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Dual aptamer-functionalized silica nanoparticles for the highly sensitive detection of breast cancer

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ABSTRACT

In this study, we synthesized dual aptamer-modified silica nanoparticles that simultaneously target two types of breast cancer cells: the mucin 1 (MUC1)(+) and human epidermal growth factor receptor 2 (HER2)(+) cell lines. Dual aptamer system enables a broad diagnosis for breast cancer in comparison with the single aptamer system. The dye-doped silica nanoparticles offer great stability with respect to photobleaching and enable the accurate quantification of breast cancer cells. The morphological and spectroscopic characteristics of the designed Dual-SiNPs were demonstrated via diverse methods such as DLS, zeta potential measurements, UV–vis spectroscopy, and fluorescence spectroscopy. Negatively charged Dual-SiNPs with a homogeneous size distribution showed robust and strong fluorescence. In addition, Dual-SiNPs did not affect cell viability, implying that this probe might be readily available for use in an *in vivo* system. Through ratio optimization of the MUC1 and HER2 aptamers, the binding capacities of the Dual-SiNPs to both cell lines were maximized. Based on Dual-SiNPs, a highly sensitive quantification of breast cancer cells was performed, resulting in a detection limit of 1 cell/100 μL, which is significantly lower compared with those reported in other studies. Moreover, the developed detection platform displayed high selectivity for only the MUC1(+) and HER2(+) cell lines. It is expected that this valuable diagnostic probe will be a noteworthy platform for the diagnosis and prognosis of breast cancer.

1. Introduction

Circulating tumor cells (CTCs) in peripheral blood are detached epithelial cells having identical or similar carcinoma characteristics to those of the primary cancer (Gupta and Massague 2006; Pukazhendhi and Gluck 2014). They have received considerable attention as valuable biomarkers for the diagnosis and prognosis of specific tumor metastasis because the detection of CTCs is a less invasive and more reliable method than currently existing conventional methods such as radiographic photography, serum tumor marker-based detection, and biopsy (Alama et al., 2014; Mostert et al., 2009). In particular, CTCs are designated as a real-time "liquid biopsy" because of their accessibility to samples from peripheral blood (Chang et al., 2014; Nadal et al., 2013). Two main strategies are currently used for the detection of CTCs, *i.e.*, immunological assays based on monoclonal antibodies and polymerase chain reaction (PCR)-based molecular assays (Pantel et al., 2008). However, such methods exhibit various problems such as being time-consuming, costly, providing false-positive or false-negative results, and requiring large instrumentation (Gazzaniga et al., 2013; Wang et al., 2014). Therefore, a relatively rapid, inexpensive, and precise detection technique for CTCs is in high demand with respect to the early diagnosis of carcinoma metastasis.

Breast cancer is one of the most common malignant tumors and the second leading cause of cancer deaths in women (Siegel et al., 2013). According to the US National Cancer Institute Surveillance, Epidemiology, and End Results database, the averaged incidence rate of breast cancer is 123.8 cases/100,000 women/year (Johnson et al., 2013). More than 90% the cancer-related deaths are due to metastatic growth (Siegel et al., 2013). Therefore, the detection and monitoring of the metastasis of the carcinoma is most important. While several biomarkers, conventional radiologic evaluation, and histological detection cannot offer sufficient information related to tumor metastasis, CTCs certainly indicate the presence and metastatic stage of the cancer (Pantel and Alix-Panabieres, 2010). It has been known that a comparatively large number of CTCs are found in the peripheral blood of patients with breast cancer (Bidard et al., 2013). Thus, the detection of CTCs in the blood is a promising method for the early diagnosis and determination of a prognosis for breast cancer patients.

To evaluate these CTCs, various biomarkers such as the epithelial cell adhesion molecule, cytokeratin-8 (CK-8), CK-18, CK-19,

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and hMAM have been utilized (Bertolini et al., 2006; Zhao et al., 2013). In particular, human epidermal growth factor receptor 2 (HER2) has been targeted for the detection of breast cancer CTCs since Slamon et al. (1987) discovered that the HER2 protein was over-expressed in breast cancer patients. Although numerous investigations for the detection of breast cancer cells based on HER2 have been conducted, they are limited to only HER2(+) cell lines (Gupta et al., 2011; Lu et al., 2010; Zhu et al., 2013). Because HER2 is over-expressed in only 15–20% of breast cancer patients, it cannot play a role as a confident biomarker for all types of breast cancer. Among the surface biomarkers for breast cancer, mucin-1 (MUC1) has been identified as an effective biomarkers, and it is abnormally over-expressed in greater than 90% of human breast carcinomas (Rakha et al., 2005; Singh and Bandyopadhyay, 2007). Multi-target methods using MUC1 and HER2 probes make the accurate and precise detection of CTCs in breast cancer possible.

Diverse studies on diagnosing breast cancer based on nanomaterials have been actively conducted because nanomaterials have numerous advantages over the existing detection tools such as their easy surface modification, good biocompatibility, and unique spectroscopic properties (Howes et al., 2014; Jeon et al., 2013; Lee et al., 2014; Singh et al., 2015). Of the effective nanomaterials, dye-doped silica nanoparticles (SiNPs) have been considered to be one of the best substances to be used for detection. Whereas some organic dyes have several limitations, including severe photobleaching, being costly and requiring time-consuming preparation, dye-doped SiNPs provide a high photostability, low-cost synthesis, great fluorescent signal, and good biocompatibility (Li et al., 2014; Wang et al., 2013). In particular, it has been verified that tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(BPY)_3$)-doped SiNPs (Dye-SiNPs) can prevent photobleaching because the positively charged $Ru(BPY)_3$ molecule is strongly confined to the negatively charged silica matrix via a coulombic interaction, which disturbs the access of potential quenching materials from the surrounding environment (Cai et al., 2013; Herr et al., 2006; Liu et al., 2014; Smith et al., 2007). Hence, the diagnosis of breast cancer using $Ru(BPY)_3$-doped SiNPs is a most robust and valuable method.

