

Article

Sensitive Visual Detection of Breast Cancer Cells via a Dual-Receptor (Aptamer/Antibody) Lateral Flow Biosensor

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Abstract

We report a novel dual-receptor lateral flow biosensor (LFB) for the rapid, sensitive, and visual detection of MCF-7 breast cancer cells as a model for circulating tumor cells (CTCs). The biosensor employs a MUC1-specific aptamer conjugated to colloidal gold nanoparticles as the detection probe and an anti-MUC1 antibody immobilized at the test line as the capture probe, forming a unique “aptamer–cell–antibody” sandwich complex upon target recognition. This design enables instrument-free, visual readout within minutes, achieving a detection limit of 675 cells. The assay also demonstrates robust performance in spiked human blood samples, highlighting its potential as a simple, cost-effective dual-mode point-of-care testing (POCT) platform. This platform supports both rapid visual screening and optional strip-reader-based quantification, making it suitable for early detection and monitoring of breast cancer CTCs.

Keywords: lateral flow biosensor; aptamer–antibody sandwich assay; MCF-7 cells; visual detection; point-of-care testing (POCT)

1. Introduction

According to the World Health Organization (WHO), cancer is projected to cause approximately 24 million new cases and 14.5 million deaths annually by 2035. This global health burden is exacerbated by the disease’s heterogeneity and its capacity for metastasis, which is responsible for an estimated 80% of cancer-related mortality [1,2]. A critical step in the metastatic cascade is the dissemination of circulating tumor cells (CTCs)—cancer cells that detach from primary or metastatic sites and enter the bloodstream [3,4]. These cells can subsequently seed new tumors in distant organs [5]. Consequently, CTCs serve as valuable biomarkers, not only indicating the presence of a tumor but also its metastatic potential [6]. Notably, CTCs can be detected in peripheral blood even during early-stage disease, highlighting their immense promise for early diagnosis, prognosis, and treatment monitoring. However, their extreme rarity among a vast background of hematologic cells presents a formidable technical challenge for their isolation and detection [7,8].

Over the past two decades, significant research efforts have been directed toward developing CTC detection technologies, leveraging advances in oncology, biology, and materials science [9–12]. A PubMed search for “Circulating Tumor Cells” yields over 35,000 publications (as of November 2024), reflecting intense and growing interest in this field [13,14]. While various sophisticated techniques exist—including line-confocal



Received: 29 December 2025

Revised: 24 January 2026

Accepted: 28 January 2026

Published: 30 January 2026

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microscopy and surface-enhanced Raman spectroscopy (SERS)—they are often hampered by operational complexity, lengthy procedures, high cost, and suboptimal accuracy, limiting their utility in routine clinical practice [15,16]. There is, therefore, a clear and pressing need for detection platforms that are rapid, simple, cost-effective, and reliably sensitive.

Biosensors have emerged as promising candidates to meet this demand, offering advantages in sensitivity, specificity, and potential for point-of-care use [17–19]. These devices typically employ specific biorecognition elements, such as antibodies or aptamers, to capture CTCs via surface biomarkers, enabling distinction from normal blood cells and reducing false positives [20–22]. For instance, electrochemical biosensors detect impedance changes from captured cells with high sensitivity but generally require complex instrumentation and skilled operators [23–25]. In contrast, lateral flow biosensors (LFBs) offer an appealing alternative due to their simplicity, rapid visual readout (typically within 10–30 min), low cost, and minimal user training, making them ideal for point-of-care testing (POCT) [26–28].

