Final Draft
of the original manuscript:

Nie, Y.; Xu, X.; Wang, W.; Ma , N.; Lendlein, A.:
The effects of oscillatory temperature on HaCaT keratinocyte behaviors.
In: Clinical Hemorheology and Microcirculation. Vol. 76 (2020) 2, 317 - 327.
First published online by IOS Press: 07.09.2020

https://dx.doi.org/10.3233/CH-209208

The effects of oscillatory temperature on HaCaT keratinocyte behaviors

Yan Nie[1, 2], Xun Xu[1], Weiwei Wang[1], Nan Ma[1, 3, *], Andreas Lendlein[1, 2, 3, *]

[1]Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, 14513 Teltow, Germany

[2]Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany

[3]Institute of Chemistry and Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany

[*] To whom correspondence should be addressed:

Prof. Dr. Nan Ma, Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstr. 55, 14513 Teltow, Germany, email: nan.ma@hzg.de

Prof. Dr. Andreas Lendlein, Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstr. 55, 14513 Teltow, Germany, email: andreas.lendlein@hzg.de

Abstract

BACKGROUND: Keratinocytes are exposed to a thermal gradient throughout epidermal layers in human skin depending on environmental temperatures.

OBJECTIVE: Here, the effect of cyclic temperature changes (ΔT) on HaCaT cell behaviors was explored.

METHODS: HaCaT cells were cultured at constant temperature (37 °C or 25 °C) or under ΔT conditions. The morphology, mechanics, cell cycle progression, proliferation, and lipid synthesis of HaCaT cells were determined.

RESULTS: ΔT conditions led to the inhomogeneous arrangement of the cytoskeleton in HaCaT cells, which resulted in enlarged size, rounder shape, and increased stiffness. Accumulation in the G2/M phase in the cell cycle, a decreased proliferation rate, and a delayed lipogenesis were detected in HaCaT cells cultured under ΔT conditions.

CONCLUSIONS: ΔT conditions resulted in the re-arrangement of the cytoskeleton in HaCaT cells, which showed similarity to the temperature-induced disassemble and re-assemble of cytoskeletons in keratinocyte in vivo. The altered cytoskeleton arrangement resulted in the cell enlargement and stiffening, which reflected the changes in cellular functions. The application of oscillatory temperature in the in vitro culture of keratinocytes provides a way to gain more insights into the role of skin in response to environmental stimuli and maintaining its homeostasis in vivo.

Keywords: oscillatory temperature, HaCaT cells, mechanics, morphology, proliferation

Introduction

The skin barrier plays an important role in response to environmental stimuli and maintaining tissue homeostasis [1]. The barrier function of skin is affected by ambient temperatures. Decreased temperature would lead to a general decrease in skin barrier function and increased sensitivity to physical or chemical stress, which might be attributed to the alterations in keratinocytes [2]. This result suggested that keratinocytes not only form the physical barrier but also act as thermal receptors in skin [3]. Keratinocytes were capable of thermal transduction and are required for normal cold (15 ºC to 30 ºC) and heat (> 43 ºC) sensation [4,5]. The transient receptor potential (TRP) superfamily serves as thermoreceptors in keratinocytes [6,7]. TRPV1 and TRPV2 are heat transducers [8], whereas TRPM8 transduce cold-dependent signals [6]. For instance, TRPM8 could mediate the Ca^{2+} shuttling from the endoplasmic reticulum to mitochondria, resulting in alterations in mitochondrial ATP and superoxide production [6].

Temperature variations could lead to robust changes in keratinocyte physiology, which drives the rhythmic skin functions [9]. The rhythms could be modulated through controlling the period, amplitude, and phase of temperature oscillations [10,11]. On the molecular level, these changes are related to changes in the translation regulatory networks, proteome networks, and metabolic networks [9,10,12]. For instance, the keratin filaments could disassemble when exposure to elevated temperature (43 °C) within 15 minutes and resemble in 60 minutes

after temperature returned to 37 °C [13]. This result may explain how temperature affects the barrier function and integrity of skin, considering keratins are the major components for withstanding the external stimuli [14,15].

In static monolayer culture, keratinocytes are maintained generally under the constant culture conditions, including nutrients, oxygen level, temperature, and humidity [16]. However, the human skin temperature is within the range of 24 ºC to 33 ºC, when unprotected [7,17]. Here, a thermal chamber was employed to control the cyclic temperature from 37 °C to 25 °C (ΔT) to mimic the temperature devotions experienced by skin keratinocytes *in vivo*. Cell cycle progression was determined using flow cytometry. Cell mechanics and morphology including cell topography, area, and aspect ratio was measured using atomic force microscopy, and confocal laser scanning microscopy. Cell proliferation potential and lipogenesis were next evaluated. We hypothesize that the application of oscillatory temperature in the *in vitro* culture of keratinocyte leads to rapid changes in cell morphology. The changes in cell morphology are attributed to the re-arrangement of cytoskeleton, which induces alterations in cell mechanics. The altered cell mechanical properties may be related to the physiological responses in keratinocytes, such as cell cycle progression and metabolism. Taken together, the oscillatory temperature mimics the variation of temperature in nature, which is required for a better understanding of the cellular response to the environmental signals (**Figure 1**).

