Interplay of chemical and thermal gradient on bacterial migration in a diffusive microfluidic device

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Living systems are constantly under different combinations of competing gradients of chemical, thermal, pH, and mechanical stresses allied. The present work is about competing chemical and thermal gradients imposed on *E. coli* in a diffusive stagnant microfluidic environment. The bacterial cells were exposed to opposing and aligned gradients of an attractant (1 mM sorbitol) or a repellant (1 mM $NiSO_4$) and temperature. The effects of the repellant/attractant and temperature on migration behavior, migration rate, and initiation time for migration have been reported. It has been observed that under competing gradients of an attractant and temperature, the nutrient gradient (gradient generated by cells itself) initiates directed migration, which, in turn, is influenced by temperature through the metabolic rate. Exposure to competing gradients of an inhibitor and temperature leads to the imposed chemical gradient governing the directed cell migration. The cells under opposing gradients of the repellant and temperature have experienced the longest decision time (∼60 min). The conclusion is that in a competing chemical and thermal gradient environment in the range of experimental conditions used in the present work, the migration of *E. coli* is always initiated and governed by chemical gradients (either generated by the cells *in situ* or imposed upon externally), but the migration rate and percentage of migration of cells are influenced by temperature, shedding insights into the importance of such gradients in deciding collective dynamics of such cells in physiological conditions. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4979103]

I. INTRODUCTION

Multiple gradients, *viz.*, chemical, thermal, pH, gaseous concentration, and mechanical stress co-exist naturally in a physiological system.[1] Interactive action of such gradients coordinates various essential biological activities such as wound healing,[2] guidance of sperm cells towards the egg,[3] cancer cell metastasis,[4] and host–microbe relationships (*viz.*, migration, colonization, secretion, and infection)[5] in living organisms.

To counter infected or diseased conditions, gradients are created within the system via external sources by drugs (skin patches, injecting drug carrier molecules/particles, and chemotherapy) or radiation and laser therapy (hyperthermia and hypothermia).[6] Such gradients imposed on cells may interact with the multiple gradients existed within the entity, and changes in the activities of the diseased cells and/or the microbes inhabited the host. *E. coli*, a Gram negative bacterium, which exists in the human gastrointestinal (GI) tract, is a highly motile microbe whose migration is constantly guided by the interaction of multiple gradients (mainly

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chemical, arising due to enzymatic secretions from the cells in the GI tract and thermal, arising due to the metabolism of cells in the GI tract) within the GI tract.[5] Even though most *E. coli* strains are non-pathogens in the human system, colonization of harmful strains can lead to diseases of the gastric and the intestinal systems.[7] Hence, it is of great importance to understand the motility and mobility of *E. coli* population under the influence of such imposed gradients for the effective design of preventive drugs and *a priori* estimation of internal therapeutic processes.

As chemical and thermal gradients are among the most important agents affecting the migration pattern of microbial cells within a physiological system, the effects of these individual gradients on *E. coli* have been reported by researchers.[5,8–15] Earlier, conventional methods such as capillary assay[8] and Boyden chamber[9] have been used to generate chemical gradients to study the chemotaxis of *E. coli* cells *in vitro*. Agar plates placed over aluminum blocks with provision for the flow of hot and cold fluids[10] were used to generate thermal gradients to study the thermotaxis of *E. coli* cells. Since the past decade, microfluidic devices has been the better system than conventional assays for the creation of stable, predictable, reproducible, and measured gradients which are not possible in conventional assays.[5] This is due to the fact that microfluidic environments can successfully mimic the requisite physiological conditions, both qualitatively and quantitatively. There have been reports on individual studies of *E. coli* cell migration due to chemical[5,11–13] and thermal gradients[14,15] using microfluidic devices.

Although there have been reports on the effect of these gradients on *E. coli* cells in microfluidic environments, there is no report on the navigation and behavior of such cells when exposed to competing thermal and chemical gradients imposed on cells, which closely mimic the physiological conditions and present during the defense mechanism by the body in conjunction with external drug molecules. A computational report[1] on migration of *E. coli* cells in the presence of competing gradients of chemical, thermal, and pH in a microfluidic device is available, but a detailed survey of the literature suggests that the robust experimental reports of such cellular migration in a fluidic microenvironment are really necessary. Understanding cellular migration under such complex gradients is a need of the hour.

Previous reports on thermotaxis suggest that at the population level (in the absence of any external chemical change in the environment), bacteria can themselves bring about a chemical change in the environment.[16] This behavior is governed by the number density of the cells at a particular location, and this can affect the thermotactic characteristics of the population.[16] It has also been reported that below a threshold population density, *E. coli* cells behave as hot temperature seeking, whereas above the threshold population density, they were cold temperature seeking.[17] It has been explained that such reversal in behavior is caused by the formation of nutrient gradients and a high Tar/Tsr ratio at high cell population density. Tar and Tsr are transmembrane chemoreceptors which play major roles in chemical and thermal sensing mechanisms of *E. coli*.[17] Both Tar and Tsr are warm seeking receptors in the absence of ligands.[16,17] Reports show that cells grown below 0.1 OD (optical density) are warm seeking since they possess an abundance of Tsr receptors (Tsr being a warmth seeking receptor; however, in the presence of ligands such as glycine or serine, it becomes insensitive to temperature). On the contrary, cells of OD 0.3 are cold seeking since they have abundant Tar receptors (Tar being warmth seeking; however, in the presence of ligands such as aspartate, it changes as cold seeking). An alternative transduction pathway through the change in intracellular or extracellular pH differences other than the chemoreceptor has also been reported.[18]

In all existent reports, the utilized attractant (aspartate/serine/glycine) was introduced into the medium under observation itself before exposing to the temperature gradient (to make it a nutrient rich medium).[17,18] In such procedures, the cells were not exposed to competing or aligned chemical and thermal gradients, which are highly plausible during radiotherapy, chemotherapy, and induced photothermal hyperthermia.[6] Several pertinent questions may arise based on the present knowledge. The behavior of bacterial cells and migration patterns of cells in a situation wherein all the possible migratory options are unfavorable are unanswered questions.

