Real-Time Temperature Measurements of HMEC-1 Cells During Inflammation Production and Repair Detected by Wireless Thermometry

Can Li, Sen Yan, Weinan He, Sheng Yang, Jianfei Sun, and Ning Gu

Abstract*—*Objective: **Cell inflammation process is reflected through real-time *in situ* cellular temperature changes. *Methods:* A wireless thermometry system for *in situ* cellular temperature measurements was used in an incubator to detect the HMEC-1 cellular temperature under lipopolysaccharide inflammation production and norepinephrine for inflammation repair. Combining the changes in cell viability, inflammatory factor levels, and ATP content caused by different lipopolysaccharide or norepinephrine doses, an obvious inflammatory response and a repair effect were obtained. Temperature variations were correlated with ATP content. *Results:* An obvious inflammatory response with a lipopolysaccharide concentration of 0.1 mg/L and an optimal repair effect with 1 μM norepinephrine were obtained. The relationship between temperature changes and ATP content were quite different during the production of inflammation in HMEC-1 cells, having an approximately linear relationship, while under conditions of inflammation repair in HMEC-1 cells, there was an obvious nonlinear relationship. *Conclusion:* During cell damage, cell thermogenesis has a linear correlation with intracellular energy. While during cell repair, there is a gradual saturation relationship between the temperature (small range) and ATP, which may be because the thermogenesis capacity of the cell is enhanced compared to conditions during cell energy storage. Additionally, there is an optimal drug concentration for cell action during cell injury and cell repair, which is not dose-dependent. *Significance:* Whether in inflammation production or treatment, there is an optimal drug concentration. The relationship between cell thermogenesis and intracellular energy reserves is related to cell processes. Quick analysis of the energy changes in different physiological process can be realized.**

***Index Terms*—Real-time cell temperature, wireless thermometry, lipopolysaccharide, inflammatory cell, cell repair.**

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C. Li, S. Yan, W. He, and S. Yang are with the School of Biological Sciences & Medical Engineering and the State Key Laboratory of Bioelectronics and Jiangsu Key Laboratory of Biomaterials and Devices, Southeast University, and also with the Collaborative Innovation Center of Suzhou Nano Science and Technology.

J. Sun and N. Gu are with the School of Biological Sciences & Medical Engineering, Southeast University, Nanjing 210018, China (e-mail: sunzaghi@seu.edu.cn; guning@seu.edu.cn).

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I. Introduction

TEMPERATURE stability and energy balance are essential for various intracellular processes. Energy metabolism disturbances are involved in the entire course of sepsis and can significantly affect the disease progression and outcome [1]. The direct exposure of vascular endothelial cells to a large number of cytokines and inflammatory mediators, and their functional and structural changes are the key to the induction of multiple organ damage and dysfunction. These cells are mediators of the activation, damage and apoptosis of the pathophysiological changes of sepsis [2]. Recent studies have reported that maintaining hemodynamic stability by exogenous norepinephrine (NE) does not aggravate vascular endothelial cell injury in sepsis patients [3]. The sensitivity to norepinephrine of patients with septic shock is greater than that to adrenaline and dopamine in tissue perfusion pressure maintenance [4]. The cardiovascular system has less adverse reactions and norepinephrine can significantly improve the mortality rate.

It is well-known that lipopolysaccharides (LPS) of gram-negative bacteria [5] can activate mononuclear cells, macrophages, and endothelial cells to synthesize and release a variety of cytokines, leading to a systemic inflammatory response [6]. Energy metabolism disturbances can occur in response to inflammatory stimuli. The detailed energy metabolic features occurring at the cellular level in the cause and treatment of acute inflammation, which may be the cause of sepsis, remain unclear.

In the human body, there are a number of 'empty' biochemical mechanisms at the level of cellular metabolism that are devoted to transforming chemically bound energy to heat without work. These processes include the uncoupling of the respiration chain and increased Na^+/K^+ - ATPase activity as well as various substrate cycles [7], [8]. Thermogenesis (adaptive increases in heat production) can develop in response to low environmental temperature, alterations in the amount or composition of the diet, and pathogenic stimuli, such as infection, injury, and inflammation [9]. Virus-infected cells exhibit altered temperature compared to uninfected cells [10].