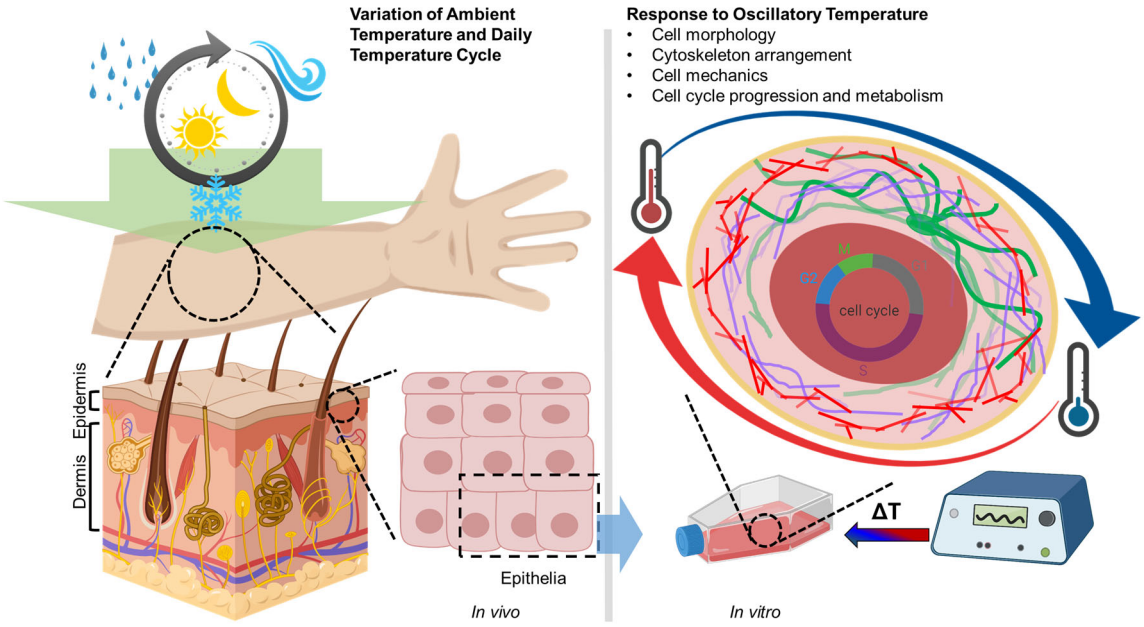

Figure 1. Schematic diagram depicting the responses of skin keratinocytes to the oscillatory temperature. Ambient temperature variations could induce changes in keratinocyte morphology and mechanics, and further resulted in a difference in keratinocyte behaviors, including changes in proliferation, differentiation, and metabolism.

Method

Cell culture

HaCaT cells were purchased from American Type Culture Collection (ATCC, USA). HaCaT were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Life Technologies, Germany) at 37 °C in a humidified atmosphere containing 95% (v/v) air and 5% (v/v) CO_2. Once reaching 80% confluence, cells were dissociated into single cells using 0.25% (w/v) Trypsin-EDTA (Gibco, Germany) and seeded onto TCP with desired density. Fresh culture medium was changed every other day. Cells were cultured

in a thermal chamber (Instec, USA) providing either periodic cooling (25 °C, 1 hour)/heating (37 °C, 1 hour) cycles (ΔT) or constant cold stress (25 °C). HaCaT cells cultured at 37 °C was used as control group.

Flow cytometry

For cell cycle analysis, HaCaT cells were seeded onto TCP at a density of 5.0 × $10^3/cm^2$ and cultured at 37 ºC, 25 ºC, or under ΔT conditions. After 2 days of culture, cells were harvested and fixed with cold 70 % v/v ethanol at 4 ºC overnight and then stained with FxCycle™ PI/RNase Staining Solution (Thermo Fisher Scientific, Germany) at room temperature for 30 minutes. Flow cytometer (MACSQuant®, Miltenyi Biotec, Germany) was applied to detect the stained cells, and the fractions of cells in different phases of the cell cycle were analyzed based on Dean-Jett-Fox model using FlowJo V10 (FlowJo LLC, USA).

Atomic force microscopy (AFM)

During the AFM experiments, HaCaT cells were maintained at the same temperature as in culture (37 °C or 25 °C), cells kept before under ΔT conditions were maintained at 25 °C. An AFM probe (qp-BioAC-10, NanoAndMore GmbH, Germany) was used to generate images of cells by QI™ mode (128 × 128 arrays of force-distance curves). Cell topography and mechanics were processed using JPKSPM Data Processing software (JPK, Germany). Hertz model was used to calculate Young's modulus from the force curve at each pixel.

Cytoskeleton staining

Microfilaments, microtubules, and intermediate filaments were stained to observe the changes in the arrangement of the cytoskeleton. Briefly, HaCaT cells were fixed with 4% (w/v) paraformaldehyde (PVA, Sigma Aldrich, Germany) for 10 minutes at room temperature and then permeabilized with 0.1% (v/v) Triton X-100 (Sigma Aldrich, Germany) for 10 minutes at room temperature. Pan Cytokeratin (ThermoFisher Scientific, Germany) was used to stain intermediate filaments at 4 °C overnight. Then secondary antibody staining was performed at room temperature for 1 hour using Goat anti-Mouse Alexa Fluor 633 (ThermoFisher Scientific, Germany). FITC-conjugated β-tubulin (Sigma Aldrich, Germany) and TRITC-Phalloidin (Sigma Aldrich, Germany) were used to stain microtubules and F-actin at room temperature for 1 hour. Then, nuclei were stained with 0.5 μg/mL 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, Germany). Cell morphology was determined using a confocal laser scanning microscope (LSM 780, Carl Zeiss, Germany). The cell spreading area and the aspect ratio of cells were analyzed using ImageJ software (National Institutes of Health, USA).