Most conventional LFBs for cell detection rely on a single type of capture probe, which can limit specificity and sensitivity. To address this issue, we developed an innovative dual-receptor lateral flow biosensor for the rapid visual detection of MCF-7 breast cancer cells as a model of circulating tumor cells (CTCs). The biosensor employs a MUC1-specific aptamer and an anti-MUC1 antibody that simultaneously recognize distinct epitopes on the target cells. This design leverages the complementary strengths of aptamers—including high stability and programmability—and antibodies—notably their high affinity and mature signaling systems—thereby significantly enhancing detection sensitivity. Our design targets the transmembrane glycoprotein MUC1, which is overexpressed on MCF-7 cells. The biosensor employs gold nanoparticles functionalized with a MUC1-specific aptamer (Apt-MUC1@AuNPs) as the detection probe, while an anti-MUC1 antibody is immobilized at the test line to act as the capture probe. This configuration forms a unique “aptamer–cell–antibody” sandwich complex in the presence of target cells, generating a visible signal. We demonstrate that this strategy facilitates sensitive, instrument-free detection with a low limit of detection. Furthermore, we validate the practical applicability of the biosensor by successfully detecting MCF-7 cells spiked into human blood samples, underscoring its potential as a simple and effective tool for point-of-care analysis of CTCs in breast cancer.

2. Experimental Section

2.1. Chemicals and Reagents

Chloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Trisodium phosphate dodecahydrate ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$), n-butanol, and magnesium chloride (MgCl_2) were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Glucose and sucrose were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA), phosphate-buffered saline (PBS), Tris-borate-EDTA (TBE) buffer, and Tris-HCl buffer (pH 8.0) were acquired from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The following bio-reagents were sourced from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China): Anti-MUC1 antibody (capture antibody) and MUC1-specific DNA aptamer (Apt). Complementary DNA strand to the MUC1 aptamer (c-Apt). The materials for lateral flow strip assembly, including polyvinyl chloride (PVC) backing cards, glass fiber conjugate pads, nitrocellulose (NC) membranes, and absorbent pads, were purchased from Shanghai Jiening Biotech Co., Ltd. (Shanghai, China), and Shanghai Jinbiao Biotechnology Co., Ltd. (Shanghai, China). The running buffer used for all lateral flow assays was $1 \times$ PBS containing 0.5% (*w/v*) BSA and 0.75 mM MgCl_2 . All chemical reagents were of analytical grade and

used as received. Prior to use, all glassware was thoroughly cleaned with aqua regia (a 3:1 *v/v* mixture of HCl and HNO₃) and rinsed extensively with ultrapure water (18.2 MΩ·cm, Millipore Milli-Q system), which was used for the preparation of all aqueous solutions.

Oligonucleotide Sequences:

MUC1 Aptamer (Apt): 5′-SH-GCAGTTGATCCTTTGGATAACCCTGG-3′

Complementary Strand (c-Apt): 5′-Biotin-CCAGGGTATCCAAAGGATCAACTGC-3′

2.2. Equipment and Instruments

The following instruments were used in this study: X-Y-Z 3D thin-film spraying instrument (Model HM3035), microcomputer-controlled automatic shearing machine (Model ZQ2002), and numerically controlled strip cutting machine (Model CTS300) from Gold Standard Biotechnology Co., Ltd. (Chengdu, China); refrigerated centrifuge (Model DH-20KR) from GallopTech Co., Ltd. (Shenzhen, China); ultrapure water system (Model Simple Q30) from Zhion Instrument Co., Ltd. (Shanghai, China); high-temperature drying oven (Model DHG9023A) from Qixin Scientific Instrument Co., Ltd. (Shanghai, China); UV-Vis spectrophotometer (Model UV-6100) from Meitaishi Instruments Co., Ltd. (Shanghai, China); colloidal gold analyzer (Model GIC-S100) from Suzhou Hexiang Instruments Co., Ltd. (Suzhou, China).

2.3. Cell Culture

The human breast adenocarcinoma MCF-7 cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco (New York, NY, USA)) supplemented with 10% (*v/v*) fetal bovine serum (Hangzhou Sijiqing, Zhejiang, China) and 1% (*v/v*) penicillin-streptomycin. Cells were maintained at 37 °C in a humidified incubator with a 5% CO₂ atmosphere. For all experiments, cells in the logarithmic growth phase were harvested, and the cell concentration was determined using a hemocytometer prior to serial dilution in the assay running buffer. The cell count used in the detection experiments was determined using the hemocytometer method.

