

A microfluidic device for efficient chemical testing using *Caenorhabditis elegans*

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Abstract The nematode worm *Caenorhabditis elegans* has been employed as a popular model organism in many fields of biological research. In this paper, we present a microfluidic device for facilitating chemical testing using *C. elegans*. For testing chemicals on chip, the device houses single nematodes in microfluidic chambers and precisely adjusts the chamber's chemical environment during experiments. Eight nematodes can be readily loaded into the chambers through separate loading channels in a quick and gentle manner. In addition, a custom-made software with a graphic user interface is also created for quantitative analysis of locomotion parameters (swimming frequency and bend amplitude) of the nematodes in response to chemical stimuli, thus greatly enhancing the efficiency of data collection. We perform proof-of-concept experiments using two chemicals, zinc ion (Zn^{2+}) and glucose, to demonstrate the effectiveness of the microfluidic device.

Keywords Microfluidics · Chemical testing · *C. elegans* · Image processing · Behavior studies · Locomotion

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1 Introduction

The nematode worm *Caenorhabditis elegans* has been employed as a popular model organism in many fields of biological research such as neuroscience, genetics, development, metabolism, and aging (Brenner 1974, Bargmann et al. 1993, Wolkow et al. 2000, Yang and Hekimi 2010, Lee et al. 2009, Wen et al. 2012). The substantial overlaps between *C. elegans* and human in genes and biochemical pathways, together with its simplicity, short lifespan, easy and inexpensive cultivation, and transparent body, make it an attractive model for *in-vivo* chemical testing and toxicity studies (Hulme and Whitesides 2011, Peredney and Williams 2000, Williams and Dusenbery 1988, Boyd et al. 2003, Wang and Xing 2008). Locomotion parameters of *C. elegans* can be used as simple yet effective indicators for assessing biological effects of chemicals/drugs on nematodes and for identifying their target behaviors and associated biochemical pathways. Conventional *C. elegans* based chemical testing is typically performed manually on petri dishes or multi-well plates (Kerr et al. 2000). Although this method is meaningful and informative, it cannot maintain a consistent exposure environment due to the chemical diffusion/evaporation and the influence from ambient environment. In addition, relatively large volumes (e.g., tens to hundreds of microliters) of chemical solutions are needed for nematode stimulation in a single micro-well or petri dish, which increases the experimental cost and may be impractical for precious drugs at their early-stage development (Kaletta and Hengartner 2006; O'Reilly et al. 2014).

Recently, microfluidic devices have become increasingly important for *C. elegans*-based chemical testing because of their unique characteristics and capabilities such as size

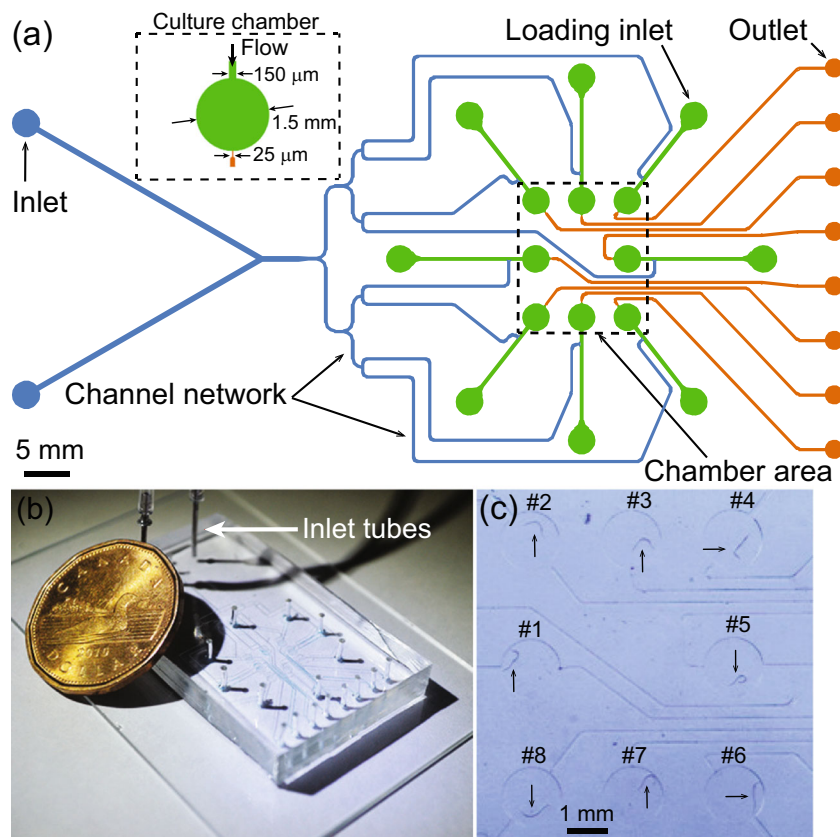
matching, low chemical consumption, precise adjustment of chemical environment, and highly parallel fluid manipulation (Chronis 2010; San-Miguel and Lu 2013; Albrecht and Bargmann 2011; Lockery et al. 2012; Chung et al. 2011; Kopito and Levine 2014; Hulme et al. 2010; Shi et al. 2010; Rohde et al. 2007). There have been several microfluidic devices reported in the literature for culture and observation of *C. elegans*. A droplet-based assay allows a homogeneous chemical environment for nematodes trapped in on-chip generated droplets; however, the presence of surfactant in the culture environment (used to maintain the droplets in oil) and the accumulation of nematode's metabolic waste in chemical environment of the enclosed sub-microliter droplets may bias the nematode's pure responses to chemical stimulation (Clausell-Tormos et al. 2008; Shi et al. 2010). For assessing a number of isolated nematodes in continuous flow environments, Chung et al. reported a microfluidic chamber array housing individual nematodes for chemical screening (Chung et al. 2011). In this design, the loading process induces mechanical stresses (immobilization and pressurization) on nematodes, which may impact their response to applied chemicals.

Hulme et al. also developed a microfluidic device with the ability to culture 16 nematodes in separate chambers (Hulme et al. 2010). This device used 16 manually-operated screw valves to control fluid flows and guide single

nematodes to isolated chambers, but the fabrication and integration of these screw valves are tedious and highly skill dependent. Carr et al. took advantage of nematode electro taxis to guide the motion of *C. elegans* in microfluidic channels and study the nematode's transient and post-exposure responses to drugs. This method, though effective, induces additional electrical stimulation to the nematodes which may affect the drug testing results (Carr et al. 2011). An automated high-throughput microfluidic system is also available in the literature for *C. elegans*-based chemical testing (Rohde et al. 2007). However, this system require complicated fluid control and device operations, which could be too difficult to operate by biologists or other users who are not familiar with the principle and operation of microfluidic devices.

In this paper, we present an easy-to-use microfluidic device for facilitating *C. elegans* based chemical testing. Our design allows eight individual nematodes to be readily loaded into microfluidic chambers through separate inlets in a quick and gentle manner without any complicated operations. In addition, we also develop a Matlab software with graphic user interface (GUI) for controlling fluid flows of the microfluidic device and quantifying the locomotion parameters of the nematodes under chemical perfusion, thus greatly facilitating the device operation and enhancing the data collection efficiency. As proof-of-concept experiments,

Fig. 1 Microfluidic device for *C. elegans*-based chemical testing. **a** Schematic layout of the microfluidic device. **b** Photograph of the fabricated microfluidic device. **c** Microscopic photograph of the nematodes loaded into the eight microchambers



we apply the device to testing the locomotion responses of *C. elegans* to different concentrations of zinc ion (Zn^{2+}) in the culture medium and glucose in the nematode diet.

2 Experimental methods

2.1 Design and fabrication of the microfluidic device

Figure 1a shows the schematic design of the microfluidic device. The layout of microfluidic channels includes three sections (colored in Fig. 1a) with different functions. (i) An inlet channel network (blue in Fig. 1a) connecting two device inlets with eight nematode culture chambers for alternating the chemical solution or culture buffer supplied to the loaded nematodes. (ii) Eight circular chambers (1.5 mm in diameter) with separate nematode loading channels (green in Fig. 1a). The diameter of these chambers is large enough to accommodate a N2 strain L4 *C. elegans* ($\sim 650 \mu\text{m}$ in length) and the height of these chambers ($\sim 50 \mu\text{m}$) is also large enough to guarantee free swimming of a N2 strain L4 *C. elegans* ($34.2 \pm 2.5 \mu\text{m}$ in diameter, $n = 8$) (Maguire et al. 2011). To prevent a nematode exiting a culture chamber under the perfusion flow, we added a narrow part of channel ($25 \mu\text{m}$ wide, $100 \mu\text{m}$ long) at the exit of each culture chamber (inset of Fig. 1a). During chemical perfusion experiments, the nematodes in different chambers are kept from escaping into the upstream loading/perfusion channels by the continuous perfusion flow of chemical solution. The nematode loading channel of each chamber has a separate loading inlet, through which a single nematode can be manually delivered into the chamber. In many previous designs (Hulme et al. 2010; Chung et al. 2011), multiple nematodes were loaded through a single device inlet, and there was always certain chance that more than one nematodes entering the same culture chamber. The separate nematode loading mechanism employed in our design eliminates the opportunity of multiple nematodes entering