Proliferation assay

HaCaT cells were seeded at a density of $5.0 \times 10^3/cm^2$ on TCP and cultured at 37 ºC, 25 ºC, or under ΔT conditions for 7 days. The cell number was measured using the FluoReporter™ Blue Fluorometric dsDNA Quantitation Kit

(ThermoFisher Scientific, Germany). The standard curve that represents the relationship between the fluorescent intensity and a series of cells with known numbers was obtained according to the manufacturer's instruction. Samples were harvested at indicated time points and were frozen at -80 °C. The frozen samples were thawed and the fluorescent probes were added to the cell lysate. The fluorescence intensity was measured by a microplate reader (Infinite 200 PRO, Tecan Group Ltd., Switzerland). The cell number was calculated from the standard curve.

Lipid synthesis

After removing the culture medium, cells were fixed with 4% (w/v) PFA for 15 minutes and washed with PBS at room temperature. Cells were then stained using 0.2% (w/v) Oil red O (Sigma Aldrich, Germany) at room temperature for 30 minutes. To extract the Oil red O-stained lipid droplets, 100% (v/v) isopropanol was used. Elusion was performed for 10 minutes at 120 rpm on an orbital shaker. For each sample, 100 µL of the eluate was transferred to a 96-well plate and the absorbance at 490 nm was measured by a microplate reader (Infinite 200 PRO, Tecan Group Ltd., Switzerland).

Statistical analysis

Data were collected from at least three independent experiments and are expressed as the mean value ± standard deviation (SD). Statistical analysis was

performed using one-way or two-way ANOVA followed by Tukey's test in GraphPad Prism software (Version 8.0.1, USA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Cytoskeleton arrangement and morphological alteration

To visualize the temperature effects on the cytoskeleton arrangement, the major components of the mammalian cytoskeleton [18,19], filamentous actin (F-actin), intermediate filament (Pan Cytokeratin), and microtubule (β-tubulin), were detected using the confocal laser scanning microscopy. The culture temperature grossly affected the arrangement of the cytoskeleton (**Figure 2A**). Constantly exposed to low temperature led to cell shrinking, the distribution of all three cytoskeleton components was restricted around the nuclear regions. In the 37 ºC and the ΔT group, β-tubulin showed a fibrous pattern and distributed all over the cytoplasm, spreading from the perinuclear area towards the cell margin. In HaCaT cells cultured at 37 ºC, F-actin stress fibers were visible and aligned with the long axis of the cell. By contrast, in HaCaT cells cultured under ΔT conditions, F-actin fibres were disintegrated and present as small fibrils or amorphous aggregate structures throughout the cell. Less long fibrils were observed at the cell periphery in ΔT groups compared to those cultured at 37 ºC. In contrast to

microfilaments and microtubules, intermediate filaments are expressed in a tissue-specific manner [18]. Keratins are the intermediate filament proteins characteristic of keratinocytes. The staining of cytokeratin showed highly organized networks, located not only around the nuclear areas but also throughout the cytoplasm. In HaCaT cells cultured under ΔT conditions, more keratin bundles and knots tended to wrap around the cell nuclei to form a cage-like structure, as compared to those cultured at 37 ºC. This might be attributed to the function of keratins playing a central role in sustaining external stresses in epithelial cells [20].

To further evaluate the temperature effects on cell morphology, the contact areas of cells with the substrate were quantified. The area of substrate contact of HaCaT cells cultured under ΔT conditions was significantly larger than control cells that were cultured at 37 ºC, while of those that were cultured at 25 ºC were significantly smaller than control cells (**Figure 2B**). Another important character of cell shape is the cell aspect ratio that has a significant influence on cell behaviors. The cell aspect ratio, which is defined as the ratio of the length of the major axis to the length on the minor axis of a single cell, was used to quantify the cell elongation. HaCaT cells cultured at 37 ºC exhibited the highest aspect ratio, and those cultured at 25 ºC showed the lowest aspect ratio (**Figure 2C**). ΔT conditions caused the HaCaT cells to become larger in size, and rounder in shape, as compared to those cultured at 37 ºC.

