Talanta 178 (2018) 116–121

Contents lists available at ScienceDirect

Talanta

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

Polyamidoamine starburst dendrimer-activated chromatography paper-based assay for sensitive detection of telomerase activity

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ARTICLE INFO

Keywords:
Paper device
PAMAM dendrimer
Amplification-free
Telomerase activity
Telomerase inhibitor

ABSTRACT

Telomerase is extensively expressed in various cancer cells and recognized as a target for cancer drug discovery. In the present study, a simple and amplification-free fluorescence assay based on polyamidoamine starburst dendrimer (PAMAM dendrimer)-activated paper device is proposed for sensitive detection of telomerase activity through hybridization of Cy5 modified single strand DNA probes with telomerase extension products. The paper substrate is fabricated by hand drawing according to a template, which is low cost, instrument free and easy operation. PAMAM is rich in amino groups on its surface and employed to immobilize the telomerase substrate (TS) primer. Highly sensitive detection of telomerase activity in HeLa cell lysate of 10 cells is achieved since the PAMAM dendrimer-activated paper surface can provide high density of binding sites for immobilization of TS primer. The experimental results also demonstrate that the assay can be employed to evaluate telomerase activity levels of various cell lines and screen telomerase inhibitors.

1. Introduction

Telomeres are located at the ends of eukaryotic chromosomes and consisted of repeated DNA sequences and protein assemblies, which are helpful for protecting chromosome from undesired degradation, recombination and/or end-to-end fusion [1,2]. Normally, the length of telomere is gradually shortened until a critically limit with cell division, resulting in replicative senescence and apoptosis [2]. Telomerase, a special ribonucleoprotein reverse transcriptase, shows RNA-dependent DNA polymerase activity that can catalyze the addition of telomeric repeats (TTAGGG)n to the ends of telomeres to maintain indefinite cell proliferation [3,4]. It is reported that telomerase expression is closely related with cell immortalization and tumorigenesis [5]. The telomerase is reactivated in over 85% of human cancer cells, but is highly depressed in most of normal somatic cells [6,7]. The reactivation of telomerase is regarded as a primary change in the physiology of cancer cell by preventing telomeric shortening to achieve malignant growth, which makes human cancer cells acquire an unlimited proliferation potential [8]. Accordingly, telomerase can be considered as a diagnostic and prognostic biomarker because of the strong relationship between the telomerase activity and cancer [9]. Therefore, developing a simple and reliable method for telomerase activity detection is extremely necessary and of great theoretical and practical importance for cancer diagnosis, prognosis, anticancer drug discovery and cancer therapy evaluation [10].

Researchers have been developed varieties of assays for detecting telomerase activity since telomerase was discovered in 1985 [11–27]. The most popular and ultrasensitive method is telomeric repeat amplification protocol (TRAP) [12]. However, TRAP usually brings about false negative results and involves the polymerase chain reaction (PCR) that is time-consuming and susceptible to contaminants [13,14]. In order to overcome the drawbacks, much effort has been made to develop PCR-free technique for telomerase activity detection, such as colorimetry [15,16], fluorescence [17,18], chemiluminescence [19,20], electrochemistry [21,22], surface-enhanced Raman scattering (SERS) [23,24] and so on [25,26]. Most of these studies rely on amplification-based technique and possess high sensitivity, but it leads to high background and poor reproducibility in the meantime [27]. Additionally, these methods are mainly based on solution-phase reaction systems and require complex equipments, which cannot be handing outside the lab to meet the requirements of point-of-care (POC) test.

In this regard, an amplification-free paper-based assay has been proposed here for telomerase activity detection. Paper-based analytical devices (PADs) have emerged as a promising technique with the advantages of low-cost, high surface-to-volume ratio, portability, ease of surface modification, biodegradability and biocompatibility [28–30]. Based on these remarkable properties, PADs have been greatly applied in biochemical sensing, environmental monitoring and POC diagnostics

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http://dx.doi.org/10.1016/j.talanta.2017.09.034
Received 15 June 2017; Received in revised form 4 September 2017; Accepted 12 September 2017
Available online 13 September 2017
0039-9140/

[31–33]. Cellulose paper is considered as an ideal platform to carry out biochemical reaction due to its high capillary action for liquid transport and low sample consumption [34,35]. The hydrophobic barriers can be patterned on papers to constrain the flow using different techniques including photolithography, wax printing, inkjet printing, etc. [36–39]. Nevertheless, the above techniques depend on customized equipment and the processes are complex, which are not suitable for use in resource-limited areas. Thus it is necessary to seek for a cost-effective, instrumental simplicity and easy operation way to construct PADs. In our previous work, a simple PAD by hand drawing has been developed for sensitive detection of polynucleotide kinase activity and inhibition on the basis of fluorescence quenching [40].

Herein, we demonstrate a polyamidoamine starburst dendrimer (PAMAM dendrimer) activated hand-drawn paper-based fluorescence assay for amplification-free telomerase detection as well as the inhibitor screening for the first time. PAMAM dendrimer is rich in amino groups on the surface, which can be used to immobilize biomolecules with high efficiency. The paper substrate is patterned according to a template using a permanent marker pen to make circular test zones with hydrophobic barriers. This hand-drawing fabrication process is fast, instrument-free and low-cost.

