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Evaluation of Quantum Dots Nanobeads Based Lateral Flow Immunoassay for Interluekin-6 Detection in Human Blood for Cervical Inflammation Monitoring

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Abstract

Sensitive detection of IL-6 in menstrual blood at home is helpful for monitoring and early diagnosis of cervical health. However, traditional gold nanoparticles based lateral flow immunoaasy is not sensitive enough for IL-6 detection in menstrual blood. Quantum dots nanobeads (QDNB) and super-quantum dots nanobeads (SQDNB) were used as labels to prepare e lateral flow immunoassay for IL-6 detection. The concentrations of fluorescent conjugates were optimized, and the analytical performance of the developed assay were evaluated. The optimized dilutions of fluorescent conjugates are 1:200 and 1:100 for QDNB and SQDNB conjugates. The limits of detection of lateral flow immunoassay used QDNB and SQDNB as labels are 0.14 and 0.16pg/mL, respectively. CV for SQDNB at 3.9 pg/mL and 62.5 pg/mL are 18.0% and 20.2% respectively. This sensitive lateral flow assay is potential for self-test of IL-6 in menstrual blood at home.

Keywords

Interleukin-6; cervical health; lateral flow immunoassay; quantum dot nanobeads, antibody

1. Introduction

Cervical health issues are one of the significant public health challenges faced by women globally [1], especially in developing countries. According to literatures, the incidence of cervical lesions remains as high as 15%–23% in China, with a clear trend of occurring at younger ages [2]. These conditions are widely distributed across populations, severely impacting women's health and quality of life. The vast majority of cervical cancers are gradually developed from chronic inflammation and lesions caused by persistent high-risk human papillomavirus (HPV) infection [3][4]. This process is long and insidious, making early detection crucial for effectively preventing disease progression and achieving primary prevention [5].

Inflammatory factors play a crucial role in the occurrence and development of cervical lesions. Among them, interleukin-6 (IL-6), as an important pro-inflammatory cytokine, has been confirmed by several studies to be closely related to cervical inflammation and precancerous lesions [6]. The upregulation of its expression level can promote the formation of a local inflammatory microenvironment, accelerating the proliferation and invasion of tumor cells. Therefore, IL-6 can serve as an effective biomarker for indicating cervical inflammation and the potential risk of lesions [7].

Currently, the mainstream method for detecting IL-6 in clinical practice is Chemiluminescent Immunoassay

(CLIA). Although this method offers high sensitivity and good specificity, it relies on large instruments and professional operators, and limits of long turnaround times. Lateral flow immunoassay is a versatile tool for biomarkers detection at point-of-care, but traditional gold nanoparticles based lateral flow assay is not sensitive enough for early disease diagnosis. More importantly, due to the low concentration of IL-6 in blood [8], they are not suitable for rapid screening self-testing at home or primary healthcare settings. Recently, the fluorescent nanoparticles, such as quantum dots, time resolve fluorescent nanospheres and upconversion fluorescent nanoparticles, are employed as novel labels for the development of highly sensitive lateral flow immunoassay [9].

In this study, we dedicated to develop a lateral flow immunoassay strip based on quantum dot nanobeads for the rapid home self-testing of IL-6 in menstrual blood. Two kinds of quantum dots nanobeads were used as labels for lateral flow assays, and the performance of assays were evaluated to obtain a more sensitive assay for IL-6 detection.

2. Experimental Section

2.1 Materials

Quantum dots nanobeads was provided by Shanghai Kundao Biotech (Shanghai, China); IL-6 detection and capture antibody were purchased from Hytest Biotech (Turku, Finland); The recombinant IL-6 protein and goat-anti-mouse antibody were obtained from Songon Biotech (Shanghai, China).

Nitrocellulose membrane (NC membrane) was purchase from Sartorius Stedim Biotech (Goettingen, Germany); polyvinyl chloride (PVC) backing card, glass fiber pad (sample pad), and absorbent pad were obtained from Shanghai Kinbio Biotech (Shanghai, China); Fluorescent strip reader (FIC-Q100N) was purchased from Helmence Precision Instrument (Suzhou, China).

2.2 Preparation of quantum dots nanobeads-antibody conjugates

a. Carboxyl nanobeads, 100 µL (10 mg/mL), EDC (carbodiimide), incubated for 0.5 h, centrifuge.
b. 100 µg detection antibody, incubated for 0.5 h, centrifuge.
c. Blocking 1% BSA/casein, 2 h, fluorecent conjugates.

2.3 Preparation of strips

a. Capture antibody 1 mg/mL, dispenser 1 uL/cm, T line.
b. Goat anti-mouse 1 mg/mL, dispenser 1 uL/cm, C line.
c. Dry Sample pad, absorbent pad assembly, cutting to strips.

2.4 Preparation of calibrators and samples

The calibrators were prepared by spiking recombination IL-6 protein in serum or buffer, with a serials of concentration of 4000, 2000,1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9. 0 pg/mL.