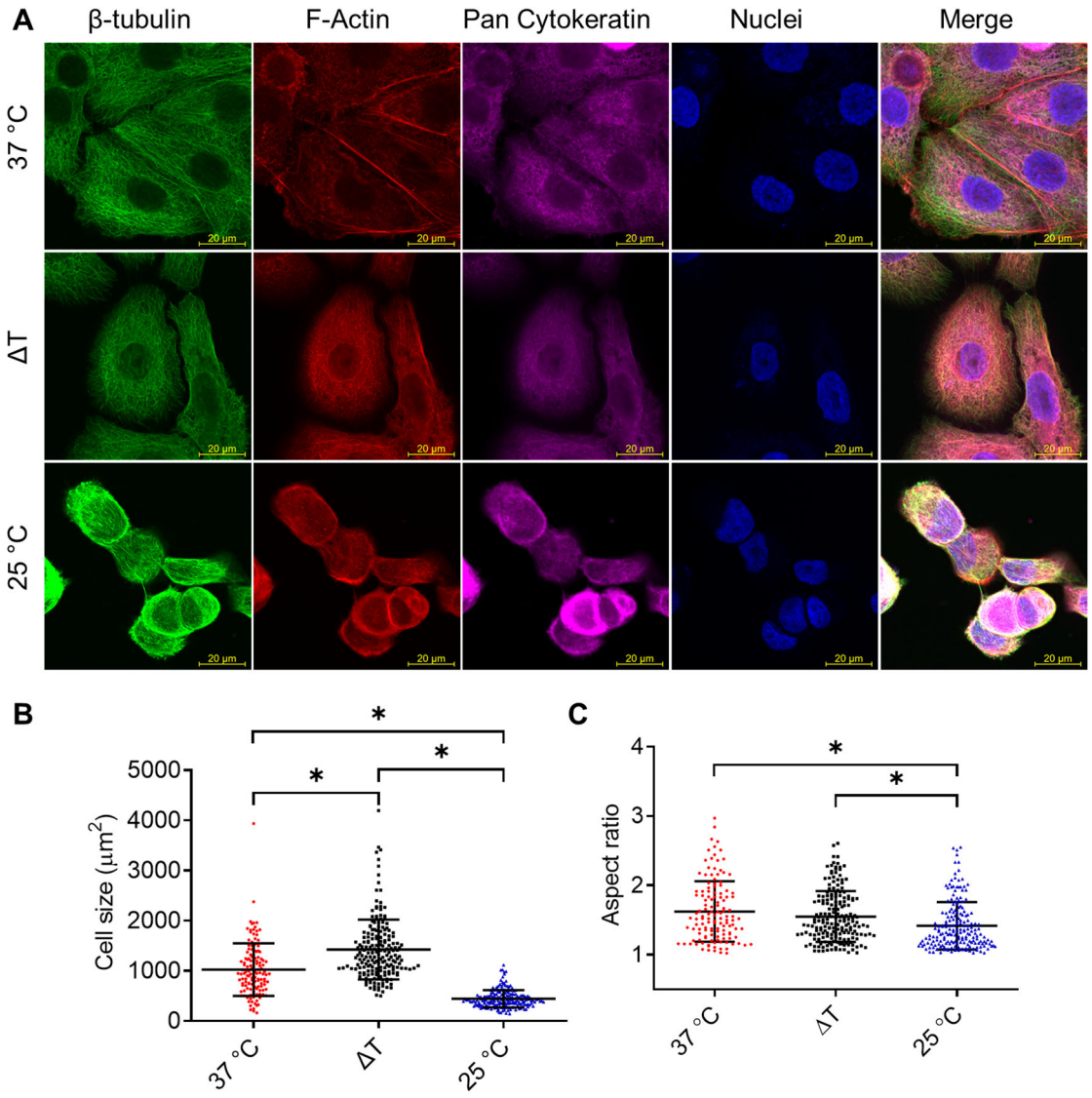

Figure 2. Arrangement of cytoskeleton in HaCaT cells. (**A**) Representative images of HaCaT cells cultured under different conditions. Three major components of the cytoskeleton, microtubule (β-tubulin, green), filamentous actin (F-actin, red), and intermediate filament (Cytokeratin, pink) were detected. Nuclei were counterstained with DAPI (blue), scale bar = 20 µm. The area of substratum contact (**B**) and the aspect ratio (**C**) of HaCaT cells was plotted (at 37 ºC, n=132; under ΔT conditions, n=189; at 25 ºC, n=195; one-way ANOVA followed by Tukey's test, * p<0.05).

Topography and mechanics

Temperature changes could not only result in the alteration of cell morphology but also cell mechanics. To directly analyze the temperature effects on HaCaT

cell elasticity, *in situ* imaging of single cells was performed with AFM. The AFM contact point images showed that the height in nuclei regions generally raised and the cell topography sloped away from the nucleus to the cell margin (**Figure 3**). A heightened edge at cell peripheries was observed in HaCaT cells cultured at 25 ºC, as compared to 37 ºC and ΔT groups. Cold exposure could lead to an increase in the height of nuclei, which might be the result of variation in the vertical nuclear-cytoskeletal arrangement [21]. Studies have demonstrated that nuclei are able to respond to external stimuli through adjusting their stiffness [22]. Young's modulus maps (**Figure 3**) showed the elasticity of cells was variable for the different points on the cell surface, as the cell membrane is highly heterogeneous [23]. At 37 ºC, HaCaT cells were more compliant. In response to low temperature, Yong's modulus of the HaCaT cell increased, which might be explained by alterations in the arrangement of cytoskeleton [24].

