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We report a nematode electrotactic-response valve (NERV) to control the locomotion of *Caenorhabditis elegans* (*C. elegans*) in microfluidic devices. This nonmechanical, unidirectional valve is based on creating a confined region of lateral electric field that is switchable and reversible. We observed that *C. elegans* do not prefer to pass through this region if the field lines are incident to its forward movement. Upon reaching the boundary of the NERV, the incident worms partially penetrate the field region, pull back, and turn around. The NERV is tested on three *C. elegans* mutants: wild-type (N2), *lev-8*, and *acr-16*. © *2011 American Institute of Physics*. [doi:10.1063/1.3570629]

Modern materials and structures fabricated using soft lithography are beginning to impact several areas in microbiology and medicine.[1] These include microscale and nanoscale devices for studying the growth, behavior and population dynamics of cells, bacteria, and microorganisms.[2–6] An inevitable operation in integrated biomicrofluidic devices is the controlled loading, trapping, and unloading of biological samples.[1–3] Microfluidic valves, particularly pinch-type and pneumatic valves, have gained popularity for controlling the flow of liquids and suspended particles.[7] In the pinch-type microvalves, actuation is achieved by pressing or releasing a flexible silicon tube[7,8] or glass beads embedded in pockets of a rigid polydimethylsloxane (PDMS) layer.[9] In pneumatic microvalves, two sets of PDMS microchannels, separated by a thin PDMS membrane, are bonded perpendicular to each other.[10] Pressure applied through the top channel (control layer) collapses the PDMS membrane and controls flow in the bottom channel (flow layer) where the experiment is performed.[10]

With recent advances in whole-animal phenotyping and screening techniques,[11–19] pneumatic valves have been adopted in integrated microfluidic systems to assist with the controlled loading, immobilization, exposure and release of worms in individual chambers.[19–22] The model organism, *Caenorhabditis elegans* (*C. elegans)*, is an ideal candidate for on-chip behavioral studies because of its microscopic size, fully mapped genome, ease of culture, availability of mutants, short lifespan, and relevance to human diseases.[19] The advantages of monolithic pneumatic valves in microfluidic worm assays is their negligible dead volume, complete sealing, linearity with applied pressure, small size, and dense integration.[10] However, monolithic pneumatic valves have some fundamental limitations. Multistep fabrication or bonding steps are needed, these valves are permanent structures within the device and these valves cannot be used to control *C. elegans* in microfluidic assays having nonfluidic (e.g., agarose gel[23] or saturated particulate[24]) media.

We hypothesized that a nonmechanical, virtual valve could be created on-chip that could be sensed by *C. elegans*. This valve would be electrically activated and would contain no moving parts. Our proposed solution stems from previous work on *C. elegans* electrotaxis[25–28] where it was shown that nematodes migrate to the negative electrode in an electric field (4 V/cm$<$E<10 V/cm for L4 stage *C. elegans*).[26] Switching the polarity of the direct current (dc) electric field caused the worms to turn around[25,26] whereas applying an ac signal (1 Hz–3 kHz) immobilized them momentarily (~20 s).[27] In previous electrotaxis experiments, locomotion response was observed while having the entire body of the *C. elegans* within an electric field. Here, we study nematode locomotion when the worm, initially contained in a microfludic area having no significant electric field, is incident upon a region containing a lateral field (E=33.33 V/cm). *C. elegans* moving through an interface where the field lines opposed their direction of movement [i.e., closed interface in Fig. 1(a)] retracted and turned around; while worms moving through an interface where the field lines were in the same direction as their movement [i.e., open interface in Fig. 1(a)] continued their progress unaffected.

Based on the above observations, we present a nematode electrotactic-response valve (NERV) to block or allow the movement of *C. elegans* in sections of a microchannel. The schematic of the valve is depicted in Fig. 1(b). The virtual valve is formed by creating parallel electric field lines within a finite section of a central channel (length=1 cm, width =350 μm, and height=80 μm). Standard soft lithography is used to fabricate the test device.[1] To create parallel electric field lines in a localized region, the central channel is microfluidically connected to a pair of electrode ports through side channels (length=500 μm, width=25 μm, and height =80 μm). The side channels are narrow enough to physically restrict the *C. elegans* from entering them. Each electrode port within a pair is placed on either side of the central channel and is laterally separated by a finite distance (d =2 mm). In a generalized case, we expect the size and shape of the central/side channels and electrode ports can be adapted for any application. Further, the microfluidic device can be surrounded by any reasonable, even number of side channels and electrode ports to allow the user to create a NERV at any location within their system.

