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PCR-free paper-based nanobiosensing platform for visual detection of telomerase activity via gold enhancement

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ABSTRACT

Telomerase activity has been demonstrated in a wide variety of most solid tumors and considered as a well-known cancer biomarker. The commonly utilized method for its detection is polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP). However, the TRAP technique suffers from false-negative results caused by the failure of PCR step. Moreover, it requires advanced equipment with a tedious and time-consuming procedure. Herein, we presented a portable nitrocellulose paper-based nanobiosensing platform for ultrafast and equipment-free detection of telomerase activity based on a simple colorimetric assay that enabled naked-eye visualization of the color change in response to enzyme activity. In this platform, hybridization was initially performed between telomere complementary oligonucleotide immobilized on gold nanoparticles (GNPs) and telomerase elongated biotinylated probe. Thereafter, the assembly was attached on activated paper strip via avidin-biotin interaction. The signal amplification was carried out by enlargement of the attached GNPs on the paper strip, forming tightly compact rod-shaped submicron structures of gold representing a visual color formation. Thanks to significant sensitivity enhancement, the color change was occurred for down to 6 cells, which can be easily observed by the naked eye. Due to the desired aspects of the developed assay including PCR-free, low cost, simple, and high sensitivity, it can be used for evaluation of telomerase activity in cell extracts for future clinical applications. Furthermore, this design has the ability to be easily integrated into lab-on-chip devices for point-of-care telomerase sensing.

1. Introduction

Telomerase is a ribonucleoprotein complex with reverse transcription function that adds repetitive nucleotide sequences of $(TTAGGG)_n$ to the end of chromosomal DNA, inhibiting telomere shortening during cell division [1]. Telomerase overexpression has been reported in over 85% of all known human cancer tissues, which is associated with cell immortalization, tumorigenesis, and other clinical outcomes [2]; hence, among more than one hundred proposed cancer biomarkers, telomerase can be considered as a distinctive and unique tumor marker.

TRAP assays are the most widely used methods in detection of telomerase activity from cell extracts and tissues [3]. However, these methods suffer from some limitations such as being costly, time-consuming, and require skillful technicians [4]. To address these issues, some alternative PCR-free assays have been developed for detection of telomerase activity such as colorimetric [5-7], chemiluminescence [6, 8], fluorescence [9, 10], and electrochemical-based assays [11, 12]. Although these methods can detect telomerase activity without PCR technique, they suffer from complicated manipulation or the employment of elaborate instruments that limit their practical applications.

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Therefore, it is of vital importance to develop a simple and cost-effective platform for detection of telomerase activity, which could be used for cancer diagnosis, prognosis, and evaluation of antitumor drugs performance [13].

Thanks to size-dependent optical properties and high extinction coefficients, gold nanoparticles (GNPs) have received considerable attention in developing colorimetric platforms for detection of biomolecules [14]. The GNP-based colorimetric assays benefit from some advantages including simplicity, sensitivity, low-cost, and easy operation. The main advantage of GNP-based assays is its ability for visual observation of color change arising from molecular recognition events [5, 15]. Paper-based diagnostic platforms benefit from some distinctive features such as cost-effectiveness, low sample volume requirements, simple fabrication, disposability, and ease of storage and transport that make them ideal candidates for development of biomedical sensing systems [16-19]. Recently, some colorimetric methods have been developed for detection of telomerase activity based on the optical properties of GNPs [5, 15]. Besides tremendous researches in this regard [20], less attention has been paid on the paper-based visual detection of telomerase activity. More recently, dual-mode detection of telomerase activity has been reported based on telomeric elongation and capturing amplification using functionalized up-converting nanoparticles (UCNPs) and methylene blue as probes on a functionalized cellulose paper [21]. Nevertheless, the main issues with UCNPs are instability of nanoparticles in some physiological buffers such as PBS and bio-functionalization of them is not straightforward [22]. The gold enhancement approach that utilizes seed-mediated growth of GNPs is a novel and efficient approach to reduce the limit of detection in lateral flow immunoassays [23-25] or immune-dot blot assay [26] and has the ability to be recruited in solid-solution phase interfaces to enhance the final visual signal as a sign of the attachment of target molecule on solid surfaces.

