# Journal of Materials Chemistry B

Accepted Manuscript






ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

# Wrapping DNA-gated mesoporous silica nanoparticles for quantitative monitoring of telomerase activity with glucometer readout

**Youmei Wang, Minghua Lu,* Jinhua Zhu, and Shufang Tian**

***Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X***

**DOI: 10.1039/b000000x**

This work reports a simple and sensitive sensing protocol for quantitative monitoring of telomerase activity based on target–responsive release of cargo from wrapping DNA-capped mesoporous silica nanoparticle (MSN) by coupling with a portable personal glucometer (PGM). To construct such an assay system, glucose molecules are initially loaded into the pores of the aminated MSN, and then the pores are sealed with a specially designed wrapping DNA. Upon addition of telomerase and dNTPs, the assembled wrapping DNA strands are prolonged with the assistance of telomerase on the aminated MSN. Accompanying the progression of telomerase, the extended DNA strands detach from the MSN owing to the formation of rigid hairpin-like DNA structures. The "molecular gates" are then opened, resulting in the release of glucose from the MSN. The released glucose molecules can be quantitatively monitored by using an external PGM. The PGM signal increases with the increment of telomerase activity. Under optimal conditions, the PGM-based sensing platform exhibits good analytical properties for the determination of telomerase activity, and allows detection of telomerase activity in the HeLa extract at a concentration as low as 80 cells $mL^{-1}$. Using somatic and tumor cell lines, the generality of the assay is evaluated with satisfactory results. The inhibition effect of 3'-azido-3'-deoxythymidine also receives a good performance in telomerase inhibitor screening research. The methodology affords good reproducibility and simple operations, thus providing a useful scheme for practical use in quantitative telomerase activity assay for clinical application.

## Introduction

Nanotechnology, the emerging research field of manipulating matter at the molecular or atomic level, has experienced tremendous growth on the successful development of new analytical tools and instrumentation for bioanalytical and biotechnological applications in recent years.[1,2] One major advantage of using nanostructures is to control and tailor their properties in a very predicable manner to meet the requirements of specific applications.[3] The application of the nanotechnology in biosensing in place of conventional sensing strategies has led to improvements in the sensitivity, simplification and assay capacity.[4,5] Nanosized silica particles have been investigated intensively and have proved to be an ideal protein host since they are highly chemically and thermally stable, they have a large surface area, a fine suspendability in aqueous solution, and are relatively environmentally inert.[6,7] Mesoporous silica nanoparticles (MSN) with high colloidal stability are receiving growing attention by the scientific community for their groundbreaking potential nanomedicine.[8,9] The nanoparticles can feature a well-defined and tunable porosity at the nanometer scale, high loading capacity, and multiple functionality for targeting and entering different types of cells.[10,11]

Recently, various cargoes-loaded MSN based on different signal-transduction principles has been developed and reported for the application of bioanalysis and biosensing.[12-14] Zhang and co-worker designed DNA-hybrid-gated MSN for dual-targeted and microRNA-responsive controlled drug delivery by using fluorescence spectrometry.[15] The multifunctional MSN was loaded with Doxorubicin and then capped with DNA hybrid. Tang et al. developed target-induced displacement reaction accompanying cargo release from magnetic MSN for fluorescence immunoassay by loading rhodamine B in the pores with biotinylated concanavalin A capped.[16] Zhang et al. reported a new electrochemical immunoassay for quantitative monitoring of biotoxin using target-responsive cargo release from the MSN by loading methylene blue with polystyrene microspheres capped.[17] In these methods, different internal or external stimuli can provoke the nanocap removal and trigger the departure of the cargo, which permits the design of stimuli-responsive drug delivery nanodevices. Thus, it is possible to load huge amounts of cargo into the mesopore voids and capping the pore entrances with different nanogates.

Another important concern for the successful development of MSN-based assay system is to adopt a simple and sensitive signal-transduction method. Personal glucose meter (PGM) is currently one of the most widely used diagnostic devices in the world because of its portable size, easy operation, low cost and reliable quantitative results.[18] Many glucose meters employ the

oxidation of glucose to gluconolactone catalyzed by glucose oxidase.[19] Compared with other detection methods (*e.g.* fluorescence, chemiluminescence and electrochemistry), the PGM-based assay protocols usually have some advantages: i) The average size is very small and approximately the size of the palm of the hand, ii) A consumable element containing chemicals that react with glucose in the drop of sample is used for each measurement, iii) The size of the drop of sample is from 0.3 to 1.0 μL, and iv) The times it takes to read a test strip may range from 3 to 60 sec. Recently, a series of methods by coupling functional DNA probes with glucometer readout were reported for the detection of different target analytes, *e.g.* metal ions, cocaine, virus and disease markers.[20-24] Inspiringly, Hou[25] and Gao[26] most recently utilized glucose as the cargo loaded in the MSN for the detection of biomolecules with glucometer readout. Molecular dynamic simulations revealed a strong preferential interaction of glucose molecules with the silica walls, which induced significant concentration gradients within the pore.[27] In this regard, our motivation of this work is to exploit a new PGM-based detection protocol for *in situ* monitoring of telomerase activity by loading glucose into mesoporous silica nanoparticles.