Similarly, the factors that would lead to the bacterial population to make a collective decision and the time necessary for it to respond to imposed interacting chemical and thermal

gradients also require proper comprehension. *E. coli* migration patterns under the influence of aligned and opposing gradients will shed insights into the behavior of the bacterial population when the host location tends to be unfavorable or favorable compared to the neighboring microenvironment which may be unfavorable or favorable. The present study attempts to understand insights into such regions of knowledge gap by employing physiology, mimicking microenvironments, and the observations are likely to replicate the migration dynamics of parasitic cells in complex physiological environments.

II. EXPERIMENTAL SECTION

A. Bacterial culture preparation

An aliquot of 500 μl of untransformed *Escherichia coli* DH5α cells (M/S. Biogenei, Bangalore, India) was used to inoculate 25 ml LB liquid broth. The culture was grown under shaking conditions (180 rpm) at 37 °C for 12 h. After incubation, the culture was centrifuged for 5 min at 6000×g and at 4 °C. Then, the sample was prepared by collecting the bacterial pellet and further suspending in distilled water to an optical density of 0.3.

B. Device design, simulation, and fabrication

The device used for generating a combined gradient under stagnant conditions of the microbe chamber consists of 5 channels (3 parallel channels at the centre and 2 channels perpendicular to the centre channels, and one on each side). The channel, having a dimension of 10 mm × 2 mm × 0.2 mm, in which the migration has been observed, contains no agarose. The dimension of the channel in the device is described in detail in the supplementary material of our previous publication.[11] The channels parallel to (on either side) the channel under investigation carry the agarose matrix and are connected to the side channels (source and sink channels).[11] The above description of the device design can create steady, long range, and flow free chemical gradients across a stagnant fluid. In addition, the device comprises of two parallel tubes to supply hot and cold water for temperature gradient generation (*cf.* Fig. 1).[15] Computations have been performed to gauge the characteristics of the generated gradient since the chemicals used in the experiments are colorless, and hence, the gradient cannot be determined quantitatively during experiments. The chemical and thermal gradient generation within the device has been already characterized and validated with the "solute transport" and "conjugate heat transfer" modules of COMSOL Multiphysics, 4.2.a., in previous reports by the same authors.[11,15] Accordingly, the same simulation protocol has been used in the present study to estimate *a priori* steady combined chemical (sorbitol/$NiSO_4$) and thermal gradient generation in the designed device. The boundary conditions are similar to those reported by the present authors.[11,15] A PDMS (polydimethysiloxane) based device was fabricated using optical and soft lithography techniques.[15]

C. Experimental methodology

The fabricated device was autoclaved before the start of the experiments. The agarose channels were filled with freshly prepared 1.5% agarose and kept undisturbed for 30 min for the formation of the matrix. Further, *E. coli* DH5α cells as obtained from the previous section (Bacterial culture preparation) were injected in the central channel under investigation through the cell inlet option provided in the device (*cf.* Fig. 1(b)) and kept undisturbed for 30 min for the population to attain the equal distribution of cells throughout the channel. The microfluidic device loaded with cells was then positioned over the stage of an inverted fluorescent microscope (Leica DMI3000 B, Leica Microsystems, Wetzlar, Germany) along with the fittings for chemical and thermal gradient generation (*cf.* Fig. 1).

Once the equal distribution of cells was achieved and confirmed qualitatively by viewing through the 62× objective of the microscope, both chemical and thermal gradients were generated simultaneously within the device by flowing respective fluids in the respective channels and tubes. A steady chemical gradient was generated by pumping a chemo–effector (1 mM

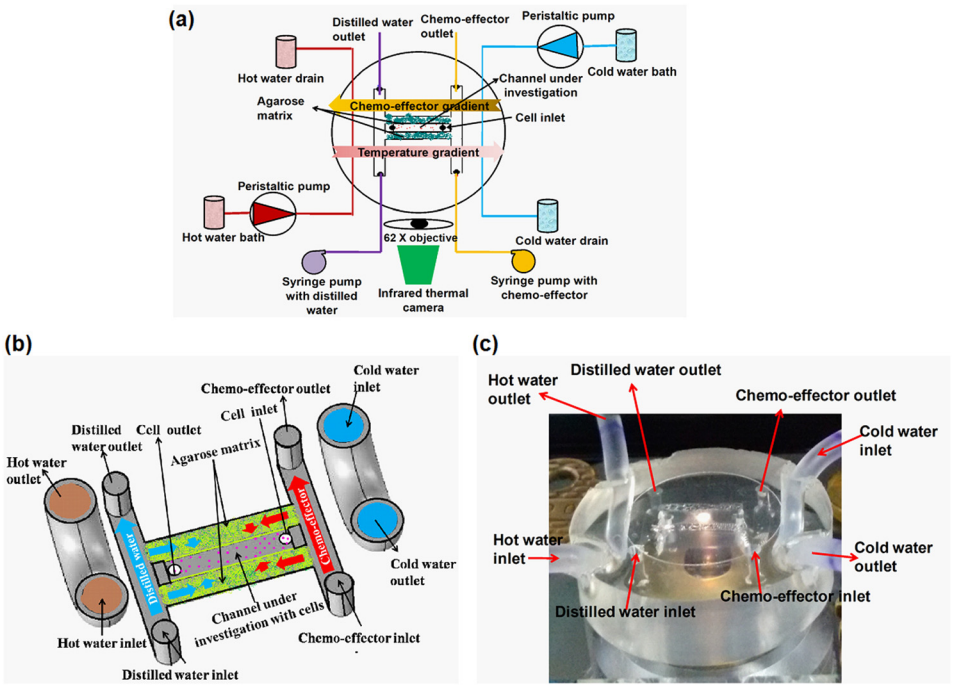

FIG. 1. (a) Sketch of the experimental setup for the cell migration study under imposed competing chemical and thermal gradients. (b) Schematic illustration of the channels in the device. (c) Schematic view of the microfluidic device under working conditions.