To meet these demands and in continuation of our previous works [12, 27, 28], herein, we provided a paper-based colorimetric nanobiosensing platform for simple, cheap, and sensitive detection of telomerase activity in cell extracts. In this way, we immobilized the telomere complementary (TC) oligonucleotide on GNPs, hybridized it by biotin-modified telomerase substrate (TS) primer elongated by the enzyme in solution phase, and attached the resultant assembly onto a paper strip, and finally the signal enhancement was carried out to detect the telomerase activity. The developed platform needs much less reagent, expertise, and time compared with standard telomerase assays.

2. Materials and methods

2.1. Materials

Chloroauric acid ($HAuCl_4$•$3H_2O$), trisodium citrate, tris(2-carboxyethyl)-phosphine (TCEP), Tween 20, Perfect-Hyb hybridization buffer (HB), Saline Phosphate buffer [0.1 M NaCl, 10 mM phosphate buffer (pH 8)], Tris–HCl, $MgCl_2$, EGTA, Bovine Serum Albumin (BSA), safe stain, nitrocellulose membrane, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), glycerol, agarose powder, and avidin were all obtained from Sigma-Aldrich. The pH 8 phosphate buffer was prepared from Na_2HPO_4 and NaH_2PO_4, both from Sigma-Aldrich; Two 10 mM pH 8 phosphate buffers including different reagents named as AGEI buffer [0.01% (w/v) SDS] and AGEII buffer [0.3 M NaCl, 0.01% (w/v) SDS] were prepared and used in DNA-GNPs conjugation step. The DNA sequences were synthesized by Sigma-Aldrich, as listed in Table 1. The distilled, deionized, and sterilized water was used during experiments. RNase/DNase free water was obtained from Thermofisher. DNA ladder and Taq DNA Polymerase 2x Master Mix were purchased from Thermofisher. CHAPS lysis buffer was prepared by mixing 1 mM EGTA, 10 mM Tris–HCl, 1 mM $MgCl_2$, 0.5% CHAPS, and 10% glycerol and stored at 4 °C before use.

Table 1
The Sequences of employed oligonucleotides in this work.

Designation	Sequence
5′-biotin-TS	5′-AATCCGTCGAGCAGAGTT-3′
5′-biotin elongated-TS	5′-AATCCGTCGAGCAGAGTTTT(AGGGTT)$_3$AGGG-3′
5′-thiolated probe	5′-A_{10}CCCT (AACCCT)$_3$AA-3′
PCR-TS	5′-AATCCGTCGAGCAGAGTT-3′
PCR-ACX	5′-GCGCGGCTTACCCTTACCCTTACCCTAACC-3′

2.2. Instruments

Nanodrop-ND 1000 Spectrophotometer (Thermo Fisher Scientific, USA) and UV–visible spectrophotometer (Bioaquarius, CE7250 model, CECIL Co. UK) were used to measure the absorbance spectrum of GNPs and DNA-GNPs conjugate. A Transmission Electron Microscope (Philips EM 208 from Phillips, USA) and Digimizer software version 4 (MedCalc Software, Belgium) were employed to measure the size of GNPs. Ultra bath Sonicator (Unisonics, Australia) was used for dispersion of GNPs. The ultrapure water (18 MΩ water) was prepared by a deionizer (Suez Water Technologies and Solutions, USA). All glass-wares were washed with an aqua regia solution, rinsed with MilliQ water, and dried inside an oven at 120 °C prior to use.

2.3. Preparation of GNPs and GNP-DNA conjugates

GNPs were synthesized by citrate reduction method [29]. Briefly, 100 mL of 1 mM $HAuCl_4$ was boiled, while homogenously stirring at 900 rpm. Then, 10 mL of 38.8 mM trisodium citrate was added to it very quickly. As a sign of successful synthesis of GNPs, five minutes later, the color of the solution turned from yellow to blurred purple and further to deep-red. Immobilization of thiolated probe to GNPs was performed as previously described [27] with some modifications. Firstly, 30 μL thiolated probe (7 nmol) was reduced with 50 μL of 10 mM TCEP during 1 h at room temperature (RT), while shaking at 120 rpm. Then, 70 μL of reduced probes were thoroughly mixed with 1 mL of GNPs; after that, 5 − 50.33 μL AGEII buffer were added incrementally followed by sonication for 10 s and incubation at room RT during 20 min, while shaking at 70 rpm. Then, the mixture was incubated overnight at RT with shaking at 70 rpm, followed by transferring the reaction to an RNase/DNase free 1.5 mL EP tube, centrifugation for 10 min at 16,000 × *g* and removing the supernatant. Then, the red oily pellet was washed two times by re-suspending in 1 mL phosphate buffer (pH 8), and the final pellet was re-suspended in 1 mL PBS and stored at 4 °C in dark for further experiments.