Telomeres are the specialized repetitive DNA sequences at the ends of the linear chromosomes, and associated proteins, that serve to maintain the integrity of the chromosomes.[28,29] Telomerase is a ribonucleoprotein reverse transcriptase adding repeated DNA sequence TTAGGG to the 3' end of telomeres.[30] In the absence of telomerase activity telomeres progressively shorten. Without telomerase, telomere shortening eventually limits the growth of cells, either by senescence, in cells with intact cell cycle checkpoints, or by crisis in cells with inactivated checkpoints. Thus, the level of telomerase activity is very important in determining telomere length in aging cells and tissues. Herein, we report a novel, portable and quantitative monitoring of telomerase activity based on target-responsive release of cargo from wrapping DNA-gated mesoporous silica nanoparticles with glucometer readout. Initially, a specially wrapping DNA is designed as the molecular gate for the construction of functional MSN. Through the interaction between the negatively charged DNA and aminated MSN with positive charge, the cargo (glucose used in this case) is loaded into the pores of MSN. In the presence of telomerase and dNTPs, the designed DNA strand is extended and then moves away from the aminated MSN through the formation of rigid hairpin-like DNA structure, thereby resulting in the release of glucose molecules from the pores. The released glucose can be monitored by using an external PGM. By determining the change in the PGM signal, we can quantitatively judge the telomerase activity.

## Experimental

### Reagents and chemicals

3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS), phenylmethylsulfonyl fluoride (PMSF), and ethylene glycol-bis (*β*-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) were obtained from Biodee Biotechnology. Telomerase kits were acquired from Innovation Beyond Limits (Germany). Tetraethoxysilane (TEOS) and *n*-cetyltrimethylammogniumbromide (CTAB) were purchased from Sinopharm Chem. Re. Co. Ltd (Shanghai, China). 3-Aminopropyltriethoxysilane (APTES) was obtained from Sigma-Aldrich (Shanghai, China). dNTPs including adenosine 5'-triphosphate (ATP), cytosine 5'-triphosphate (CTP), guanosine 5'-triphosphate (GTP) and uridine 5'-triphosphate (UTP) were purchased from Dingguo Biotechnol. Co. Ltd (Beijing, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. The oligonucleotides with specially designed wrapping DNA were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China).

Wrapping DNA: 5'-$(CCCTAA)_6$AATCCGTCGAGCAGAGTT-3'

In the wrapping DNA, the underlined sequence was the primer of telomerase. DNA stock solution was obtained by dissolving oligonucleotides in tris-HCl buffer solutions (pH 7.4). Each oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature (RT) before use. Phosphate-buffered saline (PBS, pH 7.4) solution was prepared by mixing the stock solutions of 136.7 mM NaCl, 2.7 mM KCl, 1.41 mM $NaH_2PO_4$ and 8.72 mM $Na_2HPO_4$. Extension buffer was achieved by mixing 50 mM Tris-HCl, pH 7.5, 1.0 mM EGTA, 50 mM KCl, 1.0 mM $MgCl_2$ and 0.05% Tween 20.

### Cell culture

HeLa cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 100 IU $mL^{-1}$ of penicillin-streptomycin. The cells were maintained at 37 ºC in a humidified atmosphere (95% air and 5% $CO_2$). The cancer cell densities were determined by using hemocytometer prior to experiments.

### Preparation of telomerase extract from HeLa cells grown in culture

Cells were collected in the exponential phase of growth. HeLa cells were counted, and an aliquot containing $1.0 \times 10^6$ cells was dispersed into a 1.5-mL eppendorf tube, washed twice with ice-cold PBS (0.1 M, pH 7.4), and resuspended into 200-$\mu$L ice-cold CHARPS lysis buffer containing 10 mM Tris-HCl, pH 7.5, 1.0 mM $MgCl_2$, 1.0 mM EGTA, 0.1 mM PMSF, 0.5% CHAPS and 10% glycerol. The mixture was incubated for 30 min on ice and centrifuged at 16,000*g* at 4 ºC for 20 min. The supernatant was collected as cell extract for analysis or flash frozen in liquid nitrogen at -80 ºC.

### Synthesis of aminated mesoporous silica nanoparticles (MSN)

Before modification with the APTES, MSN was synthesized according to the literature.[31] Briefly, 0.5 g of CTAB was initially dissolved in 200-mL distilled water, and then 1.75-mL sodium hydroxide (2.0 M) was slowly added into the CTAB solution with vigorous stirring for 20 min at 80 ºC. Afterwards, 2.5-mL TEOS was dropped into the above-prepared solution, and vigorously stirred for 2 h until white precipitates were obtained. Following that, the product was filtered, washed with distilled water and methanol, and dried in air. To remove the excess CTAB, MSN was refluxed for 10 h in a solution composed of HCl (37% 1.5 mL) and methanol (75 mL), and then washed with distilled water and methanol. The resulting MSN was dried for 4 h at 60 ºC in vacuum to remove the remaining solvent from the pores.