2.4. Preparation of Apt@AuNP Conjugates

The conjugation of the thiol-modified MUC1 aptamer to colloidal gold nanoparticles (AuNPs) was performed using a modified salt-aging method with an n-butanol dehydration step.

2.4.1. Concentration of Colloidal Gold Solution

Prior to conjugation, the as-synthesized colloidal gold solution was concentrated 8-fold. Briefly, 800 µL of the solution was centrifuged (8000 rpm, 4 °C, 10 min). The supernatant was carefully removed, and the AuNP pellet was resuspended in 100 µL of ultrapure water via vortexing.

2.4.2. Conjugation and Dehydration

For the conjugation reaction, 50 µL of the concentrated AuNP solution was mixed with 4 µL of the thiol-modified MUC1 aptamer (Det-Apt, 100 µM). Subsequently, 800 µL of n-butanol and 100 µL of 0.5× TBE buffer (pH-adjusted) were added to the mixture. The solution was shaken vigorously to facilitate n-butanol-mediated dehydration, a process indicated by a color change from the original pink/red of the AuNPs to a clear or pale purple solution. The mixture was immediately centrifuged in a mini-centrifuge for 20–30 s, yielding a dark red precipitate.

2.4.3. Purification of Conjugates

The supernatant was carefully discarded, and the Apt-MUC1@AuNP pellet was resuspended in 100 μ L of ultrapure water, restoring a burgundy red color. To remove unbound aptamers and reaction by-products, the conjugate solution was centrifuged (7000 rpm, 4 $^{\circ}$ C, 10 min), the supernatant was discarded, and the pellet was washed three times by repeating this resuspension–centrifugation cycle with 100 μ L of ultrapure water. Finally, the purified Apt-MUC1@AuNP conjugates were resuspended in 50 μ L of an appropriate storage or resuspension buffer (e.g., PBS containing stabilizers such as BSA and sucrose).

2.5. Fabrication of Lateral Flow Biosensors (LFB)

The LFB strips were assembled from four primary components laminated onto a polyvinyl chloride (PVC) backing card: a sample pad, a conjugate pad, a nitrocellulose (NC) membrane, and an absorbent pad. Assembly was performed as follows:

2.5.1. Pad Pretreatment

Both the sample pad and the conjugate pad were immersed in a pretreatment buffer containing 2.5% (*v/v*) Tween-20, 0.25% (*v/v*) Triton X-100, and 0.05 M Tris-HCl (pH 8.0) for 4 h. The pads were then dried overnight at 37 $^{\circ}$ C and stored at 4 $^{\circ}$ C until use.

2.5.2. Preparation and Dispensing of Capture Lines

Two capture lines were dispensed onto the NC membrane using an automated dispensing platform. The control line (C line) solution was prepared by incubating 18 μ L of streptavidin (1 mg/mL) with 12 μ L of the biotinylated complementary DNA strand (100 μ M) at room temperature for 2 h. The test line (T line) solution consisted of 30 μ L of the anti-MUC1 capture antibody. Both solutions were dispensed as separate lines. The NC membrane was then dried at 37 $^{\circ}$ C for 2 h to immobilize the capture reagents.

2.5.3. Conjugate Pad Loading and Final Assembly

The purified Apt-MUC1@AuNP conjugates (from Section 2.3) were dispensed onto the pretreated conjugate pad and dried. The absorbent pad, NC membrane, conjugate pad, and sample pad were then sequentially laminated onto the PVC backing card with a 2-mm overlap between adjacent components. The assembled sheet was cut into individual 3.0 mm wide strips using a computer-controlled cutter. Finished strips were stored in sealed bags with desiccant at room temperature prior to use.

2.6. Optimization of Assay Conditions

To achieve optimal detection performance, key experimental parameters were systematically investigated using the signal-to-noise (S/N) ratio as the evaluation metric: $S/N = (\text{signal intensity of positive sample})/(\text{signal intensity of negative sample})$. The following parameters were optimized sequentially while keeping others constant: (1) concentration of colloidal gold nanoparticles; (2) amount of MUC1 aptamer used for conjugation; (3) volume of conjugate dispensed onto the conjugate pad; (4) concentration of the anti-MUC1 antibody at the T line; (5) type of nitrocellulose membrane; and (6) composition of the running buffer. A higher S/N ratio indicated superior assay sensitivity and lower background. The detailed optimization results for each parameter are presented and discussed in Section 4.