sorbitol as an attractor or 1 mM $NiSO_4$ as an inhibitor) through one side channel and distilled water through the other side channel, in a parallel flow mode and both at a flow rate of 100 μl/h. A steady thermal gradient was simultaneously generated by pumping hot (53 °C) and cold water (30 °C) through the respective tubes inserted in the device in a counter flow mode at a flow rate of 4000 ml/h. The surrounding temperature was maintained at 30 °C. Fig. 2 gives various combinations of thermal and chemical gradients generated. Given the fact that it is not feasible to introduce the cells (at population level) in the main channel without disturbing the existing gradients, the cells were introduced before generating the gradients.

Images and videos of the cell population were captured at 15 different axial locations of the channel at a frequency of 30 min up to 2 h, and the images of the cells were analyzed for the cell count using Image J software. The thermal image of the device was captured using an infrared camera (FLIR T250, M/S. PCI middle east FZE, UAE), and the same was analyzed using the associated FLIR Quick Report software to quantify the thermal gradient generated across the channel under investigation. The cell migration behavior was studied in a combined gradient microenvironment and further quantified using two population based matrices, *viz.*, the migration rate (MR) and the apparent migration coefficient (AMC).[11] The definitions of the migration rate and the apparent migration coefficient have been reported by Murugesan *et al.*, and they are given below.[11]

$$\text{The migration rate (MR)} = \frac{\mathrm{d}\left(\mathrm{N}/\mathrm{N}_{\mathrm{avg}}\right)}{\mathrm{dt}},$$

where $\mathrm{N}_{\mathrm{avg}}$ = the number of cells irrespective of distance before the initiation of the gradient and N = the count at different times, after the initiation of the gradient, at specified distance in the said channel. If the gradient of an unknown compound is considered from these values, it will be easier to interpret whether the unknown compound is an attractant or a repellant. This facilitates the detection of the unknown compound.

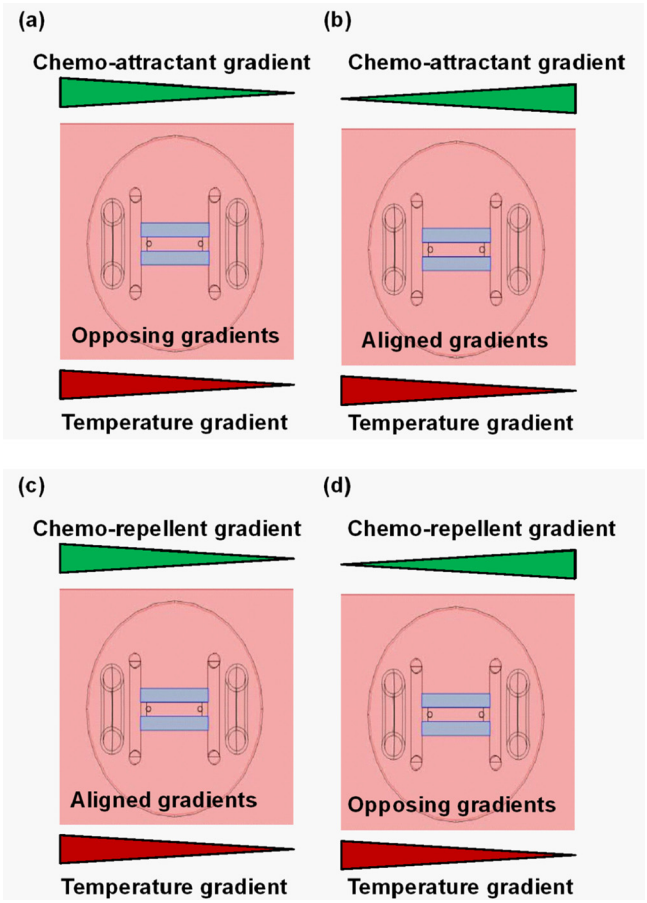

FIG. 2. Combined gradient generation within the microfluidic device. (a) Opposing gradients of the attractant (sorbitol) and temperature. (b) Aligned gradients of the attractant and temperature (c) Aligned gradients of the inhibitor ($NiSO_4$) and temperature. (d) Opposing gradients of the inhibitor and temperature.

$$\text{The apparent migration coefficient (AMC)} = \frac{\sum_{i=1}^{5}\left(1 - \left(N/N_{avg}\right)\right)}{a},$$

where a = the number of observation points and i = the point or location at which the cell count is made. If the microchannel is hypothetically divided into two halves, then AMC is the net shift of the cells from one half to another. The clock has been started around 20 min from the establishment of the gradients (i.e., 10 min) for the MR and AMC plots. The total number of cells in the channel under investigation under the key conditions of the experiment during the experimental duration (~2 h) has been given in Figs. S1 to S7 (*cf.* supplementary material) to show that the total number of cells in the channel under investigation is the same under all the conditions and that the migration results are not affected by this.

III. RESULTS AND DISCUSSION

A. Characteristics of the microfluidic environment

A steady combined chemical and thermal gradient has been generated in a stagnant microfluidic environment. It took 10 min for the device to establish chemical and thermal gradients. With an initial concentration of 1 mM of the chemo-effectors, the microfluidic device was able to generate a concentration profile ranging from 0.2 mM at one end of the channel to 0.8 mM at the other end of the channel (as obtained from simulation and illustrated in Figure 3).