Our previous studies have developed a wireless thermometry system for in situ cell temperature measurements in incubator that can measure real-time temperature on the thousand-cell level using thermal resistors. The wireless thermometry consists of twelve thermal resistors for highly concentrated, small-volume cell suspensions seeded on a sensor surface to force the cells to grow only on the surface; a current direction-switching circuit to reduce ambient noise; a multichannel acquisition system for simultaneous multichannel data acquisition; and a transceiver and signal processing system for the transmission data out from the incubator [11]. The monitoring system is designed to be used in an incubator that ensures the proper growing temperature for cells. Cellular temperature variations could be monitored with an accuracy resolution below 0.01 °C. ATP hydrolysis is coupled with significant thermogenesis. Surplus energy during ATP production is released in the form of heat from mitochondria [12]. The changes in cellular temperature and ATP content in cells may help us understand the role of energy metabolism in the course of inflammation. Cytokines and other inflammatory factors released during sepsis or injury can alter thermogenesis [13]. Low-grade hypothalamic inflammation leads to defective thermogenesis, insulin resistance, and impaired insulin secretion [14]. Some of the cytokines recruited during metabolic inflammation (e.g., IL-1, IL-18 and IL-6), as well as COX-2, may promote thermogenesis and negative energy balance [15].

Therefore, we studied the cellular temperature changes in the production of an acute HMEC-1 cell inflammation induced by LPS at different concentrations and different infection temperatures. Furthermore, we investigated the repair effects of norepinephrine treatment with different doses in inflammation-stimulated HMEC-1 cells.

LPS of different concentrations was used to induce inflammation to screen the best drug dose to obtain the most serious degree of inflammation, and norepinephrine of different concentrations was used to treat the cellular inflammation. Cellular temperature, ATP levels, inflammatory factor levels and cell activity were recorded depending on dose and/or time series. Further correlations between temperature variations and ATP contents were evaluated as well. This is the first time to utilize wireless technology to record the temperature, was first used for detection of the generation and treatment of inflammation in an incubator.

II. Experimental Method

We tested the cellular temperature changes of HMEC-1 cells under different LPS doses, combining the evaluation of the inflammatory reaction of cells with testing of the inflammatory factor levels, cell activity, and ATP content, and a best LPS dose was obtained for optimal inflammation stimulus. Then, we repaired the HMEC-1 inflammatory cell response with norepinephrine treatment at different concentrations. We detected the real-time cellular temperature during the process, as well as inflammatory factor levels, cell activity, and ATP content in order to evaluate the repair effect. Fig. 1a shows the schematic diagram of the real-time temperature measurement.

A. Cell Culture

The microvascular endothelial cell line HMEC-1 (ATCC) from human skin was cultured as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum and 1% penicillin/streptomycin at 37 °C and 5% CO_2.

B. Treatment of HMEC-1 Cells

After incubation, the HMEC-1 cells were digested and seeded on a test thin-film thermal resistor surface (10000 cells for each testing sensor). We dripped 20 μl of cell medium on the sensor surface. Owing to the liquid surface tension, all cells were fell onto the sensor surface. After approximately 2 h, when the cells were attached to the sensor surface, a culture medium of suitable volume was added. As the sensors are not translucent, the cells could not be observed using a light microscope. Hence, after more than 12 h, the growth status of the cells was investigated by SEM. The cells were fixed by ethanol gradient dehydration and immobilization, and dried before observation.

1) LPS -induced cell injury: LPS was used to replicate sepsis induced vascular endothelial cell injury in vitro. The HMEC-1 cells were then treated with LPS at 0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L for 6 h [16], [17].
2) Norepinephrine for cell repair: LPS of 0.1 mg/L was used to induce HMEC-1 cell injury for 6 h, then, norepinephrine of different concentrations (0 μM, 0.01 μM, 1 μM, and 100 μM) were used to repair the inflammation in HMEC-1 cells [18], [19].

C. Cellular Temperature Measurement

The cell temperature was measured using the wireless thermometry system (Fig. 1a). The wireless thermometry system was composed of a six well plate integrated with 12 thin film platinum resistor sensors, a signal acquisition and transmission system (in the incubator), a signal receiving system and a PC for signal processing. As reported in our previous study [11], the resistance resolution of the circuit exhibits 20 mΩ, which corresponds to no more than 0.01 °C. The resistance deviations of each channel are corrected with software compensation. The linearity between the temperature and the resistance of the sensors lies above 0.999 in the applied temperature range (30 °C–42 °C).

After the cells were confluent on the sensor surface, the temperature experiments were carried out. Before the temperature measurement, the drug was preheated until it reached room temperature (25 °C). Then, the drug was added to the cell medium at the final required drug concentration when the cell reached a steady state (i.e., small temperature fluctuations). The temperatures of the cell suspension ($t_{control}$) and cell ($t_{cell\ with\ suspension}$) were tested simultaneously. The temperature changes of pure cells were calculated in accordance with $\Delta t = t_{cell\ with\ suspension} - t_{control}$.