3. Results and Discussion

The synthesized aptamer-unmodified AuNPs were initially characterized by transmission electron microscopy (TEM). As shown in Figure 1A, the particles exhibited an ideal spherical morphology, with an average diameter calculated to be 28.9 ± 1.2 nm. Subsequent characterization by UV-vis absorption spectroscopy was performed on both the unmodified AuNPs and the aptamer-conjugated particles (AuNP-Apt). Figure 1B reveals a new absorption peak near 263 nm for AuNP-Apt, indicating successful aptamer conjugation. Furthermore, a red shift in the characteristic plasmon resonance peak was observed upon modification. This shift suggests an increase in the local refractive index and hydrodynamic radius following aptamer attachment, consistent with successful surface functionalization.

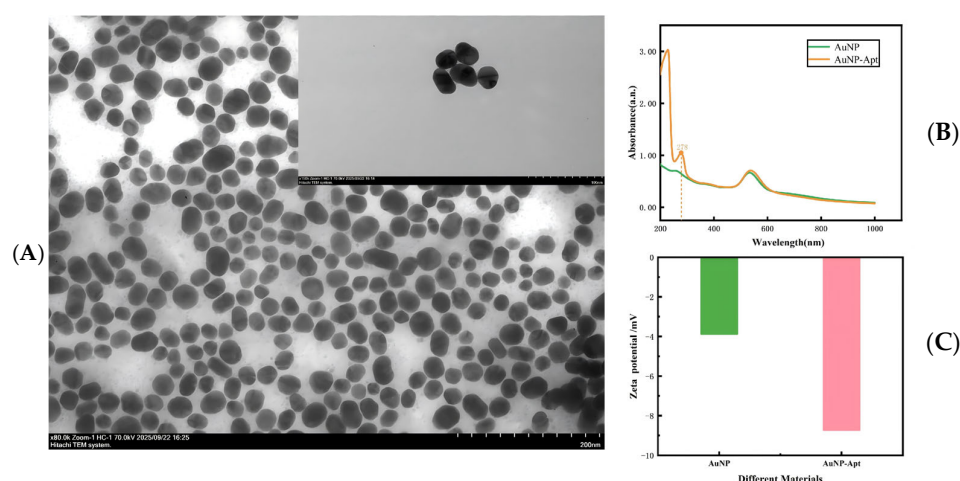


Figure 1. (A) TEM image of AuNPs; (B) UV-vis spectra of the as-synthesized AuNPs and aptamer-modified AuNP conjugates; (C) zeta potentials of the as-synthesized AuNPs and aptamer-modified AuNPs.

To further confirm the conjugation, the zeta potentials of the AuNPs and AuNP-Apt were measured. As shown in Figure 1C, the unmodified AuNPs possessed a surface charge of approximately -3.89 mV. After aptamer conjugation, the zeta potential increased to approximately -8.73 mV. This increase in electronegativity is attributed to the negatively charged phosphate backbone of the aptamer, which dissociates in aqueous solution. The significant change in surface charge provides additional evidence for the successful preparation of the AuNP-Apt conjugate.

Figure 2a illustrates the typical structure of the lateral flow biosensor (LFB), which consists of a sample pad, conjugation pad, nitrocellulose membrane, and absorption pad laminated sequentially onto a polyvinyl chloride (PVC) backing. The working principle of the dual-receptor (aptamer-antibody) LFB is depicted in Figure 2b. Upon applying a sample containing MCF-7 cells to the sample pad, the solution migrates via capillary action. It first rehydrates the gold nanoparticle-aptamer (AuNP-Apt) conjugates on the conjugation pad. These conjugates then specifically bind to MUC1 proteins on the surface of the target cells, forming mobile complexes. As the flow continues, these complexes are captured at the test line by immobilized anti-MUC1 antibody (Cap-Anti-MUC1), leading to the formation of a visible red band due to the accumulation of AuNPs. The liquid continues to migrate toward the absorption pad, which drives the flow and ensures the completion of the assay. Excess AuNP-Apt conjugates continue to flow to the control line, where they are immobilized through hybridization with complementary DNA probes, producing a second colored band. The assay result is interpreted by the presence or absence of these visual bands, as illustrated in Figure 2c. A valid test requires a band at the control line. A positive result is indicated by the appearance of bands at both the test and control lines,