Next, the aminated MSN was synthesized consulting to the literature with some modification.[32] 0.5 g of the calcined MSN was suspended in 10-mL anhydrous ethanol inside a round-bottom flask. Afterwards, 500-$\mu$L APTES (original concentration) was injected into the suspension. After stirring for 6 h at RT, the resulting mixture was filtered, washed with ethanol, and dried at 60 ºC to achieve the aminated MSN (*i.e.* APTES-functionalized MSN).

**Preparation of glucose-loading MSN capped with wrapping DNA**

The loading of glucose molecules into the pores of aminated MSN was prepared according to the literature.[25] Briefly, 5 mg of the aminated MSN was initially dispersed into 500 $\mu$L of pH 7.5 extension buffer containing 2.0 M glucose, and the resulting mixture was then gently shaken on an end-over-end shaker (MS, IKA GmbH, Staufen, Germany) for 16 h at RT. During this process, glucose molecules were diffused into the pores of the aminated MSN. Following that, 20 $\mu$L of wrapping DNA (200 $\mu$M) was added into the resulting suspension. The mixture was gently stirred for 8 h at 4 ºC. Owing to the interaction between positively charged MSN and negatively charged DNA, the wrapping DNA was attached onto the surface of the aminated MSN, and gated the glucose molecules inside. Following that, the DNA-wrapped MSN was centrifuged and washed with distilled water to remove the excess DNA strands and glucose until a low background PGM signal was obtained. Finally, the DNA-capped MSN loaded with glucose (designated as DNA-MSN) was re-dispersed into 200 $\mu$L extension buffer (pH 7.5) ($C_{[MSN]} \approx 25$ mg mL$^{-1}$) for further use.

**Telomerase activity detection in HeLa cells**

Scheme 1 represents the assay procedure of telomerase activity by using the as-prepared DNA-MSN with glucometer readout. Initially, 10 $\mu$L of the above-prepared DNA-MSN ($C_{[MSN]} \approx 25$ mg mL$^{-1}$) was injected into 200-$\mu$L PCR tube. Then, telomerase extracts diluted in lysis buffer with the respective number of HeLa cells and 10 mM dNTPs were added into the tube. The mixture was incubated for 80 min at 37 ºC. Following that, a 5-$\mu$L aliquot of the supernatant obtained by centrifugation was removed for glucose measurement using a commercialized PGM. The obtained PGM signal was registered as the sensing signal relative to telomerase activity. All measurements were conducted at RT (25 ± 1.0 ºC). Measurements are always made in triplicate.

**Monitoring of telomerase kit**

50-$\mu$L telomerase sample/standard solution was added in the well of ELISA plate, and incubated at 37 °C for 30 min. After washing with PBS, 50-$\mu$L the labeling reagent was added to each well, and the mixture was incubated at 37 °C for 30 min. Then the medium was removed, and 50-$\mu$L color development agent A and 50-$\mu$L color development agent B were added to each well. After the plate was vibrated for 10 min at 37 °C, 50-$\mu$L stop buffer was added to each well to stop the color reaction. The absorbance of each well was measured at 450 nm using a microplate reader.

**Polyacrylamide gel electrophoretic analysis**

The products reacted with trifunctional molecular beacon were characterized by a polyacrylamide (PAGE) gel (20% acrylamide, 19:1 acrylamide/bisacrylamide) with 1× tris-borate-EDTA (TBE) (pH 8.0) buffer as the separation buffer. Electrophoresis was carried out at 11 mA for 1.5 h. After Stains-All staining by EB solution for 25 min, gels were photographed by gel image system.

## Results and discussion

**Design and characteristics of DNA-MSN**

Scheme 1 shows the fabrication process of DNA-MSN-based sensing probe decorated with glucose molecules and wrapping DNA, and the assay principle toward telomerase activity. In this work, the strong preferential interaction of glucose molecules with the silica walls could induce significant concentration gradients within the pore.[33,34] Because of the largely loaded capacity of mesoporous silica, a noticeable amount of glucose molecules could be loaded into the pores. Accompanying the loading of glucose molecules in the pore, the gate was sealed by the specifically designed wrapping DNA based on the interaction of positively charged MSN with negatively charged DNA. To ensure that the synthesized MSN was of similar size and contained similar amount of glucose, we would adapt the same sythesis route of MSN and the same loading method of glucose during all the experiments. Upon addition of telomerase and dNTPs, the assembled DNA [5'-$(CCCTAA)_n$ AATCCG TCGAGC AGAGTT-3'] was extended along the 5'→3' direction, thus resutling in the formation of repeated DNA sequence (TTAGGG) at the 3' end of telomerase. The repeated DNA sequence TTAGGG at the 3' end could be complementary with the sequence CCCTAA at the 5' end to form a rigid hairpin-like DNA structure. The *in situ* synthesis of telomeric repeats at the 3' end led to the detachement of the wrapping DNA from the surface of aminated MSN. Consequently, the "gate" is triggered to open and release the pore-entrapped glucose into the solution for PGM readout. The released glucose molecules in the resulting solution were collected and detected by an external PGM. By monitoring the change in the PGM signal, we can indirectly determine the level of telomerase activity.

