Supporting Material

In-vitro Hyperthermia Studied in a Continuous Manner Using Electric Impedance Sensing

Xinwu Xie\textsuperscript{1,3,4}, Ran Liu\textsuperscript{†}, Youchun Xu\textsuperscript{1,3}, Lei Wang\textsuperscript{3}, Ziyang Lan\textsuperscript{1}, Weixing Chen\textsuperscript{1}, Haoran Liu\textsuperscript{1}, Ying Lu\textsuperscript{1,3} and Jing Cheng\textsuperscript{*1,2,3,5}

\textsuperscript{1} Department of Biomedical Engineering, School of Medicine, Tsinghua University, Beijing 100084, China
\textsuperscript{2} Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China
\textsuperscript{3} National Engineering Research Center for Beijing Biochip Technology, 18 Life Science Parkway, Beijing 102206, China
\textsuperscript{4} Institute of Medical Equipment, Academy of Military Medical Science, Tianjin 300161, China
\textsuperscript{5} The State Key Laboratory of Biomembrane and Membrane Biotechnology, Tsinghua University, Beijing 100084, China

\textsuperscript{†} This author contributed equally to the first author.

\textsuperscript{*} Correspondence should be addressed to J.C.(jcheng@tsinghua.edu.cn).

Tel: (86)-10-62772239. Fax: (86)-10-80726898.
Section S1. Culture medium impedance variation test

As the temperature could influence the background impedance of the culture medium \( (Z_b) \), we elaborated an experiment to test the medium’s impedance variation under hyperthermia \( (Z_t) \). Each cavity on the device was filled with 600 \( \mu \)L culture medium (DMEM), and the medium was then heated by the isothermal platform while the control cavities were maintained under normal indoor temperature (~30 °C). The impedance of each well was recorded as the experimental cavities’ temperature increased (~30 °C) to 60 °C and then returned to ~30 °C. Two independent repeat tests were conducted. Impedance was being measured every 16 s, and the temperatures of the two cavities were being measured every second.

As shown in Figure S1, the impedance of the ECIS device with fresh culture medium (DMEM) decreased with increasing of temperature and rose again as the temperature decreased. The maximum variation \( (Z_t) \) was ~1-2 \( \Omega \) when temperature changed between 30-60 °C, comparable to the standard control group error. The differences of impedance \( (Z_t) \) were < 1 \( \Omega \) at 45 °C and 37 °C. Thus, the impedance variation of the culture medium in our experiments (37-45 °C) was moderate for cells adherent on the ECIS device. The maximal \( Z_t \) was much smaller than \( Z_0 \) and \( Z_{b0} \), and thus the \( Z_t \) could be neglected in \( Z_b \).
Figure S1. Culture medium impedance variations (two repeats compared with control group). The solid lines are the experimental groups’ impedance (Z1, and Z2), and the average impedance of the control cavities (Z3) with standard error bars (SD). The dashed lines are temperatures (T1, and T2) of the experimental groups, and their ordinate was on the right.

Section S2. Gene expression profiling experiment

The 35K human genomic oligo arrays (CapitalBio Corp, Beijing, China) were used for the gene expression profiling experiments. Total RNA was isolated by Trizol (Invitrogen), and reverse transcription was performed by M-MLV (Takara Chemicals, Shiga, Japan). Details of the microarray experiments were the same as previous publications1, 2. Raw data were normalized using the space- and intensity-dependent LOWESS program3. The dye-swap strategy was used for hybridization experiments of each hyperthermia and control sample at the time points of -1 h (before treatment start), 20 min after treatment started, 0 h (treatment end), 1, 8, 24 h (after treatment).
Figure S2 showed the fold change of common genes that differentially expressed in all time points. Their expression level increased at 20 min after hyperthermia started, and kept up-regulated until 24 h after thermal treatment. The expression level of 5S_rRNA gene reached maximum value at the end of hyperthermia, while 7SK, FOS genes reached their top value at 1 h after treatment. The gene expression of HSPA1B, HSPA1A, HSPA6, and DNAJB1 were similar to impedance of 43 °C hyperthermia. The gene expression results demonstrated that the cells stress response to heat began immediately after treatment started (at least 20 min after that).

Figure S2. The impedance curve of A549 cells treated by 43 °C and the gene expression changes during and after the treatment. (a) Impedance curve of 43 °C (Z43C) and control (Zctr); (b) 7 Genes’ expression change fold of 43 °C hyperthermia.
compared to control; (c) Scatter plot results of the gene expression profiling experiments, red (green) plots and numbers represent the up-regulated (down-regulated) genes and counts at each time point, respectively.

Section S3. Experimental set up for morphology observation

Figure S3. Experimental set up for morphology observation. The microscopy system can provide standard cell incubation conditions, and the ITO glass can provide heat for hyperthermia experiments. (1) Microscope, (2) PID controller, (3) thermocouple, (4) Petri-dish, (5) ITO glass.

Section S4. Movies of A549 cell morphological changes in 2 h

Movie S4: A549 cell morphological changes in 2 h when the temperature was 37 °C.

Movie S5: A549 cell morphological changes in 2 h during 41 °C hyperthermia for 30 min and 1 h after treatment.

Movie S6: A549 cell morphological changes in 2 h during 43 °C hyperthermia for 30
Movie S7: A549 cell morphological changes in 2 h during 45 °C hyperthermia for 30 min and 1 h after treatment.

Section S5. PI staining result of A549 cells after hyperthermia at 45 °C for 30 min

Figure S8. PI staining result of A549 cells after hyperthermia at 45 °C for 30 min. (a) Bright field image. (b) Florescence image (red represents dead cells stained with PI).

Section S6. Impedance profiling of HaCaT cells hyperthermia by the platform

The experimental method was the same to other cell lines. The impedance curves during and after hyperthermia were displayed in Figure S9.
Figure S9. Dynamic impedance curves (Zctr, Z41 °C, Z43 °C, and Z45 °C stand for the impedance of cells under 37 °C in the incubator or at 41, 43, and 45 °C hyperthermia for 30 min, respectively) of HaCaT cells during (a) and after hyperthermia (b).

References: