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(Citation)
Microfluidics and Nanofluidics,29(6):31

(Issue Date)
2025-05-07

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100495854

Microfluidics and Nanofluidics (2025) 29:31
https://doi.org/10.1007/s10404-025-02807-6

RESEARCH

Microfluidic device integrated with a porous membrane for quantitative chemotaxis assay of plant-parasitic nematodes

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Received: 7 November 2024 / Accepted: 24 April 2025

Abstract
Plant-parasitic root-knot nematodes (RKNs) cause significant damage to plant crops by inhibiting nutrient absorption in host plants through infection. Chemotaxis is an important factor in controlling RKNs behavior as well as in understanding the mechanisms of parasitic behavior of RKNs on plants. Thus, studies on RKN chemotaxis are important for developing more environmentally friendly strategies to manage RKN infestations instead of current control methods using environmentally harmful pesticides. To better understand the chemotactic behavior of RKNs, we developed an easy-to-use microfluidic device consisting of two-layer polydimethylsiloxane (PDMS) microchannel chips and a porous hydrophilic polycarbonate membrane. The porous membrane acts both as a filter in introducing agarose gel containing nematodes to the observation chamber and as a diffuser to generate chemical concentration gradients in chemotaxis assays. We demonstrated the chemical concentration gradient was formed within 5 min in the gel-filled chamber using fluorescence substance. Using this device, we analyzed the correlation between nematode activity (chemotactic behavior and mobility) and the concentration gradients of several chemicals including KNO_3, cadaverine, and putrescine (1, 10 and 100 mM). Finally, we confirmed the repellent effect of KNO_3 and the attractive effect of cadaverine and putrescine on the RKN, *Meloidogyne incognita*, which was cultured on tomatoes, within 10 min after injecting the chemicals and quantitatively identified the correlation between nematode activity and chemical environmental conditions.

Keywords Plant-parasitic nematodes · Chemotaxis · Polydimethylsiloxane · Microfluidic device

1 Introduction

Plant-parasitic root-knot nematodes (RKNs), such as *Meloidogyne incognita*, are a major cause of global crop loss due to their ability to cause significant damage to a wide range of crops, leading to significant economic losses in the agricultural industry (Williamson et al. 2003; Singh et al. 2013; Kumar et al. 2020).

RKNs locate their host plants by chemotaxis which is directional movement along chemical gradients exuded from the plants (Reynolds et al. 2011). After invading plants through the root, RKNs induce cell swelling and the formation of feeding organs known as root knots or galls, which prevents infected plants from absorbing nutrients and water from the soil, and eventually causes growth defects or plant death (Nurul Hafiza et al. 2016; de Montanara et al. 2022; Karuri et al. 2022). Detailed studies on RKN chemotaxis are needed to develop new management methods to prevent RKNs attack on plants and inhibit their spread (Abd-Elgawad 2020, 2021; Ahmad et a. 2021; Li et al. 2022).

To study the chemotaxis of nematodes including RKNs, centimeter-scale agar plates have been conventionally used (Wuyts et al. 2006; Oota et al. 2020; Wang et al. 2021). In this method, the attractive and repellent properties of nematodes to the chemical gradients formed on agar plates are defined by tracking the behavior of nematodes. However, the on-agar-plate method has the several technical issues. One of them is that it takes several hours or more days to determine the chemotactic properties of RKNs by tracking their movement on the agar plate. In addition, it is difficult to quantitatively analyze the relation between

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Published online: 07 May 2025

chemical environmental conditions and chemotaxis properties of RKNs because the chemical conditions change over time due to three-dimensional diffusion onto the agar plate. These issues prevent us to quantitatively define the effective chemical substances and their concentration in the chemotaxis of RKNs.

