Sensors and Actuators B 262 (2018) 703–709

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

On-chip plasmonic detection of microRNA-106a in gastric cancer using hybridized gold nanoparticles

Sang-Heon Park[a,b], Jihye Lee[a,b], Jong-Souk Yeo[a,b,*]

[a] School of Integrated Technology, Yonsei University, Incheon, 406-840, Korea
[b] Yonsei Institute of Convergence Technology, Yonsei University, Incheon, 406-840, Korea

ARTICLE INFO

Article history:
Received 14 August 2017
Received in revised form 27 January 2018
Accepted 1 February 2018
Available online 2 February 2018

Keywords:
Biosensing
miRNA
Plasmon
Plasmon coupling
Core-satellite structure
Gastric cancer

ABSTRACT

For the early detection of miRNA-106a, the promising biomarker of gastric cancer, we demonstrate on-chip colorimetric biosensing platform based on the plasmon coupling of hybridized gold nanoparticles. Uniform and reproducible substrates are developed by optimizing the distribution and density of core gold nanoparticles with controlled ratio of organosilanes for optical on-chip detection. The core-satellite nanostructure is made by a conjugation between miRNA-106a and probes functionalized on gold nanoparticles, thus enabling the observation of scattering color changes and spectral shift by plasmon coupling effect with high specificity and sensitivity.

1. Introduction

Although gastric cancer is the second most common cause of cancer related deaths in the world [1], early stage detection is still lacking due to the inherent property of the cancer cells that are initially small in size and asymptomatic. The mortality rate for gastric cancer remains high due to the later stage presentation with the limited monitoring options. Thus, early detection and prognosis monitoring are very important to avoid the death from gastric cancer [2]. Current practice for screening gastric cancer completely relies on the endoscopy and computed tomography (CT) for directly diagnosing the cancer and its metastasis, however, it is difficult for many people to access the instruments and expertise for proper screening since more than two thirds of gastric cancer occurs in developing countries. Also, those instruments are not designed for mass screening and early stage detection.

There are various approaches to find the promising biomarkers in gastric cancer cells instead of screening by endoscopy and conventional CT, but few molecular markers are clinically used. Proteomics is one of the commonly used technology to find biomarkers for the detection of the cancer [3]. The protein level biomarkers such as heat shock protein 27 (HSP 27), carcinoembryonic antigen (CEA), or carbohydrate antigen 19-9 (CA 19-9) are promising biomarkers used for convenient diagnostic assays, but they have several remaining challenges such as low specificity and sensitivity to be used for clinical application and early detection, low density of target protein associated with cancer, and difficulty of forming a suitable high-affinity anti-body for the detection of the biomarkers [4,5].

Recent evidences have shown that microRNAs (miRNAs) are promising biomarkers for cancer diagnosis [6]. The miRNAs are small non-coding RNAs which play a regulatory role in gene expression at the post-transcription level [7]. Some of miRNAs showing abnormal concentration or ratio in patient serum, plasma, saliva, and urine can be used as potential biomarkers for clinical applications with physio-pathological conditions in a specific organ [8,9]. In the case of gastric cancer, miRNA-106a is up-regulated in a patient plasma and also related with cancer stage, tumour size, and proliferation of tumour cells [10–12]. To detect miRNAs, various approaches have been investigated such as electrical, optical, and mass sensors with the help of micro-electro-mechanical systems (MEMS) as well as nanotechnology [13–17]. More conventional techniques such as the polymerase chain reaction (PCR) or northern blot can detect the promising biomarker, RNA but they still have many limitations and relatively lack sensitivity. PCR method requires a measurement for a sufficient period of time using large instrument with power and also at relatively high cost. Northern

* Corresponding author at: School of Integrated Technology, Yonsei University, Incheon, 406–840, Korea.
E-mail address: jongsoukyeo@yonsei.ac.kr (J.-S. Yeo).

https://doi.org/10.1016/j.snb.2018.02.010
0925-4005/

blot also has some risks of RNA degradation and contamination of sample with RNase. Moreover, it has high risk to the experimenter and also to the biomarker because it uses UV, ethidium bromide (EtBr) to intercalate with RNA, and diethylpyrocarbonate (DEPC) as a RNase inactivation reagent [18,19]. Although many studies are being progressed, detecting specific miRNAs in gastric cancer cells with the clinically required sensitivity has remained challenging and there is no prior report on the optical plasmonic detection with nanostructure for miRNA-106a.

In this paper, we demonstrate a novel on-chip optical biosensing platform for the detection of miRNA-106a based on the principle of localized surface plasmon resonance (LSPR) of coupled core-satellite nanostructures. The LSPR occurs when the metallic nanoparticles interact with an incident light [20]. The electric field of the incident light induces the waves from the collective oscillation of conduction electrons on the surface of a metallic nanostructure, which is a phenomenon called LSPR. It can be tuned by size, shape, and material of the nanoparticles. The resonance condition also can be changed by plasmon coupling effect that is made by the coupling of nanoparticles with nanoscale gap between them [21]. When the particles are brought sufficiently close to each other, the plasmon oscillations of those nanoparticles become coupled with near-field interaction. Our sensing platform leverages this plasmon coupling effect from the assembly of gold nanoparticles (AuNPs) hybridized with miRNA of our interest, specifically miRNA-106a for gastric cancer [22–24]. The platform for the detection of miR-106a is illustrated in Fig. 1. The hybridized AuNPs are made by the pairing of complementary oligonucleotides with the sequence of miRNA. It enables the detection of miR-106a using colorimetric changes of red shifted spectra induced by plasmon coupling effect.