The experiments were independently performed in triplicate.

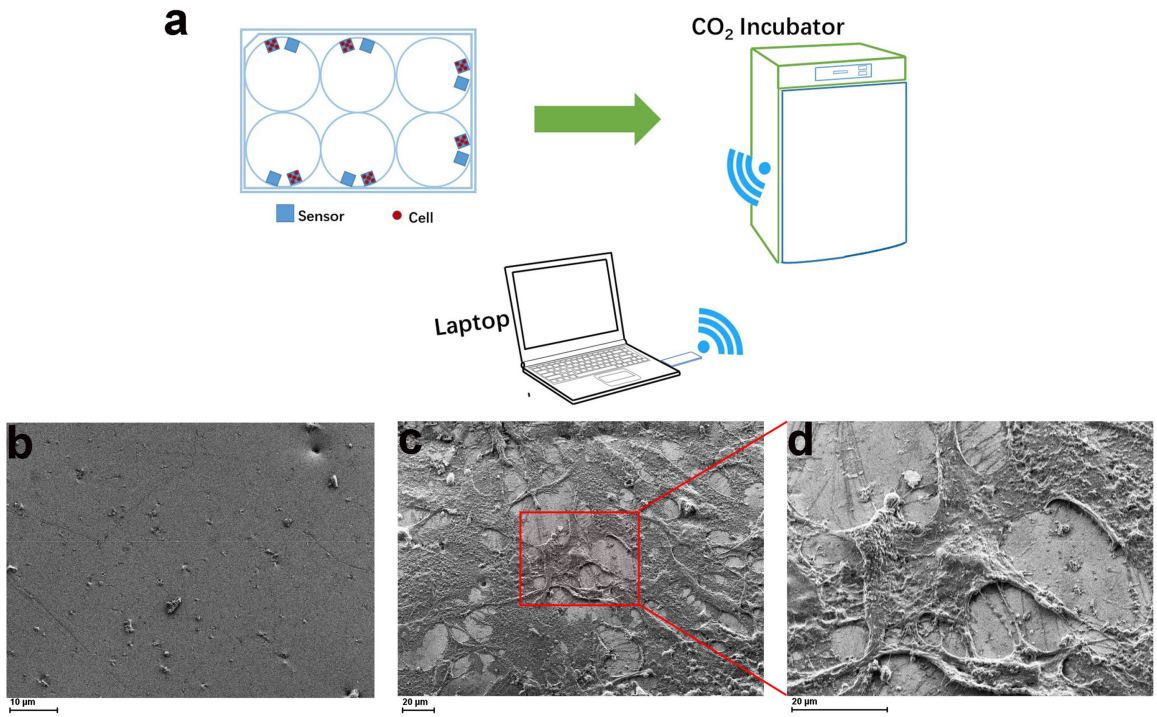

Fig. 1. Schematic diagram of the temperature measurement experiment and observation of the thermal resistors surface with and without HMEC-1 cells growing by SEM. (a) Schematic diagram of the temperature measurement experiment. (b) Thermal resistors surface without cells. (c) Thermal resistors surface with HMEC-1 cells growing. (d) Local amplification of (c).

D. Cellular Activity Measurement

The cell viability of HMEC-1 cells following treatment with LPS at different concentrations (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L) for 6 h and the cell viability following treatment with different concentrations of norepinephrine (0 μM, 0.01 μM, 1 μM, 100 μM) in the inflammatory HMEC-1 cells (induced by LPS concentration of 0.1 mg/L for 6 h) for 24 h were evaluated. The cells were seeded in a 96-well plate with 5000 cells per well. After 24 h, different concentrations of the drug were diluted into complete culture medium and were added to the well plate by replacement of culture medium. The cell viability was measured using the CCK-8 method (KeyGEN Biotech, KGA317). The experiments were independently performed in triplicate.

E. Inflammatory Factors Measurement

The HMEC-1 cells were seeded in a six well plate and treated with different concentrations of LPS (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L) for 6 h or with different concentrations of norepinephrine (0 μM, 0.01 μM, 1 μM, 100 μM) for 24 h after pretreatment with 0.1 mg/L LPS for 6 h. Then, cell culture supernatants were collected and the levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) were determined using the ELISA kits (KeyGEN Biotech, Human IL-6 ELISA Kit, Human IL-8 ELISA Kit and Human TNF-an ELISA Kit) according to the manufactures' instructions. The experiments were independently performed in triplicate.

