Supplementary Information

Trend of telomerase activity change during human iPSC self-renewal and differentiation revealed by a quartz crystal microbalance based assay

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Table S1. Methods for the detection of telomerase activity

Method	PCR-depended	Advantages (compared to Conventional TRAP)	Disadvantages (compared to Conventional TRAP)	Ref.
TRAP	+	/	/	1
TRAP-[^{32}P]	+	Avoid false negative results	Radiological hazard	2
F-TRAP	+	Nonisotopic	/	3
TRAP-SPA	+	PAGE-free, high-throughput, high signal/noise ratio	[^{3}H]-TTP and biotinylated primer required	4
TRAP/HPA	+	PAGE-free, Nonisotopic	AE-labeled probe required	5
TP-TRAP	+	High sensitive and linear result, PAGE-free	[^{3}H]-TTP and two reverse Primers required	6
TRAP-ELISA	+	High sensitive, PAGE-free and commercially available	expensive	7
TRAP with Amplifluor primers	+	PAGE-free, commercially available	expensive	8
RT-TRAP	+	Exact quantitation, real-time, PAGE-free	Real-time PCR instrument required, High-priced	9
In situ TRAP	+	Microscopic identification of individual cells expressing telomerase activity, PAGE-free	Fluorescein isothiocyanate -labeled telomerase primer required and low-throughput	10
TMA/HPA	-	Isothermic amplification, PAGE-free	AE-labeled probe required, false negative results	11
Optical/color detection	-	Macroscopic, fast	Low sensitive, modified primer/ AuNPs required	12,13
TRE	-	Real-time, label-free, information on reaction kinetics	BIACORE apparatus and biotinylated substrate primer required	14

Samples of undifferentiated hiPSCs, differentiated hiPSCs (for 1 day, 3 days and 5 days) and HeLa cells are measured with a standard TRAP assay for verification (Fig. S1). The result is consistent with figure 3 in the main text.

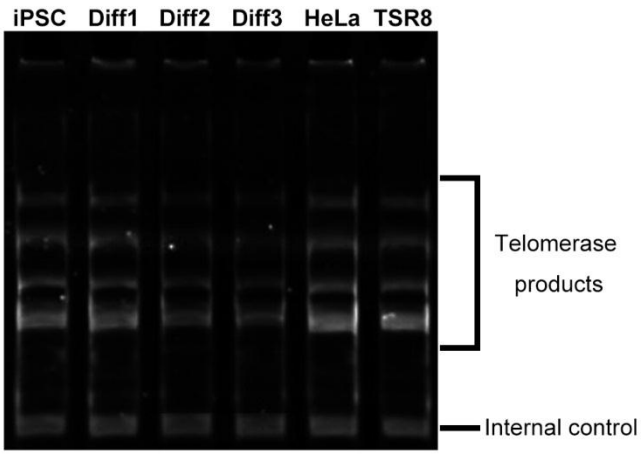

Figure S1. TRAP assay result (hiPSCs, differentiation of hiPSCs for 1 day, differentiation of hiPSCs for 3 days, differentiation of hiPSCs for 5 days, HeLa cells and positive control, from left to right) for the verification of QCM.

In order to ensure the reliability of TREAQ, we investigated the relationship between different cell lysate concentrations and Total Product Generated (TPG) according to the TRAP (Fig. S2).

(1). Measure the signal of the region of the gel lane corresponding to the TRAP product ladder bands from all samples including non-heat-treated (x) and heat-treated sample extracts (x_o), 1 × CHAPS Lysis Buffer only control (r_o), and TSR8 quantitation control (r).

(2). Measure the signal from the internal standard in non-heat-treated samples (c) and TSR8 quantitation control (c_R).

(3). Quantitate the amount of telomerase product using the following formula:

$$\mathrm{TPG} = \frac{(x - x_0)/c}{(r - r_0)/c_R} \times 100 \text{ (if 0.1 amole of TSR8 is used)}$$

Each unit of TPG corresponds to the number of TS primers extended with at least 4 telomeric repeats by telomerase in the extract in a 30 minute incubation at 30 ^{o}C

(one of the steps of PCR in TRAP).