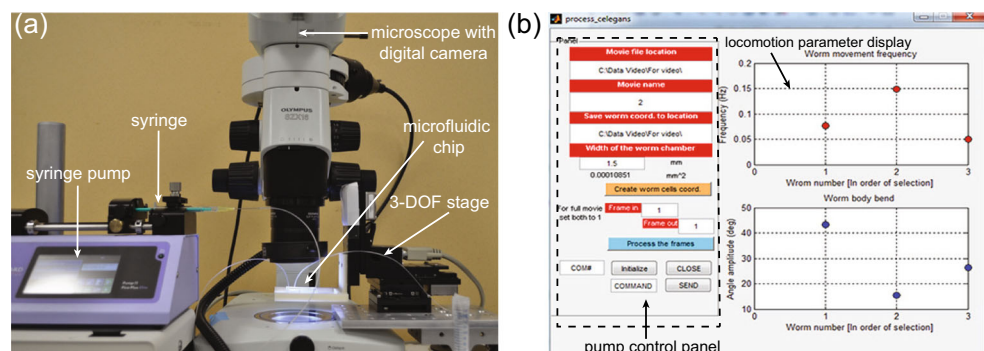
into the same chamber and ensures that each chamber is occupied by only one nematode. The detailed procedure for nematode loading will be described in Section 2.4. (iii) Eight outlet channels (red in Fig. 1a) for collecting waste solutions from the chambers.

Standard soft lithography was carried to fabricate the microfluidic device using polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning) (Xia and Whitesides 1998). Briefly, SU-8 2050 photoresist (Microchem) was spin-coated on a silicon wafer with a thickness of $50 \mu\text{m}$. The designed feature shown in Fig. 1a was then transferred to the photoresist layer via photolithography for generating a SU-8 mold. The mold was treated with tridecafluoro 1,1,2,2-tetrahydrooctyl-1-trichlorosilan via chemical vapor deposition to prevent the adhesion of molded PDMS to the mold surface. To form a 5 mm thick PDMS device, a 12 g PDMS mixture (10:1 ratio) was poured into a 100 mm petri dish with the SU-8 mold, and degassed for two hours. After fully cured at 80°C for another two hours, the PDMS piece with microfluidic channels was peeled off from the mold. The PDMS piece was then punched by a 1.5 mm biopsy punch to form inlet/outlet holes, and finally bonded with a $50.8 \text{ mm} \times 76.2 \text{ mm}$ glass slide using oxygen plasma treatment. Figure 1b shows a fabricated microfluidic device loaded with blue dye.

2.2 Experimental setup

To facilitate the locomotion analysis of on-chip cultured *C. elegans*, the experimental system should be able to scan all the chambers under a microscope and record videos of nematodes for off-line analysis of nematodes locomotion. Figure 2a shows the system setup. The microfluidic device is mounted on a three-degree-of-freedom (3-DOF) motorized stage (MP-285, Sutter) under a stereo microscope (SZX-16, Olympus). The motorized stage carries the device and scans the nematodes cultured in different chambers. A digital camera (A601f, Basler) is mounted on the microscope for video recording. A microfluidic syringe

Fig. 2 Experimental system setup. **a** Photograph of the experimental system. **b** Graphic user interface for pump control and *C. elegans* locomotion analysis



pump (Pump 11 Elite, Harvard Apparatus), controlled by a custom-made Matlab software, is utilized to regulate the flow of the chemical solution into microfluidic device. An image processing algorithm (Hulme et al. 2010) is integrated into the Matlab software with a graphic user interface (shown in Fig. 2b) to track the nematode's locomotion and extract two major locomotion parameters, swimming frequency and body angle amplitude, which will be defined in Section 2.5.

2.3 Preparation of *C. Elegans*

The wild-type N2 strain of *C. elegans* was cultivated at 20 °C following an established method (Brenner 1974). For the chemical testing experiments, nematodes were synchronized to the L4 stage of development via the following protocol (Stiernagle 2006). Briefly, 10-15 gravid adult nematodes were first transferred to a clean nematode growth media (NGM) agar plate seeded with OP50 strain of *E. coli* using a worm pick (made from a Pasteur pipette and a titanium wire). The nematodes were then left in the plate for 2 h allowing the collection of a large population of nematode eggs. The adult nematodes were then removed from the plate and the eggs with maximum two-hour difference in development were left for hatching at 20 °C. After 48 h, the nematodes reaching the L4 stage were collected for experiments.

