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Strand displacement amplification for ultrasensitive detection of human pluripotent stem cells

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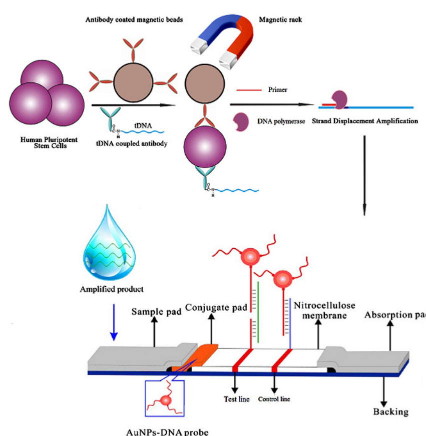
HIGHLIGHTS

- The limit of detection was 19 cells by strip reader and 100 cells by naked eye.
- This assay shows good specificity and sensitivity of whole stem cells detection.
- The gold-nanoparticle based lateral flow biosensor enables visual detection.

GRAPHICAL ABSTRACT



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ABSTRACT

Human pluripotent stem cells (hPSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), provide a powerful model system for studies of cellular identity and early mammalian development, which hold great promise for regenerative medicine. It is necessary to develop a convenient method to discriminate hPSCs from other cells in clinics and basic research. Herein, a simple and reliable biosensor for stem cell detection was established. In this biosensor system, stage-specific embryonic antigen-3 (SSEA-3) and stage-specific embryonic antigen-4 (SSEA-4) were used to mark human pluripotent stem cells (hPSCs). Antibody specific for SSEA-3 was coated onto magnetic beads for hPSCs enrichment, and antibody specific for SSEA-4 was conjugated with carboxyl-modified tDNA sequence which was used as template for strand displacement amplification (SDA). The amplified single strand DNA (ssDNA) was detected with a lateral flow biosensor (LFB). This biosensor is capable of detecting a minimum of 19 human embryonic stem cells by a strip reader and 100 human embryonic stem cells by the naked eye within 80 min. This approach has also shown excellent specificity to

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distinguish hPSCs from other types of cells, showing that it is promising for specific and handy detection of human pluripotent stem cells.

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1. Introduction

Over the past decade, stem cell research has elicited continuous scientific, commercial, and public interest [1–3]. Human pluripotent stem cells (hPSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have a gene expression program that allows them to differentiate to all types of somatic cells in response to developmental cues [4]. Meanwhile, somatic cells can reprogram to iPSCs by ectopic expression of several transcription factors, originally Oct4, Sox2, Klf4 and Myc (OSKM) [5,6]. These two types of cell conversion hold great promise for regenerative medicine and provide a powerful model system for studies of cellular identity and development [7]. Therefore, an efficient and convenient approach for the discrimination of stem cells from somatic cells is highly required in both research and clinics.

Traditional cell lysate-based methods for stem cell identification, including reverse transcription-polymerase chain reaction (RT-PCR), western blot, and northern blot, are labor intensive, time consuming, and costly. They use specific proteins and messenger RNAs (mRNAs) in stem cells as markers. It usually takes several hours even days to obtain confirmed results. Besides, protein/DNA extraction also adds complexity to the detection [8–10]. Thus, rapid whole cell-based immunoassays, such as flow cytometry and immunofluorescence, for stem cell detection have been developed [11,12]. They use specific protein markers in stem cells as target. These methods have better performance in sensitivity and specificity, but expensive, specialized equipments and highly trained personnel are still required. To avoid these disadvantages, a lateral flow biosensor for the detection of hPSCs has been developed [13]. The surface proteins were used as markers, and a red band produced by gold nanoparticle (AuNP) accumulation in test zone was used as the positive signal. Although this biosensor is visual, cheap, rapid and convenient, the low sensitivity (10,000 cells) is still a major limitation. Therefore, signal amplification and magnetic bead based enrichment emerge may be effective for improving the sensitivity.