whereas a negative result—signaling the absence of MCF-7 cells—is confirmed by a band at the control line only, with no band at the test line.

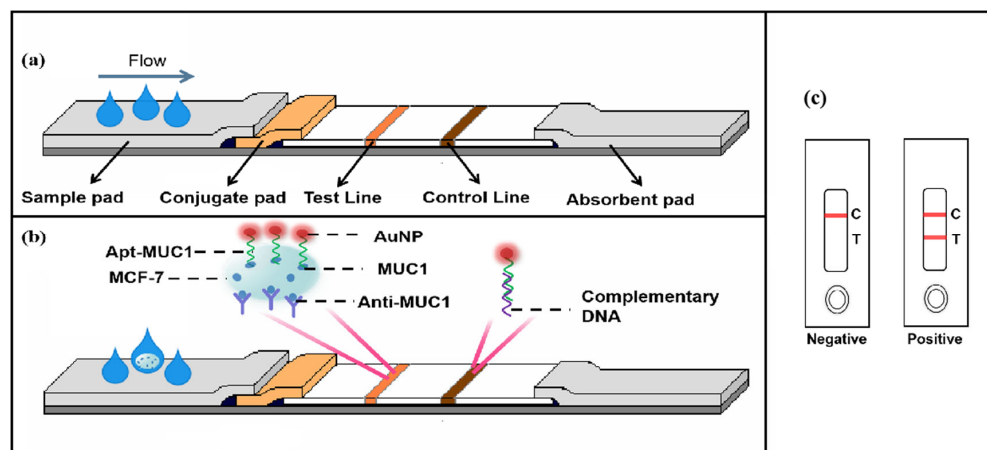


Figure 2. (a) Schematic diagram of a lateral flow biosensor; (b) the working principle of the dual receptor (aptamer–antibody) based LFB for MCF-7 cell detection using gold nanoparticles as the labeling material; (c) qualitative and quantitative results in the absence and presence of MCF-7 cells.

The analytical performance of LFBs is highly dependent on their preparation and the selected experimental parameters. To ensure optimal reproducibility and sensitivity, several key parameters were systematically optimized. First, the concentration of the colloidal gold solution was adjusted to achieve the optimal chromogenic signal. As shown in Figure 3a, the signal-to-noise (S/N) ratio was highest when the optical density (OD) of the AuNP solution was concentrated to 8-fold. Lower concentrations produced unsatisfactory color intensity, while higher concentrations increased background signal, thereby reducing the S/N ratio. Subsequent optimization focused on the conjugation and application of the AuNP-Apt probe. The molar amount of the aptamer used during conjugation was varied, with the S/N ratio reaching a maximum at 0.5 μmol , as shown in Figure 3b. The volume of conjugate applied to the pad was also critical. As illustrated in Figure 3c, color intensity improved with increasing volume up to 2.3 μL , beyond which it declined. An excessive volume leads to a surplus conjugate that cannot bind the target, increasing the potential for non-specific binding at the test line and raising the risk of false-positive signals. The false-positive rates corresponding to coupling volumes of 1.9 μL , 2.1 μL , 2.3 μL , and 2.5 μL were 0%, 0%, 0%, and 33%, respectively.

Furthermore, the nitrocellulose membrane pore size, which influences flow rate and binding efficiency, was evaluated. Three membranes (VIVID, CN140, and JN120m) with varying flow characteristics were compared. As shown in Figure 3d, the VIVID membrane provided the optimal S/N performance for this assay. The concentration of the capture antibody (anti-MUC1) immobilized on the test line was another critical parameter. Figure 3e indicates that a concentration of 1.64 mg/mL yielded the highest S/N ratio. Finally, the running buffer composition was optimized to enhance sensitivity. While Mg^{2+} ions can improve aptamer-mediated cell capture, they also increase non-specific adsorption, as seen in the initial decrease in S/N (Figure 3f). To counteract this, BSA was incorporated to block non-specific binding sites on the AuNP surface. The optimal buffer formulation was determined to be 1 $\times$ PBS containing 0.5% BSA and 0.75 mM MgCl_2 , which maximized specific signal while minimizing background interference.

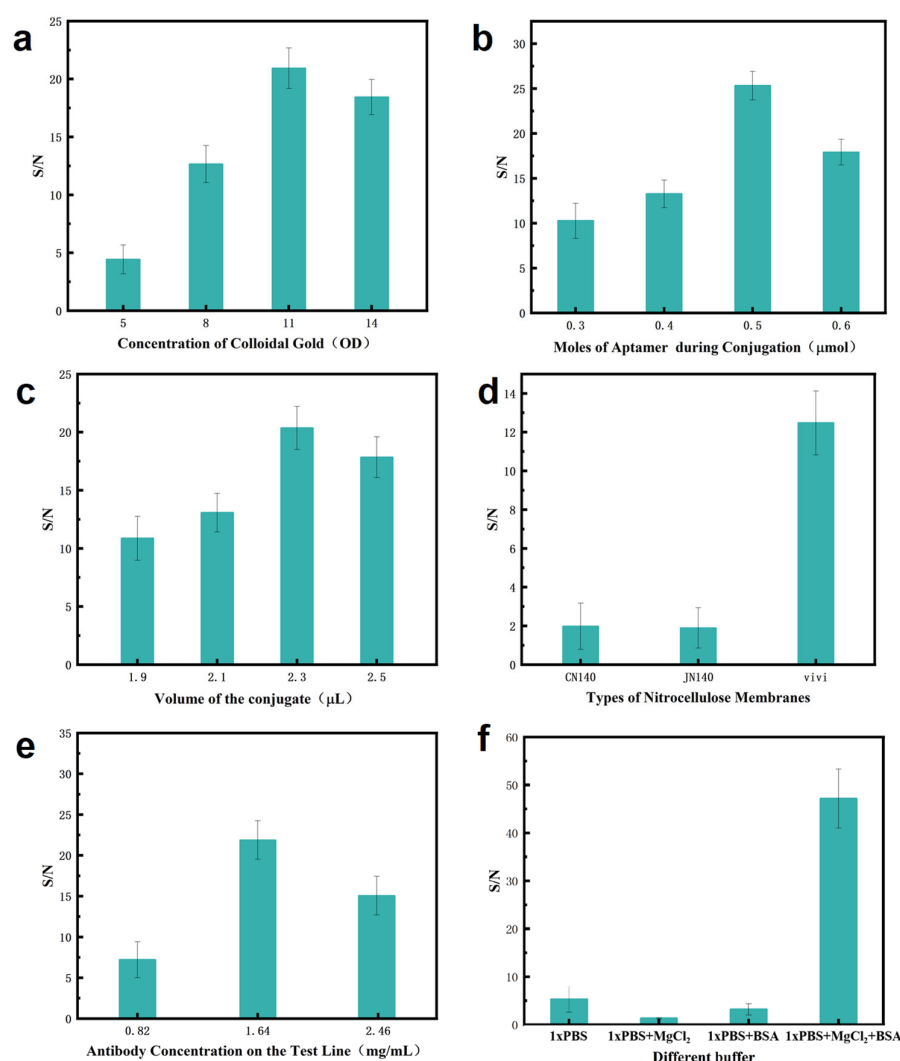


Figure 3. Optimization of experimental conditions: (a) optimization of AuNP concentration; (b) optimization of aptamer probe concentration; (c) optimization of conjugate volume; (d) optimization of nitrocellulose (NC) membrane; (e) optimization of MUC1 antibody concentration on the T line (Test line); (f) optimization of running buffer.