The device is filled with a 0.8% w/v mixture of agarose in M9 buffer. The measured resistivity of the medium is ~0.98 Ω m. Platinum electrodes are inserted into two electrode ports and a dc voltage (V=10 V) is applied (e.g.,

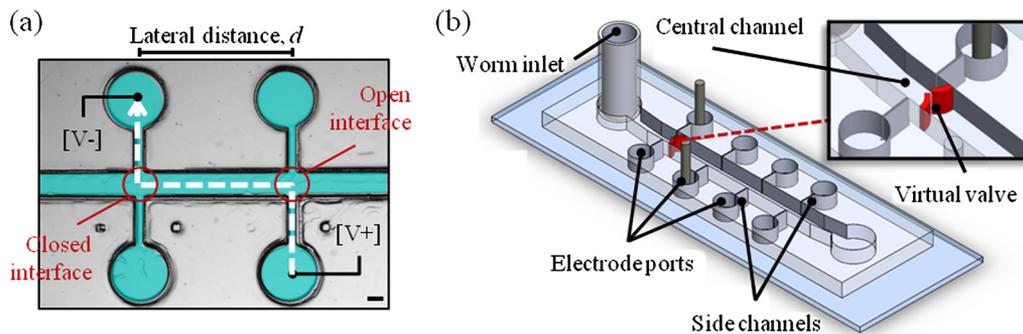[a)]Electronic mail: pandey@iastate.edu.

FIG. 1. (Color online) (a) Snapshot of the device's top-view illustrating the electrical field lines (white arrow) and interfaces. Electric field simulations show the expected field profile within the device. Small fringing effects near the interfaces can be seen with 90% of the field dissipating within 200 μm. Scale bar=175 μm. (b) Schematic of the microfluidic device and nematode NERV for controlling the locomotion of *C. elegans*.[29]

cathode=-10 V and anode=0 V). This creates an electric field parallel to the walls of the central channel that is primarily confined within the lateral distance d between the two electrode ports [Fig. 1(a)]. *C. elegans* are cultured at room temperature on nematode growth media plates seeded with *Escherichia coli* OP50. Single or multiple L4/young adults are hand-picked with a sterile platinum wire and placed into the input port. After a brief scouting time (2–4 min), the worms enter the central channel. Their sinusoidal movements are recorded with a high-speed Leica MZ16 imaging system and forward velocities are measured ($v_{in\ N2}$ =238.8 $\pm$ 17.2 μm/s, $v_{in\ lev-8}$=227.6 $\pm$ 15.4 μm/s,   and $v_{in\ acr-16}$=242.3 $\pm$ 28.9 μm/s; n=10 for each strain). Prior to reaching the NERV, there is no noticeable change in locomotion directionality which indicates that the worms did not yet sense the electrical field. Upon meeting electric field lines which are incident to its forward movement [i.e., the closed interface; Fig. 1(a)], the worm partially penetrates its body [defined as the *penetration depth* (PD)] into the field region (Fig. 2). Thereafter, the worm turns its body around and continues moving in the opposite direction. Thus a virtual wall is created to block the worm's forward movement to a given section of the device. The forward velocities measured after directional change ($v_{out\ N2}$ =245.9 $\pm$ 29.9 μm/s, $v_{out\ lev-8}$=235.8 $\pm$ 27.7 μm/s,   and $v_{out\ acr-16}$=222.5 $\pm$ 19.3 μm/s; n=10 for each strain) show no significant change (p<0.05; ANOVA test) compared to

those measured before encountering the NERV. Further, the observed worms survive during the course of the experiment ($\sim$1 h) showing that the electric field does not cause any noticeable adverse effects on the worms. This result is in accordance with current literature which shows that the worms' velocity and survivability is unaltered upon the application of an electric field.[26] By simply switching off the dc voltage, the NERV is removed and the incident worms continue their forward movement through the region. Turning on the dc voltage recreates the NERV, again blocking incident worms (at the closed interface). Switching the polarity of the applied voltage reverses the direction of the valve. The field lines, now in the same direction as the worm's forward movement, allow the worms to pass through the open interface, but blocks them from returning through the closed interface. The same experiment is conducted with multiple (6–10) *C. elegans* in the central channel, showing the NERV is capable of controlling more than one worm at a time.