F. Measurement of Cellular ATP Content

Intracellular ATP content in the HMEC-1 cells was measured using an ATP Bioluminescence Assay Kit (Beyotime Institute of Biotechnology) according to manufacturer's instructions and using the bioluminescence technique. In this study, the content of ATP of the HMEC-1 cells stimulated by different LPS concentrations (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L) compared to that of the inflammatory-primed HMEC-1 cells repaired by different norepinephrine concentrations (0 μM, 0.01 μM, 1 μM, 100 μM) were measured at four exact time points: 0, 5, 10, and 20 min after treatment according to the manufacture's instructions. The experiments were independently performed in triplicate.

G. Statistical

Statistical analysis was performed using ORINGIN Pro 9.0 (Oringinlab Corporation). Qualitative data were expressed as the mean and standard deviations (SDs). One-way ANOVA and student –Newman –Keuls tests were used for comparative statistical analysis. Two-tailed Student's t tests were used to determine p values. Statistical significance was defined as $p < 0.05$.

The fitting of temperature variations and ATP content of HMEC-1 cells under LPS stimulus compared to that of the norepinephrine/ LPS treated HMEC-1 cells was done. The fitting formula for LPS action on HMEC-1 cells during the temperature variations of 0–0.5 °C was $y = k * t + b$, where y refers to the ATP content, and t represents the temperature change of the cells. k and b are the constants in the formula. The

