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A DNA nanostructured aptasensor for the sensitive electrochemical detection of HepG2 cells based on multibranched hybridization chain reaction amplification strategy

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ABSTRACT

Sensitive detection of cancer cells is beneficial to the early diagnosis of cancer and individual treatment. In the present study, a DNA nanostructured aptasensor was used for the sensitive electrochemical detection of human liver hepatocellular carcinoma cells (HepG2) based on multibranched hybridization chain reaction amplification strategy. We established a well-designed platform by immobilizing DNA tetrahedron, a three-dimensional DNA nanostructure, on the gold electrode to capture HepG2 cells more specifically and efficiently. Meanwhile, functional hybrid nanoprobes consisted of MIL-101@AuNPs (Au nanoparticles), numerous hemin/G-quadruplex DNAzyme from multibranched hybridization chain reaction, and natural horseradish peroxidase (HRP) was designed. The hybrid nanoprobes possessed the functions of specific discernment and enzymatic signal amplification simultaneously. With the help of nanoprobes, HepG2 cells were recognized and captured to form a DNA tetrahedron-cell-nanoprobe sandwich-like structure on the electrode surface. The lower detection limit of this established cytosensor is 5 cells per ml. Moreover, it delivered a broad detection range from 10^2 to 10^7 cells per ml. The results revealed that the as-proposed cytosensor may be utilized as a powerful tool for early diagnosis of cancer in the future.

1. Introduction

Cancer, the most common deadly disease threatening the health of human in today's society, is the primary cause of death in human (Siegel et al., 2017). Especially, liver cancer has been one of the leading causes of death in China (Chen et al., 2016). During the development of cancer, circulating tumor cells (CTCs) which can circulate in the peripheral blood, are extremely rare in human blood (1–3000 CTCs per ml), playing a crucial role in tumor metastasis (Shen et al., 2017; Wan et al., 2014). Hence, it is urgent to develop an ultrasensitive and more specific method to detect these cancer cells.

To date, various techniques have been introduced into the detection of cancer cells, such as fluorescence analysis (Kang et al., 2007), flow cytometry (Paredes-Aguilera et al., 2001), inductively coupled plasma mass spectrometry (ICP-MS) (X. Zhang et al., 2016), electrochemiluminescence (H. Zhou et al., 2014), and electrochemical biosensor (Dervisevic et al., 2017; Pang et al., 2017; Sun et al., 2015, 2017, 2016; Zheng et al., 2014). Particularly, electrochemical aptasensor (aptamer-based biosensor) has attracted increasing interest owing to the advantages of fast response, simple operation, excellent sensitivity and low cost compared with other methods (Chen et al., 2014; Kashefi-Kheyrabadi et al., 2014). Aptamers, the specific DNA sequences screened by the systematic evolution of ligands by exponential enrichment (SELEX) system, can bind to cells, small molecules and proteins with high specificity (H.M. Zhang et al., 2016). Nevertheless, there are some demerits hindering the follow-up progress of aptasensor. For instance, single-stranded DNA aptamers immobilized on electrodes tend to entangle and aggregate with each other, which extremely impeded effective combination of aptamers and cells. In this way, it is also difficult to immobilize single-stranded DNA aptamers on electrodes orderly and precisely in spatial orientation.

Therefore, to deal with these challenging problems, studies have widely utilized DNA tetrahedron in the field of biosensor to detect nucleic acid (Liu et al., 2015), protein (J.P. Wang et al., 2017), small molecules (Wen et al., 2011) and viruses (Dong et al., 2015). In 2010, Fan and co-workers (Pei et al., 2010) proposed the first DNA