2.4 Nematode loading procedure

The easy-to-operate nematode loading mechanism leads to the major advantage of our design over others. To begin a loading process, a polyethylene tube with a 1 ml syringe was used to fill the entire device with M9 medium. Individual nematodes were then transferred into separate loading inlets of the microfluidic device using a worm pick. After that, a M9-medium-filled syringe was connected to a loading inlet via a polyethylene tube, and the syringe was manually pushed to induce enough fluid flow which carried the nematode from the inlet into its respective chamber. The same operation procedure was repeated eight times to load each chamber with one nematode. The whole operation process can be completed within five minutes by a user who were experienced with general nematode handling but only trained for two hours on usage of our device. Figure 1c shows the eight microfluidic chambers loaded with single nematodes (arrow-labeled).

2.5 Visual analysis of nematode locomotion

Color videos of swimming nematodes during chemical perfusion were captured at a frame rate of 20 fps and an image

resolution of 656×488 pixels. The captured videos were processed off-line using the Matlab software. To facilitate the visual analysis of nematode locomotion in each chamber, a square region of interest (ROI), approximately circumscribing the nematode-containing chamber, was firstly selected manually on the computer screen. The color ROI image was then converted into a gray-scale image (Fig. 3a). To remove the influence of the chamber edge and the region outside the chamber on image processing, the nematode-containing region was further selected to be the circular area which is concentric with the original square ROI and 1.425 mm in diameter (95 % of the chamber diameter), and the pixels outside the selected circular area were assigned with a fixed gray-scale value equal to the average gray-scale value of the four corner pixels of the original square ROI. Histogram equalization was also performed on the circular area to form a better contrast between the nematode and the background (Fig. 3b). The image in Fig. 3b was binarized via fixed-value thresholding. In order to eliminate noise points in the binarized image (Fig. 3c), a so-called 'flood-fill' algorithm was used. This algorithm produced a matrix of the image where all the pixels were labeled by a number and all the pixels that corresponded to the same group of connected pixels received the same number. Then, the area of each group of connected pixels was calculated, the index of the largest group of connected pixels was retained, and other groups of connected pixels were removed with the assumption that the nematode is the biggest group of connected pixels in the image. The resultant image is shown in Fig. 3d.

To extract the two locomotion parameters (swimming frequency and body angle amplitude), the points of nematode head, tail, and body centroid need to be identified from the nematode area (Fig. 3d). The centroid was readily calculated from the nematode area using a Matlab built-in function. In order to find the two end points, the nematode body was firstly reduced to a single spline (white line in Fig. 3e) and the corresponding pixel indices of all the points on the spline were found. Then, the two ends (head and tail) were easily identified since they were the only two points with one neighbor point. We calculated the body angle to be the one formed by the head point, the centroid, and the tail point of the nematode (Fig. 3e). There was one exemption occurring when the nematode body coiled and formed a closed loop, for which the image processing algorithm was not able to find the head and tail points. As a *C. elegans* coils is not an endpoint in our experiments, we simply ignored the image frames containing a coiled nematode. However, it is worth noting that the nematode curling frequency can also be easily extracted by simply modification of the current algorithm, which actually is already capable of detecting the nematode coil.