2.4. Cell culture and telomerase extraction

MDA-MB-231 breast cancer cells were obtained from the American Type Culture Collection. Then, the cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin and maintained in a humidified atmosphere of 95% air and 5% CO_2. The cells were harvested in the exponential growth phase by trypsin 0.25%. Almost 4 × 10^5 cells were collected in 2 mL EP tube and washed twice with cold PBS. After that, the cells were used for telomerase extraction, as previously described [12]. Briefly, the plate of cells was re-suspended in CHAPS lysis buffer and incubated for 30 min with mixing every 10 min. Then, the lysate was centrifuged at 16,000 × *g*, for 20 min at 4 °C, and the supernatant, without disrupting of the pellet, was collected into a fresh EP tube and stored at −70 °C for further use.

2.5. Telomerase extension reaction

Firstly, the cell extract was diluted serially, and 10 μL of each dilution was added to 90 μL reagent mixture containing 10 μL

biotinylated TS primer, 20 μL RNase/DNase free deionized water, and 60 μL dye-free Taq DNA Polymerase 2x Master Mix. For negative control, cell extracts were heated at 95 °C for 30 min, before adding them to the reaction mixture. Telomerase extension reaction was performed with incubation of the mixture at 37 °C for 30 min to allow the addition of telomeric repeats to 3′ end of biotinylated TS primer by telomerase. The elongation reaction was stopped through heating the reaction mixture for 5 min at 95 °C.

2.6. Conventional trap assay

Telomerase activity was also measured by the conventional telomeric repeat amplification protocol (TRAP) [30]. For this purpose, the elongated and the negative control samples were subjected to PCR and the obtained products were resolved by agarose gel electrophoresis and visualized by staining with ethidium bromide under UV light.

2.7. Analytical procedure

After preparation of GNPs-oligonucleotide conjugate, the hybridization was carried out between it and elongated (positive control oligonucleotide/real sample) or non-elongated target (negative control). Briefly, 100 μL of target and 100 μL of GNPs-DNA conjugate were mixed with 10 μL of hybridization buffer. The mixture was stirred by shaker at room temperature for 30 min. On the other hand, the paper strip was activated by incubating in 2 mg/mL streptavidin solution for 1 h, followed by blocking with 5% BSA solution for 15 min. After washing the paper, it was immersed in hybridization solution for 30 min with shaking at 100 rpm to let the hybridized assembly to attach on the paper surface via avidin-biotin interaction. The resultant strip was washed again thoroughly and then immersed in 600 μL seeding solution during 5 min. The seeding solution consisted of 100 μL of 1 mM Au^{3+} and 500 μL of 200 mM hydroxylamine hydrochloride [27, 31, 32].

Furthermore, the serially diluted concentrations of enzyme solution from 400,000 cells were used to define the detection limit of the assay. Photos of strips were captured by a smartphone camera (16 MP) and subsequently analyzed by ImageJ free software according a procedure provided by the developer (https://imagej.nih.gov/ij/docs/menus/image.html). The surface morphologies of paper strips including control (−) and control (+), prior to and after seeding were further recorded by FE-SEM.

3. Results and discussion

3.1. Characteristics of GNPs and GNP-DNA conjugates

The UV–Vis absorption spectra of GNPs and respective DNA conjugate are given in **Fig. 1a**. As seen, the characteristic surface plasmon resonance peak (SPR) of GNPs was observed at 524 nm. By DNA conjugation, a red-shift was occurred and the characteristic SPR peak of GNPs was shifted to 537 nm. The TEM image, **Fig. 1b**, showed spherical-shaped GNPs with an average diameter of 13.43 ± 1.89 nm that is desired for biosensing applications due to their high extinction coefficient values [33]. Size distribution graph of the synthesized GNPs is illustrated in inset of **Fig. 1b**, representing a uniform distribution of nanoparticles mainly in 11–15 nm range.

3.2. Conventional trap assay

We confirmed the existence and activity of telomerase in the cell extract dilutions of MDA-MB-231 human breast cancer cells using conventional TRAP Assay. As previously described in Section 2.5, for the negative controls, cell extracts were heated at 95 °C for 30 min, while TS elongated primers were used as a positive control. Our results from agarose gel electrophoresis (1.5%) (Fig. 1c) confirmed the activity of telomerase in MDA-MB-231 cell extract dilutions by observing corresponding sharp bands at 50-bp position (lane 3–11) like as the positive control (lane 1). However, the subjection of the heated sample into the reaction did not result in any clear band in that position (lane 2), confirming deactivation of the enzyme but not completely. As a matter of facts, appearing of a sharp band at just 50 bp confirms the exact activity of the telomerase which is consistent with our results in both of the positive control and the samples group.

