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Pattern-based sensing of triple negative breast cancer cells with dual-ligand cofunctionalized gold nanoclusters

Yu Tao [a], Mingqiang Li [b], Debra T. Auguste [a, *]

[a] Department of Biomedical Engineering, The City College of New York, 160 Convent Avenue, New York, NY, 10031, United States
[b] Department of Biomedical Engineering, Columbia University, New York, NY, 10027, United States

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ABSTRACT

Early detection of breast cancer is a critical component in patient prognosis and establishing effective therapy regimens. Here, we developed an easily accessible yet potentially powerful sensor to detect cancer cell targets by utilizing seven dual-ligand cofunctionalized gold nanoclusters (AuNCs) as both effective cell recognition elements and signal transducers. On the basis of this AuNC multichannel sensor, we have successfully distinguished healthy, cancerous and metastatic human breast cells with excellent reproducibility and high sensitivity. Triple negative breast cancer cells (TNBCs), which exhibit low expression of the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2, were identified. The high accuracy of the blind breast cell sample tests further validates the practical application of the sensor array. In addition, the versatility of the sensor array is further justified by identifying amongst distinct cell types, different cell concentrations and cell mixtures. Notably, the drug-resistant cancer cells can also be efficiently discriminated. Furthermore, the dual-ligand cofunctionalized AuNCs can efficiently differentiate different cells from the peripheral blood of tumor-free and tumor-bearing mice. Taken together, this fluorescent AuNCs based array provides a powerful cell analysis tool with potential applications in biomedical diagnostics.

1. Introduction

Breast cancer is the most common invasive malignancy diagnosed and the second leading cause of cancer fatality in women worldwide [1,2]. Early breast cancer detection holds great promise for effective therapy [3–5]. Among them, triple negative breast cancers (TNBCs) are an aggressive breast cancer subtype defined by low expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) [6,7]. Although TNBCs represent only 15–20% of all breast cancer cases [8,9], they are responsible for a greater proportion of metastatic cases and deaths [9–11]. The high mortality rate appears to be due to the intrinsic aggressiveness of cancer cells, as well as the lack of effective diagnostic methods and targeted therapeutic strategies [12]. Therefore, the availability of rapid and sensitive methods to identify breast cancer cells, particularly TNBCs, may provide significant insight for predicting disease conditions and cancer treatment [13,14].

Traditional techniques for cancer cell detection mainly apply molecular ligands (e.g., peptides, aptamers, and antibodies) that are highly specific to predetermined biomarkers of the target cell population [15,16]. However, the identification of TNBCs by the representative approaches (e.g., ELISA-type tests [17], gel electrophoresis [18,19], proteomics and related approaches coupled with mass spectrometry [20], RT-PCR [21], as well as immunotyping by flow cytometry [22,23]) remains challenging due to the constrains in the availability of specific molecular biomarkers that can discriminate between TNBC cells and nonneoplastic cells. In addition, no biomarker is established as a cancer screening tool that has sufficient sensitivity to distinguish between normal, cancerous, and metastatic cell types [24]. Therefore, it is still highly appealing to develop facile and efficient methods for breast cell type analysis, especially for TNBCs.

Unbiased “chemical nose” array sensors may be considered as potential alternatives for cell discrimination, allowing identification through selective recognition [25,26]. In the chemical nose strategy, a sensor array is developed to provide differential binding interactions with analytes via nonspecific receptors, generating fingerprint-like response patterns that can be statistically analyzed and utilized for discriminative identification [27,28]. Analogous to our own noses, chemical nose sensors preclude the need of prior knowledge of the analytes and are instead “trained” to identify

* Corresponding author.
E-mail address: dauguste@ccny.cuny.edu (D.T. Auguste).

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analytes [29,30]. A wealth of applications of chemical nose sensors are demonstrated, including detection of metal ions [31], volatile organic compounds [32,33], carbohydrates [34,35], amino acids [36,37], and proteins [38–45]. Recently, these strategies have been expanded to more complex systems, such as cell [46–55] and bacteria [56–61] sensing.

Various receptor systems have been employed for array-based sensing of cells, including fluorescent polymers [53], green fluorescent proteins [46,50,55], fluorescently labeled DNAs [52,54], magnetic glyco-nanoparticles [51], and gold nanomaterials [48,49]. Although these methods are capable of discerning cells, these systems generally require a large population of cells. For instance, Rotello and coworkers fabricated an array-based system for discrimination of normal, cancerous and metastatic cell types using conjugated polymer/gold nanoparticle constructs with a detection limit of higher than 20000 cells [53]. In addition, Fan and Hu applied adaptive “ensemble aptamers” that exploited the collective recognition abilities of a small set of rationally designed, nonspecific DNA sequences to identify a wide range of molecular or cellular targets discriminatively, including different cell lines with a limit of detection of 5000 cells [52]. Micrometastases remain undetectable by conventional means; detection methods for small numbers of cancer cells are a prerequisite to early intervention.

