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Visual detection of cancer cells by colorimetric aptasensor based on aggregation of gold nanoparticles induced by DNA hybridization

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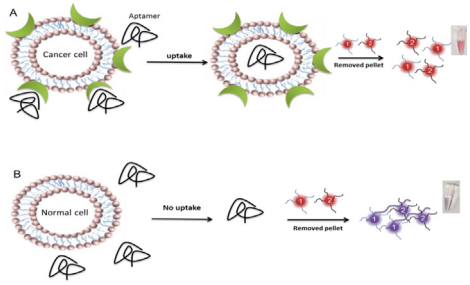
HIGHLIGHTS

- A simple and the sensitive colorimetric method for detection of MCF-7 was introduced.
- Direct cancer cells detection was developed based on aptamer–cell interaction.
- The nanobiosensor has detection limit of 10 cells for determination of MCF-7 cells.
- The proposed nanobiosensor could be extended to detect other cells.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple but highly sensitive colorimetric method was developed to detect cancer cells based on aptamer–cell interaction. Cancer cells were able to capture nucleolin aptamers (AS 1411) through affinity interaction between AS 1411 and nucleolin receptors that are over expressed in cancer cells, The specific binding of AS 1411 to the target cells triggered the removal of aptamers from the solution. Therefore no aptamer remained in the solution to hybridize with complementary ssDNA-AuNP probes as a result the solution color is red. In the absence of target cells or the presence of normal cells, ssDNA-AuNP probes and aptamers were coexisted in solution and the aptamers assembled DNA-AuNPs, produced a purple solution. UV–vis spectrometry demonstrated that this hybridization-based method exhibited selective colorimetric responses to the presence or absence of target cells, which is detectable with naked eye. The linear response for MCF-7 cells in a concentration range from 10 to 10^5 cells was obtained with a detection limit of 10 cells. The proposed method could be extended to detect other cells and showed potential applications in cancer cell detection and early cancer diagnosis.

1. Introduction

Cell recognition and detection could be useful for analyzing tissue samples or for capturing circulating tumor cells. By

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measuring the number of specific cells in human blood, clinicians could determine the onset of a specific disease or predict the patient's response to a specific treatment. The ability to detect specific cells at a very low concentration is critical for detecting circulating tumor cells. Because most solid tumor cells can be found at a concentrations up to 200 cells/ml in an average adult male (0.004% of cell population in the blood) [1]. More importantly, screening people with no symptoms to find early signs of cancer in a routine blood test would increase the chances of being cured dramatically [2,3]. However, traditional analysis techniques, such as immunohistochemistry, flow cytometry, and polymerase chain reaction (PCR) [4–6] do not meet the requirement for point-of-care (POC) diagnostics because they usually require costly instruments, long analytical time, and complicated operations [7]. Meanwhile, considering the very low quantity of stray cancer cells [1,8], screening requires a new clinical platform with high specificity and ultra-sensitivity to detect cancer cells, especially for early clinical diagnostics. Furthermore, the screening approach should be affordable to screen more people, especially in developing countries and detect cancer at early stages that would increase the survival rate tremendously. Therefore, there is a need for developing a simple, sensitive and affordable diagnostic tool to detect rare circulating cancer cells.

Coupling nanomaterials and biomolecule recognition events represents a new direction toward the development of novel molecular diagnostic tools [9]. For detection of cancer cells, not only it is important to have a specific platform, but we also need to have a highly sensitive tool as well. The advanced detection techniques Aptamers designed allow us to design aptamers with predictable structures and site-specific chemical modification to provide linkage for advanced signaling mechanism. AS1411 (26 mer, 7.8 kDa) is a GC-rich DNA aptamer that binds to nucleolin with a high affinity (dissociation constant is in the picomolar to low nanomolar range) [10,11]. Nucleolin is a phosphoprotein overexpressed in cytoplasm and on plasma membrane of the metastatic cells, and not the normal cells. AS1411 enters many different cancer cell-types via nucleolin-mediated endocytosis [12,13].