The NERV is tested on L4 stage/young adult *C. elegans* [N2 and two mutants (ZZ15 *lev-8* and JER426 *acr-16*)]. Figure 3(a) shows the percentage of worms that were blocked at the closed interface. For each strain, the average of n=21 worms for each of three repeats (i.e., N=1, N=2, and N=3) is shown. With an applied field of 33.33 V/cm, 100% of the wild-type N2, 95 $\pm$ 5% *acr-16* mutant, and 100% of the *lev-8* mutants are blocked. We further observed that the NERV does not block early stage *C. elegans* (L1–L3 stages) which pass through the electric field region even at higher electric fields (up to 45 V/cm). This is because early stage *C. elegans* have not yet developed their amphid neurons which are responsible for electrostatic behavior of nematodes.[25,26]

As mentioned, an incident worm does not immediately turn around upon encountering the opposing field lines (at the closed interface) but probes up to a penetration depth before reversing its movement [Fig. 3(b)]. For each strain, the vertical scatter plot shows the depth for all worms studied as well as the overall mean values (n=21; N=3 for each strain). The effectiveness of NERV in blocking worm's forward movement is reduced if the lateral distance d between the electrode ports is comparable or smaller than the average penetration depth for the *C. elegans* under study. Thus the separation between a pair of electrode ports should be kept larger than the penetration depth for the specific strain being studied (for the three strains studied here no significant difference (p value >0.05) is found between the average PDs).

In conclusion, the NERV device presented here is based on an interesting finding that *C. elegans* (moving in a region
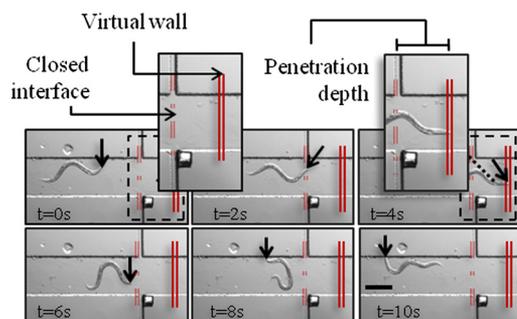

FIG. 2. (Color online) Time-lapsed images of single N2 *C. elegans* encountering the closed interface of the NERV. A worm, moving from an area of little or no electric field, is incident on the closed interface of the NERV. After probing the lateral E-field region, the worm penetrates to some depth, retracts and turns around; thus a virtual wall is formed at the closed interface. Scale bar=150 μm. (enhanced online). [URL: http://dx.doi.org/10.1063/1.3570629.1][URL: http://dx.doi.org/10.1063/1.3570629.2][URL: http://dx.doi.org/10.1063/1.3570629.3]

(a)

(b)
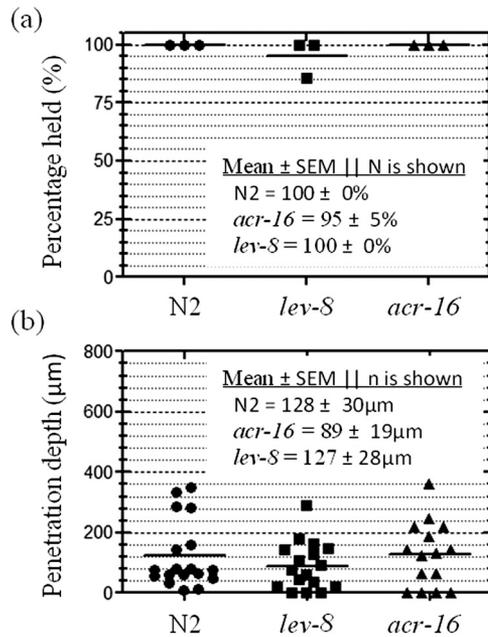

FIG. 3. (a) Percentage of *C. elegans* (n=21) blocked at the closed interface for each of the three days (i.e., N=1, N=2, and N=3) [mean± standard error of the mean (SEM)]. No significant difference (p>0.5) was found among the strains. (b) Penetration depth into the closed interface of the NERV for the three strains (mean± SEM). No significant difference (p >0.05) was found.

of no significant electric field) do not prefer to move through an interface with electric field lines that are incident to its forward movement. The NERV can block (at the closed interface) or allow (at the open interface) a *C. elegans's* forward movement in a switchable and reversible manner. This method can be used to prevent or allow nematodes from entering/exiting a microfluidic chamber or to entrap it within a certain microfluidic region by using two back-to-back valves. Unlike pressure-driven valves, there are no movable parts and no additional fabrication or bonding steps are needed. The applicability of NERVs in controlling *C. elegans* locomotion is particularly useful in worm chips having nonfluidic, conducting media (e.g., agarose gel). Since the proposed valves are electrically activated, we anticipate the incorporation of these devices in large-scale, integrated be-

havioral assays where multiple valves are individually controlled by an automated computer program.

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[29] See supplementary material at http://dx.doi.org/10.1063/1.3570629 for electric field simulations of the field profile within the device.