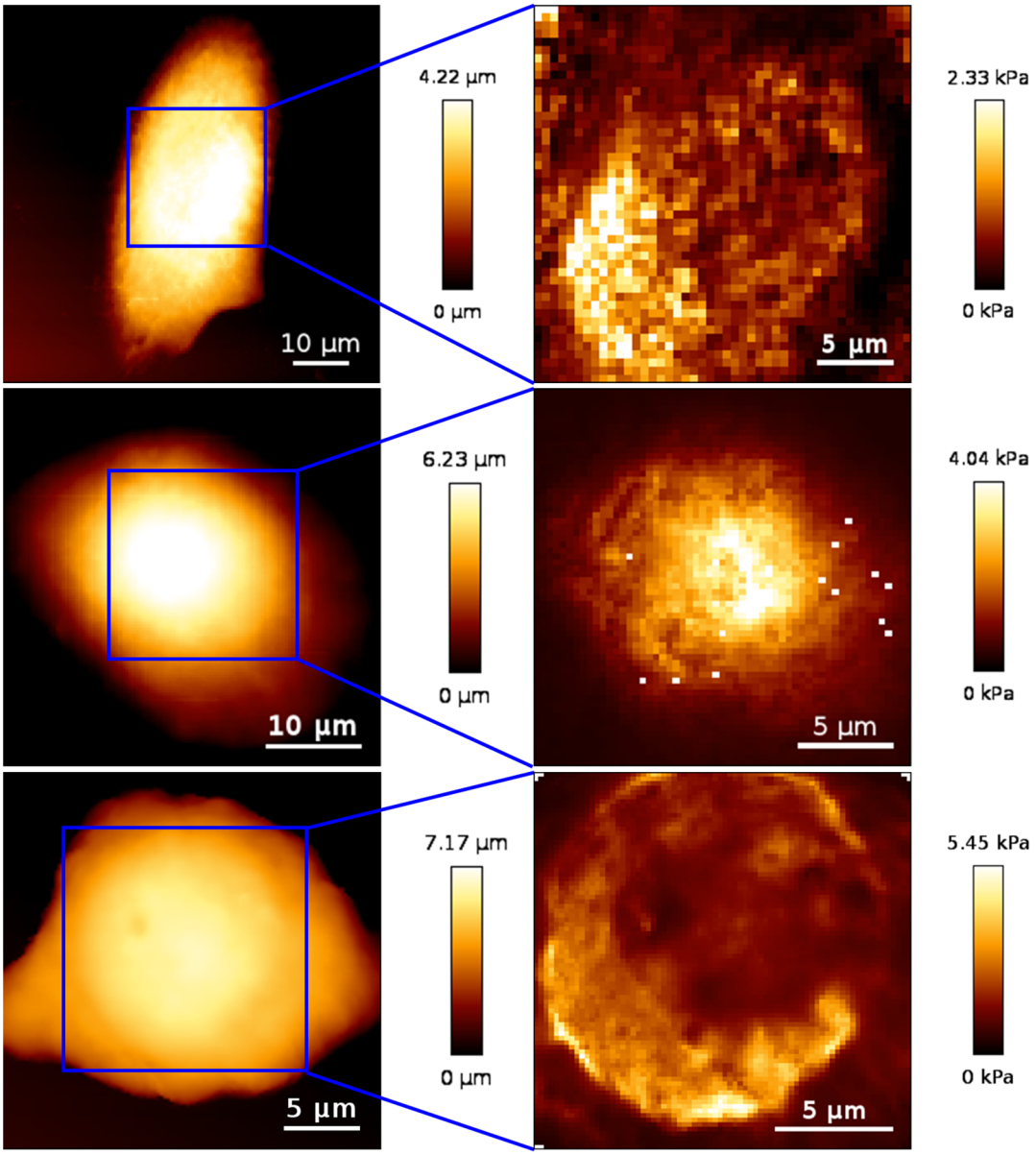

Figure 3. HaCaT cell topography and mechanics. Representative AFM QI image of a single HaCaT cell. For cells cultured at 37 ºC, the AFM measurement was conducted at 37 ºC; for cells cultured at 25 ºC or under ΔT conditions, AFM measurement was conducted at 25 ºC.

Cell cycle progression and metabolism

The temperature variations could not only induce changes in cytoskeletal organization and the mechanical properties of cells, but also lead to alteration in cellular functions [25,26]. The cell cycle distribution patterns of HaCaT cultured

under different conditions was shown in **Figure 4A**. Temperature decrease led to a decrease in the percentage of cells in S phase and an increase in G2/M phase. In HaCaT cells cultured at 25 °C, the number of cells in G0/G1 phase was higher than those cultured at 37 °C. While for those cells cultured under ΔT conditions, a decrease of cells in G0/G1 phase but an increase of cells in G2/M phase were detected as compared to those cultured under static conditions. Previous studies showed that progression through G1 and S was not blocked by "sub-optimal" (25 ˚C-33 ˚C) temperatures, but these cells failed to undergo the G2/M transition [27]. This was consisting with our result that cold exposure led to an accumulation of cells in G2/M (**Figure 4B**). More cells were accumulated in G2/M under ΔT conditions compared to those cultured at 25 °C constantly, suggesting this oscillatory temperature could influence more temperature sensitive processes that modulate the progression through G2/M phase.