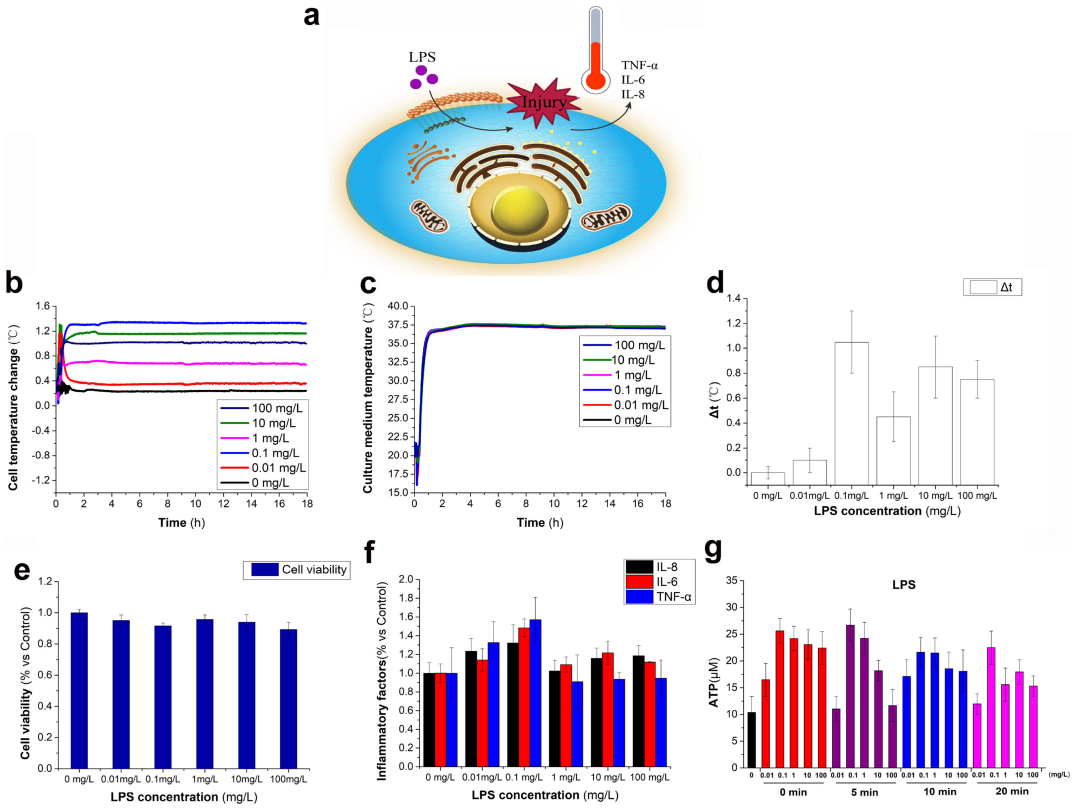

Fig. 2. Temperature measurements of HMEC-1 cells after treatment with different LPS concentrations and cell viability, secretion of inflammatory cytokines, and ATP content in different time points in HMEC-1 cells under different LPS concentration. (a) Sketch of cell injury caused by LPS. (b) Temperature changes of cell relative to medium temperature for each LPS concentration (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L). (c) Medium temperature for each drug concentration on cells. (d) Temperature rise compared to drugless condition; Δt is the temperature change of each drug concentration's action on the cells minus the temperature change of the drugless condition. (e) The cell viability induced by different LPS concentrations (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L) for 6 h. (f) HMEC-1 cells were stimulated with different concentrations of LPS (0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L) for 6 h, and the secretion of TNF-a, IL-1b, IL-6, and IL-8 was measured by ELISA. (g) Effects of LPS on the content of ATP in HMEC-1 cells. The 0 mg/L of LPS was used as control, which represents there was no drug was added. Results are given as means $\pm$SD.

fitting formula for norepinephrine repair of the inflammatory HMEC-1 cells during the temperature variations of 0–0.5 °C was $y = a - b * c^t$, where y refers to ATP content, and t represents the temperature changes of the cells. a, b and c are the constants in the formula.

III. Results and Discussions

A. SEM Observation of HMEC-1 Cells Growth on Thermal Resistors

Fig. 1b to Fig. 1c shows the growth of HMEC-1 cells on the thermal sensors, from which we can see that the sensor surface is clean when there are no cells on it. The cell growth on it made the surface appear rough. Additionally, the cell links could be seen clearly by SEM observation, which may mean that normal cell growth existed and intercellular links and communications were intact.

B. LPS Stimulus to Normal HMEC-1 Cells for Inflammation Production

Our study found that the cellular temperature was altered after LPS stimulation. The cellular inflammatory factors and cell activity reflected the inflammation level in cells, while ATP content had a correlation with temperature change. Energy metabolic disturbances occurred in response to the inflammatory stimuli, which subsequently altered the cellular temperature in vascular endothelial cells.

Evidence suggests that the stimulation of Toll-like receptor 4 (TLR4) by LPS induces the release of critical proinflammatory cytokines that are necessary to activate potent immune responses [20], [21]. Improper regulation of LPS/TLR4 signaling has the potential to induce massive inflammation and cause acute sepsis or chronic inflammatory disorders [21], [22]. These proof-of-concept cell analysis study results demonstrate that the HMEC-1 cellular temperature is increased but not in a dose-dependent manner after LPS treatments (Fig. 2). Fig. 2a exhibits the sketch of cell damage caused by LPS. Fig. 2b to 2d shows the temperature measurement results of normal HMEC-1 cells under stimulus of different concentrations of LPS. The 0.1 mg/L concentration of LPS insult led to an acute increase in cellular temperature (by more than 1.0 °C), which was maintained during the experimental period, while low or high concentrations of LPS caused a less dramatic increase in cellular temperature (by less than 1.0 °C), which was also maintained during the experimental period. Energy metabolism disturbances occurred in response to the inflammatory stimuli, which subsequently altered the cellular temperature in human microvascular endothelial cells.

The cell viability experiment results in Fig. 2e show that a LPS concentration of 0.1 mg/L in the HMEC-1 cells leads to an obvious decrease in cell viability. The levels of LPS induced inflammatory responses are shown in Fig. 2f which shows a LPS concentration of 0.1 mg/L led to the greatest increase in the secretion of TNF-α, IL-6, and IL-8. From Fig. 2g, we can see that the highest ATP level occurred at a LPS concentration of 0.1 mg/L.

The cell viability and the inflammatory factor levels had a corresponding relationship with the temperature change; that is, the LPS dose (0.1 mg/L) that caused the greatest temperature rise also led to the greatest elevation of inflammation levels (TNF-α, IL-6, IL-8) and showed the greatest decrease in cell activity assays.

From the results of above experiments, we determined that a LPS dose of 0.1 mg/L induced the greatest inflammatory response compared to other LPS concentrations in our experiment; therefore, we choose the LPS concentration of 0.1 mg/L to induce an inflammatory response in HMEC-1 cells.

C. Norepinephrine Repair for Inflammatory HMEC-1 Cells Caused by LPS

Next, we were ready to study inflammation repair. Studies have shown that dopamine and norepinephrine have a therapeutic effect on systemic and splanchnic oxygen utilization in hyperdynamic sepsis [23] and that norepinephrine may have a more favorable hemodynamic profile and improve splanchnic tissue oxygen utilization in sepsis. Therefore, we choose norepinephrine to use for inflammation rescue. After inducing an inflammatory response for 6 h using a LPS concentration of 0.1 mg/L, we used different norepinephrine concentrations for HMEC-1 cell inflammation repair.