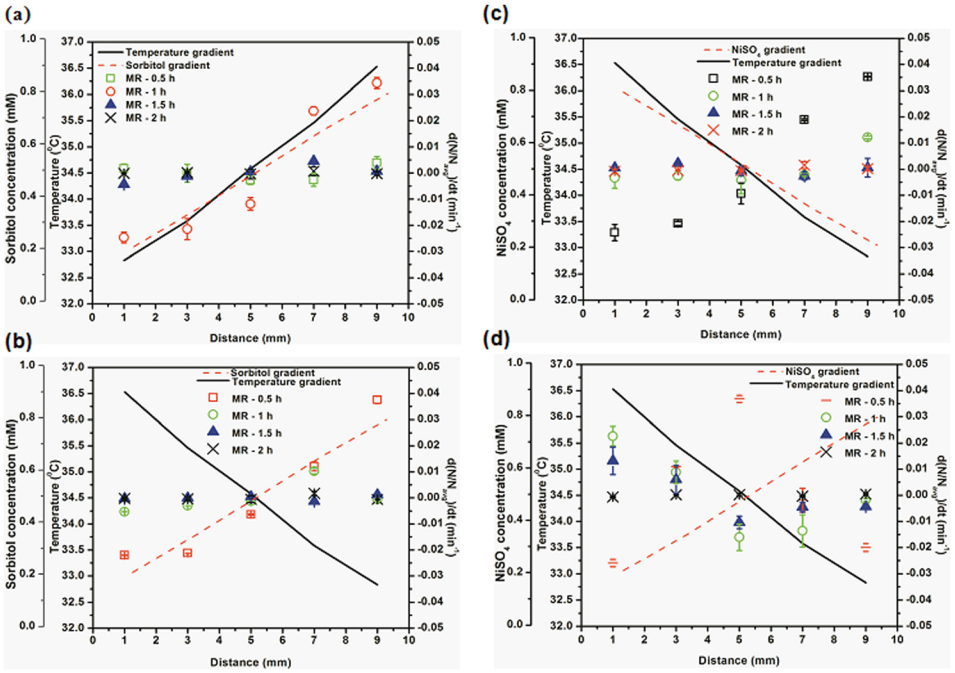

FIG. 3. Migration rate (MR) of *E. coli* DH5α cells under different interacting gradients: (a) opposing gradients of temperature and attractor (the range of the error is from $3.95 \times 10^{-5}\,\mathrm{min}^{-1}$ to $4.03 \times 10^{-3}\,\mathrm{min}^{-1}$), (b) aligned gradients of temperature and attractor (the range of the error is from $4.49 \times 10^{-7}\,\mathrm{min}^{-1}$ to $1.67 \times 10^{-3}\,\mathrm{min}^{-1}$), (c) aligned gradients of temperature and inhibitor (the range of the error is from $3.11 \times 10^{-3}\,\mathrm{min}^{-1}$ to $5.34 \times 10^{-3}\,\mathrm{min}^{-1}$), and (d) opposing gradients of temperature and inhibitor (the range of the error is from $1.43 \times 10^{-3}\,\mathrm{min}^{-1}$ to $6.19 \times 10^{-3}\,\mathrm{min}^{-1}$). The right axis in the panels is the migration rate. The data points are the average of two independent experiments. The error bar indicates half of the difference between the values obtained in the two independent experiments.

Furthermore, with the surrounding temperature maintained at 30 °C and by the flow of hot and cold water at 53 °C and 30 °C, the device was able to generate a temperature profile between 32 °C and 37 °C from one end of the channel under investigation to the other end (obtained from the simulations and also experimentally validated by thermal imaging[15] and illustrated in Fig. 3).

B. Cell migration under opposing gradients (attractant and temperature)

The initial investigation is performed for opposing gradients of the chemo–attractor and temperature, i.e., the high attractor concentration and high temperature zones are on the same side of the channel. Under the influence of such opposing gradients of an attractant (1 mM sorbitol) and temperature, the cells show no directional migration for an initial period of 30 min or so. It was after this initial lag period that the cells start migrating towards the left side of the channel under investigation, i.e., the higher concentration of the attractant and higher temperature (*cf.* Fig. 4). On the contrary, in the presence of a simple attractant gradient, the cells migrate towards the higher concentration region within 5 min of gradient generation.[11] This observation thereby poses an interesting interactivity of the thermal gradient with the chemical and its effects vis-à-vis bacterial migration. The reason behind the apparently large lag period before migration in the present case can be explained based on the metabolic activity of the cells. With a low concentration of sorbitol and low temperature present at the right end of the channel, it is possible that the cell population near that region was self-sufficient, i.e., null activity due to low attractor species and favorable temperature for high cell population density, and hence, the population remained stationary. This is due to the fact that the cell metabolic rate at lower temperature is less, and hence, sorbitol consumption is also less, which further aids in maintaining low metabolic activity.[16,17]