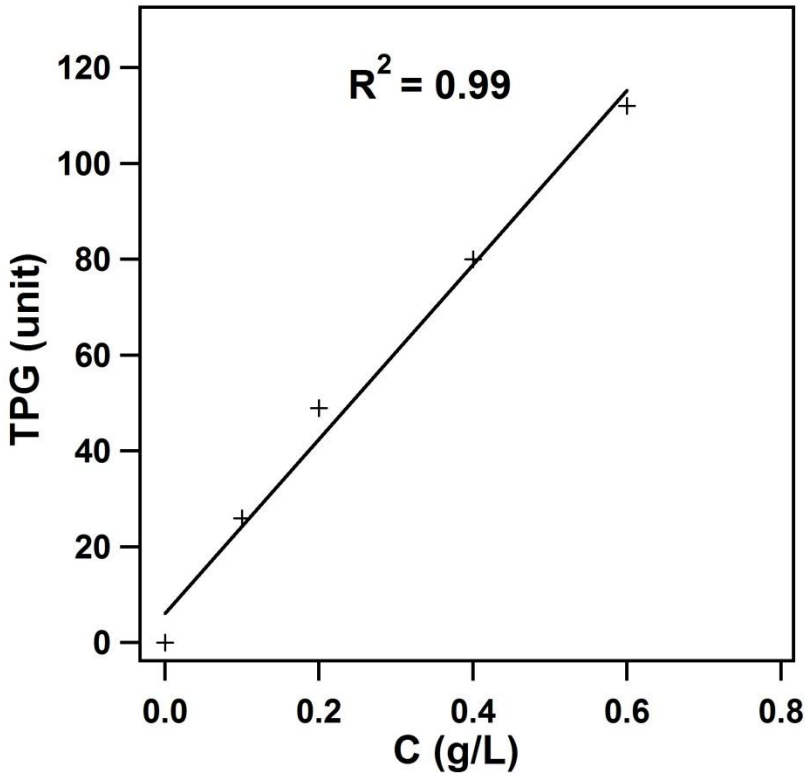

Figure S2. A linear relationship (y = 181.81x + 6.13) between TPG and the cell lysate concentration, which indicated the favorable specificity compared with traditional methods.

In order to prove the binding of telomerase, three experiments were conducted as the following table.

Table S2. Control experiments on gold chips

Primers	EG_3	Cell lysate + dNTP	F_T
-	-	+	150 Hz
+	-	+	105 Hz
-	+	+	30 Hz
+	+	+	78 Hz

For the experiment without primers and EG_3 on gold chips, the nonspecific

protein adsorption on whole area triggered 150 Hz frequency shift. But with primers on gold chips, only the remaining sites were available for the nonspecific protein, which triggered 105 Hz frequency shift. When only EG_3 exists, 30 Hz frequency shift was triggered as the whole chips were blocked by EG_3.

As two substrates (gold and polymer) could be applied to TREAQ, we used HeLa cells to choose a better substrate for TREAQ. Firstly, F_T for HeLa cells on gold surface was less than F_T for HeLa cells on polymer surface (ΔF_T = 27 Hz), which meant the frequency signal had been amplified on polymer surface compared with gold surface. We deduced that this amplification could be attributed to the better immobilization of DNA primers. Secondly, nonspecific protein adsorption, an important phenomenon at the interface of biosensor, could be avoided on polymer surface. In this case, leaving out the EG_3 blocking step could save time and also improved the immobilization of DNA primers. For the two reasons above, we supposed that TREAQ based on polymer-coated chips was superior to that on gold chips (Fig. S3).