As shown in Fig. 1, we designed a dual aptamer (MUC1 and HER2)-modified SiNP (Dual-SiNP) for the detection of breast cancer cells. This is the first time that both types of biomarkers for breast cancer have been targeted utilizing aptamers, which enables a wide-ranging diagnosis of breast cancer. First, a series of surface modifications was conducted on the core silica particles (Fig. 1A), and then their morphological and spectroscopic characteristics were analyzed through various techniques, such as transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential measurement, ultraviolet–visible (UV–vis) spectroscopy, and fluorescence spectroscopy. The actual detection was performed in the following two steps: magnetic bead-based separation and incubation with Dual-SiNPs (Fig. 1B). The low cytotoxicity, high sensitivity, and selectivity of the developed detection system were verified through a methylthiazol tetrazolium (MTT) assay and fluorescence measurements. This newly developed diagnostic system could be a good platform for the diagnosis and prognosis of the metastasis of breast cancer.

2. Materials and methods

2.1. Functionalization of PEG-SiNPs with the dual aptamers

Prior to the immobilization of the dual aptamers on the SiNPs, the surfaces of the PEG-coated SiNPs (PEG-SiNPs) were modified by avidin. The cyanogen bromide activation method was introduced to attach avidin to the particles (March et al. 1974). Three milligrams of PEG-SiNPs were incubated under gentle stirring in 3 mL of a 2 M sodium carbonate solution for 15 min at room temperature (RT). Thereafter, 3 mL of cyanogen bromide (CNBr) in acetonitrile (0.8 g/mL) was added to the reaction solution. The mixture was stirred for an extra 15 min at RT, and then the activated particles were gathered by centrifugation, which was followed by washing with chilled distilled water twice as well as with chilled phosphate buffered saline (PBS, pH 7.4) twice. Furthermore, the resulting particles were incubated with an excess amount of avidin (100 μL of 1 mg/mL avidin in PBS) under stirring

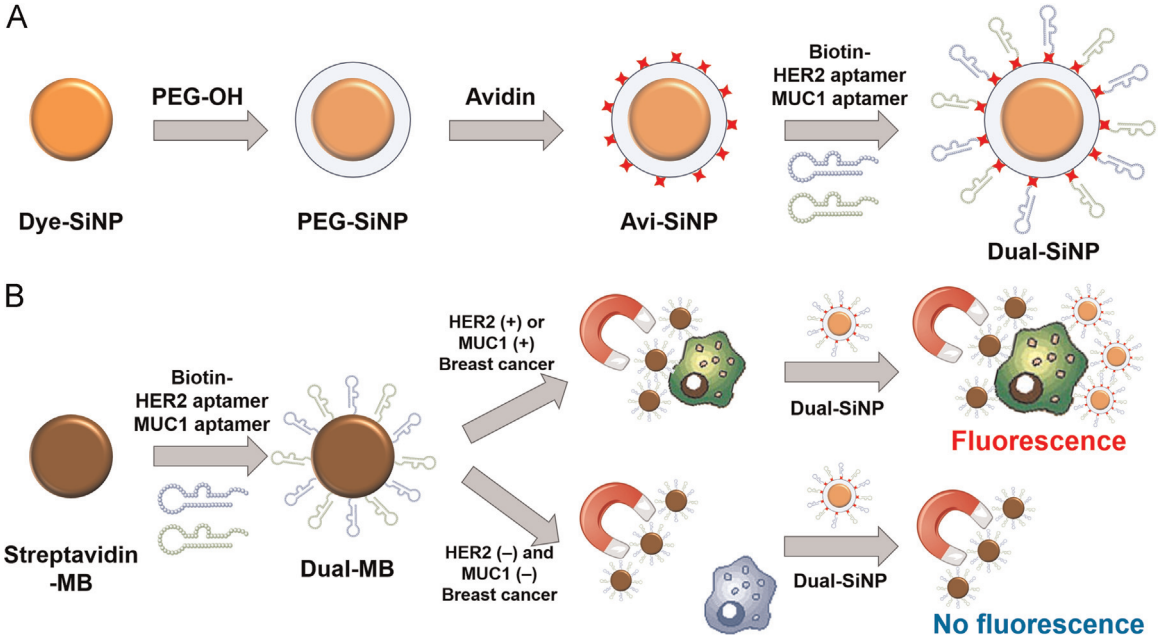

Fig. 1. (A) Modification processes of the silica nanoparticles. First, Dye-SiNPs were synthesized via the reverse microemulsion technique, and then, they were coated with PEG. Through CNBr activation, avidin was immobilized onto the PEG-SiNPs, and MUC1 and HER2 aptamers were functionalized by biotin-avidin interactions. (B) Schematic illustration of the selective detection for only MUC1(+) and HER2(+) breast cancer cells by Dual-SiNPs. MUC1(+) or HER2(+) breast cancer cells were selectively separated using dual aptamer-modified magnetic beads (Dual-MBs), and then, they were detected with Dual-SiNPs.