Norepinephrine is a potent and commonly used α-adrenergic agent in cases of septic shock [24] and is also known as a hormone and the neurotransmitter released from the axon terminals of sympathetic postganglionic neurons in direct relation to its effector cells [25]. Our results demonstrated that the inflammatory HMEC-1 cellular temperature was not altered in a dose-dependent manner in the HMEC-1 cells after norepinephrine treatment (Fig. 3). Fig. 3a exhibits the sketch of the repair of norepinephrine to damaged cell caused by LPS. The temperature measurement results (Fig. 3b to 3d) show that the middle concentration (1 μM) of norepinephrine rescue led to an obvious increase in cellular temperature (by more than 0.4 °C), which stayed high during the experiment period. In contrast, low or high concentrations of norepinephrine caused less of an increase in cellular temperature (by less than 0.1 °C), which was also maintained throughout the experimental period.

The cell viability (Fig. 3e) and the inflammatory factor levels (Fig. 3f) had a corresponding relationship with the temperature change. Low and medium concentration of norepinephrine (0.01 μM, 1 μM) had repair effects on inflammatory HMEC-1 cells. Most notably, the norepinephrine dose (1 μM) that caused the greatest temperature rise also led to the most obvious decline in inflammation levels (TNF-α, IL-6, and IL-8) and enhanced the cell activity. From the inflammation levels and cell viability, the inflammatory reaction of the cells declined under norepinephrine treatment at a concentration of 1 μM, while high norepinephrine concentration had the opposite or no treatment effect.

The ATP levels at each time point (Fig. 3g) show that during the action of norepinephrine repair, the ATP level rose and had an upward trend during the experimental time. Among the different norepinephrine concentrations, the ATP levels of the inflammatory HMEC-1 cells were maintained at a high level under a norepinephrine concentration of 1 μM.

D. The Different Relationship of Temperature Changes and ATP Content in Different Time Points Under Cell Damage by LPS and Cell Repairmen by Norepinephrine

Fig. 4 shows the relationship between temperature change and ATP content in different time points under cell damage by LPS and cell repair by norepinephrine. From Fig. 4a to 4d, we can see that during the temperature change range of 0–0.5 °C, the cellular ATP content of different time points presented an obvious linear relationship with cellular temperature (the correlation coefficients are 0.98765, 0.99667, 0.94499, 0.9686) under cell stress by LPS. On the other hand, when the cells are repaired with norepinephrine, they have a nonlinear correlation (Fig. 4e to 4h, the correlation coefficients are 0.50151, 0.76996, 0.63939, 0.99958), which is quite different from the inflammatory situation.

The wireless thermometry system for in situ cell temperature measurements in an incubator used in our study has been proven to monitor cellular temperature in real time, with an accurate resolution of less than 0.01 °C. Combining the levels of inflammatory factors and cell viability results, we found that during inflammation, cells are releasing heat as viability is decreasing. IL-6 and TNF-α have been implicated as key mediators in the inflammation, morbidity, and mortality associated with sepsis. Lisa R. Leo found that IL-6 is a key mediator of fever and food intake, whereas TNF is responsible for the initial hypothermia and lethality of sepsis [26]. While in the process of cell repair, cells are also releasing heat as the viability is increasing.

Transient or permanent damage of cell membranes during trauma will lead to massive increases in extracellular ATP, and rapid formation of adenosine. There is also regulated release of ATP [27]. Here, the ATP level was highest under the most obvious inflammatory response caused by LPS. ATP levels were also high under the repair of norepinephrine (1 μM) in the inflammatory HMEC-1 cells, which corresponded to the temperature change. However, the tendency of ATP levels to vary along with time may be related to cell state. Cells can die either by energy-dependent apoptosis or by necrosis [28]. In the present study, in the process of cell damage, the temperature changes are different depend on the different injury degrees. While only a very significant repair process can cause significant temperature changes in the process of cell repair. With LPS -induced cell damage, there was a linear relationship between the temperature (in a small range) and ATP content, that is, cell thermogenesis had a linear correlation with intracellular energy, which may be because apoptosis is an active process requiring energy, whereas necrosis has been considered to be a passive process [29]. So through cellular temperature measurement, the cell death mode

