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Platinum nanoparticles encapsulated metal−organic frameworks for electrochemical detection of telomerase activity

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Contents

General

Materials and reagents. Zirconium chloride ($ZrCl_4$), 2-aminoterephthalic acid (NH_2-H_2BDC), dimethylformamide (DMF), polyvinylpyrrolidone (PVP, MW 40k), and hydrogen hexachloroplatinate (IV) hexahydrate (H_2PtCl_6•$6H_2O$) were purchased from Sigma-Aldrich Inc (USA). Methanol was purchased from Nanjing Chemical Reagent Co., LTD (China). Carboxylic graphene oxide (CGO, purity > 99.8%, carboxyl ratio > 5.0 wt%, single layer ratio > 80%) was purchased from Nanjing XFNano Materials Tech Co. Ltd. (Nanjing, China). The deoxynucleotide mixture (dNTPs) was purchased from Sangon Inc. (Shanghai, China). Phenylmethylsulfonyl fluoride (PMSF), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS), ethylene glycol bis(aminoethyl ether)-*N,N,N,N*-tetraacetic acid (EGTA), glycerol, and Tween 20 were purchased from Sigma-Aldrich Inc (USA). The telomerase ELISA kit containing a bottle of telomerase standard solution (80 IU L^{-1}) was from Innovation Beyond Limits (Germany). HeLa, CEM, U87, MOF-7, HaCaT and HEpG2 cells were from KeyGen Biotech. Co. Ltd. (Nanjing, China). Buffer solutions involved in this study were as follows: A 0.1 M H_3BO_3-NaOH buffer (pH 11.0) was used as detection solution, and Tris-HCl buffer (0.1 M) containing 0.1 M NaCl and 5 mM $MgCl_2$ (pH 7.4) was prepared as DNA stock solution. The washing cancer cell buffer was prepared with 0.1 M PBS (pH 7.4) containing K_2HPO_4 and KH_2PO_4. A 10 mM Tris-HCl (pH 8.3) buffer solution was employed to wash electrodes. Ultrapure water (> 18 MΩ, Millipore) was used in all experiments. All DNA oligonucleotides used in the experiment were offered by Sangon Inc. (Shanghai, China). The sequences of DNA oligonucleotides are given below:

TS primer: 5'-NH_2-TTTTTTTTTTAATCCGTCGAGCAGAGTT-3'

Capture DNA (cDNA): 5'-COOH-$(CH_2)_6$-TTTTTTTTTTAACCCTAACCCT-3'

Apparatus. The morphology of UiO-66-NH_2 and Pt@UiO-66-NH_2 was investigated by using scanning electron microscope (SEM) from an S-4800 scanning electron microscope (Hitachi, Japan).

The size and morphology of UiO-66-NH_2 and Pt@UiO-66-NH_2 was further characterized by using transmission electron microscopy (TEM) on a JEOL JEM-2010 transmission electron microscope operating at an accelerating voltage of 200 kV. Crystal structure of the sample was characterized by X-ray diffraction (XRD) using a Cu sealed tube (λ = 1.54178 Å) at 40 kV and 40 mA. UV-vis spectra were performed on UV-3600 UV-vis-NIR spectrophotometer (Shimadzu Co., Kyoto, Japan). Surface area analysis of the sample (3 mg) was obtained with the outgassing for 6 h at 150 ^{o}C on the Micromeritics ASAP2020 at 77 K. Thermogravimetric analyses (TGA) were performed on a STA 449 C (Netzsch, Germany). The infrared spectra were carried out using a Vector 22 Fourier transform infrared spectrometer (Bruker Optics, Germany). Dynamic light scattering were obtained from Dynamic Light Scattering BI-200SM (Brookhaven, USA). X-ray photoelectron spectroscopy (XPS) was carried out using an ESCALAB 250 spectrometer (Thermo-VG Scientific Co., U.S.A.) with ultra-high vacuum generators. Electrochemical experiments, including cyclic voltammetry (CV) and linear sweep voltammetry (LSV), were performed on CHI 660D electrochemical workstation (Shanghai CH Instruments, China) with the three-electrode setup consisting of a glassy carbon working electrode (GCE), a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode.