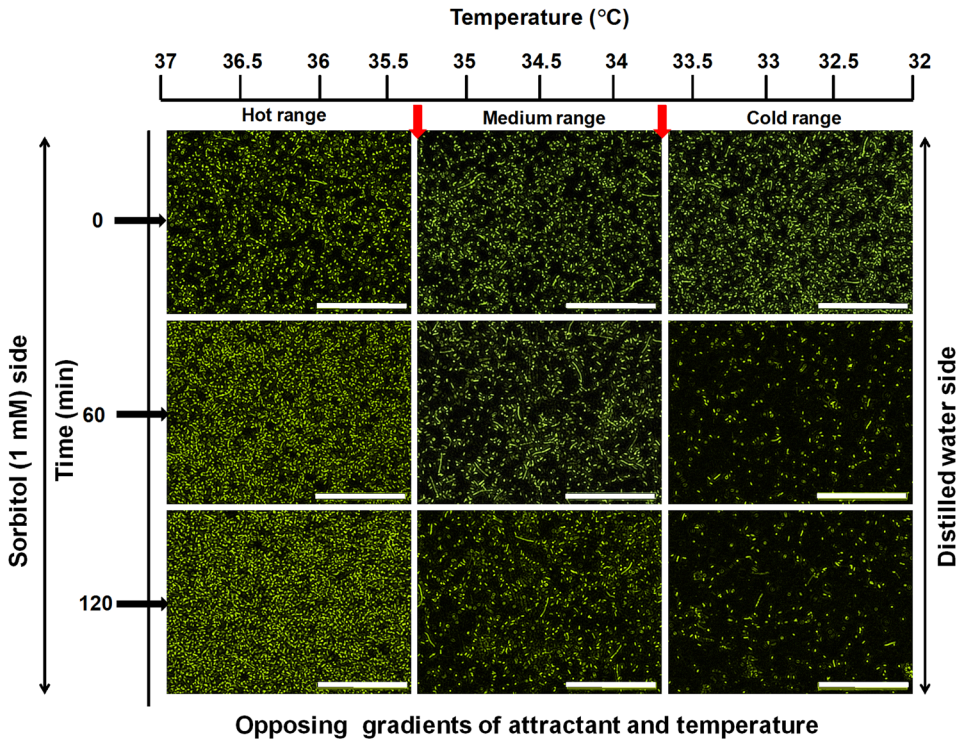

FIG. 4. Migration behavior of *E. coli* population under opposing gradients of temperature and chemo–attractant (left side sorbitol and high temperature; right side distilled water and low temperature). Each row shows photographs at specified times. All the experiments are repeated twice. Background corrections were done using "Image J software (National Institute of Health, Maryland, USA)." The scale bar is 100 μm.

Similarly, the cells at the left end of the channel (higher attractor concentration and higher temperature) are also self-sufficient with both nutrient and temperature being high. While high temperature requires higher metabolic activity,[16,17] the abundance of the attractor provides the necessary impetus for this requirement. However, the cells migrate from higher temperature when exposed to the temperature gradient alone,[15] and this is due to that the population creates an *in situ* nutrient gradient since at higher temperature, the cell metabolism rate is high and most of the nutrients are utilized.[16,17]

This leads to the formation of a nutrient gradient from a higher temperature side to a lower temperature side, and the cells migrate to counterbalance this gradient. However, in the present case (opposing gradients of the attractant and temperature), the cells at higher temperature are exposed to enough sorbitol to consume and hence the cells need not migrate to counter any sort of nutrient scarcity. Thus, all zones of the channel are nutrient balanced during the initial lag phase in accordance to the local temperature, i.e., favorable conditions exist on both the sides of the channel. As time progresses, the cells at the low concentration attractor end begin to sense highly favorable conditions, i.e., higher concentration of attractant and higher temperature. The range of temperatures exist in the channel is 32 °C–37 °C. As mentioned in the Bacterial culture preparation section, the favorable growth temperature for *E. coli* DH5α is 37 °C. This triggers cell migration towards the higher sorbitol side of the channel to improve their growth and metabolism. In this case, higher temperature is favorable because bacteria in the presence of abundant nutrient have no reason to be cold seeking. Hence, it can be inferred that both the thermal and chemical gradients influence each other and the joint effect governs cellular migration. It can be further proposed that for therapeutics employing hyperthermia to obliterate diseased cells or pathogens, establishing a nutrient source at the site might be a useful tool to prevent migration of the cells from the target site.

The MR (*cf.* Fig. 3(a)) and the AMC (*cf.* Fig. 5(a)) indicate that around 30 min after the gradients have been established, the cells started migrating and reached the steady state at

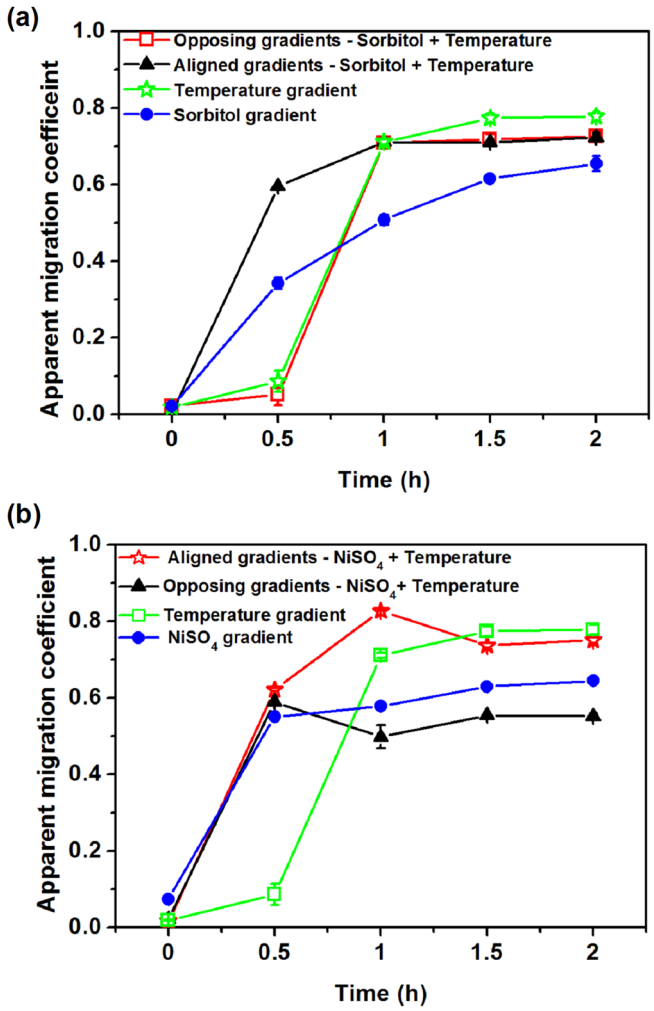

FIG. 5. Comparison of the apparent migration coefficient (AMC) of *E. coli* DH5α cells under aligned and opposing gradients of temperature and attractor/inhibitor with the AMC of the same species of cells under simple attractor/inhibitor and simple temperature gradients for (a) sorbitol (1 mM) and temperature gradient (32 °C to 37 °C) (the range of the error is from 1.22×10^{-4} to 0.02813) and (b) $NiSO_4$ (1 mM) and temperature gradient (32 °C to 37 °C) (the range of the error is from 5.80×10^{-4} to 0.03056). The data points are the average of two independent experiments. The error bar indicates half of the difference between the values obtained in the two independent experiments.

