The construction of an interfacial valve-based microfluidic chip for thermotaxis evaluation of human sperm

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Thermotaxis has been demonstrated to be an important criterion for sperm evaluation, yet clinical assessment of thermotaxis capacity is currently lacking. In this article, the on-chip thermotaxis evaluation of human sperm is presented for the first time using an interfacial valve-facilitated microfluidic device. The temperature gradient was established and accurately controlled by an external temperature gradient control system. The temperature gradient responsive sperm population was enriched into one of the branch channels with higher temperature setting and the non-responsive ones were evenly distributed into the two branch channels. We employed air-liquid interfacial valves to ensure stable isolation of the two branches, facilitating convenient manipulation of the entrapped sperm. With this device, thermotactic responses were observed in 5.7%-10.6% of the motile sperm moving through four temperature ranges (34.0-35.3 °C, 35.0-36.3 °C, 36.0-37.3 °C, and 37.0-38.3 °C, respectively). In conclusion, we have developed a new method for high throughput clinical evaluation of sperm thermotaxis and this method may allow other researchers to derive better IVF procedure. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4866851>]

I. INTRODUCTION

The meeting of a sperm and oocyte is the beginning of a new individual. In mammals, several mechanisms of attraction are believed to act as selection guides for competent sperm during their long and obstructed migration to the oocyte locating within the oviduct.¹ The chemotaxis mechanism, whereby responsive sperm swim towards a higher concentration of biomolecules secreted from the oocyte compartment is well studied by us and another group in sperm screening and analysis,^{2,3} whilst thermotaxis, the directed movement of cells along a temperature gradient, is another important long-range selection mechanism, while less well studied in human sperm evaluation.

Bahat *et al.* used a modified Zigmond chamber to analyze the directionality of tracks of rabbit and human sperm swimming between two wells held at different temperatures, demonstrating that spermatozoa respond by positive thermotaxis to small temperature differences

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(0.5 °C or less).⁴ Recently, they used a Lucite tube device with a controllable temperature gradient to show that only a small fraction of human sperm, which are capacitated and ready-to-fertilize, have thermotactic responses, and that portion of sperm can respond to both ascending and descending temperature gradient within a wide temperature range.^{5,6} Despite these advances, a convenient and efficient observable device with precise control of temperature gradient is still need to be developed for human sperm thermotaxis screening.

Microfluidic devices have been used increasingly in assisted reproductive technology (ART) because of their advantages in small sample requirements, precise micro-environment control and potential for automation, including for the analysis of sperm behavior,^{3,7,8} sperm motility and selection,^{9–11} cumulus cell removal,¹² *in vitro* fertilization (IVF),¹³ and embryo culture.¹⁴ Regarding sperm thermotaxis, Ko *et al.* enriched motile mouse sperm in a straight 13 mm long microfluidic channel using an optimal 2 °C temperature difference.¹⁵ But the number of sperm being analyzed in their experiment was too low to have a correct thermotaxis assay. We have previously developed microdevices to evaluate both sperm motility and chemotaxis in a straight micro-channel connected to two selection branch channels.² Recently, we developed novel microfluidic devices which integrate the entire IVF process, including oocyte positioning, sperm motility screening, fertilization, rapid medium replacement and debris expulsion, as well as long-term embryo culture.^{16,17} Apart from sperm chemotaxis, the environmental condition-directed motion of other kinds of cells, such as electrotaxis of cancer cells,^{18,19} chemo-electrotaxis of T cells,²⁰ and shear stress-guided migration of cancer cells and bacteria,²¹ were also investigated using various microsystems. Yet up until now there are no reports about any microdevices for the on-chip screening and trapping of thermotactic sperm.

Here, we describe the development and application of a microfluidic chip for the screening, enumeration and capture of thermotactic sperm. Human sperm were permitted to swim freely along a micro-channel, exposed to a highly controlled lateral temperature gradient, and then separated into two identical branches held at two temperatures and quantified. We made novel use of an air-liquid interfacial valve to trap the selected human sperm in both branches, enabling the collection of the sperm for further purposes. By examining sperm responses under different temperature ranges, we found that only a small portion of sperm (5.7%-10.6%) showed thermotactic responses depending on the temperature range and demonstrated that our chip can be well used in the thermotaxis evaluation of human sperm.