Synthesis of UiO-66-NH_2 and Pt@UiO-66-NH_2. The UiO-66-NH_2 was synthesized according to the procedure reported by Lillerud group with minor modifications.[S1] In a typical synthesis, 40 mg of $ZrCl_4$, and 31 mg of NH_2-H_2BDC were dissolved in 10 mL of DMF in a 20 mL Teflon liner at room temperature, and then 28 μL of water was added and placed in oven at 120 °C for 24 h. After cooling to room temperature, the solid MOFs were washed with fresh DMF.

Different from the conventional two-step method to synthesize NP@MOF composites by adding the pre-synthesis sized NPs into the MOF precursors, we have here employed one-step method to synthesize Pt@UiO-66-NH_2 by mixing both the Pt and MOF precursors. Briefly, 40 mg of $ZrCl_4$, 31 mg of NH_2-H_2BDC and 0.2 g PVP were dissolved in 10 mL DMF-ethanol (v/v = 4:1) mixed solution in a 20 mL Teflon liner. After dissolved thoroughly, 14 μL of 0.25 g mL^{-1} H_2PtCl_6 solution was

added into Teflon liner, and then allowed to react at 120 °C for 24 h without stirring. The product was collected by centrifugation and then washed with fresh DMF. Finally, MOF nanoparticles were suspended in water for characterization and functionalization with DNA.

Polyacrylamide hydrogel electrophoresis. The mixtures of 10 μL TS solution (10 mM), 10 μL dNTPs (10 mM each) and 10 μL cell extract were incubated at 37 ^{o}C for 90 min. The 10% native polyacrylamide gel electrophoresis (PAGE) was performed with 5×Tris-Borate-EDTA (TBE) buffer. The loading sample was prepared by mixing 7 μL of DNA sample and added 1.5 μL 6×loading buffer, 1.5 μL UltraPower dye, respectively, and then kept for 3 min so that the dye could integrate with DNA completely. The gel was injected in 1×TBE buffer and carried out at 90 V for 90 min, and then scanned with Molecular Imager Gel Doc XR (BIO-RAD, USA).

Bioconjugation of Pt@UiO-66-NH_2 with cDNA. Pt@UiO-66-NH_2-cDNA was prepared via the conjugation between -NH_2 groups of Pt@UiO-66-NH_2 and -COOH groups of cDNA. An aqueous solution of DNA (10 μM, 0.5 mL) was added to 500 μL of 400 mM EDC and 100 mM NHS in ultrapure water and reacted for 20 min at room temperature. Then, 200 μL Pt@UiO-66-NH_2 aqueous solution (1 mg mL^{-1}) was injected into the above aqueous solution. The mixture reacted for 4 h at room temperature. After washing three times at 10000 rpm for 20 min, the unbound DNA and excessive EDC and NHS were removed. The resulting Pt@UiO-66-NH_2-DNA conjugate was resuspended in 0.1 M PBS (pH 7.4) and stored at 4 °C for characterization and analysis.

Cell culture and Telomerase extraction. HeLa cell (Human cervix adenocarcinoma), HaCaT (immortalized human epidermal cells), HEpG2 (human liver cancer cells) and U87 (human glioma cell line) were cultured using Dulbecco's minimal essential (DMEM) medium supplemented with 10% fetal bovine serum (FBS), MCF-7 (human breast cancer cells) and CEM (acute lymphocytic leukemia cell) were grown in Roswell Park Memorial Institute (RPMI 1640) medium 1640. All the cells were maintained at 37 ^{o}C in a humidified atmosphere (95% air and 5% CO_2) and were kept in logarithmic growth.

The telomerase extracts were prepared in the exponential phase of growth. Cells were counted using a Beckman cell counter, about 10^6 cells were transferred into an 1.5 mL EP tube and washed twice with ice-cold PBS (0.1 M, pH 7.4) by centrifugation at 2000 rpm for 5 min at 4 ^{o}C. Then, the cells were resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercapto ethanol, 0.5% CHAPS, 10% glycerol), and kept for 30 min on ice. The lysate was centrifuged at 12,000 rpm for 20 min at 4 °C. The resulting extract was carefully transferred into a fresh tube without disturbing the pellet and frozen at −80 °C or used immediately. For the control experiment, the telomerase extract was heat-treated at 95 °C for 10 min prior to the detection.