To overcome these technical issues, the microfluidic technologies based on MEMS (micro-electro-mechanical systems) have great advantages thanks to scale effects (Judy 2021; Beebe et al. 2002). Using the microfluidic devices, we can precisely control not only the position of small biological samples including cells (Roggo et al. 2018), bacteria (Mao et al. 2003; Nagy et al. 2015), and nematodes (Qin et al. 2007; Lockery et al. 2008; Jung et al. 2012) but also their environmental conditions such as chemicals and temperature with high special and time resolution by integrating with MEMS components (Rondelz 2005; Galletto et al. 2006). More recently, the applications of microfluidic device in chemotaxis assay of plant-parasitic nematodes including RKNs have been reported (Hida et al. 2015; Beeman et al. 2016). Compared with the agar-plate methods, these microfluidic devices have enabled us to efficiently define the chemotaxis of the RKNs by confining nematodes to the micro chamber. Nevertheless, several technical issues still remain: manipulability of RKNs less than 1 mm and long preparation time because of millimeter-scale diffusion path in these microfluidic devices.

In this study, we developed a poly-dimethylpolysiloxane (PDMS) microfluidic device integrated with a porous membrane to efficiently and quantitatively analyze the chemotactic properties of the RKNs. Using this device, we can simply manipulate the RKNs with a micropipette and observe their mobility in detail under an optical microscopy. Furthermore, this device allows us to rapidly generate chemical concentration gradients in chemotaxis assays thanks to the short diffusion path corresponding to the thickness of the porous membrane. To demonstrate the developed on-chip method for chemotaxis assay, we tested three chemicals at different concentrations in chemotaxis assay: KNO_3, cadaverine, and putrescine. These substances, which are present in soil as fertilizers or plant secretions, have been reported to have an attractive and repellent on *M. incognita* (Castro et al. 1991; Oota et al. 2020). Using this analytical method, we quantitatively defined that *M. incognita* RKNs are repellent to KNO_3, whereas attractant to putrescine and cadaverine.

2 Materials and methods

2.1 Design of microfluidic device

Figure 1 shows a schematic of the fabricated microfluidic device used for the chemotaxis assay of RKNs. The device consists of two PDMS layers with a porous membrane sandwiched between them (Fig. 1a). The top PDMS layer has a nematode inlet (2 mm in diameter), two chemical reservoirs, and a suction port to introduce nematodes into the device. The chemical reservoir (1000 μm wide, 70 μm high, and 4000 μm long) is connected to two inlets (2 mm in diameter) via microchannel (100 μm wide, 70 μm high, and 500 μm long). The bottom PDMS layer contains an observation chamber (3000 μm wide, 70 μm high, and 6000 μm long) for nematode activity analysis. The suction port and chemical reservoirs in top PDMS layer and the observation chamber in the bottom PDMS layer were partially overlapped through the porous membrane. In contrast, the nematode inlet and observation chamber were directly connected without the porous membrane (Fig. 1b). In this study, we used a porous membrane with a diameter of 10 μm, which is smaller than the body width of RKNs (tens of micrometers). The porous membrane serves two roles: it acts not only as a filter when introducing nematodes into the observation chamber, but also as a chemical diffuser in chemotaxis assay.

For the chemotaxis assay, gel medium containing RKNs was introduced into the observation chamber by aspirating through the suction port. During the gel introduction, the nematodes were retained in the observation chamber by filtering via the porous membrane. After the gel solidified, the nematodes moved in the observation chamber because of physical support by the gel medium. Then, a chemical solution was injected into the chemical reservoir and the chemical concentration gradient was created in the observation chamber by diffusion through the porous membrane (Fig. 1c). Finally, the responses of RKNs to the chemical concentration gradient were quantitatively analyzed by investigating mobility and distribution of RKNs in the observation chamber.