II. MATERIAL AND METHODS

A. Materials and reagents

Polydimethylsiloxane (PDMS, Sylgard 184) was from Dow Corning Inc. (Midland, MI). SU-8 photoresist was from MicroChem Inc. (Newton, MA). Human tubal fluid (HTF) was from Millipore (Temecula, CA). Mineral oil was from Sigma-Aldrich (St. Louis, MO).

B. Microfluidic chip design and fabrication

Figs. 1(a) and 1(b) show a microfluidic assay unit and its operational elements. Each unit has four functional regions: a sperm inlet pool (I1, 3.2 mm diameter), connecting channel (100 μm width, 500 μm length), thermal gradient channel and an air inlet (I2, 3.2 mm diameter). The thermal gradient channel is the main part of the device, comprising a straight channel (1.5 mm in width, 1.4 mm in length), and 2 symmetric bevel branches leading to chambers N1 and N2, each at an angle of 60° to the straight channel. The line distance between the center of the sperm inlet pool I1 and the chamber centers, i.e., average sperm swimming distance (dashed-dotted line in Fig. 1(b)), was ~ 4.5 mm, allowing sperm with an *in vitro* average VSL (straight-line velocity) bigger than $5 \mu\text{m}\cdot\text{s}^{-1}$ to reach the branches within 15 min. Sperm samples were first screened by motility with the straight channel and after this, thermotaxis screening was applied on those which meet the clinical motility evaluation criterion.²² The integration of motility screening and thermotaxis screening was thus made possible with the current design. The branch areas (N1, N2) are designed to fit within the field of view of a 20 $\times$ microscope

FIG. 1. Microfluidic device for human sperm thermotaxis assay. (a) Top view of one microfluidic assay unit filled with blue dye. (b) Schematic drawing of the assay unit after interfacial valve closure. Sperm are not drawn to scale. (c) Detailed illustration of the right branch after interfacial valve closure. (d) Microscopic image of the right branch after interfacial valve closure.

objective lens (dashed line box in Fig. 1(b), $830\ \mu\text{m} \times 630\ \mu\text{m}$). All micro-channels were $20\ \mu\text{m}$ in height, matching the depth of field of the objective lens and providing a clear image of sperm inside the channel. The connecting channel between the sperm inlet I1 and the thermal gradient channel was designed that sperm entry was symmetrical on the center line of the channel and also designed to maintain stable fluidic resistance during valve closure.

Sperm trapping is achieved by novel use of a slow positive air flow at air inlet I2, making the air-liquid interface move slowly backwards down the thermal gradient channel until sperm entry and exit from the branches are stopped. Figs. 1(c) and 1(d) show that two $30\ \mu\text{m}$ -diameter-pillars at the entrances of the bevel branches (N1, N2) and nearby convex channel sidewalls facilitate the complete and symmetrical closure of interfacial valves at both branches.

Three independent sperm assay units are aligned symmetrically along the chip centre line for replication of tests (Figs. 2(b) and 2(e)). The microdevice was constructed with an upper PDMS layer and a lower glass layer, following standard photolithography and micromolding procedures.²³ Briefly, SU-8 photoresist was patterned onto a 4 inch silicon wafer to form a master. Liquid PDMS prepolymer solution (base+curing agent in a proportion of 10:1) was poured onto the master, cured at 70°C for 1.5 h, and then peeled off the master, producing the final replica with micro-channels. The upper layer was diced from the replica, and through-holes (I1 and I2) were punctured. Standard glass slides (B270, Schott, Germany) were cut in half ($76.2\ \text{mm} \times 12.2\ \text{mm} \times 1\ \text{mm}$) by a dicing machine (DAD321, Disco, Japan) to form the lower layer. Both layers were treated in an oxygen plasma generator for 45 s at 25 W (Femto, Diener Plasma-Surface-Technology, Germany) and bonded irreversibly together. The silicon tubes (inner diameter 1 mm and outer diameter 3 mm) were then inserted to air inlets I2 and sealed by daubing and curing liquid PDMS prepolymer.