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nanostructure-based electrochemical biosensor by using DNA tetrahedron. In order to anchor DNA tetrahedron to the surface of the gold electrode through the formation of Au-S bond, the three vertices of DNA tetrahedron were linked with thiol. The fourth vertex was expanded a pendant single strand DNA to bind target (Chen et al., 2018; Lin et al., 2015; Liu et al., 2015; Pei et al., 2014). This nanostructure showed 5000-times greater affinity than single strand oligonucleotides. Moreover, DNA tetrahedron was ordered in vertical orientation on account of high mechanical rigidity without the help of 6-mercapto-1-hexanol (MCH). The introduction of DNA tetrahedron could reduce the non-specific adsorption of the developed biosensor (Pei et al., 2010). In addition, Miao's group constructed a cytosensor with DNA tetrahedron nanostructure to detect Hela cells sensitively (Meng et al., 2016). Also, Tan's group reported a portable electrochemical aptasensor for the detection of exosomes using DNA nanotetrahedra (S. Wang et al., 2017).

Meanwhile, sensitivity is another urgent problem that needed to be solved. Various strategies have been introduced to enhance the sensitivity of biosensor including nucleic acid amplification techniques (Ge et al., 2014; Yu et al., 2015) and functional hybrid nanoprobes (Zheng et al., 2014). Firstly, among the nucleic acid amplification techniques, hybridization chain reaction (HCR) gained wide attention owing to an enzyme-free, simple, and isothermal amplification process (Bi et al., 2017; Lan et al., 2016; Liu et al., 2013; Lu et al., 2015; Qian et al., 2015; Zhou et al., 2013). In a typical HCR, the initiative strand triggers hybridization events between two DNA hairpins, resulting in countless repeating units. Zuo's group exploited multibranched HCR (mHCR) to establish an electrochemical detection platform for MCF-7 cells (G.B. Zhou et al., 2014). In their work, they reported that mHCR can produce multiple branched arms for multivalent binding and multiple biotin that avidin-HRP can attach for signal amplification. Secondly, functional hybrid nanoprobes have played a great role in improving sensitivity of biosensors. Among multiple nanoprobes, metal-organic framework (MOF) has become one of the most rapid developing fields due to the distinct advantages of high surface areas, favorable chemical stability, and uniform pore sizes (Ling et al., 2016; Yang et al., 2017). In particular, some MOFs with enzyme-mimicking activities have been developed as a tool to construct sensors. Recently, Fe-MOFs have been proved to have intrinsic peroxidase-like activity (Hu et al., 2017; Jiang et al., 2015; Zhang et al., 2014). Hence, Fe-MOFs are efficient tools for increasing sensitivity of biosensors.

In the present study, a well-designed biosensor platform was proposed for HepG2 cells based on DNA tetrahedron as the approach of recognition and capture, HCR as the signal amplified strategy, and MIL-101(Fe) (a typical type of Fe-MOF) as the functional hybrid nanoprobes. Firstly, TLS11a, the specific aptamer for HepG2 cells, was built into one of DNA tetrahedron sequences to efficiently capture cells on the gold electrode. By doing so, the specificity and capture efficiency of this cytosensor were improved because individual aptamers were distributed at defined nanoscale distances and hindrance effect was decreased. Then, the synthesized MIL-101(Fe)@AuNPs were attached to cells on the gold electrode with the assistance of aptamers to fabricate a sandwich-like structure. In this work, the signal amplification has become reality by coupling with MIL-101(Fe)@AuNPs, numerous hemin/G-quadruplex DNAzyme, and natural HRP to catalyze the oxidation of hydroquinone (HQ) in the presence of H_2O_2. Therefore, this DNA nanostructured aptasensor for the sensitive electrochemical detection of HepG2 cells has potential in the detection of cancer cells and the early clinical diagnosis of liver cancer.

2. Experimental

2.1. Preparation of nanoprobes

MIL-101 was synthesized according to the reported method (Xu et al., 2017). Firstly, the mixture of 0.675 g $FeCl_3{\cdot}6H_2O$ and 0.206 g p-phthalic acid (H_2BDC) were dissolved in 15 ml N,N-dimethylformamide (DMF) to a clear solution with the help of ultrasound. Then, the solution was removed to a 50 ml Teflon-lined stainless-steel autoclave and maintained at 110 °C for 20 h. After cooling to room temperature (RT), the yellow products were collected by centrifuging and washed by DMF and ethanol for three times. Next, the products were held in ethanol at 70 °C overnight. After that, the obtained MIL-101 was isolated from ethanol and stored in ultrapure water for further use.