3.3. Principle of the assay for detection of telomerase activity

Principle of the employed detection strategy is represented in Fig. 2. As illustrated, after elongation of biotinylated TS primers by telomerase, hybridization of them with reporter probe immobilized on GNPs was carried out in solution. The reporter probe is complementary to the elongated sequence via the enzyme activity. Avidin activated and BSA-blocked paper strips were then soaked into solution containing the hybridized assembly of “GNPs-reporter probe-biotinylated capture probe” to allow the attachment of it on the paper surface via avidin-biotin interactions. Since each avidin molecule itself has four binding sites for attachment to biotin; thus, it is possible that more than one assembly being captured by each avidin embedded on strip. Subsequently, the paper strip was washed and the enlargement of attached GNPs on strip was performed via hydroxyl amine hydrochloride seeding as the main step of detection strategy. For this purpose, the freshly prepared solutions of hydroxylamine and gold precursor were mixed and added to the microtube containing the paper strip. Our investigations represented that the seeding time of 5 min is sufficient enough to differentiate between positive and negative results. In the final step, a smartphone was used to on-site image recording, and then analysis of results was carried out via ImageJ software. The washing condition in each step was optimized to prevent the possible background signal. The best condition was obtained for 3 times washing of paper strip by pipetting the water on its surface.

The TS primer without elongation, due to the heat treatment of enzyme, was employed as a negative control (Ctr (-)). The SEM image of the paper strip in experiments containing Ctr (-) after seeding did not reveal any nanoparticle (Fig. 3a). In addition, due to the acidic nature of seeding solution, the fibril nature of paper was broken up after the seeding procedure and showed an amorphous structure. On the other hand, the SEM image of the paper strip in the experiment containing elongated TS primer, as a positive control (Ctr (+)), showed a considerable number of spots on its surface as a sign of the attachment of GNPs assembly (Fig. 3b). So that, after subjection to the enlargement reaction, tightly compact rod-shaped submicron structures of gold were observed (Fig. 3c). The reason for such morphology of gold nanostructures can be attributed to the highly face-selective surface reaction of GNPs in the reduction of Au^{3+} ions via hydroxylamine [34]. Although, we employed a freshly prepared seeding solution in the assay; further investigations showed that this solution is stable over two weeks at 4 °C, without the formation of any nanoparticles within it. Other studies confirmed that the presence of primary GNPs is necessary for catalytic enlargement reaction, especially within the time window used for enhancement here. So the developed assay benefits from both high sensitivity and specificity.

3.4. Analytical performance of bioassay

The analytical performance of developed bioassay was studied by the implementation of serially diluted fractions of the extracted enzyme from 400,000 MDA-MB-231 breast cancer cells in elongation of biotinylated TS primer and its subsequent utilization in the bioassay. The evaluated corresponding cell numbers were 400,000, 200,000, 100,000, 50,000, 25,000, 12,500, 6250, 625, 62, 6, and 3 cells. The captured photos of paper strips for each experiment are given as the circles within Fig. 4, which show that the visual detection of telomerase activity could be achieved down to 6 cells. Furthermore, it is clear that

