Sensors and Actuators B 223 (2016) 9–14

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Hyaluronic acid-coated magnetic nanoparticles-based selective collection and detection of leukemia cells with quartz crystal microbalance

Yaping Zhou [a,*], Qingji Xie [b]

[a] College of Pharmacy, Zunyi Medical University, Zunyi 563000, PR China
[b] Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form 9 September 2015
Accepted 11 September 2015
Available online 14 September 2015

Keywords:
Hyaluronic acid-coated magnetic nanoparticles
Leukemia cell
Quartz crystal microbalance

ABSTRACT

We have synthesized hyaluronic acid-coated magnetic nanoparticles (HA-MNPs) to selectively collect and detect of leukemia cells (CCRF-CEM) in combination with quartz crystal microbalance (QCM) measurement. Based on the specific binding of HA with CD44 receptors overexpressed on cell surface, HA-MNPs were successfully employed for selectively extracting CCRF-CEM cells from the complex matrices including human plasma samples. The collection and detection conditions for CCRF-CEM cells were optimized. Under optimized conditions, a detection limit of 8×10^3 cells mL^{-1} was obtained. The HA-MNPs-based magnetic separation combined with sensitive QCM measurement provides a simple, rapid and economical method for collecting and detecting leukemia cells, and this method may have great potential for wider applications in biomedical research and clinical diagnostics.

1. Introduction

In the past several years, leukemia has become one of the major human cancers that ultimately result in death. Facile collection methods and accurate detection techniques for leukemia cells are of critical importance for diagnosis, prevention and treatment of leukemia. As far as we know, current detection techniques for leukemia include karyotyping [1], flow cytometry [2], PCR [3], and so on. However, complexity, high cost and the time-consuming feature of those techniques limit their applications. Therefore, it is still an essential need to develop a simple, rapid and economical detection method for leukemia.

Hyaluronic acid (HA), which is a negatively charged linear polysaccharide and the main component of the extracellular matrix, has been extensively investigated as an attractive tumor-specific targeting vector in cell imaging [4,5] and drug delivery system [6–8]. HA could bind specifically to the CD44 receptors that overexpress on many types of tumor cell surfaces and play an important role in cell migration, tumor cell invasion and metastasis. Hence, HA has been frequently utilized as the targeting moiety for diagnosis and therapy of the cancers that have a high expression of CD44, such as ovarian cancer [9], breast cancer [10], acute leukemia and colon cancer [11]. In addition, when HA binds with CD44, this binding-mediated signal could trigger many cytological activities such as structural changes in the membrane and tumor cell migration. Disrupting the CD44-HA interaction by introducing anti-CD44 antibodies, hyaluronan oligomers or hyaluronidase may result in proliferation inhibition and apoptosis of tumor cells.

Recently, multifunctional magnetic nanoparticles (MNPs) have attracted increasing attention for their successful applications in biomedical field such as cell labeling and imaging [9,12], bioseparation [13–16], magnetic resonance imaging [17,18], drug delivery [19,20], and hyperthermia [13]. Due to the unique properties of HA mentioned above, MNPs modified with HA have been widely used for cancer cell imaging by MRI [9,10,21] and targeted drug delivery [22], but none of them has been used for cell extraction to date. The magnetic interactions over space and physical barriers make the magnetic extraction of cells very simple and effective. Obviously, employing HA-coated MNPs (HA-MNPs) to selectively bind the leukemia cells that overexpress CD44 receptors and then separating the cells from the crude samples with an external magnetic field, is a promising extraction method for leukemia cells. Compared with the methods reported previously [23–25], the proposed method presents some advantages

* Corresponding author.
E-mail address: yaping20302@163.com (Y. Zhou).

http://dx.doi.org/10.1016/j.snb.2015.09.063
0925-4005/

including simple operation procedure, low cost, mild conditions as well as high stability, making it a good candidate for biomedical applications.

Quartz crystal microbalance (QCM) as a sensitive transducer can detect the changes in mass loading and interfacial properties of the electrode surface [26,27]. It has been widely used in label-free bioanalysis of proteins, bacteria and cells, due to its satisfactory performance, e.g. high sensitivity, dynamic monitoring and facile operation. Also, QCM can provide much process information on the cellular attachment, spreading, proliferation and apoptosis on many surfaces for better understanding the cell–surface interactions [28]. Furthermore, the cellular responses to drugs and particles can be detected by QCM [29–31]. The premise of those studies is that the cells are able to adhere and spread on the surfaces. But for nonadherent cells, for instance, leukemia cells, the detection of cells with QCM should rely on the immobilization of magnetic particles-bound cells on QCM electrode surface under a magnetic field.

Herein, we prepared HA-MNPs via electrostatic interaction and utilized them to perform the selective extraction and detection of leukemia cells (CCRF-CEM) in combination with QCM measurement. The collection and detection conditions for CCRF-CEM cells were optimized, and the extraction and detection capabilities of the proposed method in complex matrix were evaluated using cell mixture and human plasma samples. To the best of our knowledge, this is the first time that the leukemia cells have been extracted with HA-coated MNPs and then detected by QCM.