Electrochemical detection of Telomerase. Prior to the experiment, the GCEs were prepared by polishing with 1.0 and 0.05 μm alumina oxide suspension followed by sonication in water on chamois leather, washed and dried at room temperature. Firstly, 1 mg CGO was dispersed in 1 mL of water by sonication for 60 min. Then, 5 μL of CGO suspension (1 mg mL^{-1}) was spread onto the GCE surface. After dried, 20 μL of 400 mM EDC and 100 mM NHS was cast onto the GCE surface for 30 min. Subsequently, different concentration of TS primer was added on the functionalized electrode for 4 h. Finally, the modified surface was washed with buffer solution (10 mM Tris-HCl pH 8.3), dried in nitrogen gas and stored in air prior to use.

For telomerase extension reaction, the telomerase primer modified GCE was incubated with 20 μL of telomerase reaction solution containing 10 μL of telomerase extracts or telomerase (20 IU L^{-1}) and 2 mM dNTPs in 1×TRAP buffer (20 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween 20, 1 mM EGTA, BSA 0.1 mg mL^{-1}), and incubated at 37 °C for 90 min to allow the extension reaction by telomerase. After that, the electrode was rinsed thoroughly with 20 mM Tris-HCl buffer (pH 8.3). Subsequently, 20 μL of Pt@UiO-66-NH_2-cDNA was placed onto the resulting electrode surface and incubated at 37 °C for 90 min, then washed by Tris-HCl buffer (20 mM, pH 8.3) for electrochemical measurements. The potential range was set from −0.4 V to 0.6 V and the scan rate is 50 mV s^{-1}.

Supplementary Figures

Size distribution of Pt@UiO-66-NH_2

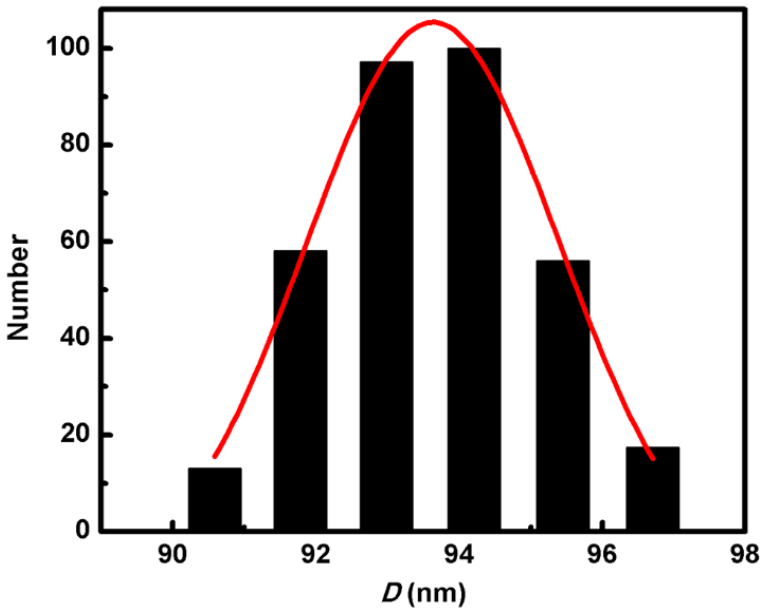

Fig. S1 Size distribution of Pt@UiO-66-NH_2 measured by DLS.