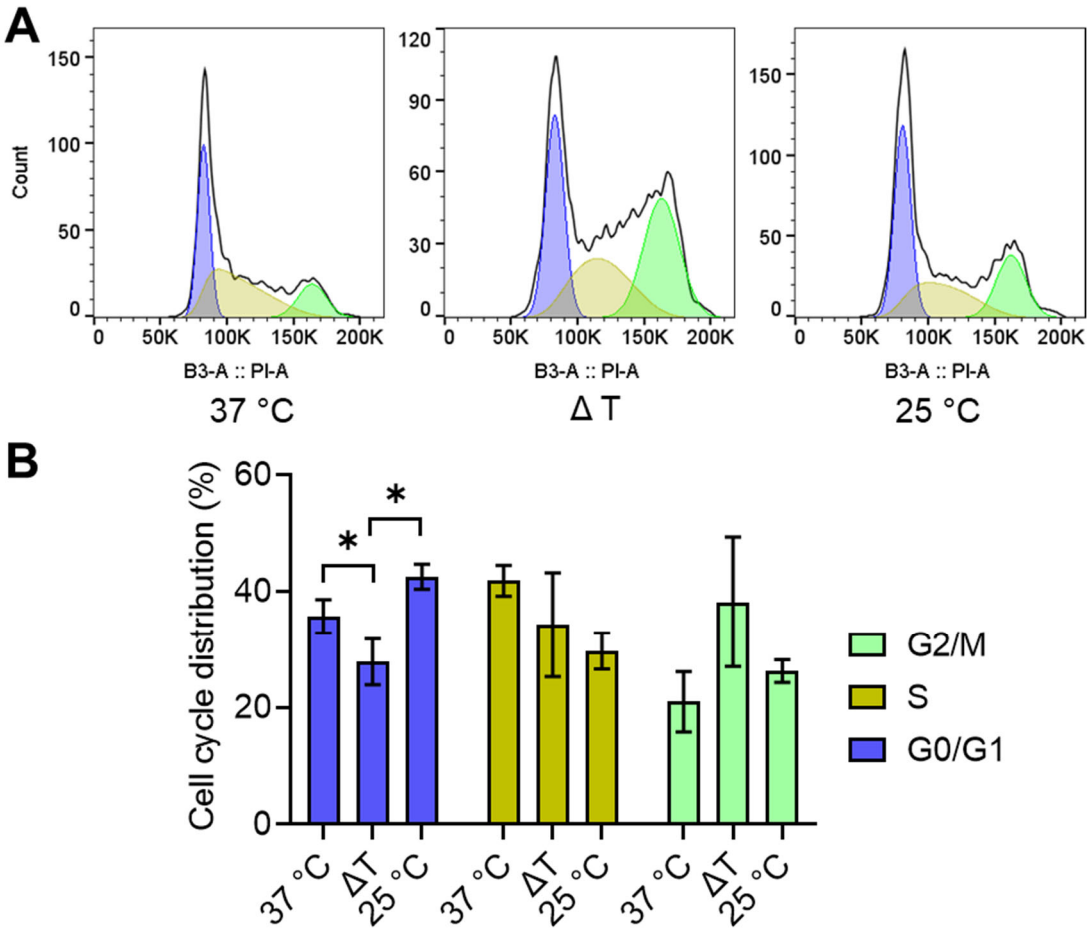

Figure 4. Cell cycle analysis of HaCaT cells cultured under different conditions. (**A**) Representative cell cycle distribution of HaCaT cells cultured at different conditions. The cells were analyzed using flow cytometry after 48 hours of cultivation. (**B**) Bar graphs showing the percentage of cells in the indicated phases of the cell cycle. (one-way ANOVA followed by Tukey's test, n=3, * p<0.05).

As shown in **Figure 5A** HaCaT cells cultured at 37 ºC rapidly proliferated from day 1 to day 4, but from day 4 to day 7, the proliferation almost ceased as the proliferative rate dropped tremendously. This might be a result of cell reaching confluence overtime. Although the proliferation was relatively slow in HaCaT cells cultured under ΔT conditions, the proliferation rate of those cells gradually increased with time. This might be the result of a cold-induced delay in entering

mitosis in cells and then the cells re-enter mitosis and complete division as temperature increased [27]. For those cultured at 25 ºC continuously could not proliferate, and cell death was observed after day 4. Keratinocytes are able to differentiate from the proliferative layer to the spinous and granular layers that are able to synthesize lipid, and end up with the non-living stratum corneum [28]. The synthesis of neutral lipids could be an indicator of the spontaneous differentiation of HaCaT cells *in vitro*. Lipid drops could be observed in HaCaT cells 7 days after cultured at 37 ºC, while a delayed formation of lipid drop was found in HaCaT cells cultured under ΔT conditions or at 25 ºC (**Figure 5B**). The rate of lipogenesis, especially of neutral lipids, was sensitive to the changes of culture temperature [29].

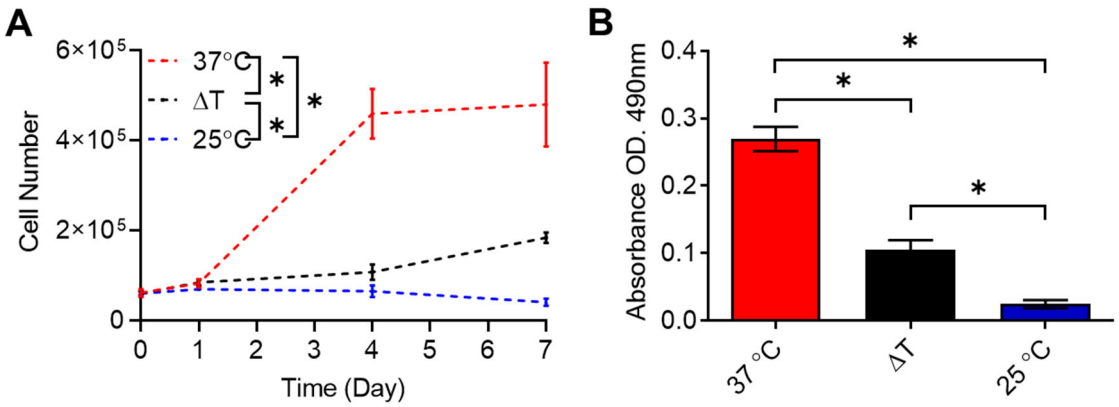

Figure 5. Temperature effect on HaCaT cell proliferation and lipid synthesis. (**A**) Proliferation potential of HaCaT cells (two-way ANOVA followed by Tukey's test, n=3, * p<0.05). (**B**) Synthesis of neutral lipids in HaCaT cells (one-way ANOVA followed by Tukey's test, n=5, * p<0.05).