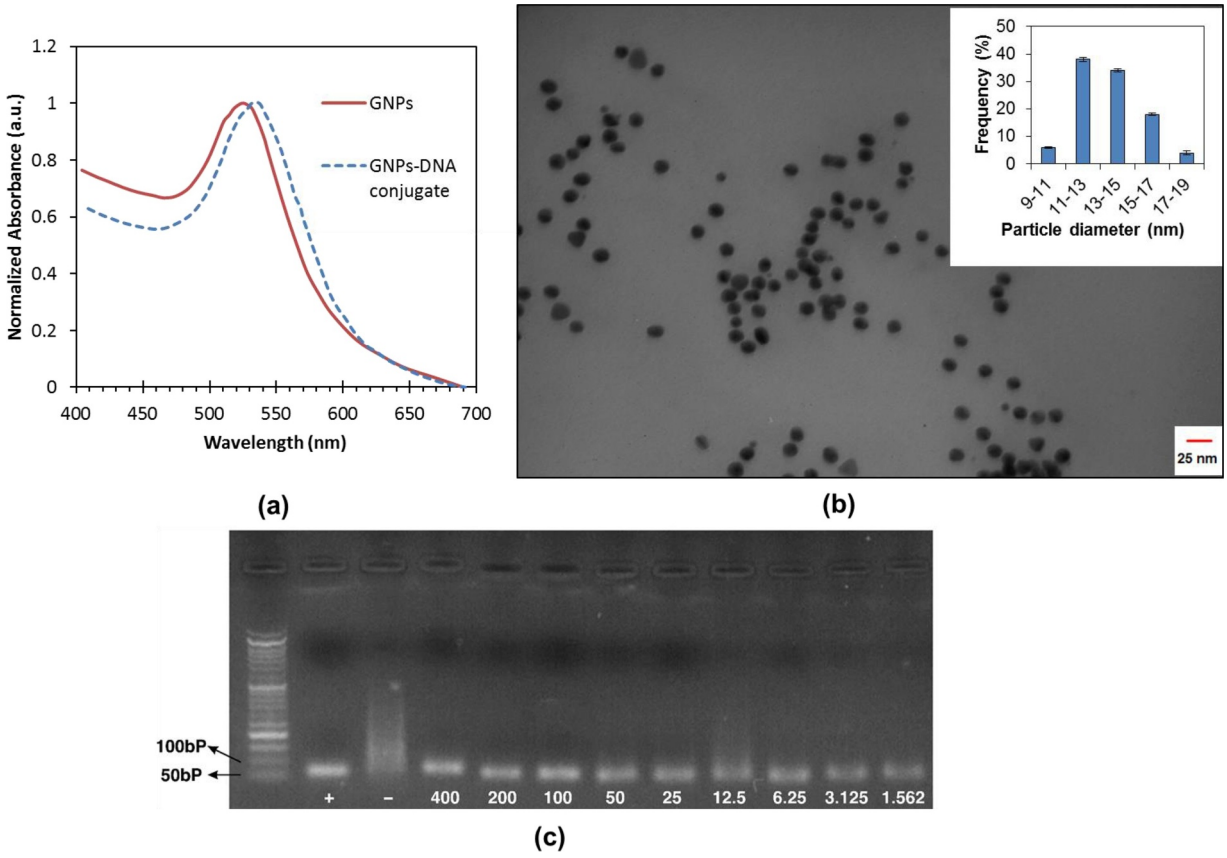

Fig. 1. (a) UV–Vis spectra for GNPs and GNP-DNA conjugate, and (b) TEM image of GNPs and size distribution graph of the synthesized GNPs (c) Agarose gel electrophoresis analysis of telomerase activity from different cancer cell numbers in the range of 400,000 to 1562 along with positive (+) and negative (-) controls.

