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Functionalized paper microzone plate for colorimetry and up-conversion fluorescence dual-mode detection of telomerase based on elongation and capturing amplification

An Chen Fu[a,1], Yue Hu[a,1], Zi-Han Zhao[a], Ruilin Su[a], Yilin Song[a], Dong Zhu[a,b,c,*]

[a] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, 210023, PR China
[b] Jiangsu Key Laboratory for Functional Substance of Chinese Medicine, PR China
[c] State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine, PR China

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ABSTRACT

As an important cancer biomarker and therapeutic target, telomerase has attracted special attention especially concerning its detection and monitoring. Here, we established a portable cellulose paper substrate for the detection of telomerase based on colorimetry and up-conversion fluorescence dual-mode methods The sterile cellulose paper substrate functionalized with telomerase substrate oligonucleotide were used as the capturing substrate, on which the telomerase extended telomeric repeats, and the corresponding telomeric repeat complementary oligonucleotide labelled with methylene blue or the up-conversion nanoparticles (UCNPs) were employed as the colorimetric or up-conversion fluorescence reporting nanolabels. The extended telomeric repeats subsequently facilitate capturing of the more reporting nanolabels via hybridization between telomeric repeat and its complementary oligonucleotide, inducing a significant amplified signal in the color and up-conversion intensity. Colorimetric functionality allows rapid preliminary discrimination of telomerase activity by the naked eye, and the low background up-conversion fluorescence increases the sensitivity of this method. Moreover, the developed cellulose paper device is more stable and easily stores the tests results compared with majority solution-phase systems.

1. Introduction

Telomere, unique nucleic acids complex, is becoming one of the most promising cancer markers due to its strong association with cellular immortality and carcinogenesis [1–9]. Thus the precise and sensitive detection of telomerase activity is crucial for telomerase based cancer diagnosis and therapeutics. Over the past decades, various techniques have been built up to detect the activity of the telomerase, and the most commonly used detection is based on the polymerase chain reaction (PCR) [10]. However, it remains some disadvantages including the expensive equipment and reagents, time-consuming and the limits of polymerase [11]. Several PCR-free protocols have also been designed and successfully employed in telomerase activity detection such as optical sensor [12], surface plasmon resonance [13], fluorescence strategies [14], electrogenerated chemiluminescence [15] and so on [16,17]. Recently, nanotechnologies such as telomerase-substrate-oligonucleotide modified gold nanoparticles sensor and silicon nanowire assay based on field-effect devices have also been applied to telomerase detection [18–20]. Although these methods were ingeniously designed and some of them can partly make up the shortage of PCR-based technique, most of these new methods still needed complicated and time-consuming protocols. More important, these efforts, however, mostly focused on solution-phase reactions, which usually are unstable and not practical for handling outside of the lab. Therefore, they are not suitable for point-of-care (POC) diagnostics, while which is essential to on-site medical care for the prevention and control of serious diseases [21].

On the other hand, repeat amplification protocol such as rolling circle amplification procedure can bring signal enhancement by generating a long single DNA strand with multiple repeat units based on isothermal DNA replication technique [22–24]. Recently, repeat amplification protocol has been used in fluorescence enhancement in situ imaging of microRNA in tumor cells [25,26] and tumor-specific delivery of drugs [27]. While telome-

* Corresponding author at: School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, 210023, PR China.
E-mail address: dongzhu@njucm.edu.cn (D. Zhu).
[1] These authors contributed equally to this work.

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rase is a unique ribonucleoprotein which can synthesize and can add tandem telomeric repeat (TE) (TTAGGG)n [28,29], which could be utilized as signal enhancement.