In vivo studies showed that heat or cold treatment could compromise the skin barrier homeostasis [30,31]. The barrier function could be recovered after the

removal of thermal stimuli, which was related to the temperature induced Ca^{2+} influx in keratinocytes [30,31]. In addition , the physiological responses of skin to temperature variations are also related to the metabolic adjustment in keratinocytes [32]. In our study, HaCaT keratinocytes showed alterations in cell morphology and mechanics through cytoskeleton re-arrangement and preserved their proliferative capacity in response to the oscillatory temperature, suggesting the *in vitro* cultured keratinocytes are capable of adapting the thermal stimuli. Taken together, both the *in vivo* and the *in vitro* skin keratinocytes are able to precisely adapt to daily cyclic alterations in the ambient environment to maintain the skin homeostasis.

Conclusion

The present study explored the adaptation of *in vitro* cultured keratinocytes to the oscillatory temperature. The oscillatory temperature (from 37 ºC to 25 ºC) induced altered organization of cytoskeletons, which was related to the cold-induced cell enlargement and stiffening. The delayed progression through the G2/M phase in the cell cycle, prolonged HaCaT cell life-span, and hindered lipogenesis were partly attributed to the changes in cell mechanics. This might also be the result of temperature oscillation-induced change in transcriptional/translational regulation and metabolism, which need to be further studied in the future. Taken together, our findings showed the altered behaviors

of keratinocytes in response to the oscillatory temperature, which advanced our understanding of how the human skin senses and responds to environmental stimuli. We established a versatile cell culture system by controlling the environmental temperature and directing cell development. This method can be combined with various imaging technology simultaneously and enable long-term observation. Our biophysical means is simple, straightforward and cost-effective in achieving the effective cell phenotype transformation. Through precise control over the temperature, the dynamic in vitro cell culture systems provide us a unique platform to mimic in vivo microenvironments for developing in vivo-like skin equivalents in the future.

Acknowledgment

This work was financially supported by the Helmholtz Association of German Research Centers through program-oriented funding and through Helmholtz Cross Program Initiative "Technology and Medicine Adaptive Systems, and by the Federal Ministry of Education and Research, Germany through the Program Health Research (grant no. 13GW0098, as well as project number 0315696A "Poly4BioBB"). Yan Nie is grateful for the support from the China Scholarship Council (CSC, No. 201706205049).

References

[1] Niehues H, Bouwstra JA, El Ghalbzouri A, Brandner JM, Zeeuwen PL and van den Bogaard EH. 3D skin models for 3R research: The potential of 3D reconstructed skin models to study skin barrier function. Exp. Dermatol. 2018;27(5):501-511.

[2] Engebretsen KA, Johansen JD, Kezic S, Linneberg A and Thyssen JP. The effect of environmental humidity and temperature on skin barrier function and dermatitis. J. Eur. Acad. Dermatol. Venereol. 2016;30(2):223-249.

[3] Kida N, Sokabe T, Kashio M, Haruna K, Mizuno Y, Suga Y, Nishikawa K, Kanamaru A, Hongo M, Oba A and Tominaga M. Importance of transient receptor potential vanilloid 4 (TRPV4) in epidermal barrier function in human skin keratinocytes. Pflugers Arch - Eur J Physiol. 2012;463(5):715-725.

[4] Liu X, Wang H, Jiang Y, Zheng Q, Petrus M, Zhang M, Zheng S, Schmedt C, Dong X and Xiao B. STIM1 thermosensitivity defines the optimal preference temperature for warm sensation in mice. Cell Res. 2019;29(2):95-109.

[5] Sadler KE, Moehring F and Stucky CL. Keratinocytes are required for normal cold and heat sensation. bioRxiv. 2020.

[6] Bidaux G, Borowiec A-S, Prevarskaya N and Gordienko D. Fine-tuning of eTRPM8 expression and activity conditions keratinocyte fate. Channels. 2016;10(4):320-331.

[7] Bidaux G, Borowiec A-S, Gordienko D, Beck B, Shapovalov GG, Lemonnier L, Flourakis M, Vandenberghe M, Slomianny C, Dewailly E, Delcourt P, Desruelles E, Ritaine A, Polakowska R, Lesage J, Chami M, Skryma R and Prevarskaya N. Epidermal TRPM8 channel isoform controls the balance between keratinocyte proliferation and differentiation in a cold-dependent manner. Proc Natl Acad Sci U S A. 2015;112(26):E3345-E3354.

[8] Talagas M, Lebonvallet N, Berthod F and Misery L. Lifting the veil on the keratinocyte contribution to cutaneous nociception. Protein Cell. 2020;11(4):1-12.

[9] Spörl F, Schellenberg K, Blatt T, Wenck H, Wittern K-P, Schrader A and Kramer A. A Circadian Clock in HaCaT Keratinocytes. J. Invest. Dermatol. 2011;131(2):338-348.