C. Construction of the temperature gradient system

The temperature gradient system is shown in Fig. 2. The chip positioning chamber was sandwiched by two aluminum alloy tanks filled with glycerol to increase heat capacitance and achieve fast temperature exchange. The tanks were independently heated by resistive heaters and were heat-insulated by surrounding polymethyl methacrylate (PMMA). The temperature of the two chamber walls were independently measured by two thermistors (Pt100, Heraeus Materials Technology Co., Germany) and controlled by two digital temperature controllers

FIG. 2. Temperature gradient control system. (a) View of the sperm thermotactic assay system. A-syringe pump, B-digital temperature controller, C- temperature gradient generator, D- inverted microscope. (b) Top view of the custom-made temperature gradient generator. (c) Cross-section of the chip positioning chamber, whose position is illustrated in dashed line in (e). (d) Computational results of the temperature field on the cross-section. (e) Schematic drawing of the temperature gradient generator. In (b), (c), and (e), numbers indicate 1-glycerol, 2-aluminum alloy tank, 3-resistive heater, 4-thermistor, 5-microfluidic channel, 6-PDMS upper layer, 7-glass lower layer, 8-chip positioning chamber, and 9-PMMA case, respectively.

(SR91, Shimaden Co., Japan). Heat was conducted to form a temperature gradient by the air. Air was chosen as heat conduction medium because of its low heat conductivity for a high temperature gradient and its compatibility with microscopic observation. The microfluidic chip, placed in the enclosed “chip positioning chamber,” was surrounded by air with 1 mm gap between the edge of the chip and the side wall of the chamber, and 1 mm gap between the chip and the bottom of the chamber. The lower glass layer of the microdevice was thin and has high heat conductivity, allowing a closely similar temperature gradient to form in the liquid medium inside the channel.

The temperature gradient on the lower glass layer of the chip was directly visualized by infrared (IR) imaging (A40, FLIR Systems, Boston, MA), while the temperature difference in the medium was measured by inserting thermoelectric couple through the PDMS into the channel. All measurements were taken when the chip was in its working position after 15 min of stabilization and the channel was prefilled with HTF.

D. Temperature gradient computational analysis

We built finite element models (COMSOL 3.5, Comsol AB, Burlington, MA) and used 2D simulations to study the heat conduction and temperature distribution in the cross-section of the “chip positioning chamber.” The model was based on the simplified steady-state heat conduction equation

$$\nabla \cdot (-k\nabla T) = Q, \quad (1)$$

where k is the thermal conductivity, T is the temperature, and Q is the heat source. Material properties were similarly modeled as reported in previous studies^{24,25} and listed in detail in Table S-1 in the supplementary material.²⁶ Constant temperature boundary conditions were applied for the sidewalls of the tanks. Heat flux boundary conditions with a heat transfer coefficient of $25 \text{ W}\cdot\text{m}^{-1} \text{ K}^{-1}$ and an infinite temperature of 25.0°C were applied for the upper and lower boundary of the apparatus. Continuous temperature boundary conditions were applied to interior boundaries and steady-state temperature field profiles were plotted.

E. The collection and preparation of human sperm

Human semen samples were obtained from healthy donors after 3 days of sexual abstinence (Center of Reproduction Medicine, Peking University Third Hospital). The experimental plan was approved by the ethical committee of Peking University Third Hospital. Semen samples with normal sperm density, motility, and morphology²² were allowed to liquefy for 60 min at 37°C . The spermatozoa were then separated from the seminal plasma by centrifugation ($100\times g$, 10 min). For capacitation, capacitating medium HTF was added and the sperm suspensions were incubated under an atmosphere of 5% CO_2 at 37°C for 1 h.²⁷ The sperm concentration was adjusted to 5×10^6 to 40×10^6 cells/ml with HTF medium before loading onto the chip.

F. Human sperm thermotactic assay

The microfluidic device was washed with 75% ethanol, rinsed with deionized water, dried in a vacuum oven, and finally treated in the plasma generator for hydrophilic surface modification. The micro-channel was filled with HTF (pre-equilibrated overnight under 5% CO_2 , 37°C) through the sperm inlet I1 and small residual air bubbles were driven out of the channels by manual pressing. The sperm inlet pools were then covered by mineral oil to prevent evaporation of the medium.