2.5 Detection procedures

In a typical detection procedure, a mixture of 50 µL of fluorescent antibody conjugates solution and 50 µL of samples was loaded into the sample hole. After immunological reaction of 15 min, the test strips were measured using a fluorescent reader, and the fluorescent photos was captured by a cellphone camara under UV light illumination.

a. Optimization of the concentration of fluorescent conjugates

To optimize the fluorescent conjugates dilution factors, prepare 2 types of fluorescent IL-6 monoclonal antibodies conjugates (QDNB, SQDNB) were diluted with buffer at ratios of 1:200, 1:400, and 1:800. Then, calibrators of IL-6 were prepared by diluting the recombinant IL-6 protein with dilution buffer at concentration of 1000 pg/mL and 4000 pg/mL, and dilution buffer was used as negative control.

b. Preparation of serial calibrator and measurement of calibration curve.

To create the calibration curve, first prepare calibrators of 0, 1, 3.9, 15.6, 62.5, 250, 1000, 4000 pg/mL using serial dilution, then, mix 50 µL fluorescent solution and 50 µL sample together and load onto the test strip, place it in the fluorescence analyzer after 15 minutes, record C and T value. A total of 3 Measurement of samples

Typically, 50 µL of fluorescent solution and 50 µL of sample was mixed together, and loaded into the samples hole. After running for15 min, the test strips were qualitatively and quantitatively measured resptectively. For qualitatively measurement, the fluorescent photos were record with a cellphone camera under UV-light illumination. For quantitative measurement, the test strips were detected with a fluorescent test reader (FIC-Q100N, Hangzhou Helmence Precision Instrument, China), and the fluorescent intensities of T and C lines were recorded.

c. Determination LODs of assays

For qualitative measurement, the limit of detection (LOD) was determined as the lowest concentration of IL-6 to produce signals distinguished from the control samples. For quantitative measurement, the LOD of assay was defined as the concentration of blank samples signals plus 3 times of standard deviation for 10 times repeated measurements a fluorescent strip reader.

d. CV precision and recovery experiment for the selected fluorescent condition

For precision measurement, use 50 µL of the selected fluorescent condition (SQDNB620) to mix with 50 µL sample (3.9 pg/mL and 62.5 pg/mL) and load onto the test strip, place it in the fluorescence analyzer after 15 minutes, record C and T value, repeat for 5 replicates.

For recovery experiment, add 20 pg/mL and 40 pg/mL to the 15.6 pg/mL sample to create sample with concentrations of 35.6 pg/mL and 55.6 pg/mL, place it in the fluorescence analyzer after 15 minutes, record C and T value.

3. Results

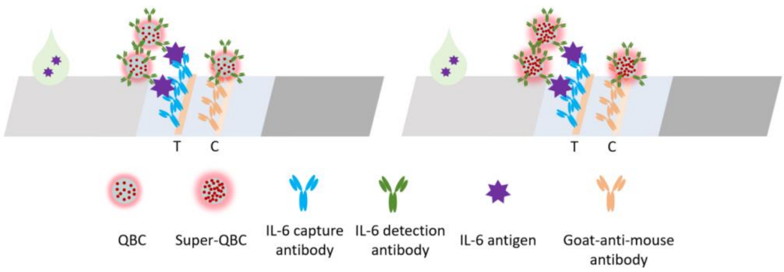

Figure 1. Schematic illustration of quantum dots nanobeads and super-quantum dots nanobeads lateral flow assay for IL-6 detection.

Fluorescent quantum dot nanobeads (QDNB) and super-quantum dot nanobeads (Super-QDNB) were employed as label for fluorescent lateral immunoassay, in this study. As shown in Fig 1, the lateral flow immunoassay strip composed of a sample pad, NC membrane and absorbent pad overlap with each other. The test line and control line were respectively immobilized with IL-6 capture antibody and goat-anti-mouse antibody. In a typical assay reaction, the samples are mixed with quantum dot nanobeads conjugates, which have been surface-modified with an IL-6 detection antibody. The mixture is then applied to the sample pad of the test strip. The liquid carries the complexes forward. As the liquid flows past the test line (T line), which is immobilized with the IL-6 capture antibodies. When the samples contain the IL-6 antigen, the T line would specifically capture the IL-6 antigen within the quantum dot-antibody conjugates. Consequently, a sandwich structure, comprising two antibodies, the IL-6 antigen, and quantum dot nanobeads, becomes immobilized on the T line and results to the increase the fluorescent intensity of T line. The control line (C line) captures excess quantum dot nanobeads and also emits fluorescence, thereby validating the experimental procedure.

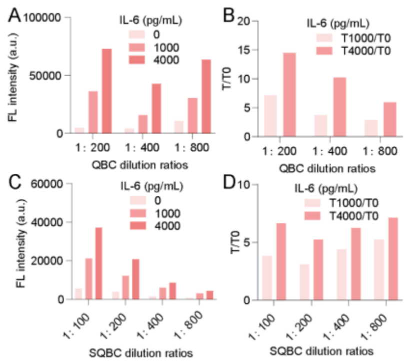

Figure 2. Concentration of fluorescent QDNB conjugates vs fluorescent intensity (A) and T/C ratio (B). Concentration of fluorescent Super-QDNB conjugates vs fluorescent intensity (A) and T/C ratio (B).