Secondly, AuNPs was synthesized according to the previous method with some modification (Chen et al., 2014). 100 ml 0.01% chloroauric acid ($HAuCl_4$) was placed in a three-necked flask and heated to boiling with continuous stirring. Then, 3 ml sodium citrate dehydrate aqueous solutions (10 mg/ml) were added immediately and quickly. When the color of solution turned to wine red, it was kept boiling and stirred for 15 min and left to cool down to RT. The obtained 100 ml AuNPs was washed with ultrapure water and collected by centrifugation, and finally dispersed in 10 ml water for further use.

Thirdly, 100 μl of the MIL-101 solution was added into 1 ml poly (ethylenimine) (PEI) solution (4 mg/ml). Then, the mixture was stirred gently at RT overnight, to obtain the NH_2-functionalized MIL-101, followed by centrifuging and washing with water twice. The resulting MIL-101 was suspended in 1 ml ultrapure water. Subsenquently, 4 ml of AuNPs was put in MIL-101 and stirred at 4 °C for 12 h, to form MIL-101@Au through Au-N bond. Meanwhile, the 20 μl of thiolated sequences H_0 was diluted with 20 mM tris(2-carboxyethyl) phosphine hydrochloride (TCEP) to a concentration of 10 μM, and incubated for 1 h at RT. Thereafter, 20 μl of H_0, H_1 and H_2 were mixed with the concentrations of 10, 100 and 100, respectively. The prepared hybridization mixture was heated to 95 °C, kept for 5 min, and rapidly cooled to RT to react overnight.

Finally, 60 μl of hybrid products and 100 μl HRP solution (1 mg/ml) were added into above MIL-101@Au, kept stirring at 4 °C for 2 days. Subsequently, hemin solution (0.1 mM) was introduced into the above solution and incubated for 2 h. The washing step was repeated twice. The resulting nanoprobes were re-suspended in phosphate buffered saline (PBS) solution for further use.

2.2. Preparation of DNA tetrahedron

The DNA tetrahedron was assembled from three thiolated DNA strands of 61 nucleotides (a, b and c), and one aptamer-linking DNA strand of 125 nucleotides (d). The thiolated DNA strands (a, b, and c) were incubated with TCEP (10 mM) in TM buffer (500 mM Tris-HCl, 80 mM Mg^{2+}, pH 8.0) for 1 h at RT. First, 5 μM of equimolar quantities of nucleotides (a, b, c, d) were mixed in TM buffer. Second, the mixture was heated to 95 °C for 10 min, and rapidly cooled to 4 °C for more than 10 min. The assembly of DNA tetrahedron was proved by 3.5% agarose gel electrophoresis in TBE buffer (89 mM Tris-Boric Acid; 2 mM EDTA) at 100 V.

2.3. Fabrication of electrochemical aptasensor

Firstly, the gold electrode was immersed in a piranha solution (H_2SO_4:30% H_2O_2 = 3:1) for 15 min. Then, the gold electrode was polished with alumina slurry (particle size: 0.05 μm) to a mirror-like surface. After that, the gold electrode was cleaned by ultrasonication for 2 min in water, ethanol, and water, respectively. The cleaned gold electrode was further electrochemically activated in 0.5 M H_2SO_4 solution. Afterward, it was rinsed with ultrapure water and dried with nitrogen.

Secondly, ten microliters of 1.25 μM DNA tetrahedron was dropped into the surface of clean gold electrode, incubated at 4 °C overnight, followed by washing with water to remove unbound DNA tetrahedron. After the nanostructure was immobilized on the gold electrode through the Au-S bond, the aptasensor is ready for HepG2 cells capture. Then, the modified electrode was incubated with a 200 μl PBS solution with a