Aptamers were conjugated with nanomaterials to enhance cancer cell detection. For instance, the density of cell-surface targets for aptamers is not always abundant, especially for cancer cells in the early stages of development. Therefore, multivalent binding versus single aptamer binding has been studied to increase cell specific signaling. The large surface area and variable sizes allow nanomaterials to serve as multivalent ligand scaffolds [14,15]. To make the assay colorimetric, gold nanoparticles were utilized because of their bio functionalization, bio stability, and spectral properties. Due to the plasmon resonance of gold nanoparticles, they possess strong distance-dependent optical properties. Once the gold nanoparticles come into proximity with one another, their absorption spectra shift and their scattering profile changes result changes in color and absorption spectra of the sample [15,16]. As a result, many techniques have been developed based on gold nanoparticles aggregation to detect ions, genes and proteins [17–21]. Considering unique properties of the aptamers and gold nanoparticles, we have developed an assay system that is colorimetric in nature and shows excellent selectivity between target and control cells. This method is based on hybridization between aptamer and two sets of gold nanoparticles functionalized with single-stranded DNA probes in supernatant (probe 1 and 2 – AuNPs). In this study since both probes had partially complementary sequences to specific site of aptamer, cross-linking of nanoparticles was induced by hybridization under target cell free condition. This phenomenon resulted in gold nanoparticle aggregation and produced a purple solution. In the presence of target cells, the specific binding of AS 1411 to the target cells triggered aptamer removal from solution, no aptamer was remained in the solution to assemble DNA-AuNPs aggregation that resulted a red color solution.

2. Materials and methods

2.1. Materials and reagents

Chloroauric acid ($HAuCl_4\_4H_2O$) and sodium citrate ($Na_3C_6H_5O_7$) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Merk. Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and penicillin/streptomycin were purchased from Gibco (USA). Deionized water with a resistivity greater than 18 MΩ cm was acquired from a Millipore Milli-Q system. Phosphate-buffered saline (PBS) was prepared by mixing 8 g NaCl, 0.2 g KCl, 1.42 g Na_2HPO_4 and 0.27 g KH_2PO_4 in 1 L of twice distilled water. All oligonucleotides used in this work were synthesized by Shanghai Generay BiotechCo. Their bases sequences are shown as follows:

Probe sequence (probe 1): 5′-SH-$(CH2)_6$ CCACCACCACCAC -3′
Probe sequence (probe 2): 5′- AACCACCACCACC$(CH2)_6$-SH-3′
AS1411 sequence: 5′-GGTGGTGGTGGTTGTGGTGGTGGTGG-3′

All oligonucleotides stock solutions were prepared with TE Buffer and kept frozen until used. To make a TE Buffer, 1 ml of 1 M Tris–HCl (pH7.5) and 0.2 ml EDTA (0.5 M) was added to deionized water to a total volume of 100 ml of solution. All chemicals were of analytical grade and used without further purification.

Cells and cell culture human cell lines used in this study were MCF-7 cells (human breast cancer cell line), A549 cells (human lung cancer cell line), AGS cells (human gastric cancer cell line), and primary fibroblast cells (from normal human skin).

2.2. Apparatus

Absorption spectra were determined using Perkin–Elmer lambda25 spectrometer. TEM images were taken with a transmission electron microscope (Zeiss, EM10C, 80 KV, Germany) on a copper grid.

2.3. Synthesis of gold nanoparticles

Fifty ml aqueous solution of hydrogen tetrachloroaurate (III) tetrahydrate (1 mM) was heated to boiling while being stirred in a round-bottom flask with a reflux condenser. 10 ml of trisodium citrate (38.8 mM) was then added into the solution rapidly. The solution was boiled again for another 10 min and the color of the solution changed from yellow to red. The heating was stopped but the stirring continued until it reached room temperature [22], The AuNPs solution was then stored in at 4 °C. The TEM imaging analysis determined the diameter and dispersion state of our synthesized AuNPs. Using the extinction coefficient ($2.7 \times 10^8\ M^{-1}\ cm^{-1}$) at 520 nm [23], the concentration of the AuNPs solution was calculated to be about 4.4 nM according to Beer's law. The diameter of the synthesized AuNPs was about 25 nm.

2.4. Preparation of DNA-Modified AuNPs

The coupling of thiolated probe to gold nanoparticles was demonstrated by the higher affinity of thiol to AuNPs. Thiolated probe (1 OD) and 1 ml of the gold nanoparticles solution was incubated at room temperature for 16 h [24]. The solution was transferred into 0.1 M NaCl, 10 mM phosphate buffer (pH 7) and kept at room temperature for 40 h. To remove unreacted probes,