Nucleic acid is cheap, stable, and can be chemically synthesized in high purity and modified with relative ease. Moreover, the virtue of high amplification efficiency, simplicity, extensively validated standard operating procedure and good reproducibility gives nucleic acid more superiority to other signal molecules. Nucleic acid amplification, especially isothermal amplification, is a valuable molecular tool not only in basic research but also in application oriented fields [14–16]. With these advantages, various strategies for signal conversion from protein/small molecules to nucleic acid have been reported [17–19]. These strategies include two types, one involves specific DNA binding with target protein/ small molecules, and the other uses modified DNA conjugated to protein/small molecules. The former only suits for some special protein/small molecules, such as transcription factors and proteins with known aptamers. The latter is a general DNA-based protein detection platform. For example, thiol-modified random DNA sequence can conjugate to protein A and the complementary DNA modified with fluorescent molecule is used as signal reporter for multiple detections of green fluorescent protein (GFP), *Renilla* luciferase (RL), and heat shock factor (HSF) [19]. Herein, we established a platform for stem cell detection by using two types of antibodies specifically binding to surface markers of stem cells. Briefly, magnetic beads coated with one type of antibodies were used to enrich target cells and carboxyl-modified template DNA (tDNA) conjugated to another type of antibodies was used as amplification template. Based on the pre-analytical sample enrichment by magnetic beads and isothermal strand displacement amplification (SDA) for signal amplification, the specificity and sensitivity of this biosensor was highly improved.

In this study, a simple and reliable biosensor for hPSC detection was constructed, in which the surface proteins, SSEA-3 and SSEA-4, were used to mark human pluripotent stem cells (hPSCs). Magnetic beads coated with antibodies for SSEA-3 were used for hPSC enrichment. Carboxyl-modified tDNA sequence conjugated to antibodies for SSEA-4 was used as template for SDA. The amplified single strand DNA (ssDNA) was detected with a lateral flow biosensor (LFB) (Fig. 1). The LFB has the following features: (i) high sensitivity (limit of detection of 100 cells), (ii) short detection time (within 1 h), (iii) no cell lysis needed for the detection, (iv) visual detection by the naked eye, and (v) no need for complex and expensive instrumentation.

2. Materials and methods

2.1. Reagents and chemicals

Human SSEA-3 and SSEA-4 antibodies were purchased from R&D Systems (Minneapolis, USA). Epoxy-modified magnetic beads and magnetic rack were purchased from INNOSEPBIO (Zhengzhou, China). Oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology (Shanghai, China). Klenow (exo^-) DNA polymerase, Nt.BbvCI nicking enzyme, deoxynucleoside triphosphates (dNTPs) were purchased from New England Biolabs (New England, USA). Bovine serum albumin (BSA) and $HAuCl_4$ were purchased from Sigma–Aldrich (Steinheim, Germany). mTeSR™1 medium was purchased from STEMCELL Technologies Inc. (Vancouver, Canada). Matrigel™ hESC-qualified matrix was purchased from BD Biosciences (San Diego, USA). DMEM/F-12 medium was purchased from Hyclone (Logan, USA). Nitrocellulose (NC) membranes were purchased from Shantou Ealon (Shantou, China). All other reagents were purchased from standard commercial sources and were of analytical grade. The strip reader was purchased from Kinbio (Shanghai, China). All buffer solutions used in this study were prepared using ultrapure water (resistance of 18 MΩ).

2.2. Construction of lateral flow biosensor

Thirty microliters of 100 μM DNA probes (test line probe and control line probe) was dispensed onto the nitrocellulose membrane simultaneously with a lateral flow dispenser (Shanghai Kinbio, Shanghai, China). The membrane was then dried at room temperature for 12 h. Fiberglass was used as sample pads after being soaked in sample pad buffer (0.5% Triton, 1% BSA, 2% sucrose, 50 mM boric acid, pH 8.0). It was dried and stored in low-humidity at room temperature. Sample pad, conjugate pad, nitrocellulose membrane and absorbent pad were attached along the long axis of an adhesive plate with an overlap of 1–2 mm, and cut into 4-mm-wide strips using a paper cutter (Fig. 1B).

2.3. Cell culture

For culture ware coat, diluted Matrigel™ was used, 1 mL per well of 6-well plate. The plate was swirled to spread the solution

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