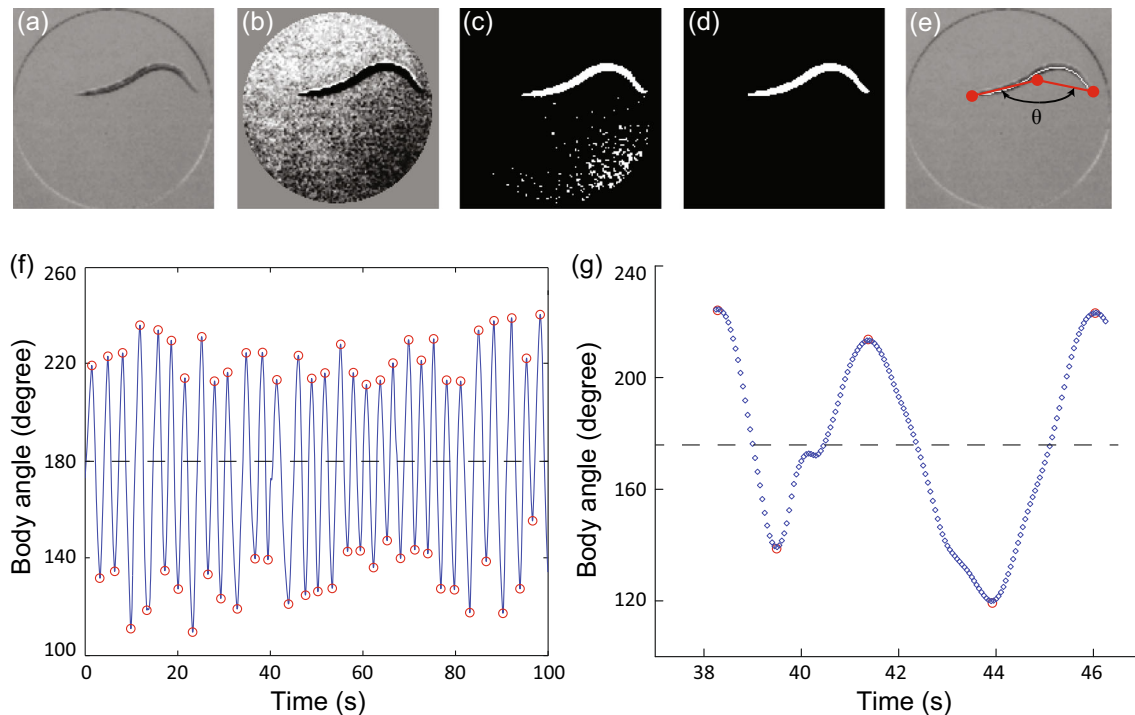


Fig. 3 Data extraction procedures for locomotion analysis of *C. elegans*. **a** The grayscale image converted from an original color image. **b** To facilitate image binarization, histogram equalization was performed to enhance the contrast between the nematode and the background. **c** The nematode was clearly identified in this binary image. **d** The noise

points were eliminated from **c** via the ‘flood-fill’ operation. **e** The head, tail and centroid points of the nematode were identified for calculating the body angle (θ). **f** Body angle oscillation as a function of time. **g** Blow-up of body angle oscillation as a function of time in bending two cycles

Figure 3f shows an example set of the body angle data of a nematode during 100 s swimming. One can see that the body angle oscillates around 180° , and one oscillation corresponds to a body stroke: two successive bending of the nematode body along opposite directions. By counting the number of body angle oscillations (or body strokes) around 180° per unit time, the swimming frequency was determined. The body angle amplitude was calculated as the average of the peak and valley amplitudes of the body angle waveform, with respect to 180° , within two minutes. This amplitude value reflects the strength of the nematode swimming, and is correlated with contraction forces of striated muscle in the nematode’s body wall (Nahabedian et al. 2012).

2.6 Statistical analysis

All statistical analyses were performed using SPSS 21.0 software (IBM Corporation, Somers, NY). Unpaired, two-tail Student’s *t*-test was used for comparison between chemical treated groups and control group. One-way ANOVA test was used for comparison among multiple control groups for quantification of device-to-device variation, and the post-hoc comparisons were performed using the Tukey method. Graphical results were reported as Tukey boxplots.

3 Results and discussion

3.1 Temporal characteristics of chemical delivery

Using our microfluidic device, two chemical solutions can be alternately perfused into individual chambers with short transition time, which could be beneficial to studies on the nematode’s response to different chemical environments. Without additional tedious manual operations to deliver chemicals, we employed two computer-controlled syringe pumps, each of which held a solution-filled syringe to infuse a solution into one device inlet at a pre-defined constant flow rate. The switch of chemical solutions delivered to the chambers was then achieved by simply alternating infusions of the two syringe pumps.

We tested the transition time from one solution to another by alternating the perfusion of a colored dye and a clear M9 buffer and measuring the change in color intensity of the solution in each chamber. We define the chemical transition time of each chamber as the time required for the normalized intensity of the solution in the chamber to rise from 2 % to 98 % of its steady value or fall from 98 % to 2 %. Obviously, this transition time is a function of the infusion flow rate of the syringe pump. Figure 4 shows