2.2 Fabrication of microfluidic device

The top and bottom PDMS layers were fabricated using a MEMS soft lithography process (McDonald et al. 2000). The device molds were fabricated using a standard photolithography process. Initially, a 70 μm thick negative photoresist (SU-8 3050, MicroChem, Newton, MA, USA) layer was formed on a 25 mm square silicon substrate by spin-coating. After softbaking on a hotplate at 95°C for 26 min, the SU-8 layers were exposed to ultraviolet (UV) light in a

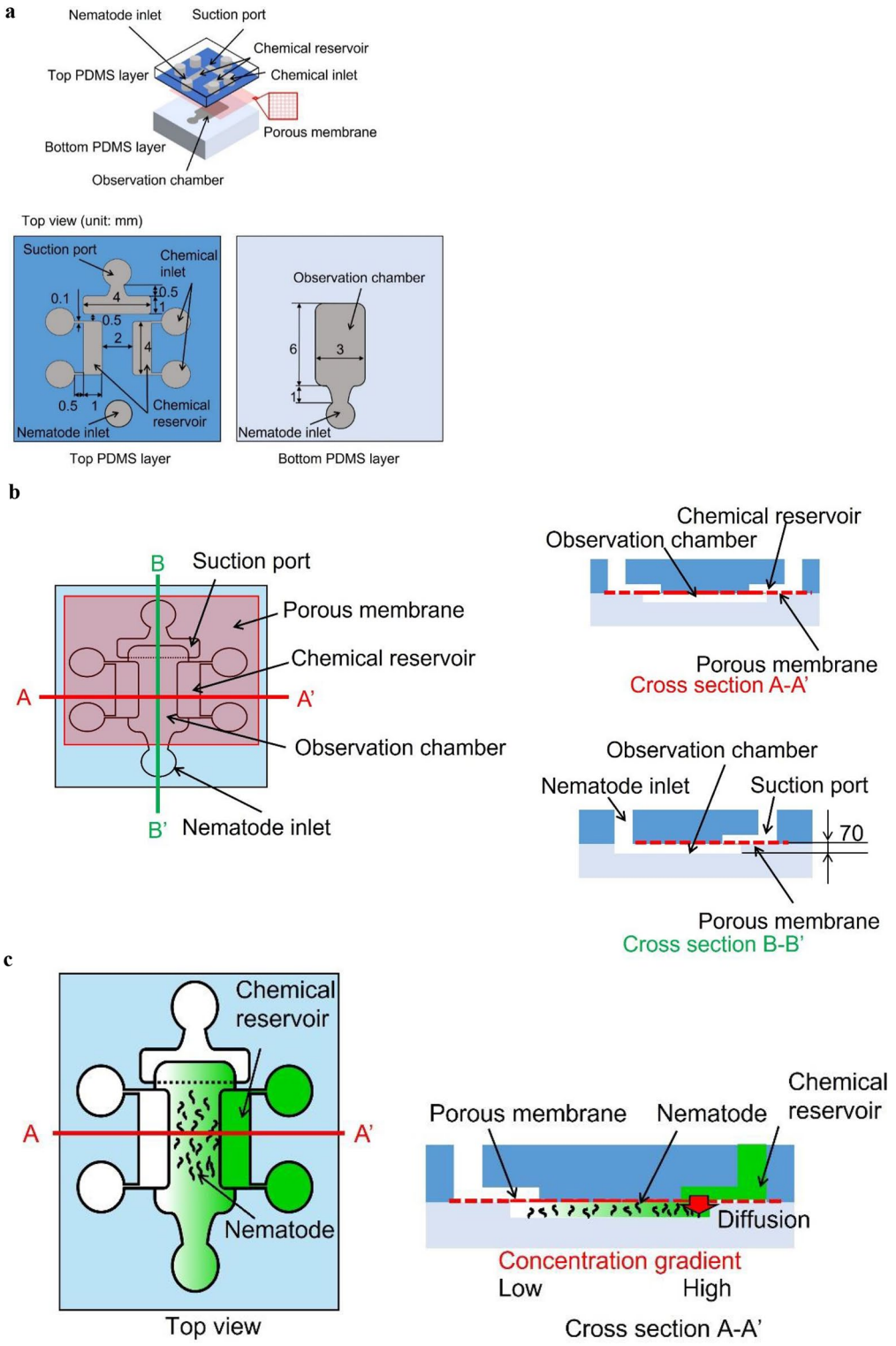

Fig. 1 PDMS Microfluidic device integrating with a porous membrane for chemotaxis assay of the nematodes. **a** Schematic illustration of the microfluidic device and the dimensions of each component in the device. **b** Illustration of assembled microfluidic device. **c** Generating the chemical concentration gradient in the observation chamber

designed pattern using a maskless exposure system (PALET, NEOARK CORPORATION, Japan). Then, the SU-8 layers were heated on a hot plate at 95°C for 5 min to expedite the curing of the UV-exposed area. Finally, the SU-8 molds were structured by development.