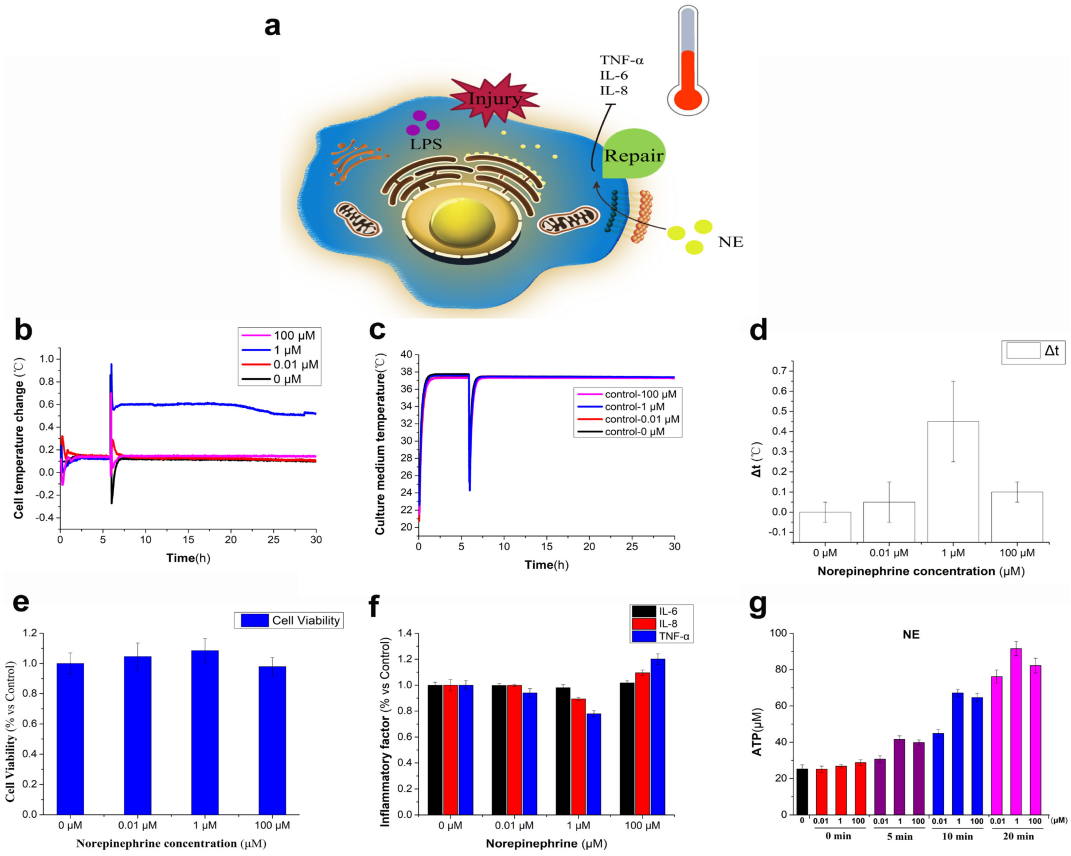

Fig. 3. Temperature measurements, cell viability, secretion of inflammatory cytokines, and ATP content of HMEC-1 cells after 24 h treatment with different concentrations of norepinephrine after receiving 0.1 mg/L LPS pretreatment for 6 h. (a) Sketch of repair to damaged cell caused by LPS. (b) Cell temperature changes of relative to medium temperature regarding each norepinephrine concentration (0 μM, 0.01 μM, 1 μM, and 100 μM). (c) Medium temperature for each drug concentration action on cells. (d) Temperature rise compared to the drugless condition; $\triangle$t is the temperature change of each drug concentration's action on the cells minus that of the drugless condition. (e) The 24 h cell viability of HMEC-1 cells following treatment by different norepinephrine concentrations (0 μM, 0.01 μM, 1 μM, and 100 μM) after a pretreatment of LPS at 0.1 mg/L for 6 h. (f) HMEC-1 cells were treated with different concentrations of norepinephrine (0 μM, 0.01 μM, 1 μM, and 100 μM) for 24 h, and secretion of TNF-a, IL-1b, IL-6, and IL-8 were measured by ELISA. (g) Effects of norepinephrine on the content of ATP in HMEC-1 cells after a pretreatment of LPS of 0.1 mg/L for 6 h. 0 μM of norepinephrine was used as control. Results are given as means $\pm$SD.