To realize our design, the successful preparation of DNA-MSN-based sensing probe is very crucial. Figure 1A shows typical transmission electron microscopic (TEM, JEM-2100, JEOL Ltd., Japan) image of the as-synthesize MSN, and the average size was about 200 nm. Meanwhile, we could also observe that there were a large number of pores on the silica nanoparticles. Nitrogen adsorption-desorption isotherm (ASAP 2000 Instrument, Micromeritics, Norcross, GA, USA) of the as-synthesized MSN showed a typical type of curve with a specific surface area of 478.1 m$^2$ g$^{-1}$ (Figure 1B), and a narrow pore distribution with a Barrett-Joyner-Halenda (BJH) average pore diameter of 4.4 nm (inset of Figure 1B), which was obviously larger than that of glucose molecule (equivalent to 1.0 nm in diameter). Therefore, a large number of glucose molecules can be loaded inside the MSN.

An additional question to be answered herein was whether the designed wrapping DNA could be assembled onto the aminated MSN by the electrostatic adsorption. To this end, the DNA-MSN after each step was monitored by microelectrophoresis (Microelectrophoresis Apparatus MK II, Rank Brothers Ltd, England). As shown from Figure 1C-a, the zeta potential of the

newly prepared MSN was -28.9 mV. When APTES was modified onto the MSN, however, a positive zeta potential (+12.4 mV) was acquired (Figure 1C-b), indicating that APTES was conjugated onto the MSN. Inspiringly, when wrapping DNA was assembled again onto the aminated MSN, the zeta potential became negative (-32.1 mV) (Figure 1C-c). To further demonstrate that the wrapping DNA could be assembled onto the MSN, we also used UV-vis absorption spectrometry (Techcomp, UV/vis 1102, China) to investigate the preparation process of functional MSN (Figure 1D). Almost no characteristic peaks were observed for the unmodified MSN (curve '*a*'). After the formation of the aminated MSN, the adsorption curve was slightly changed relative to curve '*a*' (curve '*b*'). Significantly, an obvious characteristic peak at 265 nm was achieved when the wrapping DNA was assembled onto the surface of aminated MSN (curve '*c*'), which was corresponded to DNA molecules.[35] These results revealed that the wrapping DNA could be assembled onto the aminated MSN through the electrostatic adsorption technique.

**Control tests**

Logically, another question to be produced was whether the assembled DNA on the aminated MSN could be extended with the help of telomerase and dNTPs. To verify this point, we used gel electrophoresis to investigate the detached rigid hairpin-like DNA structure from the aminated MSN before and after incubation with telomerase and dNTPs, respectively (inset in Figure 2). As seen from lane 2, the base number of the wrapping DNA was ~60 bp, which was in agreement with our design. Lane 3 shows the gel electrophoresis of the supernatant from the DNA-wrapped MSN suspension in the absence of telomerase and dNTPs after shelving for 80 min at 37 ºC. No spots were appeared, indicating that the designed wrapped DNA could not be detached from the aminated MSN in the absence of telomerase and dNTPs. Favorably, the supernatant showed an extensive product of ~80 bp (lane 4) when incubating the DNA-MSN with telomerase and dNTPs for 80 min at 37 ºC, which was about 4 TTAGGG segments longer than that of the designed wrapping DNA (lane 2). The results indicated that the assembled DNA could be moved away from the aminated MSN with the aid of telomerase and dNTPs via the formation of long DNA strand.

The assembled DNA could block the release of glucose from the aminated MSN or not. For comparison, we prepared two types of glucose-loaded MSN sensing probes with and without the wrapping DNA by using the same method. The corresponding supernatant in the extension buffer was detected by using an external PGM at the different-shelving times at 37 ºC. Figure 2 shows the PGM signal of the supernatant (versus the release time). As shown from Figure 2a, the PGM signal of the supernatant without the wrapping DNA increased with the increasing time, and tended to a constant value after 30 min, indicating that the loaded glucose rapidly released out in the absence of the wrapping DNA. In the presence of wrapping DNA, however, the PGM signals were not almost changed (close to zero) (Figure 2b). The results suggested that the specifically designed wrapping DNA could be used as the 'biogate' for blocking the cargo into the aminated MSN.

**Optimization of experimental conditions**

In this work, the detectable PGM signal mainly derived from the loaded glucose molecules in the aminated MSN. Undoubtedly, the sensitivity of DNA-MSN-based sensing probe was dependant on the amount of the loaded glucose. Thus, we investigated the effect of using different-concentration glucose during the DNA-MSN preparation on the PGM signal. Initially, 10 $\mu$L of DNA-MSN suspension loading with different-concentration glucose ($C_{[MSN]} \approx 25$ mg mL$^{-1}$) was initially dispersed into 200-$\mu$L PCR tube. Then, telomerase and dNTPs (excess) was added into the resulting suspension, and incubated for 2 h at 37 ºC in order to ensure the adequately extensive reaction of the wrappind DNA on the DNA-MSN. Afterwards, the released glucose molecules were monitored by using the PGM. As seen from Figure 3a, the PGM signal increased with the increment of glucose concentration, and tended to level off after 2.0 M glucose. High-concentration glucose might cause the change of viscosity of extension buffer. Considering this point, 2.0 M glucose was selected for the DNA-MSN preparation in 500 $\mu$L of extension buffer containing 5 mg of the aminated MSN.