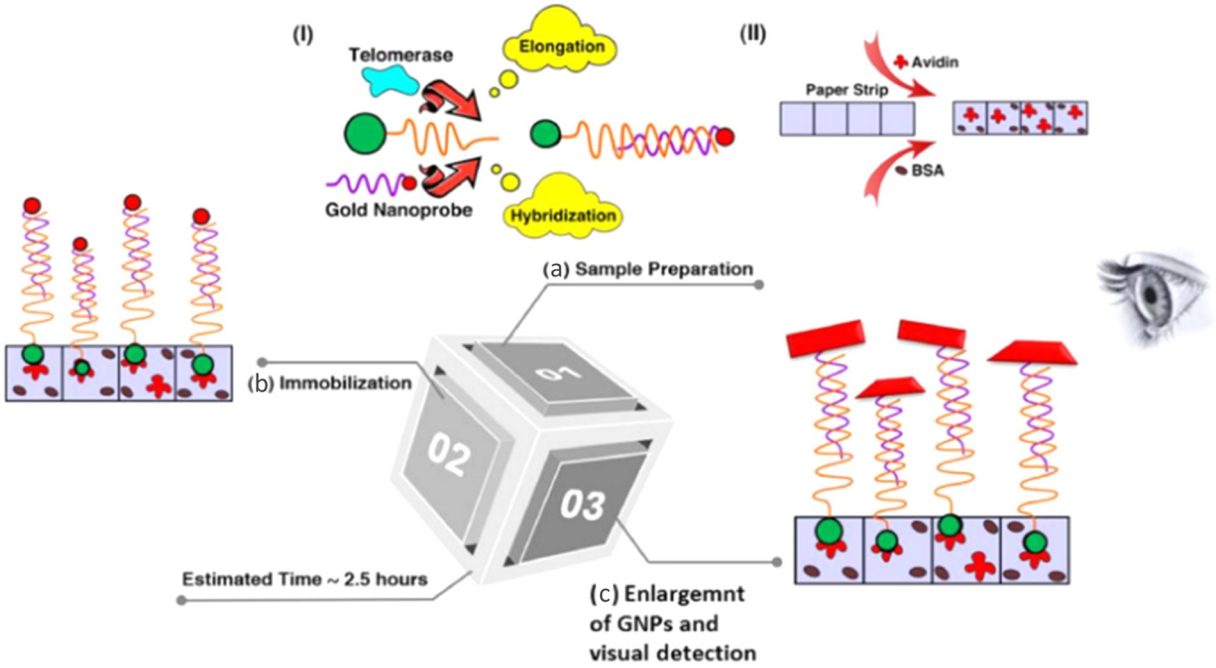

Fig. 2. Principle of telomerase activity detection based on GNPs signal enhancement on a paper strip (a): I. extension of biotinylated TS primers by telomerase, followed by solution phase hybridization of GNP-DNA conjugate with elongated TS primer, and II. Surface modification of paper strips (b) biotin-labeled capture probe binding with the avidin presented on paper strip, (c) Signal amplification by GNPs seeding and enlargement and visual detection (Drawings are not in scale).

by increasing the telomerase concentration, the color of paper strips change from light-red to light-red-blue and finally to black-red., which is in good agrement with optical properties of seperated, aggreagated, and thightly compacted GNPs, respecctivly. This is a notable achievement in telomerase activity detection in cancer cell extracts via the naked-eye. Furthermore, to achive a quantitaive measurement, the gray

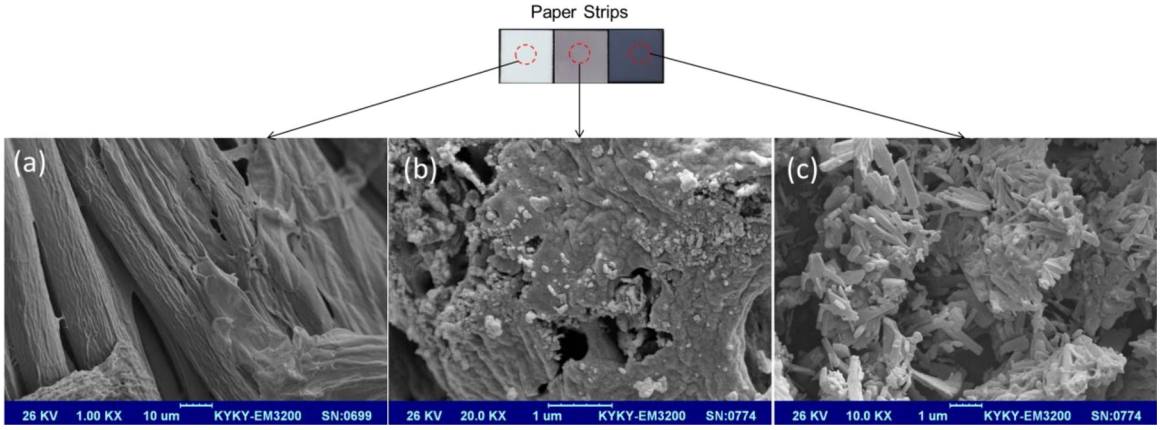

Fig. 3. SEM images of paper strips employed in the experiments including (a) Ctr (-) or negative sample, (b) after binding GNP-probe-elongated TS primer assembly onto the paper strip or Ctr (+), (c) after signal amplification by seed mediated growth of the bound GNPs.

scale analysis via ImageJ software was also performed, and the results were adjusted via background intensity as defined in Eq. (1):