2. Materials and methods

2.1. Reagents

Whatman cellulose chromatography paper (Grade 1) and PAMAM dendrimer (generation 5.0, the terminal is -NH_2) were purchased from Sigma-Aldrich Company (St. Louis, USA). CHO-labeled TS primer (5′-CHO-T_{10}-AATCCGTCGAGCAGAGTT-3′) and fluorescence dye (Cy5) labeled ssDNA capture probe (CP: 5′-Cy5-CCCTAACCCTAA CCCTAACCCT-3′) were synthesized and purified by HPLC (Sangon, Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris), ethylene glycol tetraacetic acid (EGTA), phenylmethanesulfonyl fluoride (PMSF), β-mercaptoethanol, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS), and Tween-20 were purchased from Beijing DingGuo Biotech. Co., Ltd. (Beijing, China). Sodium periodate ($NaIO_4$), lithium chloride (LiCl) and other reagents were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All solutions were prepared with Milli-Q water (18.2 MΩ cm).

2.2. Preparation of PAMAM dendrimer-activated paper substrate

The paper was firstly treated by $NaIO_4$ to form aldehyde groups for the immobilization of PAMAM dendrimer according to our previous work [40]. Subsequently, the CHO-modified paper samples with a dimension of 20 × 60 mm (width × length) were immersed into 0.05% PAMAM dendrimer solution and incubated overnight at room temperature under shaking. After washed with 30 mL Milli-Q water for three times, the PAMAM dendrimer-activated paper was dried in an oven at 37 °C, and then patterned with a template to make circular test zones (Fig. S1).

2.3. Cell culture and telomerase extraction

Six human cancer cell lines (HeLa, A549, MCF-7, HepG2, SW480 and SW620) and one human normal liver cell line (HL-7702) were purchased from Shanghai Cell Bank, CAS (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum in a humidified 5% CO_2 incubator at 37 °C. All kinds of cells were collected during the exponential phase of growth and washed with 1 mL ice-cold phosphate buffer saline (PBS) (2 times). Next, 1 × 10^6 cells were resuspended in 200 μL ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH = 7.5, containing 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% Chaps, and 10% glycerol), and incubated on ice for 30 min. The cell lysates were centrifuged at 12,000 rpm under 4 °C for 20 min, respectively. The supernatants were carefully collected and used immediately or frozen at − 80 °C.

2.4. Telomerase extension on PAMAM dendrimer-activated paper substrate

In a typical assay, 1.5 μL aliquot of TS primer at a certain concentration were pipetted on the test zone and incubated at 37 °C under 60% humidity for 1 h, then washed with 30 mL 20 mM Tris-HCl buffer (pH = 8.0, 3 times). Telomerase extracts were diluted with lysis buffer. And different dilution ratios of cell extracts were added into telomerase extension solution containing reaction buffer (20 mM Tris-HCl, pH = 8.3, containing 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween-20, 1 mM EGTA and 0.1 mg mL^{-1} BSA) and 1 mM dNTP. Then 1.5 μL aliquot of the above mixture was transferred to TS primer modified test zone and incubated at 37 °C under 60% humidity for 2 h. Subsequently, the paper was washed with 30 mL 20 mM Tris-HCl buffer (pH = 8.0, 3 times) and 30 mL Milli-Q water (3 times), respectively, and dried in air.

2.5. Detection of telomerase activity

After the telomerase extension reaction completed, 1.5 μL aliquot of 300 nM CP in hybridization buffer (20 mM Tris-HCl, pH = 7.4, containing 5 mM $MgCl_2$) was transferred onto test zone and incubated at 45 °C under 60% humidity for 30 min. After washed and dried as previously described, the paper substrate was adhered to a piece of glass slide by the use of double-sided tape. The fluorescence image was acquired by LuxScan-10K fluorescence microarray scanner (CapitalBio Ltd., Beijing, China). And the fluorescence intensity was calculated using Image J software (National Institute of Health, USA). For inhibition of telomerase, 3′-Azido-3′-deoxythymidine (AZT) or BIBR 1532 was performed in the presence of 5000 HeLa cells.

3. Results and discussions

3.1. Assay principle

PAMAM dendrimer is a type of nanomaterials using ethylenediamine as the core and the surface is rich in amino groups. In this case, generation 5.0 of PAMAM dendrimer is used which has 128 amino groups on its outer surface. As illustrated in Fig. 1, the paper substrate was first functionalized with aldehyde groups for immobilization of PAMAM dendrimer. Next, the CHO-labeled TS primer was transferred onto the surface of PAMAM dendrimer-activated paper through covalent attachment, following by the telomerase extension reaction. The presence of telomerase would catalyze the addition of telomeric repeats (TTAGGG)n to the ends of TS primer to generate the telomerase reaction products (TRPs). TRPs can be further recognized by CP, which is designed complementary with the telomeric repeats. The fluorescence intensity on the paper substrate relied on the quantities of TRPs and reflected the telomerase activity. In the absence of telomerase, TS primer cannot be extended and CP cannot be hybridized, only showing the signal in a nonspecific adsorption on the paper surface. When telomerase was introduced, more telomerase would lead to higher fluorescence intensity.

The modification of paper was first characterized by scanning electron microscope (SEM) and Fourier transform infrared (FT-IR) spectroscopy. It can be seen from Fig. S2 that the morphology of cellulose paper exhibits negligible change after PAMAM dendrimer modification. As shown in Fig. 2A, a new absorption band at 1726 cm^{-1} assigned to C=O stretching appears after the paper was treated with $NaIO_4$, indicating that aldehyde functionalization of the paper substrate was achieved. The carbonyl peak weakens obviously while the paper substrate was activated by PAMAM dendrimer. X-ray photoelectron spectroscopy (XPS) is further employed to characterize different