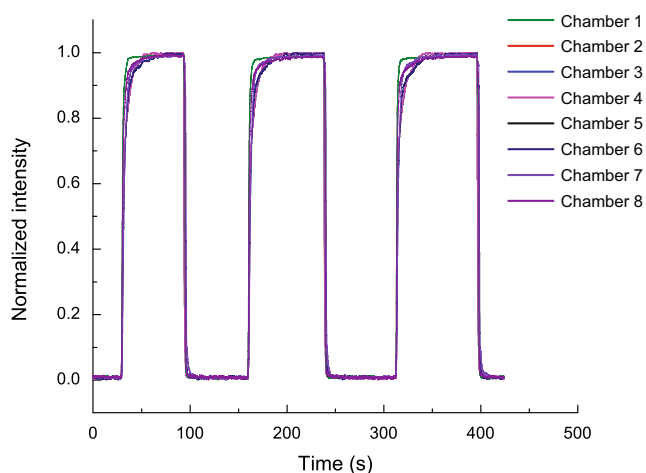


Fig. 4 Stepwise chemical delivery in eight chambers as a function of time

the normalized intensity of the solution in eight chambers during multiple alternations between M9 buffer and the colored dye (flow rate: 1000 $\mu\text{l/h}$), and the transition time of the eight chambers is in the range of 6–40 s. The standard deviation of steady-state chemical concentrations in the eight chambers is less than 0.7 %, demonstrating a constant chemical environment for all the on-chip cultured nematodes. The variation in transition time among different devices may also alter the nematode's swimming parameters and thereby we further utilized three more different devices to verify this variation (Fig. S1). The results turn out all four devices (including the one shown in Fig. 4) are capable of completely renew the chamber environment with new solutions within 40 s (Table S2). The variation in the chemical transition time for different chambers is due to the different lengths of channels from the inlets to different chambers. As typical nematode perfusion time in chemical testing ranges from minutes to hours, this relatively short variation in chemical transition time is less likely to induce significant device-to-device variations of the final biological results (Chung et al. 2011).

We have demonstrated that our device is capable of delivering chemicals to individual chambers with short transition time and maintaining the chemical concentration at a constant level. Here, we would also point out a potential issue of the device material, PDMS, for chemical-testing applications. PDMS is well-known for its ability to absorb small hydrophobic molecules due to its network polymer structure, which could be problematic in experiments of testing certain hydrophobic chemical/drug molecules (Toepke and Beebe 2006; Wang et al. 2012). This problem can be overcome by chemically modifying

inner surfaces of the PDMS channels or using alternative materials for device construction. Coating the inner surfaces of PDMS channels with parylene or paraffin wax was found to be effective for reducing molecule absorption (Sasaki et al. 2010, Ren et al. 2010). Devices made from the mixture of PDMS and polyethylene oxide (PEO) shows a long-term hydrophilic surface and protein absorption is thus significantly reduced (Yao and Fang 2012; Wong and Ho 2009). While it is possible to reduce the PDMS absorption for certain molecules, in cases where more strict chemical testing experiments are needed, using alternative materials for device fabrication may prove to be better options. The alternative materials such as polyurethane (PU) and polystyrene (PS) show little absorption problem and have been widely used as biomaterial substrates (Berthier et al. 2012). Thus, these materials can also be utilized for making our microfluidic devices via various molding/embossing processes (Domansky et al. 2013, Wu et al. 2012, Young et al. 2011).

3.2 Zinc ion induced defects in nematode locomotion

For both chemical testing experiments, chemical solutions were perfused into the microfluidic device at a flow rate of 400 $\mu\text{l/h}$ to maintain a constant chamber environment. We experimentally demonstrated that this flow rate did not induce significant changes in locomotion parameters of N2 nematodes. Since different groups of worms are analyzed on different devices, the device-to-device variation in worm and device handling may alter the final measured nematode swimming parameters. To quantify the device-to-device variation, we conducted four groups of control experiments with control solution (pure M9 buffer) following the experimental procedure used in both chemical testing experiments (each with $n = 8$). No statistical difference was revealed in comparisons of data sets from different devices for both measured nematode swimming parameters (Fig. S2). It is also worth noting that the experiment environment was maintained at room temperature (21 °C) and a cool-light illumination was used for microscopic imaging to minimize the temperature variation.

To demonstrate the capability of our microfluidic device for *C. elegans* based chemical testing, we used Zn^{2+} as a model chemical (Wang et al. 2007). Zinc is one of the most prevalent metals in the environment, and the exposure of *C. elegans* to Zn^{2+} leads to multiple biological defects affecting lifespan, reproduction, chemotaxis plasticity, and locomotion behavior (Wang et al. 2007). For the locomotion behavior, the exposure to Zn^{2+} may affect the nematode's functions of neurons and muscle cells, which are important

for adopting correct cell fate, forming appropriate cellular contacts, and assembling repertoire of signaling proteins into presynaptic and postsynaptic structures; these effects could eventually result in defects in the nematode's locomotion (Loria et al. 2004; Wang et al. 2007).