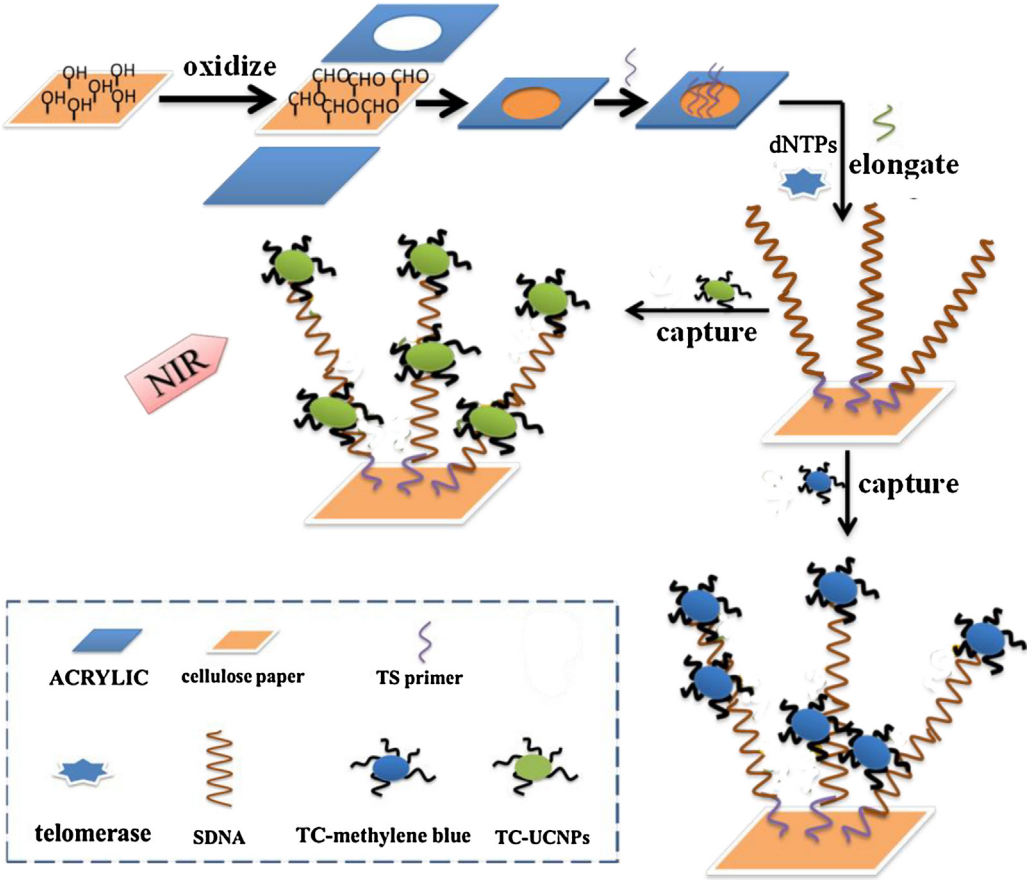
Functionalized cellulose paper has been constructed as immunoplates which have the advantages of low-cost, high stability, visibility, biodegradability, portability and excellent chemical compatibility with many applications [30–32]. Since the microfluidic paper-based analytical protocol was first reported by Whitesides group [33], paper-based biodetection systems have been proved very promising technologies for public health, environmental and especially point-of care testing (POCT) fields [34–38].

Herein, we present a colorimetry and upconversion fluorescence dual-mode detection for telomerase based on telomeric elongation and capturing amplification by using functionalized upconversion nanoparticles (UCNPs) and methylene blue as probes on a functionalized cellulose paper microzone plate. This assay simultaneously holds the merits of both colorimetry and upconversion based techniques, i.e. fast read-out, easy operation as fast preliminary screening with naked eye and high sensitivity. UCNPs can be excited by near-infrared (NIR), avoiding the generation of any interfering bio-background luminescence [39,40]. On the prepared paper microzone plate, each test zone required only about 5 μL of sample, and an entire assay can be completed in less than two hours. As depicted in Scheme 1, a paper microzone plate was firstly prepared by covering two perforated acrylic plates, forming a paper-based zone that could load sample solutions. And then the telomerase substrate (TS) primer is firstly dropped on the preprocessed sterile cellulose paper. The TS primer can be extended with the telomerase and further produce a long single DNA (denoted as SDNA), which can capture more probes to induce more sensitivity. The TC-oligonucleotides labelled by the methylene blue or the UCNPs could hybridize with the SDNA, resulting in observable color change and up-conversion fluorescence signal under NIR excitation. And the signal intensity was related to the amount of SDNA, which reflected the activity of the telomerase. While in the absence of telomerase, the TC-oligonucleotides cannot hybridize with SDNA since no telomeric repeat is added. Thus both the color and up conversion fluorescence intensity of the final precipitates can imply telomerase activity. The prepared paper-based assay was portable, low-cost, handy, and visible with naked eyes or fluorescence with the help of a NIR laser.

2. Experimental section

2.1. Chemicals and materials

4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), sodium dodecyl sulfate (SDS), 1-ethyl-3-(3-dimethlyaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were from Shanghai Sangon Biotechnology Inc. Sodium chloride (NaCl), ammonium fluoride (NH_4F), sodium periodate ($NaIO_4$), Lithium chloride (LiCl) were from Aladdin. Bovine serum albumin (BSA), dNTPs and RNase inhibitor were purchased from Nanjing KeyGen Biotechnology Co., Ltd. Amino group functionalized telomerase substrate oligonucleotide (TS primer, 5′-$NH_2(CH_2)_6$TTTTTTTTTTAATCCGTCGAGCA-3′,telomeric repeats(TE, 5′-$NH_2(CH_2)_6$TT$(TTAGGG)_9$-3′) and telomeric repeat complementary oligonucleotide(TC,5′-CCCTAACCCTAAAAAA$(CH_2)_3NH_2$-3′,5′-



Scheme 1. The illustration of the prepared paper-based assay by applying elongate and capture amplification for colorimetry and up-conversion fluorescence dual-mode detection of telomerase based on modified paper microzone plate.