around 1 h (after gradients are established). The AMC values show that the migration of the cells increased by 0.07 in the combined opposing gradient (attractor based) system compared to the simple attractor gradient, but the AMC value of the combined opposing gradient (attractor based) was reduced by 0.05 compared to the simple thermal gradient. This difference exists since in the case of the simple sorbitol gradient, even though the cells had ample exposure to the growth substance, the surrounding temperature was maintained at 30 °C. Under such conditions, the activity of the cells is low since the favored temperature of the *E. coli* DH5α cells is ∼37 °C.

On the other hand, the higher cell migration rate in the simple temperature gradient compared to the opposing combined gradient exists because in the former case, the cells in higher temperature regions experience a high metabolism rate.[16,17] This in turn pushes the cells towards starvation in the absence of any growth medium. This forces the population to migrate promptly to the colder end to protect the population from starvation via reduced metabolism. In the case of opposing gradients (attractant and temperature), the cells migrate from lower sorbitol and lower temperature range (right side of the channel) towards the higher temperature

range to improve their metabolism rate since the abundance of growth medium or sorbitol is present.[16,17] Unlike the simple temperature gradient, in the case of opposing gradients of the attractant and temperature, there is always a less and steady concentration of sorbitol present in the right side of the channel. Due to that reason, the cells will not be facing starvation conditions. Hence, the cell migration rate is little less compared to the simple temperature gradient condition. From observations, it can be stated that even though in the case of the combined opposing (attractant and temperature) gradient the effect of the chemical change holds major ground, the quantification and analysis reveal that cells in a combined gradient environment will be influenced by temperature to varying extents and the migration rate will be affected, if not the direction of migration.

C. Cell migration under aligned gradients (attractant and temperature)

Under the influence of aligned gradients of the attractant and temperature, the bacteria cells start migrating within 5 min from the onset of establishing the gradient. Furthermore, the cells showed directional migration towards the side of the channel where a high concentration of the attractant and low temperature exist (*cf.* Fig. 6). The cells in a combined aligned gradient environment prefer the lower temperature side similar to the case of a simple thermal environment (thermal gradient alone).[15] For the thermal gradient alone, the cells migrated towards lower temperature to reduce the metabolism rate and to balance the *in situ* generated nutrient gradient.[15–17]

Unlike the previous case, in the present case, the cells can collectively converge to a decision easily between two choices. It can be either a highly favorable side (higher sorbitol concentration which satisfies their nutrient consumption requirement and lower temperature for controlling their metabolism) or a highly unfavorable side (less sorbitol concentration, which is insufficient for their nutrient consumption requirement and higher temperature, which will enhance the metabolism rate, in turn, augmenting the nutrient deficit). Since the feasible choices

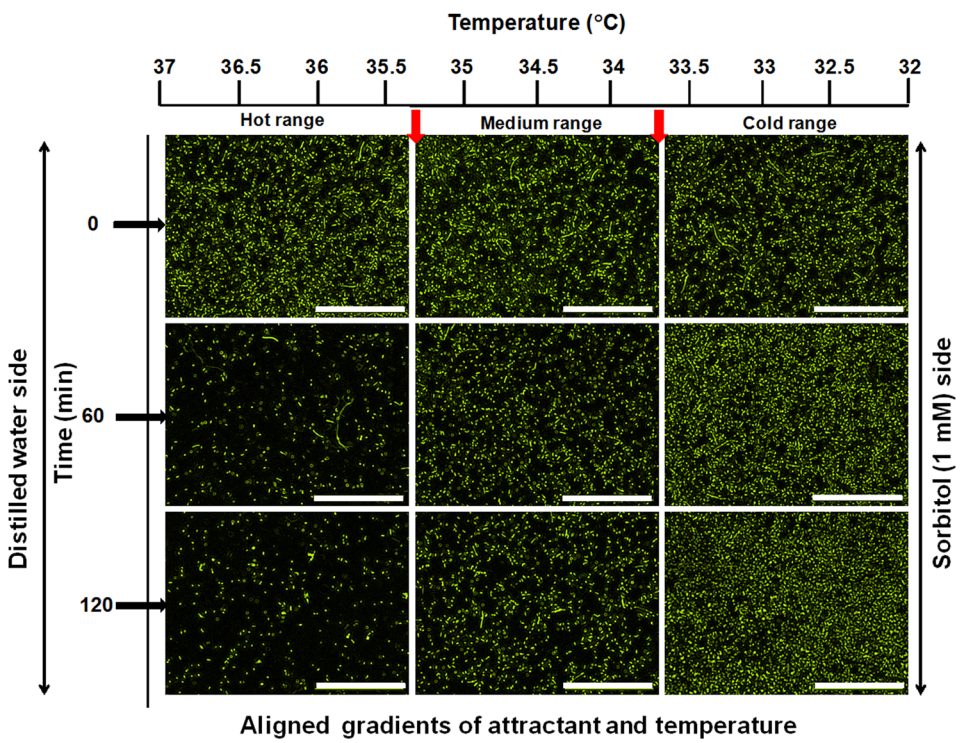

FIG. 6. Migration characteristics of *E. coli* cells under the influence of aligned gradients of temperature and chemoattractant. Each row shows photographs at specified times. All the experiments are repeated twice. Background corrections were done using "Image J software (National Institute of Health, Maryland, USA)." The scale bar is 100 μm.

are not complicated, the decision time required by the population is less and the migration rate is high from the very beginning (*cf.* Fig. 5(a)) compared to opposing gradients of sorbitol and temperature, simple sorbitol gradient, and simple temperature gradient.