[10] Saini C, Morf J, Stratmann M, Gos P and Schibler U. Simulated body temperature rhythms reveal the phase-shifting behavior and plasticity of mammalian circadian oscillators. Genes Dev. 2012;26(6):567-580.

[11] Sandu C, Dumas M, Malan A, Sambakhe D, Marteau C, Nizard C, Schnebert S, Perrier E, Challet E, Pévet P and Felder-Schmittbuhl M-P. Human skin keratinocytes, melanocytes, and fibroblasts contain distinct circadian clock machineries. Cell. Mol. Life Sci. 2012;69(19):3329-3339.

[12] Abraham U, Schlichting JK, Kramer A and Herzel H. Quantitative analysis of circadian single cell oscillations in response to temperature. PLoS One. 2018;13(1):e0190004.

[13] Morley S, Dundas S, James J, Gupta T, Brown R, Sexton C, Navsaria H, Leigh I and Lane E. Temperature sensitivity of the keratin cytoskeleton and delayed spreading of keratinocyte lines derived from EBS patients. J. Cell Sci. 1995;108(11):3463-3471.

[14] Ramms L, Fabris G, Windoffer R, Schwarz N, Springer R, Zhou C, Lazar J, Stiefel S, Hersch N, Schnakenberg U, Magin TM, Leube RE, Merkel R and Hoffmann B. Keratins as the main component for the mechanical integrity of keratinocytes. Proc. Natl. Acad. Sci. U. S. A. 2013;110(46):18513.

[15] Biggs LC, Kim CS, Miroshnikova YA and Wickström SA. Mechanical forces in the skin: roles in tissue architecture, stability, and function. J. Invest. Dermatol. 2020;140(2):284-290.

[16] Wong T-Y, Chang S-N, Jhong R-C, Tseng C-J, Sun G-C and Cheng P-W. Closer to Nature Through Dynamic Culture Systems. Cells. 2019;8(9):942.

[17] Smith ADH, Crabtree DR, Bilzon JLJ and Walsh NP. The validity of wireless iButtons® and thermistors for human skin temperature measurement. Physiol. Meas. 2009;31(1):95-114.

[18] Omary MB, Ku N-O, Tao G-Z, Toivola DM and Liao J. 'Heads and tails' of intermediate filament phosphorylation: multiple sites and functional insights. Trends Biochem. Sci. 2006;31(7):383-394.

[19] Schiffhauer ES, Luo T, Mohan K, Srivastava V, Qian X, Griffis ER, Iglesias PA and Robinson DN. Mechanoaccumulative elements of the mammalian actin cytoskeleton. Curr. Biol. 2016;26(11):1473-1479.

[20] Salas PJ, Forteza R and Mashukova A. Multiple roles for keratin intermediate filaments in the regulation of epithelial barrier function and apico-basal polarity. Tissue Barriers. 2016;4(3):e1178368.

[21] Neelam S, Hayes PR, Zhang Q, Dickinson RB and Lele TP. Vertical uniformity of cells and nuclei in epithelial monolayers. Sci. Rep. 2016;6(1):1-10.

[22] Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R and Burridge K. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. Nat. Cell Biol. 2014;16(4):376-381.

[23] Marguet D and Salomé L, Lateral Diffusion in Heterogeneous Cell Membranes. in: Bassereau P, and Sens Ps, Editor. Physics of Biological Membranes, Springer International Publishing, Cham, 2018, pp. 169-189.

[24] Rico F, Chu C, Abdulreda MH, Qin Y and Moy VT. Temperature modulation of integrin-mediated cell adhesion. Biophys. J. 2010;99(5):1387-1396.

[25] Ipponjima S, Umino Y, Nagayama M and Denda M. Live imaging of alterations in cellular morphology and organelles during cornification using an epidermal equivalent model. Sci. Rep. 2020;10(1):1-12.

[26] Gdula MR, Poterlowicz K, Mardaryev AN, Sharov AA, Peng Y, Fessing MY and Botchkarev VA. Remodeling of three-dimensional organization of the nucleus during terminal keratinocyte differentiation in the epidermis. J. Invest. Dermatol. 2013;133(9):2191-2201.

[27] Rieder CL and Cole RW. Cold-shock and the Mammalian cell cycle. Cell Cycle. 2002;1(3):168-174.

[28] Proksch E, Brandner JM and Jensen JM. The skin: an indispensable barrier. Exp. Dermatol. 2008;17(12):1063-1072.

[29] Ponec M, Gibbs S, Weerheim A, Kempenaar J, Mulder A and Mommaas AM. Epidermal growth factor and temperature regulate keratinocyte differentiation. Arch. Dermatol. Res. 1997;289(6):317-326.

[30] Denda M, Sokabe T, Fukumi-Tominaga T and Tominaga M. Effects of Skin Surface Temperature on Epidermal Permeability Barrier Homeostasis. J. Invest. Dermatol. 2007;127(3):654-659.

[31] Shahzad Y, Louw R, Gerber M and du Plessis J. Breaching the skin barrier through temperature modulations. J. Controlled Release. 2015;202:1-13.

[32] Schmidt A, von Woedtke T and Bekeschus S. Periodic Exposure of Keratinocytes to Cold Physical Plasma: An *In Vitro* Model for Redox-Related Diseases of the Skin. Oxid. Med. Cell. Longevity. 2016;2016:9816072.