Analytical performances

To evaluate the sensitivity and visual limit of detection (LOD) of the lateral flow biosensor, sample solutions spiked with known concentrations of MCF-7 cells (0, 675, 1350, 2690, 5380, 10,760, and 21,520 cells) were analyzed using test strips from a single batch. The visual LOD was defined as the minimum number of cells required for a stable visual distinction between positive and negative signals. Results were assessed visually 15 min after application. The visual LOD was determined to be 675 cells, defined as the lowest concentration producing a distinct colored band at the test line (T line). For quantitative analysis, the signal intensity was measured using a strip reader and expressed as the test-to-control (T/C) ratio. As shown in Figure 4, the T/C ratio exhibited a good linear relationship with the cell concentration in the range of 0 to 2690 cells. Table 1 compares the performances of several representative biosensors for CTC detection with respect to key metrics, including the detection limit (LOD), assay time, cost, and operational simplicity. The biosensor offers rapid detection (≤ 15 min), low cost, and simple visual operation, making it well-suited for point-of-care CTC screening. While its sensitivity may not match some lab-based methods, it offers an efficient and economical complementary approach for the bedside detection of CTCs.

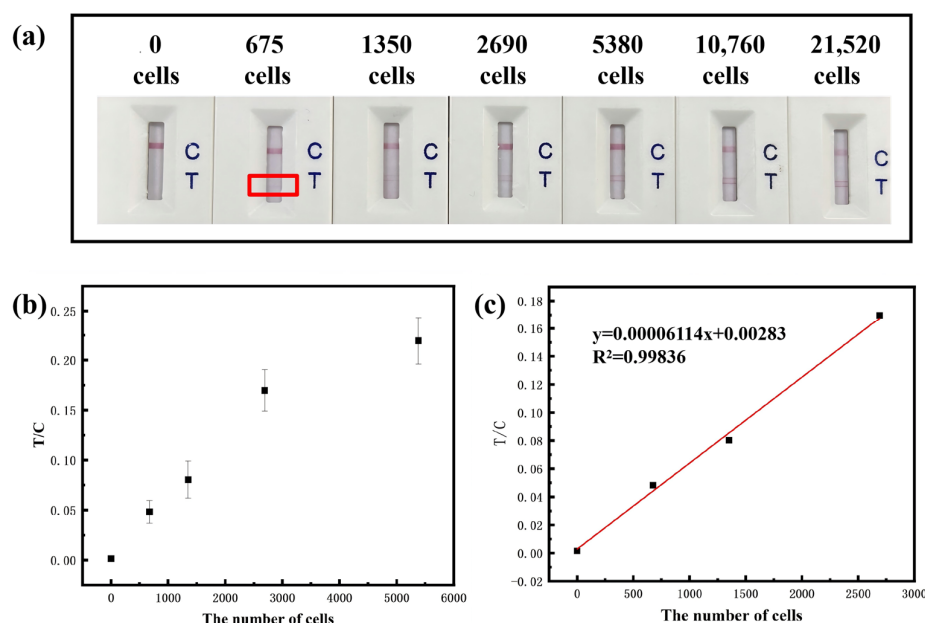


Figure 4. Detection of MCF-7 in the buffer system: (a) photos of strips with different numbers of MCF-7 cells, with the red box indicating the visual detection limit (LOD) of MCF-7; (b) spot map of MCF-7 under optimal experimental conditions; (c) calibration curve of MCF-7, with error bars representing the standard deviation of three independent measurements.

Table 1. Comparison of typical biosensors for detecting CTCs.