The MR (*cf.* Fig. 3(b)) and AMC (*cf.* Fig. 5(a)) values for the present case indicate that unlike opposing gradients of temperature and chemical, the cell population reaches a steady state at around 30 min after the initiation of gradient generation and remains steady till the end of observations (2 h in this case). Comparison of aligned gradients, opposing gradients, simple sorbitol gradient, and thermal gradient (*cf.* Fig. 5(a)) reveals that temperature holds a major role in determining the rate of migration and also the percentage of cells that eventually migrate. However, in spite of temperature being a major factor, it is the nutrient gradient (over and above the existing chemical gradient) which is generated as an aftermath of the thermal gradient which seems to regulate the migration in all the cases with the attractant as the chemo–effector. The lag time of 30 min is observed before initiation of migration in the simple temperature gradient because it took the cells ∼30 min to encounter starvation, whereas in the case of opposing gradients of the attractant and temperature, the lag time is caused due to the existence of favourable conditions on both sides of the channel.

D. Cell migration under aligned gradients (inhibitor and temperature)

Under the influence of aligned gradients of temperature and inhibitor (i.e., 1 mM $NiSO_4$), the cells migrate towards the side with a lower concentration of the inhibitor and lower temperature (*cf.* Fig. 7). This behavior is naturally expected since at the opposite end, the conditions are highly unfavorable (i.e., high concentration of the inhibitor and higher temperature). Under such unfavorable conditions, the cells will eventually die and hence the population migrates towards the favorable side of the channel for survival. The MR (*cf.* Fig. 3(c)) and AMC (*cf.* Fig. 5(b)) values indicate that the population reaches the steady state at around 30 min from the generation of the gradient and remains steady till the end of observation.

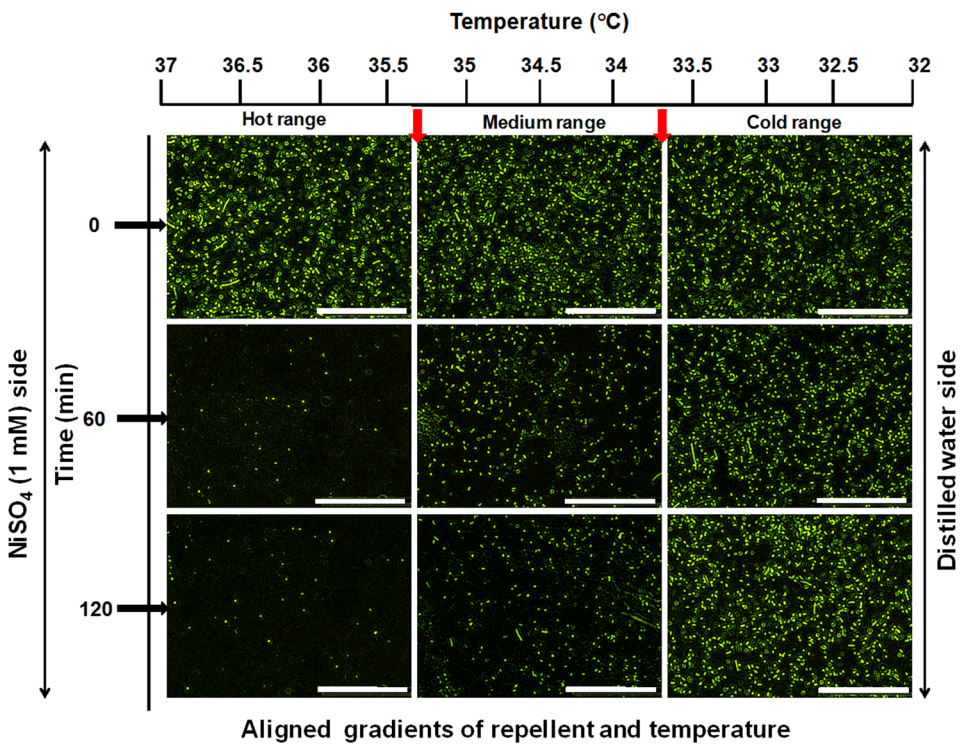

FIG. 7. Migration characteristics of *E. coli* cells under the influence of aligned gradients of temperature and chemical repellent. Each row shows photographs at specified times. All the experiments are repeated twice. Background corrections were done using "Image J software (National Institute of Health, Maryland, USA)." The scale bar is 100 μm.

The AMC for cells in the presence of aligned gradients of $NiSO_4$ and temperature is 0.75, and this value is higher compared to cell migration under the inhibitor gradient alone[11] but nearly similar to the temperature gradient alone[15] condition (*cf.* Fig. 5(b)). Since the chemo–effector is an inhibitor, the cell migration begins promptly once the gradients are established. In this scenario, cell migration has been initiated by the imposed inhibitor gradient unlike the competing gradients of the attractor and temperature where migration is initiated due to the nutrient gradient generated by the cells *in situ* by virtue of the thermal component. Hence, in the presence of an inhibitor, the role of the imposed gradients plays the major role and not the gradient locally formed by the cells after interacting with the existing gradients.

E. Cell migration under opposing gradients (inhibitor and temperature)

In the case of opposing gradients of temperature and inhibitor, the cells initially (the first 60 min after gradient generation) gather near the regions at the center of the channel, which is caused by the unfavorable conditions at both ends (*cf.* Fig. 8). However, after the initial clustering at the center up to 60 min, the population shows directional migration towards the side at higher temperature and lower inhibitor concentration.