XPS characterization

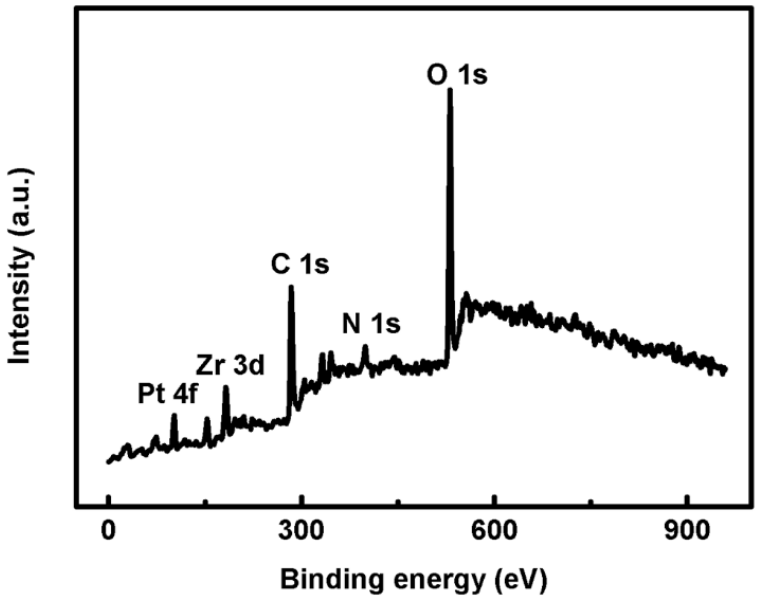

Fig. S2 XPS survey spectrum of Pt@UiO-66-NH_2.

TGA characterization

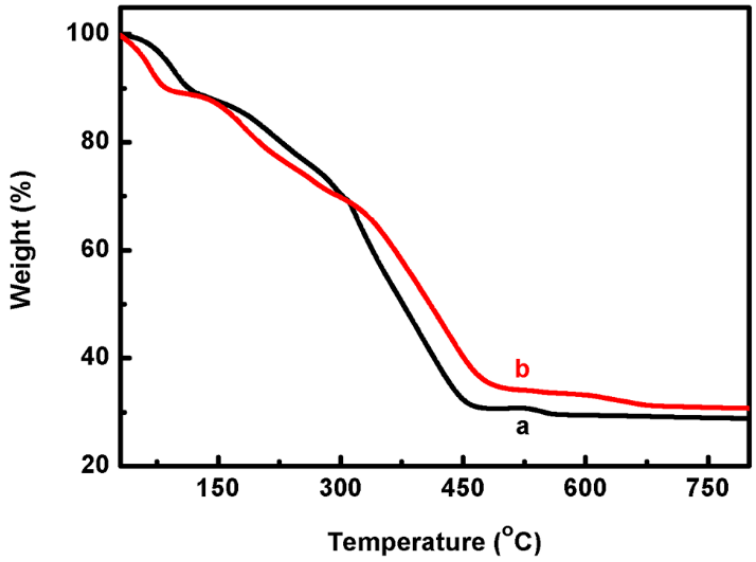

Fig. S3 TGA spectra of (a) UiO-66-NH_2 and (b) Pt@UiO-66-NH_2.

FT-IR characterization

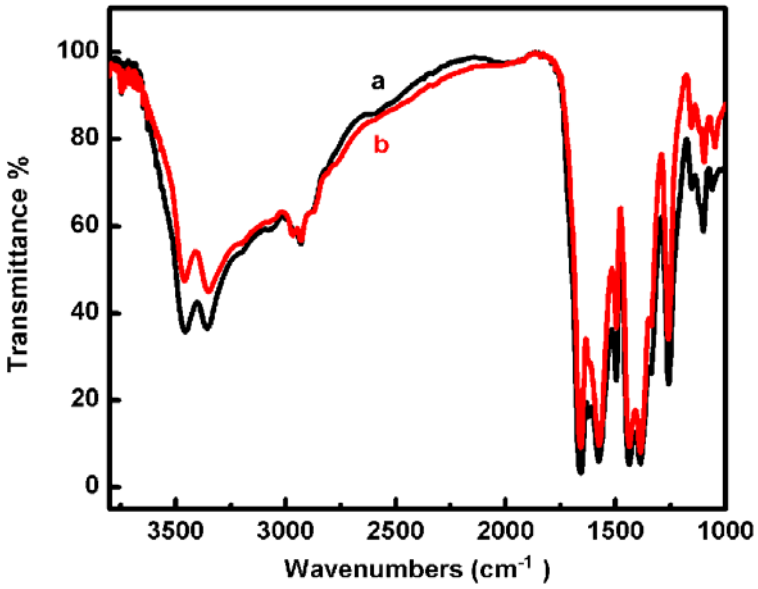

Fig. S4 FT-IR spectra for (a) UiO-66-NH_2 and (b) Pt@UiO-66-NH_2.