$$G.\ S\ intensity = \frac{I_s - I_b}{I_b} \times 100 \quad (1)$$

In which I_s and I_b represent gray scale intensity for paper strip for each sample and background (Ctr(-)), respectively. Representing the results as G. S. intensity *vs.* the cell numbers in logarithm scale, as seen in Fig. 4a, show that there is ascending relation between them. These observation are in good agreement with the fact that at low concentrations of target, there are few number of GNPs within the strip and their growth cause color change from light-red to red-blue. On the other hand, at high concentrations of telomerase, due to presence of many GNPs within the strip, their growth in a tightly compact form results black-red color with more increase in the grayscale intensity. In fact, different mechanisms could be considered for the growth of GNPs: (1) at low concentrations of telomerase, because of the low number of elongated sequences, the growth mechanism is the growth of separated GNPs forming likely separated spheres. On the other hand, at high concentrations of enzyme the binding of GNPs, attached on the same elongated chain, to each other during growth step is possible which results in gold nanorods and thus higher color changes. This will influence the color change intensity, a phenomenon that is in good agreement with the obtained results. Based on Fig. 4a, it may conclude that for concentrations higher than 25,000 cell number, such binding events occur in practice. Although a gradual increase in black-red color of paper strip is observed from 50,000 to 200,000 resulting considerably black-blue color for 200,000; however, red color formation for 400,000 maybe due to the formation of new GNPs on the primerly formed gold nanorods. The exact examination of such nano/micro strucures is not the subject of this manuscript and it can be perfomed by further SEM imaging. Since herein the gray scale intensities of strips were utilized for quantification, so the attained results are to somewhat less affected by such color changes. Furthermore, a direct relationship was achieved between the grayscale intensity of paper strips and the logarithm of the cell numbers in the range of 6–2500 with correlattion coefficeint (R^2) of 0.91 (Fig. 4b). It is worthy to note that color formation was occurred down to 6 cells in this design, which can be easily observed by the naked eye. In contrary, for negative control sample as well as for the lower amounts of enzyme, no clear color change was observed. Since the color change arises from the catalytic effect of primary GNPs as seeds for further growth and subsequent color change; thus, the availablity of GNPs, and hence the actvity of telomerase is necessary to attain a color change. Furthermore, in the present design, the thiolated reporter probe is attached to the elongated section of

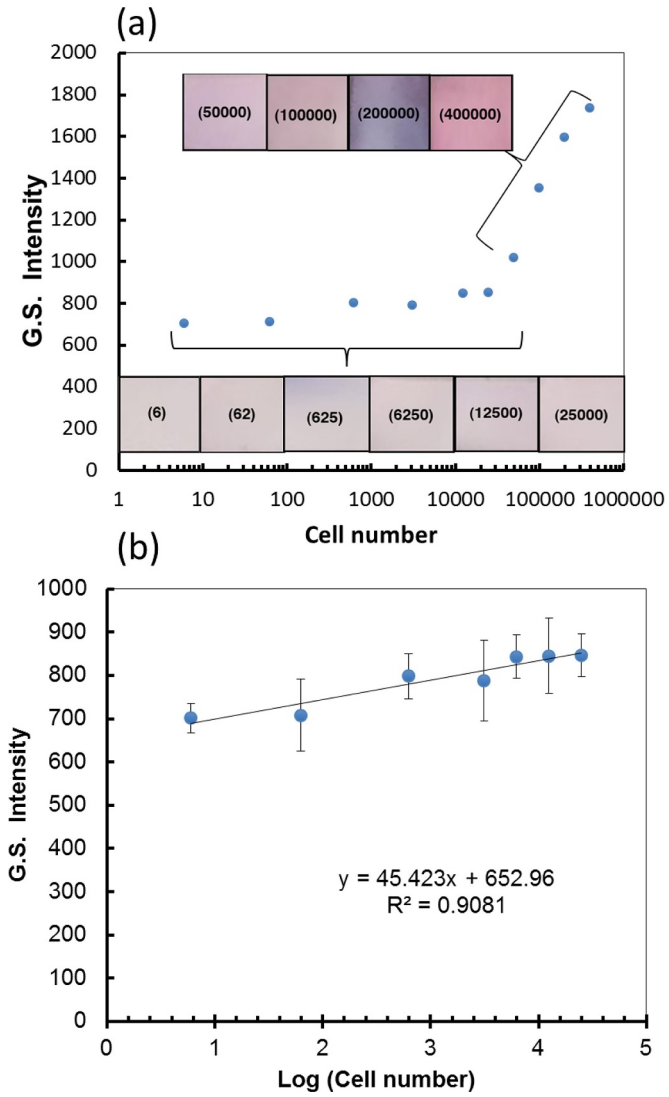

Fig. 4. (a) The obtained results for G.S. intensity *vs.* Cell number, the recorded photos of paper strips were also shown, (b) Linear correlation between the G.S. Intensity and Logarithm of cell numbers in the range of 6–2500 cells (the data show mean ± SD for $n = 3$).

Table 2
Comparison of the current work with some of the latest approaches for detection of telomerase activity.

