells, with a correlation coefficient of 0.9955, according to the equation

$$\text{current } (\mu\text{A}) = 0.0641(\text{cell number}/1000) + 0.497\ (\mu\text{A})\ (n = 3)$$

The limit of detection (calculated as the concentration of cells corresponding to 3 times the standard deviation of the estimate)

was 4000 cells in 700 μL of sample. The reproducibility of the method shows a relative standard deviation (RSD) of 7%, obtained for a series of three repetitive assay reactions for 100000 cells.

In addition, the ability of the method to discriminate HMy2 in the presence of PC-3 cells was also demonstrated. Figure 3B shows the values of the analytical signal after incubation with AuNP/αDR for mixtures of HMy2 and PC-3 cells at different ratios (100% corresponds to 200000 cells). The presence of PC-3 cells does not significantly affect the analytical signal coming from the recognition of HMy2 cells, and once again, a good correlation was obtained for the signal detected and for the amount of positive cells on the electrode. This could pave the way for future applications to discriminate, for example, tumor cells in tissues or blood, as well as biopsies, where at least 4000 cells express a specific marker on their surface.

Further technological improvements, such as reducing the size of the working electrode, could lead to a reduction in the volume of sample required for analysis, thereby allowing the detection of even lower quantities of cells. In addition, amplification strategies could be implemented; for example, micro-/nanoparticles could be simultaneously used as labels and carriers of AuNPs, making it possible to obtain an enhanced catalytic effect (more than one AuNP per antibody would be used) that would produce improved sensitivities and detection limits.

The developed methodology could be extended for the discrimination/detection of several types of cells (tumoral, inflammatory) expressing proteins on their surface, by using specific monoclonal antibodies directed at these targets. For example, the methodology could be applied for the diagnosis of metastasis. Metastatic tumor cells can express specific membrane proteins different from those in the healthy surrounding tissue, where they colonize. It could also be used for those primary tumors that exhibit specific tumor markers or overexpress others than those that are normally absent or have very low expression in healthy tissues. The breast cancer receptor (BCR) could possibly fall into this category, as it appears at low levels in healthy cells, but is overexpressed in some types of breast cancer. With a positive response, the identification of tumor cells could be very useful for an early treatment of the patient with monoclonal antibodies specifically targeted against this cancer receptor.

CONCLUSIONS

A novel cell sensor design has been developed coupled with a new electrocatalytic detection method for AuNPs, making possible specific identification of tumor cells. The proposed cell sensor is a rapid and simple cell detection device, on which cell growth occurs in situ, followed by detection on the same platform. The method's electrochemical detection mode is a more sensitive alternative to the direct detection of AuNPs reported earlier for DNA or protein sensing. Chronoamperometric plotting of the analytical signal is much simpler than the stripping analysis or differential pulse voltammetries described in previous reports.[17−19,21] In our particular example, we show that HLA-DR molecules on the surface of HMy2 cells are recognized by specific antibodies, previously conjugated with AuNPs. Their catalytic effect on hydrogen ion reduction is measured, allowing for specific cell identification, with a detection limit of 4000 cells.

In conclusion, we have demonstrated a tumor cell sensor based on gold nanoparticle immunoconjugates that, in combination with a screen-printed electrode, provides an efficient sensing platform, achieving both detection and identification of analytes. This strategy uses the electrocatalytic properties of gold nanoparticles to impart efficient transduction of the cell-binding event. A rapid, efficient, and generalized identification and quantification of cells is made possible by the use of this cell sensor. We have shown here that it can be used in the detection of surface molecules on tumoral cells, but more research is under way to determine whether this cell sensor could also be used for the detection of intracellular antigens and in cell suspensions. This would increase the possible uses of this device even more. The robust characteristics of the nanoparticles and transducing platform, combined with the diversity of surface functionality that can be readily obtained using nanoparticles, make this approach a promising technique for biomedical diagnostics. This approach could pave the way for further applications, such as the detection of cancer or inflammatory cells in diagnostic procedures (e.g., in needle aspiration biopsies in the operating room), using a simple miniaturized system. This method can also be extended to DNA detection based on conjugated AuNPs. As the cell sensor is produced by standard screen-printing fabrication, it can be readily mass produced at low cost and as disposable units. The use of magnetic nanoparticles could soon bring inherent signal amplification in a manner similar to that previously reported for DNA[19] or protein detection.[21,22]

Finally, efforts are under way to fabricate a lab-on-a-chip system that will include the use of magnetic particles as antibody immobilization platforms and could be used for protein and cell analysis. We envisage that the resulting cell sensor will be broadly applicable to sensing different cells, biomarkers, and biological species with enhanced sensitivity and specificity. Furthermore, it will be a truly portable, low-cost, easy-to-use device for point-of-care use.

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SUPPORTING INFORMATION AVAILABLE

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

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