The microfluidic chip was first set in the temperature gradient generator to develop a horizontal temperature gradient and a syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) was connected to air inlet I2. A positive-pressure driven flow of $2 \text{ ml}\cdot\text{h}^{-1}$ was generated to pressurize the air-liquid interface into the channel and was stopped just before the interface reached the branches, as illustrated in Fig. 4(a) (multimedia view). The temperature gradient was allowed to reform and equilibrate for 5 min, and then $0.5 \mu\text{l}$ of prepared sperm were pipetted into sperm inlet I1 through the mineral oil. The sperm were then able to swim freely in the micro-channel while experiencing the thermal gradient. The sperm thermotactic responses were examined at 15 min after being loaded onto the device. For sperm enumeration, both branches were video recorded for 6 s at a rate of 15 frames/s, in (random) sequence at a maximum interval of 5 s, using an inverted microscope (DM-IRB, Leica Microsystems GmbH, Wetzlar, Germany) under a $20\times$ objective lens ($\text{NA} = 0.40$).

To trap the sperm present in each branch, positive-pressure air was pumped at $2 \text{ ml}\cdot\text{h}^{-1}$ until the air-liquid interface closed the entrances to the branches (see Fig. 4(d)). Branch closure typically took 15 s.

G. Sperm enumeration and data statistics

The separated frame of the videos was extracted using MATLAB 2010a program (Mathworks Inc., Natick, MA). We marked every sperm manually in each branch in one frame and counted the number of sperm automatically, distinguishing the background noise and any immobile parts of the image by comparing with the image ten frames later. The number of sperm in each branch was typically between 100 and 400, which varied according to sperm motility.

For convenience in evaluating sperm thermotaxis, we defined two parameters “Thermotaxis Index” (TI) and “Thermotaxis Percentage” (TP) by the following equations:

$$TI = N_1/N_2, \quad (2)$$

$$TP = (N_1 - N_2)/(N_1 + N_2) \times 100\%, \quad (3)$$

where N_1 and N_2 are the numbers of sperm in the channel branches held at higher and lower temperature, respectively. For the control isothermal experiments (without temperature gradient) the definition of TI is different, N_1 and N_2 are the numbers of sperm in the left and right branches, respectively. TI represents sperm thermotactic behavior directly; while TP is an estimate of the percent of thermotactic sperm in the whole motile sperm population.

Each sperm sample was tested sequentially 2-3 times in all 3 assay units located on the same chip and the mean values of TI and TP from the repetitive assays were calculated as the valid TI and TP value of this sample. We independently replicated each temperature setup 7-12 times, with sperm samples from different donors. The student's T-tests were performed to assess the thermotactic response difference between the experiment groups and control groups. SPSS software (SPSS Inc.) was used for data analysis.

III. RESULT AND DISCUSSION

A. Temperature gradient simulation and measurement

The homemade temperature gradient generator (Figs. 2(b) and 2(e)) formed a highly controllable temperature gradient in the microfluidic chip. The cross-sectional temperature distribution of the chip positioning chamber was simulated by the 2D finite element model (Figs. 2(c) and 2(d)). Although the temperature distribution along the vertical direction in the chip positioning chamber was non-uniform, an almost linear lateral temperature gradient was established within the entire space of the 3 mm-wide by 20 μ m-high microfluidic channel with a linearity of 0.99, shown as blue and green dashed lines in Fig. 3(b).

Fig. 3 shows the IR images and thermistor measurements perfectly match the simulation results with a lateral temperature gradient of $1.3 \pm 0.1^\circ\text{C}$ over the 3 mm channel width. Temperature differences in rabbits of $\sim 1.6^\circ\text{C}$ (Ref. 28) or $\sim 2^\circ\text{C}$ (Ref. 4) and in mated pigs of $\sim 0.7^\circ\text{C}$ (Ref. 29) between the sperm reservoir site at uteri-isthmus junction and the fertilization site at the oviduct ampulla close to the time of ovulation have been reported. Thus, a temperature difference of 1.3°C was chosen to match physiological conditions reported in mammalian thermotactic zones. Based on our measurement, there is no temperature difference across the channel width in the control 35.0-35.0 $^\circ\text{C}$ group.