Target	LOD	Time	Cost/Need for Large-Scale Instruments (Yes/No)	Simplicity	Type	Ref.
MCF-7	2 cell/mL	2 h	Yes	Moderate	electrochemical biosensor	[23]
Hep G2	10 cell/mL	0.5 h	Yes	Moderate	fluorescent biosensor	[29]
MCF-7	3 cell/mL	0.5 h	Yes	Moderate	Microfluidic biosensor	[30]
MCF-7	675 cell/mL	0.4 h	No (≈2 RMB)	Simple	Lateral flow biosensor	This paper

Reproducibility

The reproducibility of the biosensor was assessed by preparing and evaluating multiple independent batches of test strips. Using the optimized protocol, sample solutions containing 834 cells and 10,000 MCF-7 cells were analyzed with test strips from six different batches, whose preparation spanned a period of more than four weeks. The quantitative results, measured as the test-to-control (T/C) ratio, are summarized in Figure 5. The relative standard deviations (RSDs) for 834 and 10,000-cell samples were 14.50% and 6.54%, respectively. The lower RSD at the higher target concentration demonstrates acceptable batch-to-batch consistency, indicating that the fabrication process yields strips with good reproducibility for quantitative detection. In addition, the results from the six batches of test strips suggest that they maintain stable performance for a period of time after preparation. Based on our laboratory's routine empirical observations, the test strips placed in a sealed bag with desiccant and stored at room temperature can maintain their functionality for up to 3 months.

Specificity Assessment

The specificity of the lateral flow biosensor for MCF-7 cells was evaluated through cross-reactivity testing. Human umbilical vein endothelial cells (HUVECs) were used as a non-target control to assess potential non-specific binding. As shown in Figure 6, a high concentration of HUVECs alone generated a negligible signal at the test line, confirming minimal non-specific adsorption. Furthermore, in a mixed-cell sample containing HUVECs spiked with 5000 MCF-7 cells, the detection signal for the target cells remained robust and unaffected by the presence of the non-target cells. These results collectively demonstrate

5 μ L detection volume required by our sensor is highly compatible with the volume of such enriched samples, thereby positioning it as a rapid and cost-effective downstream tool for the detection and quantification of enriched CTCs. This integrated approach would help mitigate the influence of complex matrices present in large-volume whole-blood samples.

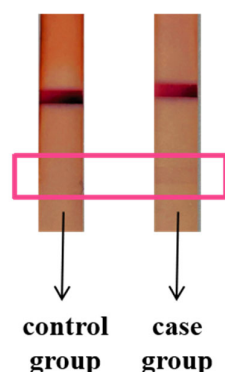


Figure 7. Detection performance in real samples.

4. Conclusions

In this work, we successfully developed a lateral flow biosensor (LFB) for the rapid, sensitive, and quantitative detection of MCF-7 cancer cells. Under optimized assay conditions, the biosensor achieved a visual detection limit of 675 cells within 15 min. Compared to established but more complex techniques such as laser confocal microscopy and surface-enhanced Raman spectroscopy (SERS), the proposed LFB offers a significantly simpler, faster, and more cost-effective platform. This makes it suitable for the qualitative and quantitative analysis of circulating tumor cells (CTCs) in blood, as demonstrated by its performance in spiked human blood samples.

Looking forward, the convergence of advances in nanotechnology [31,32], microfluidics [33,34], and bioinformatics is poised to further enhance the capabilities of LFB platforms. Although the direct application of LFBs for CTC detection in clinical blood samples is still emerging, their inherent advantages—including simplicity, low cost, and rapid results—present considerable potential. Such platforms could pave the way for new point-of-care tools for cancer monitoring, ultimately contributing to more precise, personalized, and accessible diagnostic and therapeutic strategies.

Author Contributions: Y.Z.: review and editing, performing the experiments, writing the paper. J.W.: review and editing, data curation, data analysis. M.M.: review and editing, data analysis. Y.H. and J.L.: review and editing, organizing the research background. H.L., X.Z., and G.L.: conceiving and designing the experiments, funding, investigation, review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by the National Key Research and Development Program of the Ministry of Science and Technology, China (Grant number: 2022YFB3207200), and the Startup Foundation for Advanced Talents of Linyi University, China (Grant number: Z6122047).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Linyi University (protocol code: LYU20230056; date of approval: 11 March 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no competing financial interests.

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