Solutions with two different concentrations of Zn^{2+} (0.3 mM and 0.6 mM) were prepared by dissolving zinc nitrate salt in M9 buffer. We loaded the microfluidic device with N2 nematodes at their L4 stage. For the two Zn^{2+} solutions and the control solution (pure M9 buffer), we perfused 16 nematodes with each solution for one hour and took a two-minute video for each nematode right after the chemical

exposure. During the one-hour perfusion, no food was supplied to the nematodes. A previous study has shown that the lack of food does not impact the locomotion behavior of nematodes within the first four hours (Boyd et al. 2003). We analyzed the videos using the Matlab software and quantified the two locomotion parameters. As shown in Fig. 5b, when exposed to Zn^{2+} solutions (0.3 mM and 0.6 mM), the nematodes swam significantly slower than the control group ($p < 0.001$), and the frequency spread in these two groups is smaller than the control group, pointing to a smaller variability of nematodes' swimming frequency; however, no significant difference was

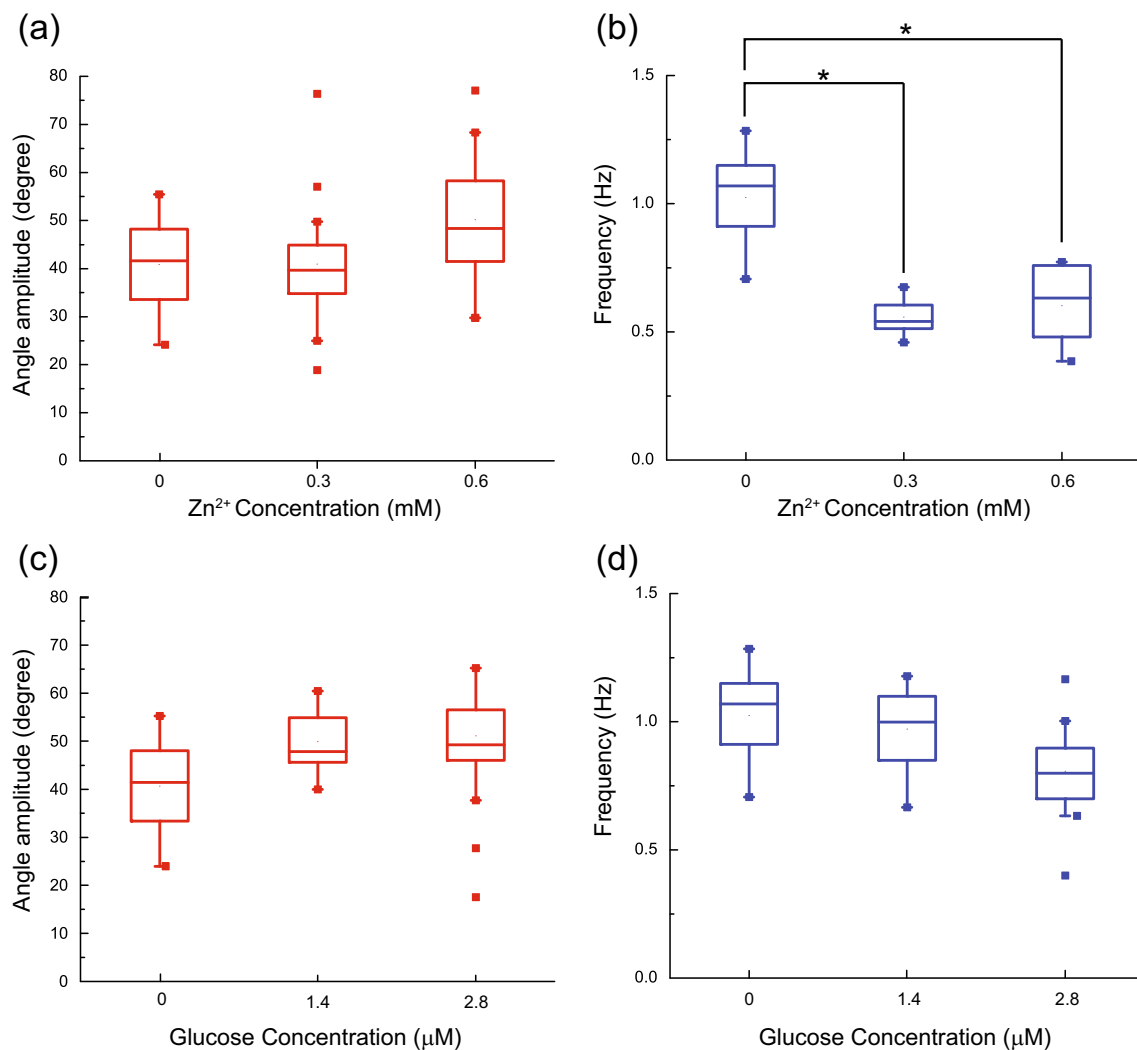


Fig. 5 Box plot of locomotion changes of *C. elegans* induced by chemical stimuli (Tukey-style whiskers). **a** The angle amplitude of *C. elegans* after one-hour zinc ion exposure. **b** The swimming frequency of *C. elegans* after one-hour zinc ion exposure. **c** The angle amplitude