FIG. 3. Simulation, measurement and imaging of temperature gradients. (a) IR imaging of the glass bottom of the device after stabilization at a temperature gradient of 36.3-35.0 $^\circ\text{C}$. (b) Results of temperature measurement on the chip by IR imaging (solid lines) and thermistor (fixed spots, ■: 35.0-36.3 $^\circ\text{C}$, ▲: 35.0-35.0 $^\circ\text{C}$, ●: 36.3-35.0 $^\circ\text{C}$) were consistent with the computational results (dashed lines). The profile of the microfluidic channel indicates its position in the temperature field.

FIG. 4. Step-by-step demonstration of the progressive closure of the interfacial valve. (a) When the valve is open, the air-liquid interface remains still allowing sperm to swim into either branch. (b) and (c) The interface was pushed towards the sperm inlet by a positive air influx (shown as white arrows). (d) An almost flat interface was formed to seal the entrances of the two branches simultaneously, with the interface pinned to the narrow connection due to surface tension. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4866851.1>] [URL: <http://dx.doi.org/10.1063/1.4866851.2>].

Real-time IR imaging also showed that the temperature gradient in the chip chamber could be established rapidly from room temperature of 25 °C within 15 min and when a chip was inserted into the chamber the gradient could be stable within 5 min. Compared to other chip-based temperature gradient systems,^{15,30} our thermal gradient system possesses an accurate and high temperature gradient ($0.43\text{ }^{\circ}\text{C}\cdot\text{mm}^{-1}$) in a stationary flow field and also has the advantages of providing multiple simultaneous tests, being reusable and having high reproducibility among different chips.

B. Trapping of thermotactic sperm by interfacial valve closure

Figs. 4(a) to 4(d) show the process of interfacial valve closure. Sperm were introduced at the inlet with the valve open, and the interface remained stationary for 15 min (Fig. 4(a)) to allow the sperm to swim freely into the branches on either side of the thermal gradient. Valve closure entailed a positive air influx at the air inlet, pushing the air-liquid interface down the main channel towards the branch channel inlets. Because the branches had one inlet and no outlet, and the velocity of interface was slow (average velocity of $100\text{ }\mu\text{m}\cdot\text{s}^{-1}$), there was no flow inside the branches and accumulated sperm were efficiently captured without being flushed out. The shape of the interface was adjusted by specific micro-structures at the branch channel entrances to finally form an almost flat interface and sealing both branch entrances and entrapping equal volumes of media in each branch cavity. Sperm cannot swim across an air interface, ensuring stable isolation of the two branches from the main channel, facilitating convenient manipulation of the entrapped sperm.

Since surface tension and viscosity dominate microfluidics, creating and controlling interfaces and using their interface properties are essential.³¹ Immiscible interfaces have been used previously as fluid stop valves during sample separation,³² and during extraction and purification of nucleic acids,³³ yet in microfluidics they have not been used to restrict cell motion. This

is the first report of creating an air-liquid interface as a microfluidic valve to restrict sperm motion. The valve has the following advantages: (1) having no moving parts on the chip, (2) needing no additional energy except a positive influx, (3) being inexpensive and easy to fabricate, and (4) being convenient for on/off switching. For these advantages, the interfacial valve would have great potential to be applied into microfluidic systems for convenient and low-damage trapping of small quantity of biological cells or molecular. Here, valve closure after sperm swimming into branches trapped the thermostatic sperm successfully and easily and caused no damage to the sperm.

The shape of the interface is passively controlled and is determined by its surface energy, yet here care was taken to design appropriate micro-structures to insure equal liquid volume in both branches. As shown in Fig. 4(b), one 100 μm -diameter-pillar in the middle of the main channel, and two abreast concave interfaces rather than a larger parabolic one, were employed to avoid the blocking of the sperm entrance from the connecting channel. Fig. 4(d) shows that two 30 μm -diameter-pillars at the entrances of the bevel branches (N1, N2) and nearby convex channel sidewalls facilitate the complete and symmetrical closure of interfacial valves at both branches. The edge-to-edge distances were 70 μm between two pillars and 85 μm between the pillar and the sidewall, which are over 10 times larger than the size of human sperm head, and the micro-pillars have no significant influence on sperm entering the branches as shown in Fig. 4. With some improvement on the structure design, the interfacial valve will also be used to facilitate the retrieval of selected sperm from the chip for further research or clinical IVF treatment.