Polyacrylamide hydrogel electrophoresis

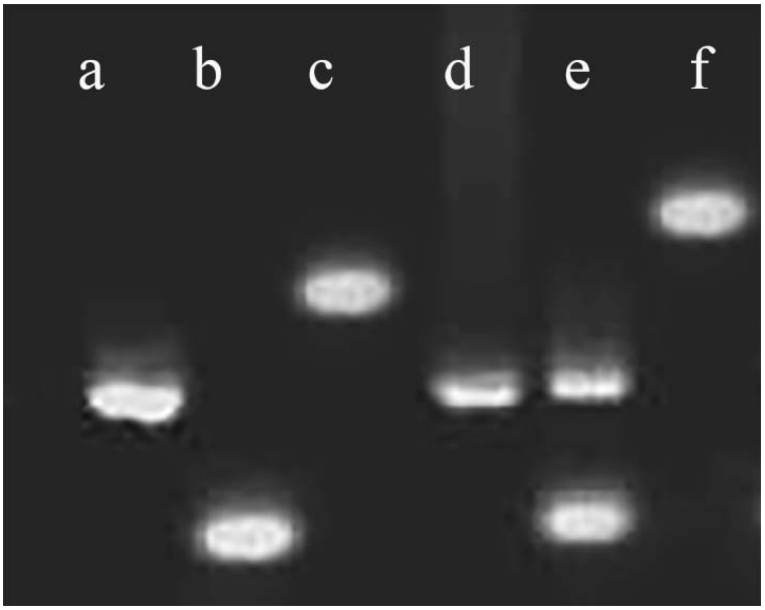

Fig. S5 PAGE analysis of 10 μM TS (lane a), cDNA (lane b), TS + cell extract (lane c), TS + the heated extract (lane d), TS+ cDNA (lane e), and TS + cell extract + cDNA (lane f) in the presence of dNTPs.

Optimization of detection condition

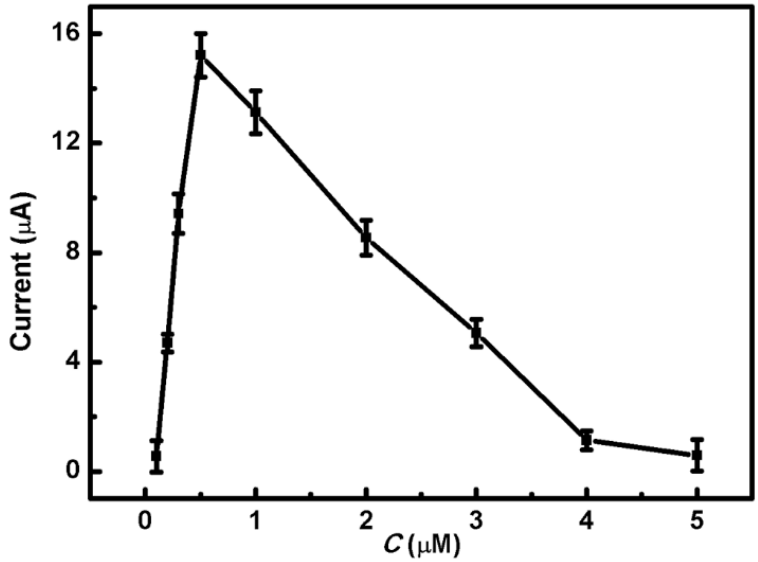

Fig. S6 Effect of the amount of TS primer on the current response to telomerase activity of 1 × 10^6 cell mL^{-1}.

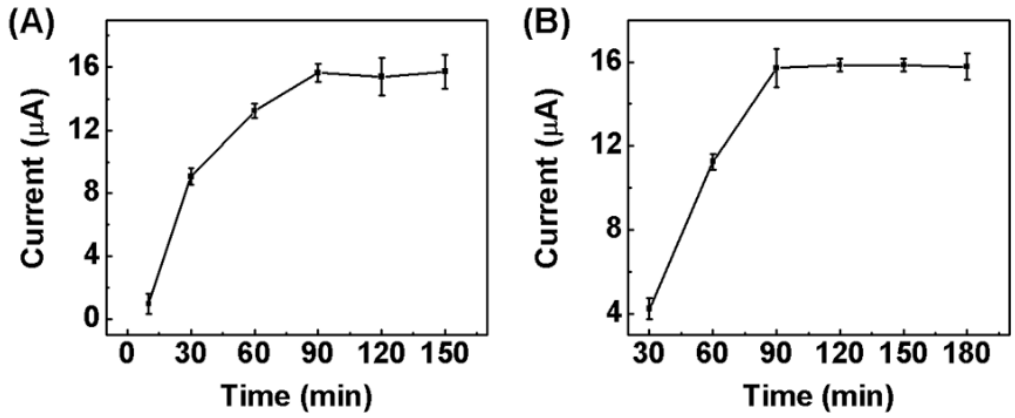

Fig. S7 Effects of (A) incubation time for telomerase extension reaction, and (B) the hybridization reaction time between cDNA and the extended TS on the current response of the biosensor to telomerase activity of 1×10^6 cell mL^{-1}.