Detection technique	Detection limit	References
Electrochemical detection via dopamine loaded liposomes	10 cells	[12]
Fluorescence detection via up-converting nanoparticles immobilized on functionalized paper	5 cells/μl	[21]
Fluorescence detection via controllable aggregation of quantum dots	9 cells	[47]
Colorimetric strategy via enzyme-aided cyclic amplification	25 cells	[48]
Fluorescence assay via polyamidoamine starburst dendrimer activated paper	10 cells	[47]
Assay via portable pH meter-based read-out	20 cells	[48]
Fluorescence detection via conformation-switchable nanoprobe	59 cells/mL	[38]
Paper-based visual detection of telomerase activity via GNPs seeding and enlargement	6 cells	The present work

biotinylated TS primer that provides sufficient selectivity and specificity in the detection of telomerase activity and prevents the occurrence of false-positive results. Although the exact number of repeated units of elongation via enzyme is not clear but the elongation of even one unit is sufficent enough to ahcive the hybridization events.

To the best of our knowledge, a very few number of studies have been reported using a paper-based platform for detection of telomerase activity. For instance, a UCNP-based assay has been developed employing UCNP conjugated probes that react with the telomerase product, and the telomerase primer was linked to the paper via formation of a secondary amine group between the amine-terminated primer and aldehyde group of the paper substrate. Although a high sensitivity was reported but such assays suffers from the lack of stability of UCNPs, complicated prepration procedure of UCNPs and corresponding DNA conjugates, as well as low efficiency of DNA hybridyzation in solid surfaces [22, 35]. The requirement for the application of a laser source for the read-out [35] is another disadvantage of UCNP-based method. On the other hand, our method takes the advantages of the stability of employed tags, lower cost, and stright-forward preparation of GNPs and GNP-ssDNA conjugates. Similarly, a polyamidoamine starburst dendrimer (PAMAM)/paper-based assay has been developed by Zhang et al. [36], in which the assay needs functionalisation of the paper substrate with PAMAM polymers and application of the fluorescent microscopy for recording the read-out. These steps are avoided in our method to simplify the assay procedure. It should be noticed that the extension of telomere primer followed by hybridization with the signal probes attached on the solid phase can limit the reaction efficiency due to the steric hindrance, which has been observed in the previous works [35, 36]. Nevertheless, these limitations are avoided in the present work by performing the telomere elongation and the hybridization in the solution phase as reported elsewhere [12, 37].

In terms of simple operation, high sensitivity, visual read-out, and low run-time, the developed platform is more intresting compared to some previously reported works in detection of telomerase activity (Table 2). The stability of visual read-outs on the paper is another important factor that cannot be offered by the solution-phase telomerase detection assays. The GNP-based solution methods for telomerase activity detection have been reported in litrature, in which GNPs were used as signaling tags of the telomere elongation. Most of these methods are equipment-dependent and are not applicable for the point-of-care settings [4, 6, 38-46]. Besides the favorite characteristics of the developed GNPs paper based platform, the employed imaging system, and the environmental light conditions are both very important to enhance the repeatability and reliabilities of attained data. In this work we tried to keep the light conditions constant by carrying the experiments in light controlled conditions. However, we believe that developing a "dark box" for image capturing by a smart phone equipped with a flash light can further improve the results.

4. Conclusions

In summary, we presented a novel colorimetric paper-based platform for highly sensitive detection of telomerase activity based on signal enhancing via GNPs seeding and enlargement. The proposed assay could detect telomerase activity in down to 6 MDA-MB-231 human breast cancer cells, with a broad detection range from 6 to 25,000 cells. The performance of the proposed bioassay can be attributed to the high sensitivity of GNPs seeding and enlargement approach, along with biotin-avidin interactions. The described method has a number of desirable characteristics: it is a PCR-free, low cost, simple, and highly sensitive with stable final signals, which makes it possible to measure telomerase activity via smartphone-based point-of-care platform for further clinical studies, especially in resource-limited situations.

CRediT authorship contribution statement

Tohid Mahmoudi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Abbas Pirpour Tazehkand:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing. **Mohammad Pourhassan-Moghaddam:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Mohammadreza Alizadeh-Ghodsi:** Conceptualization, Writing - original draft. **Lin Ding:** Methodology, Writing - review & editing. **Behzad Baradaran:** Supervision, Project administration, Funding acquisition. **Sajad Razavi Bazaz:** Software, Visualization. **Dayong Jin:** Supervision, Project administration, Funding acquisition. **Majid Ebrahimi Warkiani:** Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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