Additionally, the sensitivity of DNA-MSN-based sensing probe depends on the blocking time (*i.e.* the loading time of glucose) of the wrapping DNA with the aminated MSN during the loading process of glucose. Figure 3b shows the effect of the assembling time between the wrappin DNA and the aminated MSN on the PGM signal of the DNA-MSN (1000 HeLa cells mL$^{-1}$ used as an example). The PGM signal increased with the augmentation of assembling time from 0 to 16 h, and reached to the balance after 16 h. Longer assembling time did not significantly increase the output signal, suggesting that the pores were largely sealed by the wrapping DNA after 16 h. hence, 16 h was employed for the assembly of the wrapping DNA on the aminated MSN.

Figure 3c represents the time-denpendent signal change upon polymerization reaction of telomerase with the assembled DNA on the aminated MSN (*i.e.* the released time of glucose from the MSN). The PGM signal increased with the increasing enzymatic reaction time, and reached an equilibrium after 80 min. The results revealed that the polymerization reaction accompanying the cargo release could reach a dynamic balance. To save the assay time, 80 min was used for the release of glucose from the aminated MSN, *i.e.* the detached time of rigid hairpin-like DNA structure.

**Evaluation of telomerase activity in HeLa cells**

Since the telomerization for wraping DNA was controlled by the content of telomerase in the cell lysate samples, the detachment of wrapping DNA, and the PGM signal, all were related to the number of cancer cells. To validate the sensitivity of the newly developed telomerase assay, cell extracts extractd from $1.0 \times 10^6$ HeLa cells were serially diluted with lysis buffer and used as a source of telomerase. Under the optimal conditions, the ability of quantitative analysis of telomerase was investigated by performing the telomerase extracts from various concentrations of HeLa cells based on target-responsive controlled release of glucose from the aminated MSN. The telomerase activity in the HeLa extracts from 0 to 6000 cells mL$^{-1}$ was monitored with

three measurements each in parallel. The PGM signal of the supernatant from the system analyzing variable numbers of HeLa Cell is shown in Figure 4a. As shown from Figure 4b, the PGM signals increased with the an increase in the number of HeLa cells, confirming that the as-prepared DNA-MSN could reponse to telomerase to trigger the release of glucose from the MSN with glucometer readout. A linear dependence between the PGM signal and the logarithm of the HeLa cell concentration was achieved in the range from 100 cells to 5000 cells. The linear regression equation was $y = 7.3298 \times \lg x - 33.929$ (cell number $mL^{-1}$) with a correlation coefficient of 0.9941 ($n = 21$). The detection limit (LOD) was 80 HeLa cells $mL^{-1}$ as calculated in terms of the rule of 3 times standard deviation over the blank signal.

**Precision, reproducibility and generality**

To investigate the reproducibility of determinations, we repeatedly assayed three HeLa cell with different numbers using identical batched of DNA-MSN sensing probes. Experimental results indicated that the coefficents of variaion (CVs) of the intra-assay between three runs were 8.9, 7.6 and 9.2% for 150, 2000, and 4000 HeLa cells $mL^{-1}$, respectively, whereas the CVs of the inter-assay with various batches were 11.3, 8.7 and 9.8% toward the above-mentioned telomerase. With the exception of the slightly increased CV for the 150 HeLa cells $mL^{-1}$ in the inter-assay experiment, the other CVs indicated that the as-prepared DNA-MSN could be used repeatedly, and further verified the possiblity of batch preparation.

Next, the newly devleoped assay system was applied for the detection of telomerase activity with different cancer cells, *e.g.* K562 cell, U937 cell, 239A, PC-3 cell and HepG2, which were selected to extract telomerase. Each extract was individually added to the extension buffer. Following that, the PGM signal was recorded after incubation for 80 min at 37 ºC. As shown in Figure 4b, the sensing probe showed significant PGM signal increase in response to telomerase extracts of all cancer cells relative to the blank lysis buffer. The results revealed that the telomerase could be detected in human cancer cells.

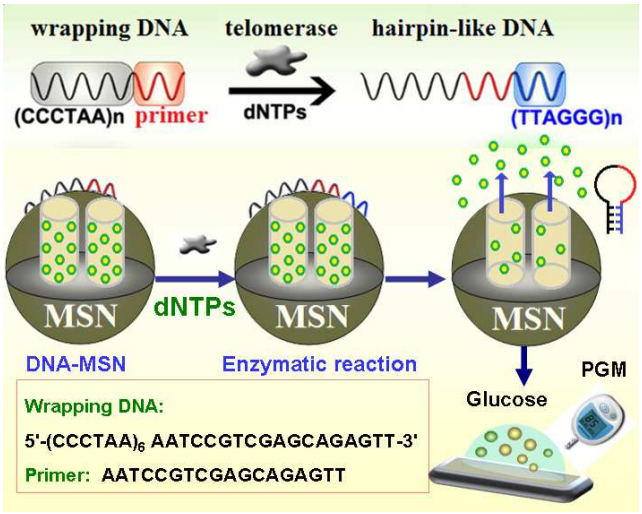
**Inhibition assay**

Using 3'-azido-3'-deoxythymidine (AZT) as a model inhibitor toward telomerase enzymatic activity, the validity of the as-prepared DNA-MSN in screening the inhibition of telomerase was evaluated through adding different-concentration AZT into the extension buffer in the telomerase extension reaction step. As seen from Figure 5a, the PGM signals decreased gradually with the increment of AZT level and incubation time, indicating the potential application of the developed assay system for studies of telomerase inhibition.