Standard curve of enzymatic activity

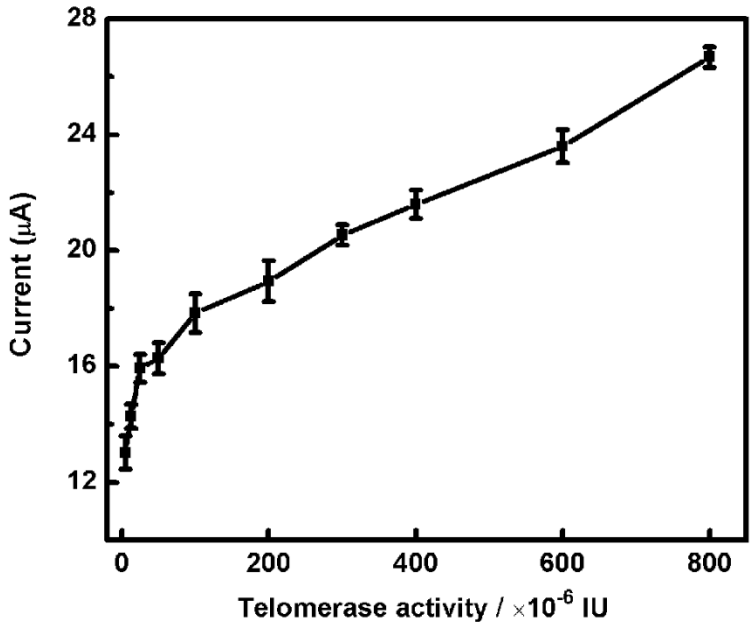

Fig. S8 Current responses of the designed biosensor to different concentration of standard Telomerase from 40 IU L^{-1} to 800 IU L^{-1}.

Telomerase activities in different cell lines

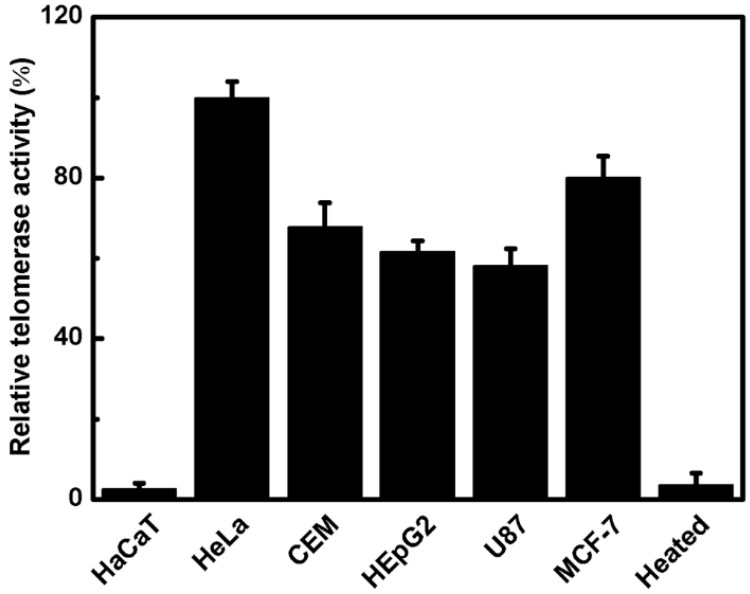

Fig. S9 Solid-state electrochemical assay to analyze telomerase activities in different cell lines. The telomerase activity of each cell line and negative heated control was normalized to HeLa cells. All the cell extracts were equivalent to 2000 cells.

Supporting Reference

[S1] J. H. Cavka, S. Jakobsen, U. Olsbye, N. Guillou, C. Lamberti, S. Bordiga and K. P. Lillerud, *J. Am. Chem. Soc.*, 2008, **130**, 13850–13851.