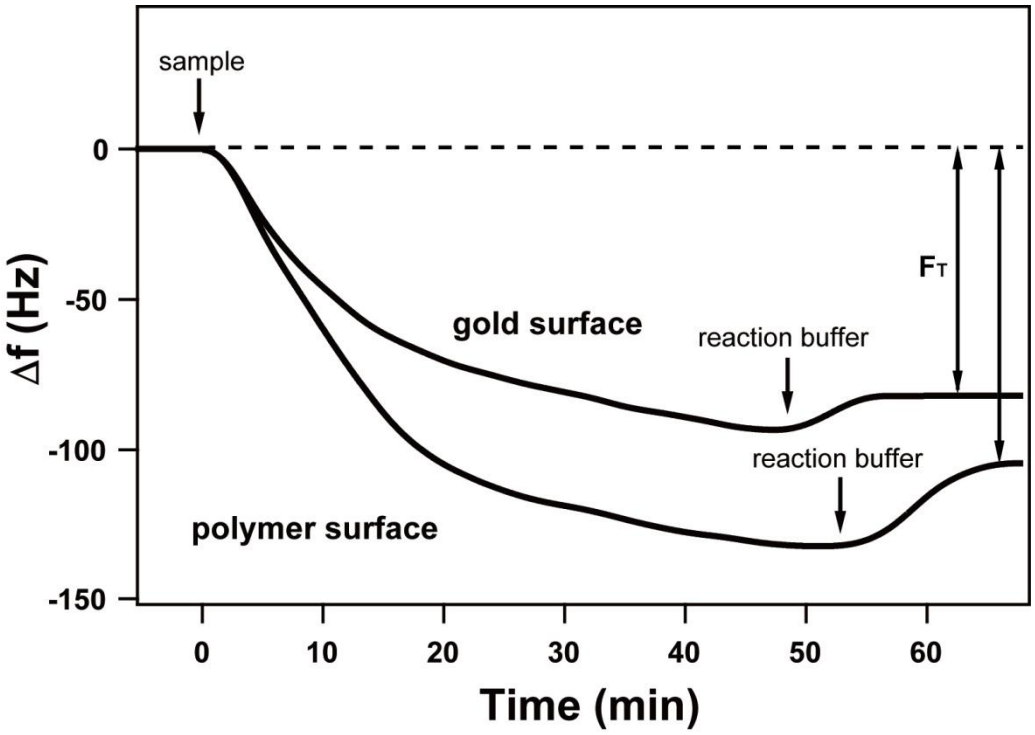

Figure S3. Real time QCM curves for HeLa cells on different substrates.

Detailed calculation process by "solidified liquid layer" (SLL) model.

1. The calculation of the film on the chip in the dry state.

According to Sauerbrey equation:

$$\Delta f = \frac{-2f_0^2 \Delta m}{A\sqrt{\mu_q \rho_q}} \qquad (1)$$

ρ_q -- density of quartz (ρ_q = 2.648 g/cm^3)

μ_q --Shear modulus of quartz for AT-cut crystal ($\mu_q = 2.947 \times 10^{11}$ g/cm×s^2)

f_0 -- Resonant frequency (Hz)

Δf -- Frequency change (Hz)

Δm -- Mass change (g)

A -- Piezoelectrically active crystal area (Area between electrodes, cm^2)

Equation (1) could be deduced next into equation (2)

$$\Delta m = C\frac{-\Delta f_n}{n} \qquad (2)$$

$f_n = nf_0$, n = 1, 3, 5, 7, 9, 11, 13;

Constant C is 17.7 ng cm^{-2} Hz^{-1} for an AT-cut, 5 MHz quartz.

The mass increase in the air is calculated by equation (2).

$$-\Delta f_n = An + Bn^2 \qquad (3)$$

Density ρ value in the air is 1.3, in the liquid is 1.1.

The thickness increase in the air is calculated by equation (3).

2. The calculation in the liqid by "solidified liquid layer" model.

$$-\Delta f_n = An + Bn^2 \qquad (4)$$

$$A = 2f_0^2 \rho_f T_f / Z_q \qquad (5)$$

$$B = -4\pi f_0^2 \rho_L T_L J_f / Z_q \qquad (6)$$

We plug in the experiment result Δf_3, Δf_5, Δf_7 into equation (4) and calculate the A value by fitting. The thickness of film in the liquid could be calculated by equation (5).

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