2. Materials and methods

2.1. Materials

Acetone (99.5%), (3-Aminopropyl) triethoxysilane (APTES, 99%), reagent alcohol anhydrous, trimethoxy (octadecyl) silane (OTMS, 90%), and isopropyl alcohol (IPA, 99.7%) were purchased from Sigma-Aldrich. Gold nanoparticles (AuNPs) with 50 nm in diameter and 30 nm in diameter were obtained from BBI Solutions. Probe 1, probe 2, miRNA-106a (miR-106a), test A, miRNA-21, and random miRNA oligonucleotides were synthesized by Bioneer Co. Ltd with a HPLC method for purification. 0.01 M tris-HCl (hydroxymethylaminomethane) buffer (pH 7.5), dithiothreitol (DTT) were purchased from Thermo Scientific. Quartz substrate was obtained from Duran Group (Germany). Nap-10 column was purchased from GE Healthcare.

Before using the quartz substrate, it was treated with O_2 plasma at 40 sccm, 400 mTorr, and 100 W for 60 s followed by sequentially rinsing with the cleaning steps of acetone, IPA, and DI water for 5 min, respectively.

2.2. Simulation of electric field distribution on the AuNPs

In order to analyze the experimental data, we have performed the simulation of electric field distribution on the AuNPs by using a finite-difference time-domain method (FDTD) from Lumerical Solution, Inc. FDTD simulation of our plasmon coupling configuration provides further insight into the plasmon coupling based biosensing. We used the 50 nm and 30 nm gold nanostructures with the plasmon coupling condition using a gap of 8.4 nm. The gap size is estimated by assuming a base size of 0.3 nm for 28 bases. The gold nanostructure uses the "Au (Gold)-CRC" material model included in the default material database from Lumerical Library. The media of the surrounding environment is air. The FDTD mesh setting is auto non-uniform with 3 order mesh accuracy. The boundary condition of FDTD is perfectly matched layer (PML) and allows symmetry on all boundaries. The override mesh is added on the circle with 5 nm size in x, y, z-direction. The excitation source is a plane wave with TM polarization with an amplitude of 1. The source of the light is illuminated to the nanostructure. The plane wave source covers a wavelength range of 400–700 nm. The type of the plane wave is Bloch/Periodic. The direction of the source is z-axis with backward direction. The x, y, z normal monitors are set for monitoring the electric field distribution and peak wavelength on the resonance condition. The monitors record the data based on standard Fourier Transform.

Table 1
Size and sequence of miRNA-106a, probe 1 and 2.

Name	Length	Sequence (5'–3')
miR-106a	22 mer	AAAAGUGCUUACAGUGCAGGUAG
Probe 1	12 mer	Thiol – GGGCUACCUGCA
Probe 2	12 mer	AGCACUUUUGGG – Thiol

2.3. Pre-modified mixed layer on quartz substrate

We immobilized the core AuNPs on the glass substrate with a proper density at detection area to make optical on-chip detection. AuNPs with 50 nm in diameter were bound to the glass substrate using a mixed layer of APTES and OTMS. APTES has the amino (NH_3^+) terminal group and OTMS has the methyl (CH_3) terminal group. The capped AuNPs were bound to the amino group and blocked by the methyl group via electrostatic interaction [25]. The mixtures of 50 mM organosilanes (APTES/OTMS = 1:0, 5:1, 3:1, 3:2, 1:1, 0:1) were dissolved in anhydrous ethanol for 3 h. By these experiments, we found the appropriate ratio of the APTES to OTMS to make the proper density of core AuNPs that provide green color, thus enabling to observe the clear colorimetric changes and spectral shift by the AuNPs based on plasmon coupling effect. The high concentration of the APTES leads to the agglomeration of AuNPs. On the other hand, the core AuNPs are not bound to the modified quartz substrate when the concentration of OTMS is high. We have selected the optimal density of core AuNPs by comparing their colours, spot-to-spot deviations, and the substrate-to-substrate deviations (Fig. 2(g)). When the mixing ratio of APTES to OTMS is 3:2, intensities at the peak wavelength on the detection area were evaluated at 5 spots on the 1.5 × 1.5 quartz substrate. The spot-to-spot deviation in a substrate was measured as 352.10 ± 38.48 and the substrate-to-substrate deviation was measured as 294.39 ± 76.89. However, when the mixing ratio of APTES to OTMS is 3:1, intensities at the peak wavelength deviated much less with 412.39 ± 17.88 for spot-to-spot and with 353.96 ± 37.66 for substrate-to-substrate on the detection area of the substrate. Thus, we determined that the 3:1 mixing ratio of APTES to OTMS provides more uniform and reproducible green color with plasmon resonance wavelength of nearly 550 nm from single core AuNPs.

2.4. Bio-functionalization on AuNPs with oligonucleotides

The probe 1, probe 2, and target miR-106a which are summarized in Table 1 were purified with DTT and Nap-10 column for preparatory experiment. The satellite AuNPs were pre-functionalized with the probe 2 for 12 h of incubation in room temperature. The probe 2 is 12 mer long oligonucleotide with the sequence complementary to a part of target miR-106a sequence. The core AuNPs with 50 nm in diameter were functionalized with the probe 1 that is 12 mer long oligonucleotide with the sequence complementary to the other part of target miR-106a sequence.