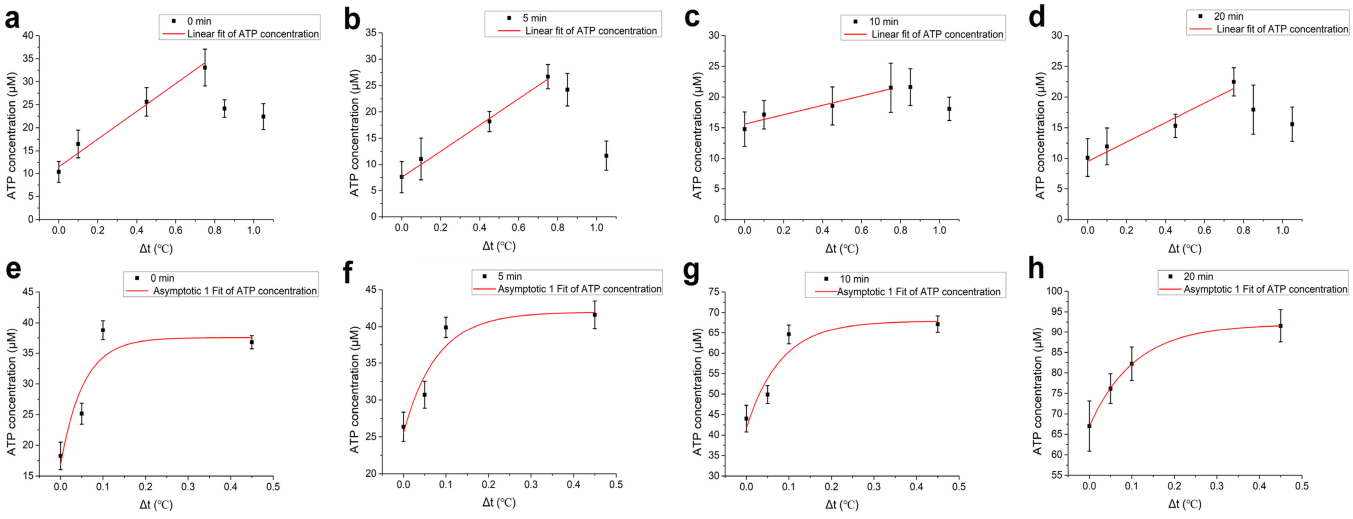

Fig. 4. The relationship between a small temperature change and ATP content of HMEC-1 cells at different time points under the stimulus of LPS or LPS and norepinephrine repair. (a), (b), (c), and (d) are the relationships between the temperature change and ATP content of 0 min, 5 min, 10 min, and 20 min under LPS stimulation to normal HMEC-1 cells. The red lines are linear fitting of the two parameters. (e), (f), (g), and (h) are the relationship between temperature change and ATP content of 0 min, 5 min, 10 min, and 20 min under norepinephrine repair in inflammatory HMEC-1 cells. The red lines are a non-linear fitting of the two parameters.

may be determined. During cell repair, there is a gradual saturation relationship between the temperature (small range) and ATP content, which may be because the cell thermogenesis capacity is enhanced compared to the cell energy storage. Cell repair is not like cell damage, which requires much energy to mediate apoptosis. Additionally, there is an optimal drug concentration for cell action during cell injury and cell repair, which is not dose-dependent during cell damage and repair. The cellular metabolic status conforms to the temperature variation, and they may jointly reflect the cellular energy change and the cell state.

IV. Conclusion

In conclusion, our results demonstrated that a LPS concentration of 0.1 mg/L, which induces a most obvious inflammatory response among experimental concentrations, increased the cellular temperature. The inflammatory reaction of HMEC-1 cells induced by a higher or lower LPS concentration in these experiments exhibited smaller temperature increases as well as a lesser inflammatory response as measured by inflammatory factor levels. There was a linear relationship between the temperature (in a small range) and ATP content during cell injury. For the repair of inflammatory HMEC-1 cells, the norepinephrine concentration of 1 μM exhibited the best repair effect in cell inflammation induced by a LPS concentration of 0.1 mg/L. The above process of norepinephrine repair of the inflammation-stimulated HMEC-1 cells was accompanied with an obvious cellular temperature rise, while higher or lower norepinephrine concentrations exhibited no temperature change. A repair effect of norepinephrine reflected that the inflammatory factor levels decreased and cell viability was evaluated compared with untreated conditions. There was a gradual saturation relationship between the temperature (small range) and ATP. The relationship between cellular temperature and cellular metabolism is an interesting topic that requires more intensive investigation. During cell injury, cell thermogenesis had a linear correlation with intracellular energy; however, cell repair may involve enhanced cell thermogenesis capacity compared to cell energy storage. Additionally, there was an optimal drug concentration for cell action during cell injury and cell repair, which was not dose-dependent. Detailed effects of the different cell stressors on cellular metabolic balancing could help us better understand the pathophysiological processes of many diseases apart from acute inflammations.

Acknowledgment

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