The MR (*cf.* Fig. 3(d)) and AMC (*cf.* Fig. 5(b)) values indicate that the cells reach the steady state at around 1.5 h since gradient initiation and remain steady till the end of observation. Also, there is a reduction in the number of cells migrated from one half of the channel to the other half. This reduction in cell migration can be attributed to the state of confusion experienced by the cells in the presence of two unfavorable regions at opposite ends. During the initial period, the cells at higher temperature and low inhibitor concentration sense that the current location is unfavorable and begin to migrate away from it. The same event occurs at the opposite end of low temperature but a high inhibitor concentration. However, once the population gathers at the central region, the intercellular communication leads the population to reach o

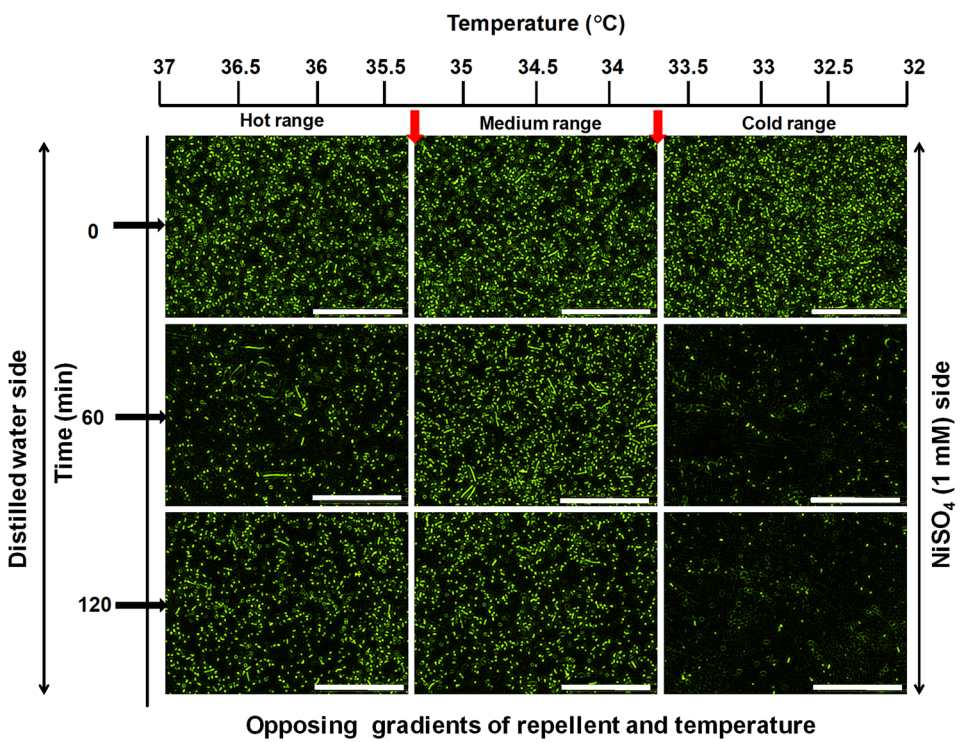

FIG. 8. Migration characteristics of *E. coli* cells under the influence of opposing gradients of temperature and chemical repellent. Each row shows photographs at specified times. All the experiments are repeated twice. Background corrections were done using "Image J software (National Institute of Health, Maryland, USA)." The scale bar is 100 μm.

consensus with respect to the degree of favorability and directed migration begins away from the region of higher inhibitor concentration.

It is for the case of opposing gradients of temperature and inhibitor that the population utilized the longest lag time (60 min) to begin unidirectional migration among all the studied combinations. It can be inferred that even though the thermal component also directs the migration behavior, finally it is the inhibitor which dominates and governs the direction of migration. Hence, in competing gradients of the inhibitor and temperature, the imposed chemical gradient dominates over the other effects.

IV. CONCLUSIONS

The effects of competing gradients (temperature and chemical) imposed on a population of *E. coli* DH5α cells in a stagnant and diffusion based microfluidic environment have been reported. The cells were subjected to opposing and aligned gradients of chemo–effectors (1 mM sorbitol as an attractant and 1 mM $NiSO_4$ as an inhibitor) and temperature. The cell migration characteristics under the influence of opposing and aligned thermal and chemical gradients were compared with respect to observations under the effects of simple chemical and thermal gradients. From the observations, it can be concluded that the cells exhibit preferential choice towards chemical gradients over the thermal gradient (within the temperature range of 32 °C to 37 °C, chosen to keep the system close to the physiological scenario) in a competing and stagnant fluidic microenvironment. Cell population migration is always observed to be initiated only due to the chemical gradient (either an imposed gradient or a nutrient gradient generated by the cells themselves due to thermal effects), both qualitatively and quantitatively. The temperature gradient is only observed to modulate the rate of migration in a competing gradient environment and does not play any major separate role in directing the migratory event. In the case of competing gradients of the attractant and temperature, the imposed thermal gradient is observed to have a more improved role of importance since the governing nutrient gradient is formed due to the higher metabolism rate, which in turn is a manifestation of higher temperature. In the case of competing gradients of the inhibitor and temperature, the imposed chemical gradient holds the absolute decisive role in the final migration behavior. The present observations hold tremendous potential in clinical therapies involving radiation, chemical, and thermal techniques involving diseased cells which are prone to migration, similar to bacterial populations. From such observations, it can be proposed that for obtaining such therapies based on diseased cell necrosis, an efficient protocol to prevent migration would be to inject an attractant at the hyperthermia/radiotherapy/chemotherapy location while maintaining a preventive barrier of inhibitor drugs around the diseased tissue.

SUPPLEMENTARY MATERIAL

See supplementary material for the total number of cells in the channel under investigation under the key conditions of the experiment.

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