C. Human sperm thermotactic assay

Fig. 5 shows initial tests, with the left chamber set at 35.0 °C and the right chamber at 36.3 °C, with sperm collected over 15 min. In a second test series, the temperatures of the two sides were reversed, while in a third control series temperatures were identical on both sides (35.0 °C). Each test series were independently repeated using sperm from 7 to 12 donors. The Thermotaxis Index (*TI*) is defined as the ratio of the number of sperm in the higher temperature branch over that in the lower temperature branch, thus a *TI* of >1.0 is expected if sperm display positive thermotaxis, <1.0 for negative thermotaxis, and a *TI* of approximately 1.0 for no thermotactic response. Sperm exhibiting positive thermotaxis would accumulate towards the higher temperature channel wall and would be depleted from the lower temperature channel wall.

Fig. 5 illustrates that the left and right sides of the thermogradient platform perform identically and give equivalent results regardless of the thermal gradient direction across the width of the channel. Thermotactically responsive sperm in test series 1 and 2 were attracted to the higher temperature of 36.3 °C. The *TIs* for the series of tests were 1.27 ± 0.08 (test 1), 1.24 ± 0.04 (test 2, reverse gradient), and 1.02 ± 0.03 (test 3, no gradient), respectively, with statistical significance ($P < 0.05$). A significant fraction of sperm travelled towards higher temperature compared with the control group, with this sub-population of responsive sperm defined by the Thermotaxis Percentage (*TP*). The *TP* value for each test group was $11.3\% \pm 3.1\%$, $10.5\% \pm 2.0\%$, and $0.6\% \pm 1.2\%$ ($\pm\text{SEM}$, standard error of mean), respectively, with statistical significance ($P < 0.05$). The mean percentage of thermotactically responsive sperm is $\sim 11\%$.

Compared with previous thermotaxis assays,^{4,5} our microfluidic chip-based assay has several advantages. First, the chip requires only minute numbers of sperm (~ 5000 cells) for each assay, which may particularly enable tests for oligozoospermia in which only a few sperm are available. Second, microfluidic channel walls perpendicular to thermal gradient served to restrict sperm lateral motion, allowing thermotactic sperm to be enriched in the branch at the preferred temperature, so as to be captured by interfacial valve for further purposes (Fig. 1(c)). Moreover, the present chip for thermotactic sperm screening has high potential for integration with our previously developed chips for sperm motility and chemotaxis screening,² and for the entire IVF process^{16,17} which would fully mimic physiological conditions of female reproductive tract and achieve an automated IVF-on-a-chip system.

FIG. 5. Human sperm thermotactic behavior. (a) Comparison of Thermotaxis Index (TI) over 15 min collection time. (b) Comparison of Thermotaxis Percentage (TP) over 15 min collection time. Error bars represent SEM. Asterisks above the columns indicate statistically significant differences with $P < 0.05$.

D. Thermotactic response of human sperm in different temperature ranges

A series of experiments involving four different temperature ranges with 1.3°C isogradient, i.e., four left to right side temperature ranges of $34.0\text{--}35.3^{\circ}\text{C}$, $35.0\text{--}36.3^{\circ}\text{C}$, $36.0\text{--}37.3^{\circ}\text{C}$, and $37.0\text{--}38.3^{\circ}\text{C}$, were examined. Then independent experiments with reverse orientation temperature gradients (right to left) were examined. The average TIs in the initial and reverse tests are shown in Fig. 6 and are detailed in Table S-2 in the supplementary material.²⁶ Corresponding control groups with both branches at the lower temperature ($34.0\text{--}34.0^{\circ}\text{C}$, $35.0\text{--}35.0^{\circ}\text{C}$, $36.0\text{--}36.0^{\circ}\text{C}$, and $37.0\text{--}37.0^{\circ}\text{C}$) were employed.