To optimize the performance of lateral flow immunoassay, the concentrations of fluorescent conjugates were optimized, as show in Fig 2. This presents the performance of two different concentrations of two fluorophores (QDNB and SQDNB) after a 15-minute incubation period at concentrations of 0, 1000, and 4000 pg/ml. For QDNB conjugates, three concentrations were evaluated: 1:200, 1:400, and 1:800. The results indicated that the 1:200 concentration exhibited the optimal fluorescent response, displaying the highest FL intensity (Fig 2A) and T/T0 (Fig 2B). In the case of SQDNB, four concentrations were measured: 1:100, 1:200, 1:400, and 1:800. The table of FL intensity clearly demonstrates a positive correlation between intensity and concentration. The 1:100 concentration of SQDNB showed the highest FL intensity. Consequently, the QDNB concentration of 1:200 and the SQDNB concentration of 1:100 were selected for subsequent experiments.

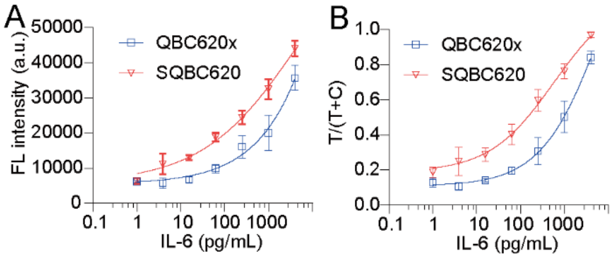

Figure 3. Calibration curves of lateral flow assay for IL-6 detection with fluorescent intensity (A) and T/(T+C) (B) as signals.

This is the calibration curve generated after fluorescence detection with QDNB at a 1: 200 concentration and SQDNB at the 1: 100 concentration. It can be observed that QDNB and SQDNB exhibit highly similar performance, with the FL intensity detected by both increasing exponentially with increasing IL-6 sample concentration. Furthermore, SQDNB demonstrates a more pronounced response as depicted in Fig 3A. The T/(T+C) values were also calculated. The computational results are analogous to the FL intensity values, indicating a positive correlation between the performance of both fluorescence probes and the IL-6 sample concentration. Notably, as the fluorescence concentration increases, the magnitude of intensity differences observed with SQDNB is more pronounced compared to QDNB. In addtion, the limits of detection of lateral flow immunoassay used QDNB and SQDNB as labels are 0.14 and 0.16 pg/mL, indicating high sensitivity. CV for SQDNB at 3.9 pg/mL and 62.5 pg/mL are 18.0% and 20.2% respectively, indicating the functional sensitivity of in vitro diagnostic reagents is about 62.5 pg/mL.

4. Discussion

Cervicitis is a wide-spread chromatic inflammation disease in women and is potentially developed to a cervical cancer. Early diagnosis of cervicitis is important for disease therapy and preventing the cancer development. Measurement of the inflammation biomarker IL-6 in menstrual blood is a promising strategy for early diagnosis of cervicitis. However, the lower concentration of IL-6 in blood is challenge for traditional gold nanoparticles based lateral flow immunoassay.

In this study, a fluorescent lateral flow immunoaasy using QDNB and SQDNB as labels were developed for highly sensitive detection of IL-6 in samples. After the optimization of the concentration of fluorescent conjugates, the IL-6 in samples can be measured in 15 min with LODs of 0.14 and 0.16 pg/mL using QDNB and SQDNB based lateral flow immunoassays. The SQDNB based lateral flow immunoassay demonstrated higher sensitivity than the QDNB based assay. Furthermore, the precision of the developed SQDNB based assay were evaluated with CVs of 18.0% and 20.2% at concentration of 3.9 and 62.5 pg/mL. The recovery rates of the SQDNB based assay were 128.5% and 119.6% with samples at concentration of 15.6 pg/mL adding 20 pg/mL and 40 pg/mL of IL-6 antigens.

The developed fluorescent lateral flow immunoassay has advantages of easy operation for at home self-test, and sensitive enough for IL-6 detection in menstrual blood. The developed lateral flow immunoassay is promising for non-invasive detection of cervicitis to benefit women's health. Nevertheless, the precision of lateral flow immunoassay is still not enough for quantitative measurement of IL-6 in menstrual blood. In the future, the developed lateral flow immunoaasy can be evaluated with the menstrual blood to verified the relation of the concentration of IL-6 with cervicitis disease.

5. Conclusion

In this study, we have developed quantum dots nanobeads based lateral flow immunoassay for IL-6 detection. After the optimization, the SQDNB based immunoassay demonstrated higher sensitivity than the QDNB based assay. This highly sensitive lateral flow immunoassay for IL-6 detection is promising for early diagnosis of cervicitis disease at home.

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