**Eliminating the interference of endogenous glucose**

Logically, another concern arises as to whether the DNA-PGM could be applied for the determination of the sample containing endogenous glucose. Because of the presence of endogenous glucose, it might interfere with the final assay results. To tackle this problem, the glucose concentration in an unknown sample must be monitored by using the PGM before measurment. When the background signal plus the generated signal by the telomerase exceeds the upper limit of the PGM readout, however, an appropriate dilution should be made. In this case, the background signal can be subtracted from the signal obtained in the subsequent actual test. As seen from Figure 5b, the telomerase activity in HeLa cell extracts containing different-concentration glucose based on the subtracted strategy were comparable to those in glucose-free HeLa cell extracts.

**Inserting Graphics**




**Scheme 1** Schematic illustration of wrapping DNA-capped mesoporous silica nanoparticles (MSN) loaded with glucose for quantitative monitoring of telomerase activity by using an external PGM.

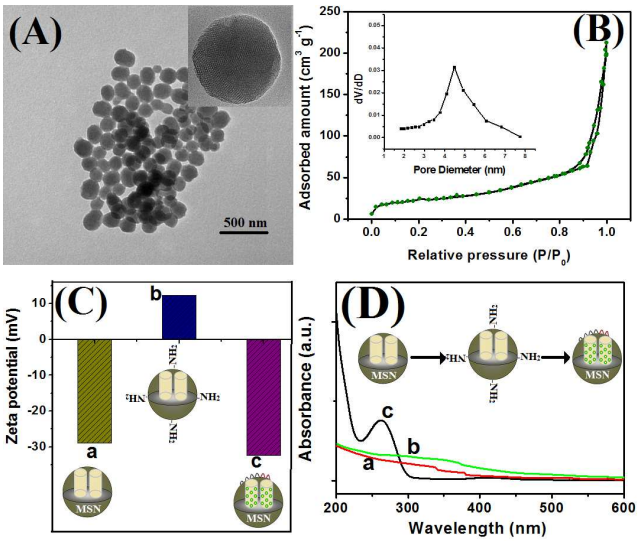



**Fig. 1** (A) TEM image of the as-synthesized MSN (inset: magnification image); (B) nitrogen adsorption-desorption isotherms (inset: pore size distribution); and (C) zeta-potentials and (D) UV-vis absorption spectra of (a) MSN, (b) APTES-functionalized MSN and (c) DNA-MSN.

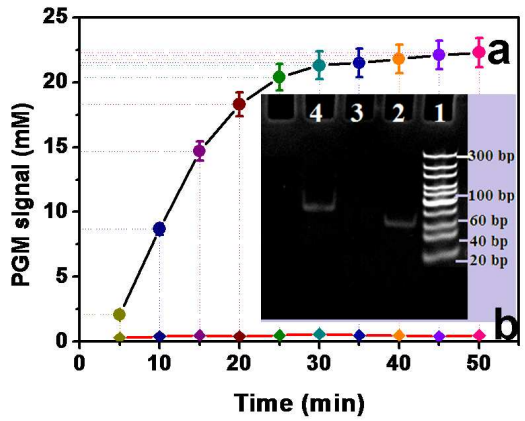



**Fig. 2** Study of the entrapping capacity (PGM signal *vs.* release time) of DNA-MSN toward cargo (glucose) molecules into the MSN by the wrapping DNA: (a) the aminated MSN loading with glucose without the wrapping DNA and (b) DNA-MSN loading with glucose with the wrapping DNA in pH 7.5 extension buffer [*Inset*: gel electrophoresis (lane 1: DNA ladder; lane 2: wrapping DNA; lane 3: DNA-MSN without telomerase and dNTPs; lane 4: DNA-MSN with telomerase and dNTPs)].

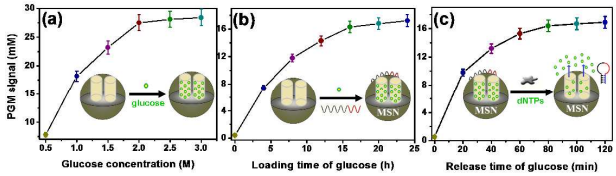



**Fig. 3** Dependence of the PGM signal on (a) glucose concentration, (b) loading time of glucose and (c) release time of glucose (1000 HeLa cells $mL^{-1}$ used in this case).