Fig. 6 shows that the TIs were larger than 1 for all the four temperature ranges of $34.0\text{--}35.3^{\circ}\text{C}$ (1.12 ± 0.02), $35.0\text{--}36.3^{\circ}\text{C}$ (1.25 ± 0.4), $36.0\text{--}37.3^{\circ}\text{C}$ (1.13 ± 0.03), and $37.0\text{--}38.3^{\circ}\text{C}$ (1.22 ± 0.07) ($\pm\text{SEM}$), with statistical significance to the corresponding control groups ($P < 0.05$), indicating that responsive sperm were thermotactically attracted to higher temperatures in all these ranges on our chip, consistent with previous study.⁶ TP values in the four different temperature ranges are $5.7 \pm 1.1\%$, $10.6 \pm 1.8\%$, $5.8 \pm 1.3\%$, $9.4 \pm 2.7\%$ ($\pm\text{SEM}$), respectively.

Under the natural condition, there are three widely reported screening mechanisms of mammalian sperm in female oviducts: screening of sperm motility, chemotaxis and thermotaxis. However, only the screening of sperm motility is currently included in the clinical assessment. To obtain the optimum sperm for IVF, the other two selection mechanisms may need to be taken into consideration. Chemotaxis screening was realized with a microfluidic device with potential application for clinical assessment, yet there is still no microfluidic systems reported for convenient, low-cost and damage-free screening of sperm thermotaxis. The temperature of

FIG. 6. Thermotactic responses of human sperm to different temperature differences. *TIs* in different temperature ranges after 15 min collection. Error bars represent SEM. Asterisks above the columns indicate statistically significant differences with $P < 0.05$. The *TIs* are larger than 1 in four temperature ranges with statistical significance ($P < 0.05$), illustrating that 5.7%-10.6% motile human sperm exhibit thermotactic response, regardless of the absolute temperature range.

female tract in human may be $\sim 37^\circ\text{C}$. We demonstrated here that our device worked well in different temperature ranges of $34.0\text{--}35.3^\circ\text{C}$, $35.0\text{--}36.3^\circ\text{C}$, $36.0\text{--}37.3^\circ\text{C}$, and $37.0\text{--}38.3^\circ\text{C}$ and the most appropriate temperature range for thermotaxis assessment could be chosen based on the real temperature gradient in human oviduct. In conclusion, we have integrated the convenient screening and efficient trapping of thermotactic sperm on our microfluidic device which shows a great potential for applications in clinical evaluation and collection of thermotactic sperm.

Apart from the evaluation of the thermotactic ability of sperm, the microfluidic system we reported here may also be used to investigate the migration of other types of cells under different temperature gradient. Cell migration is a universally existing phenomenon in the mammal tissues and organs, which could be directed by the complicated *in vivo* conditions, such as chemical signals and physical parameters.²⁰ In fact, there have been some microfluidic devices constructed to study external force-directed cell migration. The electrotactic behavior of lung cancer cells and oral carcinoma cells were previously analyzed, respectively, using ordered three-dimensional scaffolds with direct current electric fields¹⁸ and micro-channels with different electric fields but comparable flow velocities.¹⁹ And T cell migration responding to the co-existing chemical gradient and direct current electric field was also measured.²⁰ Rupprecht *et al.* fabricated a tapered channel to analyze the detachment kinetics and shear stress-dependent motion of cells.²¹ Similar to the above-mentioned microsystems, our device is also an external force field generation system capable of testing the cell functions. The temperature control system we developed here provides a highly controllable and accurate temperature gradient in a stationary flow field and the introduction of the interfacial valve facilitates the separation and statistics of the screened cells. These microfluidic systems, once organically combined and used, may offer more functional, highly controllable and easily observable platforms for the migration analysis of a large set of cell types under various environmental conditions.

IV. CONCLUSIONS

In this work, we have developed a novel microfluidic system to study human sperm thermotaxis on a chip. The temperature gradient in the microfluidic channel was established and accurately controlled by an external temperature gradient control system. Thermal responsive human sperm were found with a tendency to accumulate in the higher temperature area in the four tested temperature ranges. The developed device trapped the thermotactic sperm fraction in a discrete branch by a novel air-liquid interfacial valve. We believe that this is the first time that both the thermotactic human sperm screening and trapping were achieved on a single microfluidic chip. These results suggest that this automated microfluidic assay may have great

potential for human sperm thermotaxis testing, and may become an additional tool for sperm evaluation and cell thermotaxis studies.

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