of *C. elegans* after 48-hour glucose exposure. **d** The swimming frequency of *C. elegans* after 48-hour glucose exposure. The sample sizes are $n = 16$ (two devices) for all the chemical tested groups, and $n = 32$ (four devices) for the control groups

observed between the two groups of Zn^{2+} treated nematodes. This result is in good agreement with the observations from a previous study (Wang and Xing 2008). The body angle amplitude data does not reveal any significant correlation with the Zn^{2+} concentration, indicating that the strength of nematode swimming may not be influenced by Zn^{2+} (Fig. 5a).

3.3 Glucose did not affect nematode locomotion

High glucose levels in diet are related to many human diseases such as obesity, type-II diabetes, and cardiovascular diseases (Venn and Green 2007). Due to its 50–80 % genes homologous to human genes and conserved insulin responsive pathway, *C. elegans* has been extensively utilized for studying the lifespan reduction in response to glucose stresses (Lee et al. 2009; Murphy et al. 2003). In addition, it also has been revealed that there is a clear correlation between the swimming parameters of adult *C. elegans* and its whole lifespan (Hsu et al. 2009; Huang et al. 2004). Thus, we speculated that this correlation also exists at the L4 larva stage, and thus the swimming parameters of L4 larva of *C. elegans* can be affected by glucose exposure.

As a proof-of-concept experiment, we applied our microfluidic device to quantifying the effect of diet glucose level (0 μ M, 1.4 μ M and 2.8 μ M) on the locomotion of *C. elegans*. In order to manifest the long-term biological effect of glucose during nematode development, we cultured N2 nematodes on 35 mm agar plates from the egg stage to the L4 stage. Besides nematode food (*E. coli*), 3 ml of M9 buffer with 1.4 μ M or 2.8 μ M D-(+)-glucose was added to the plates before nematode culture was started. When the nematodes reached the L4 stage, they were loaded into the microfluidic device for locomotion analysis. While capturing two-minute videos of nematode locomotion, the microfluidic device continuously perfused the nematodes with M9 buffer. In this experiment, we only used the microfluidic device for culturing *C. elegans*, and no on-chip glucose perfusion was performed. It is also feasible to modify our current device design for nematode culture and glucose perfusion both on chip, from the egg stage to the L4 stage (approximately 2 days at 20 °C); this requires the perfused solution to contain both glucose and *E. coli* food, and a mechanism preventing channel clogging by *E. coli* would need to be integrated into the microfluidic device (Hulme et al. 2010).

Figure 5c and d illustrate the experimental data of the nematode swimming frequency and the body angle magnitude after glucose exposure. There is no significant difference in both locomotion parameters extracted from the

control group and the experimental group treated with 1.4 μ M glucose, though the angle amplitude of nematodes increased slightly and the swimming frequency decreased. As the glucose concentration increases to 2.8 μ M, the angle amplitude of nematodes further increased and the swimming frequency further decreased comparing to the control group; however, there is still no statistical difference revealed.

It has been previously demonstrated that the locomotion capacity of mid-adult N2 adults could be decreased by high glucose diets through the pathway of ectopic apoptosis (Choi 2011). Cell corpses from ectopic apoptosis were found via fluorescence staining in nematodes at day 10 of adulthood, which corresponded to the glucose-induced locomotion reduction. However, the ectopic apoptosis was not found in at the L4 stage. Thus, it is reasonable that, due to the lack of responsible pathway (ectopic apoptosis), the young L4 nematodes we used displayed normal locomotion capacity after glucose treatment.

4 Conclusion

We reported an easy-to-use microfluidic device for carrying out on-chip culture, chemical perfusion, and locomotion analysis of *C. elegans*. Different from the existing microfluidic devices, our design involved a separate nematode loading mechanism allowing the easy loading of single *C. elegans* into individual culture chambers. This design significantly lowered the technical barrier of using the microfluidic device by biological researchers and other layperson users who are not familiar with microfluidic technology. Using the current device, the loading of eight nematodes was completed within 5 min by a user with two-hour training of the device usage. The Matlab software we developed also allowed automatic visual analysis of nematode locomotion and microfluidic pump control, which make the device operation straightforward and improves the efficiency of data collection. Using this microfluidic device, we examined the locomotion changes of N2 *C. elegans* induced by Zn^{2+} and glucose. We believe this device holds the potential to become a useful tool for facilitating *C. elegans*-based chemical screening.

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