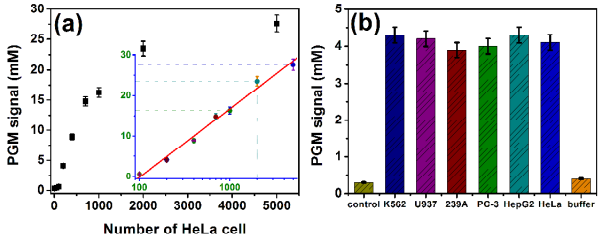



**Fig. 4** (a) The relationship between the PGM signals and different-concentration telomerase equivalent to different amounts of HeLa cells. A linear standard curve from 100 to 5000 HeLa cells $mL^{-1}$ was obtained with a detection limit of 80 cells $mL^{-1}$. (b) PGM responses of the DNA-MSN to the telomerase extracts from 200 cells of different kinds of cancer cells.

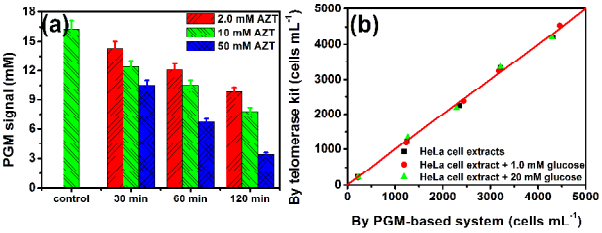



**Fig. 5** (a) Inhibition of telomerase activity in 1000 HeLa cells by using different-concentraion AZT at the different inhibition times. (b) Comparison of the assayed results for HeLa cells in the absence and presence of various-concentration glucose by using the DNA-MSN sensing system and commercialized telomerase kit

## Conclusions

In the present study, we demonstrated the ability of a simple and sensitive sensing protocol of quantitative monitoring the telomerased activity by target-induced release of cargo (glucose used in the case) from DNA-gated mesoporous silica nanocontainers. Design of specifically wrapping DNA could be utilized as the 'biogate' for the blocking and releasing of cargo from the MSN. Compared with the standard instrumental sensing methods, the PGM-based assay system is low-cost, rapid, portable and user-friendly, which is more available for the public use especially in major developing countries. More importantly, the assay does not require sophisticated equipment and sample pretreatment, thus representing a versatile detection method.

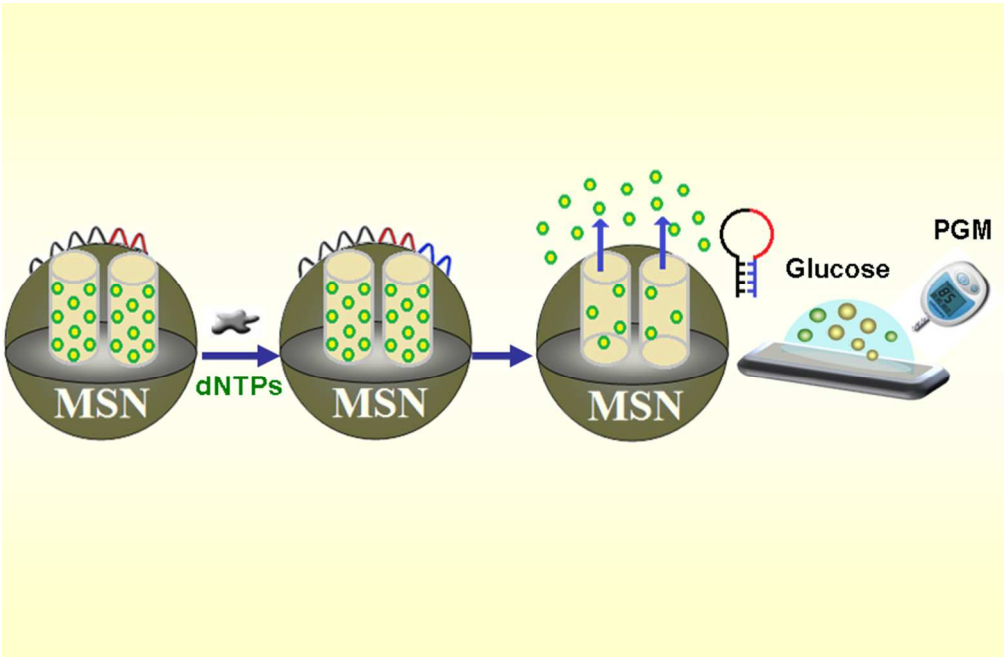
Support by the National Natural Science Foundation of China (grant no.: 21305029), High-Level Talent from Henan University (grant no.: B2012156), and State Key Laboratory of Cotton Biology Open Fund (grant no.: CB2013A22) is gratefully acknowledged.