2. Experimental

2.1. Chemicals and instruments

Iron (III) chloride hexahydrate, iron (II) chloride tetrahydrate, poly(diallyldimethylammonium chloride) solution (PDDA, Mw 200,000–350,000, 20 wt.% in water) and hyaluronic acid sodium salt from *Streptococcus equi* were purchased from Sigma–Aldrich. Phosphate-buffered saline (PBS) solution (pH 7.4), RPMI 1640 medium and fetal bovine serum were obtained from Invitrogen. IMDM medium was obtained from Thermo Scientific. Biological grade water (>18 MΩ cm) was used throughout the experiments. Other reagents were of analytical grade or better quality.

An AT-cut 10 MHz piezoelectric quartz crystal with gold electrodes (5 mm diameter) formed uniformly on both sides were used. It was mounted in a Kel-F cell sealed by two O-rings [32], with only one side of the crystal wafer exposed to the liquid. A coin-like neodymium iron boron magnet of 5 mm in diameter and 3 mm in thickness was fixed on the back of the QCM electrode (ca. 5 mm away from the crystal surface exposed to liquid). A research QCM (RQCM, Maxtek Inc.) was used to record the resonant frequency of the quartz crystal. An inverted optical microscope (Nikon TMS) equipped with a CCD camera was used for taking images of cells. A Fourier transform infrared (FT-IR) spectrometer (Varian 3100) and a Hitachi H-7650 transmission electron microscope (TEM) were used to characterize the nanoparticles.

2.2. Cell culture

CCRF-CEM cells (T cell, human acute lymphoblastic leukemia), Raji cells (B cell, human Burkitt's lymphoma) and HL-60 cells (human promyelocytic leukemia cell line) were obtained from ATCC (American Type Culture Collection). CCRF-CEM and Raji cells were cultured in RPMI 1640 medium supplemented with 10% FBS, whereas HL-60 cells were cultured in IMDM medium supplemented with 20% FBS. The cells were incubated at 37 °C in water-saturated atmosphere containing 5% CO_2, and collected after centrifugating at 1000 rpm for 5 min and redispersing in cell media for three times. The cell density was determined by a hemocytometer.

2.3. Preparation of HA-MNPs

First, MNPs (Fe_3O_4) were synthesized by coprecipitating iron salts according to our previous report [30]. Second, HA-MNPs were prepared via electrostatic interactions. The MNPs prepared above were dispersed in PDDA aqueous solution by ultrasonication and the positively charged PDDA-modified MNPs were obtained. Then 1 mL of PDDA-modified MNPs solution (3 mg mL^{-1}) was added dropwise into 3 mL of HA aqueous solution (1 mg mL^{-1}) with mild stirring. The electrostatic attraction between the negatively charged HA molecules and positively charged MNPs resulted in the production of HA-MNPs. After 4 h, the particles were collected by magnetic separation and washing with water several times. Finally, the HA-MNPs were obtained and dispersed in water for use.

2.4. Magnetic extraction and QCM measurement

The process of cell magnetic extraction with HA-MNPs and then detection by QCM is shown in detail in the Supporting Information (Scheme S-1). A certain volume of HA-MNPs suspension was added into the cell suspension in a centrifuge tube for a period of incubation, then a magnetic field was introduced to the tube side, and after a few minutes the nonmagnetic materials were removed with a pipette. To complete the wash process, the magnetic field was removed and the magnetic materials were redispersed in fresh PBS for three times. Finally, the magnetic materials were dispersed in 20 μL PBS for following QCM measurement.

Supplementary scheme related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2015.09.063.

1 mL of PBS was filled in the QCM detection cell, when the resonant frequency of QCM became steady, the magnetic sample prepared above was added into the QCM detection cell and the frequency shift was recorded. After experiment, the crystal was regenerated by trypsin treatment for 24 h, followed by washing with hydrogen peroxide–sulphuric acid mixture (v/v, 1:3) and water in sequence for several times.

3. Results and discussion

3.1. Characterization of HA-MNPs

FT-IR spectra of MNPs, PDDA-modified MNPs and HA-MNPs in the range of 4000–600 cm^{-1} are shown in Fig. 1A. After modifying the MNPs with PDDA, the absorption peaks at ~2936 and ~1465 cm^{-1} attributed to the stretching vibration and bending vibration of C—H appeared, indicating the successful modification of PDDA on MNPs. The absorption peak at ~1036 cm^{-1} corresponding to the C—O stretching vibration of HA could be observed in the FT-IR spectrum of HA-MNPs, demonstrating the presence of HA in HA-MNPs. The size of the nanoparticles was characterized by TEM. From the TEM images shown in Fig. 1B, we could see that the MNPs were nearly spherical in shape and had a diameter of ~13 nm (upper left in panel B). After modifying the MNPs with PDDA, the obtained nanoparticles (PDDA-modified MNPs) had an increased diameter of ~80 nm (upper right in panel B). Obviously, HA-MNPs had a core–shell structure with a diameter of ~110 nm, and the thin HA shell of ~15 nm could be clearly seen from the TEM image of HA-MNPs (bottom in panel B). In addition, we observed that the HA-MNPs could be well attracted to precipitate on the glass bottle wall by magnets (Fig. 1C), showing their excellent magnetic property.