Generally, biosensors often possess two basic functional components: recognition units and transducers, which are usually divided into two parts and composed of two distinctive elements [30]. We here construct an array based cell identification system by using seven dual-ligand cofunctionalized gold nanoclusters (AuNCs) as both effective cell recognition elements and signal transducers (Scheme 1). Compared with organic dyes and quantum dots based fluorescent probes, AuNCs are more promising for bioanalysis due to their simple synthesis, favorable biocompatibility, strong photoluminescence and high stability for long-time observations [62–65]. This sensing system is designed utilizing one species with dual roles, which offers extra advantages in terms of function integration and low cost. As we know, cell membrane surfaces of different cell populations are comprised of distinctive types and amounts of integral membrane proteins, carbohydrates and amphipathic phospholipids [53,66]. The as-prepared AuNCs showed similar fluorescence properties but distinctive surface properties, characterized by their unique surface charge states and chemistry. These differences of AuNCs may provide differential binding affinities and hence diverse interactions with individual cell lines [67], resulting in distinct fluorescence responses for each cell type. The additive responses offer an efficient means of identification. This sensing array is capable of efficiently distinguishing healthy, cancerous and metastatic human breast cells with excellent reproducibility and high sensitivity, including TNBC cells. In addition, the versatility of the sensor array is further demonstrated by identifying amongst distinct cell types, different cell concentrations, cell mixtures, and discrimination of unknown cell samples. Furthermore, the dual-ligand cofunctionalized AuNCs can identify nonresistant and drug-resistant cancer cells, and differentiate cells from the peripheral blood of tumor-free and tumor-bearing mice, providing new opportunities for cancer diagnosis.

2. Materials and methods

2.1. Materials and instrumentation

11-mercaptoundecanoic acid (MUA), 6-mercapto-1-hexanol (MH), (11-mercaptoundecyl)-N,N,N-trimethylammonium bromide (NSH), 4-mercaptobenzoic acid (MBA), (2-mercaptoethyl)amine (MEN), glutathione (GSH), mercaptosuccinic acid (MSA), 3-mercaptopropionic acid (MPA), tetrakis (hydroxymethyl) phosphonium chloride (THPC), and gold(III) chloride hydrate ($HAuCl_4$) were purchased from Sigma-Aldrich. Dulbecco's phosphate buffered saline (PBS) was purchased from Invitrogen (Carlsbad, CA). All other reagents were all of analytical reagent grade and used as received. Nanopure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment. TEM images were recorded using a JEOL 2100 transmission electron microscope operating with an accelerating voltage of 200 kV. Zeta potential measurements were performed with a ZetaPALS zeta potential analyzer. Fluorescence measurements were carried out by using a microplate spectrofluorometer GeminiXPS. Mass spectrometry (MS) experiments were carried out using the Waters ESI-Q-TOF MS (m/z 70–1000), which can quantify the surface thiolate ligands on the dual-ligand cofunctionalized gold nanoclusters. NaCN (20 mM) was used to etch the purified dual-ligand cofunctionalized gold nanoclusters (100 nM, 1.0 mL) to liberate the thiolate ligands from the gold nanocluster surface. Each type of thiolates with the concentration range from 0 to 100 μM was prepared for the quantification curve. DTT (20 mM) was conducted to avoid the formation of disulfide bonds of thiolates. The etching sample (5.0 μL) was dispersed in 1:1 water/ethanol solution (5.0 μL). Each mixture was then injected into a capillary trap column. After that, the MS was operated with a spray potential of 3.0 kV. NanoLockSpray source was utilized for accurate mass measurement, with the lock mass channel sampled every 30 s. Data acquisition was carried out in data direct analysis mode. The concentration of thiolate was calculated according to the MS signal of sample and thiolate quantification curve.

2.2. Cell culture

MDA-MB-231, MDA-MB-436, MDA-MB-157, MCF7, SKBR3, MCF10A, HCC1806, HCC1569, Hs578T, MDA-MB-468, DU4475, HUVEC, PC3, 3T3 and 4T1 cells were all obtained from American Type Culture Collection (ATCC, Manassas, VA). MDA-MB-231, MCF7, MDA-MB-436, HUVEC, PC3 and 3T3 cells were cultured in DMEM (Corning, Corning, NY), supplemented with 10% FBS (Life Technologies, Carlsbad, CA) + 1% Pen/Strep (Life Technologies, Carlsbad, CA). SKBR3 cells were cultured in McCoy5a (Life Technologies, Carlsbad, CA) + 10% FBS. MCF10A cells were cultured in DMEM/F12 (Life Technologies, Carlsbad, CA) + 5% Horse Serum (Life Technologies, Carlsbad, CA) + 20 ng/mL of Epidermal Growth Factor (EGF) (Peprotech, NJ) + 0.5 mg/mL of Hydrocortisone (Sigma-Aldrich, St. Louis, MO) + 100 ng/mL Cholera Toxin (Sigma-Aldrich, St. Louis, MO) + 10 mg/mL of Insulin (Sigma-Aldrich, St. Louis, MO) + 1% Pen/Strep (Life Technologies, Carlsbad, CA). MDA-MB-157 cells were cultured in L-15 (ATCC) + 10% FBS. HCC1806, HCC1569, MDA-MB-468 and DU4475 cells were cultured in RPMI-1640 (Corning) + 10% FBS. Hs578T cells were cultured in DMEM, supplemented with 10% FBS + 10 mg/mL of Insulin. 4T1 cells were cultured in RPMI-1640 + 10% FBS + 1% Pen/Strep. All cell lines were grown in a humidified atmosphere (5% CO_2) at 37 °C.

2.3. Synthesis of dual-ligand cofunctionalized AuNCs

Dual-ligand cofunctionalized AuNCs were synthesized according to previous reports with slight modification [40,68]. Firstly, 100 μL of NaOH (1 M) solution and 24 μL of THPC (8%, wt) solution were mixed with 8 mL of ultrapure water under violent stirring for 5 min, and then, 400 μL of $HAuCl_4$ (Au(III), 24 mM) solution was added rapidly. The color of the solution turns from light-yellow to brown in 1 min, indicating the formation of small gold nanoparticles. At this point, 100 μL of thiolate (R-SH, 10 mM) solutions was added to obtain different thiolate-protected gold nanoparticles. After stirring for another 15 min at room temperature, the solution was cooled to 4 °C for further use. After aging for 1 day, 1 mL of thiolate-protected gold nanoparticles stock solution was