## Notes and references

*Institute of Environmental and Analytical Science, School of Chemistry and Chemical Engineering, Henan University, Kaifeng 475004, Henan, P.R. China. Fax/Tel.: +86 371 23881589; E-mail: minghua.luhnu@gmail.com (M. Lu)*

1 R. Mo, T. Jiang, J. Di, W. Tai and Z. Gu, *Chem. Soc. Rev.,* 2014, **43**, 3595-3629.
2 S. Zeng, D. Baillargeat, H. Ho and K. Yong, *Chem. Soc. Rev.,* 2014, **43**, 3426-3452.
3 Y. Xu and B. Zhang, *Chem. Soc. Rev.,* 2014, **43**, 2439-2450.
4 S. Kleijn, S. Lai, M. Koper and P. Unwin, *Angew. Chem. Int. Ed.,* 2014, **53**, 3558-3586.
5 N. Li, P. Zhao and D. Astruc, *Angew. Chem. Int. Ed.,* 2014, **53**, 1756-1789.
6 M. Zhu, Y. Zhu, B. Ni, N. Xie, X. Lu, J. Shi, Y. Zeng and X. Guo, *ACS Appl. Mater. Interfaces,* 2014, **6**, 5456-5466.
7 X. Ma, S. Sreejith and Y. Zhao, *ACS Appl. Mater. Interfaces,* 2013, **5**, 12860-12868.
8 M. Colilla, B. Gonzalez and M. Vallet-Regi, *Biomater. Sci.,* 2013, **1**, 114-134.
9 Q. He and J. Shi, *Adv. Mater.,* 2014, **26**, 391-411.
10 C. Argyo, V. Weiss, C. Brauchle and T. Bein, *Chem. Mater.,* 2014, **26**, 435-451.
11 W. Mai and H. Meng, *Integr. Biol.,* 2013, **5**, 19-28.
12 P. Nadrah, O. Planinsek and M. Gaberscek, *J. Mater. Sci.,* 2014, **49**, 481-495.
13 Z. Tao, *RSC Adv.,* 2014, **4**, 18961-18980.
14 S. Shi, F. Chen and W. Cai, *Nanomedicine,* 2013, **8**, 2027-2039.
15 P. Zhang, F. Chen, R. Zhou, J. Cao, J. Li, C. Burda, Q. Min and J. Zhu, *Angew. Chem. Int. Ed.,* 2014, **53**, 2371-2375.
16 D. Tang, B. Liu, R. Niessner, P. Li and D. Knopp, *Anal. Chem.,* 2013, **85**, 10598-10596.
17 B. Zhang, B. Liu, J. Liao, G. Chen and D. Tang, *Anal. Chem.,* 2013, **85**, 9245-9252.
18 X. Ma, Z. Chen, J. Zhou, W. Weng, O. Zheng, Z. Lin, L. Guo, B. Qiu and G. Chen, *Biosens. Bioelectron.,* 2014, **55**, 412-416.
19 Q. Wang, H. Wang, X. Yang, K. Wang, F. Liu, Q. Zhao, P. Liu and R. Liu, *Chem. Commun.,* 2014, **50**, 3824-2826.
20 Y. Xiang and Y. Lu, *Anal. Chem.,* 2012, **84**, 1975-1980.

21 Y. Xiang and Y. Lu, *Anal. Chem.,* 2012, **84**, 4174-4178.
22 Y. Xiang and Y. Lu, *Chem. Commun.,* 2013, **49**, 585-587.
23 L. Yan, Z. Zhu, Y. Zou, Y. Huang, D. Liu, X. Jia, D. Xu, M. Wu, Y. Zhou, S. Zhou and C.Yang, *J. Am. Chem. Soc.,* 2013, **135**, 3748-3751.
24 J. Xu, B. Jiang, J. Xie, Y. Xiang, R. Yuan and Y. Chai, *Chem. Commun.,* 2012, **48**, 10733-10735.
25 L. Hou, C. Zhu, X. Wu, G. Chen and D. Tang, *Chem. Commun.,* 2014, **50**, 1441-1443.
26 Z. Gao, D. Tang, M. Xu, G. Chen and H. Yang, *Chem. Commun.,* 2014, **50**, 6256-6258.
27 A. Lerbret, G. Lelong, P. Mason, M. Saboungi and J. Brady, *J. Phys. Chem. B,* 2011, **115**, 910-918.
28 Y. Li and V. Tergaonkar, *Cancer Res.* 2014, **74**, 1339-1644.
29 V. Sekaran, J. Soares and M. Jarstfer, *J. Med. Chem.,* 2014, **57**, 521-538.
30 M. Wilsdorf and H. Reissig, *Angew. Chem. Int. Ed.,* 2014, **53**, 4332-4336.
31 R. Qian, L. Ding and H. Ju, *J. Am. Chem. Soc.,* 2013, **135**, 13282-13285.
32 E. Climent, R. Martinez-Manez, F. Sancenon, M. Marcos, J. Soto, A. Maquieira and P. Amoros, *Angew. Chem. Int. Ed.,* 2010, **49**, 7281-7283.
33 A. Ziemys, M. Ferrari and C. Cavasotto, *J. Nanosci. Nanotechnol.,* 2009, **9**, 6349-6359.
34 A. Ziemys, A. Grattoni, D. Fine, F. Hussain and M. Ferrari, *J. Phys. Chem. B,* 2010, **114**, 11117-11126.
35 B. hang, B. Liu, J. Zhou, J. Tang and D. Tang, *ACS Appl. Mater. Interfaces,* 2013, **5**, 4479-4485.




229x150mm (96 x 96 DPI)