For the soft lithography process, a PDMS precursor (Sylgard 184, Dow Corning, Midland, MI, USA), which was a mixture of the silicone elastomer and curing agent (10:1 weight mixing ratio), was poured into the mold and degassed in a vacuum chamber for 1 h. The degassed PDMS precursor was baked to cure for 1 h at 85°C. After peeling off the cured PDMS from the mold, holes for the chemical and nematode inlets were punched. In this study, we used a hydrophilic polycarbonate porous membrane (10 µm pore diameter, 16 µm thick, IsoporeTM; Merck, Darmstadt, Germany). The microfluidic device was manually assembled by sandwiching the porous membrane between the top and bottom PDMS layers. Both the PDMS layer and the porous membrane had smooth surfaces, thus they were bonded together by van der Waals force without further surface treatment (Fig. 2).

2.3 Chemical gradient analysis

To quantify the chemical concentration gradient formed in the observation chamber, we used fluorescent fluorescein (Wako Pure Chemical Industries Ltd., Osaka, Japan), which has the similar diffusion characteristics to those of the target substance because their molecular weight is close. We have previously developed a protocol to allow the RKNs to move in the microchannel by introducing the optimized gel medium (Hida et al. 2015). Thus, we analyzed the time-course concentration gradient in the chamber filled with the gel under the same condition as follow. First, 20-µL 1% (w/w) ultra-low-melting agarose gel (A2576 Agarose Type IX-A, Sigma-Aldrich, St. Louis, MO, USA) was introduced into the observation chamber by aspirating through the suction port with a micropipette. After solidifying the gel in a refrigerator for 15 min at 5°C, the device was stored at room temperature (RT, 25 ± 2°C) for at least 10 min to allow it to return to RT. Then, we injected 40-µL of aqueous fluorescein into one of the chemical reservoirs and measured the time-course fluorescence intensity in the chamber using a fluorescence microscope (IX73, Olympus, Tokyo, Japan) at RT. We captured florescence images every minute for 10 min and analyzed the distribution of fluorescence intensity in the chamber using image analysis software ImageJ (Schneider et al. 2012). The fluorescence intensity of the aqueous fluorescein in the microchannel is proportional to its concentration (Hida et al. 2015). Thus, we can estimate the chemical concentration distribution generated in the microchamber by measuring the fluorescence intensity. Based on the measurement results using fluorescein solution, we estimated the developmental process of concentration gradient when using target substances in chemotaxis assays. During the development of the concentration gradient, the diffusion rate is proportional to the diffusion coefficient, which is inversely proportional to the molecular weight of the solute. Thus, the time required for a concentration gradient of the target substance to form in the observation chamber can be estimated by comparing the measurement results using the fluorescein (332.31 g mol^{-1}).

2.4 Nematode chemotaxis assay

In this study, RKNs (*M. incognita*) were cultured on tomatoes (*Solanum lycopersicum* cv. Pritz) as hosts (Ejima et al. 2011; Nishiyama et al. 2014). Tomato seedlings were inoculated with RKNs every 3 days during weeks 4–5 of growth for a total of six inoculations. At each stage, approximately 120,000 s-stage larvae (J2) were inoculated into the tomato seedlings. After inoculation, tomato plants were grown in a hydroponic system, and J2-stage nematodes were collected 2–3 days later (Lambert et al. 1992). Finally, the cultured nematodes were stored in a dark place at approximately 5°C to maintain their vital functions.

To prepare for the chemotaxis assay, we mixed 20 µL nematode suspension with 20 µL liquid agarose gel heated to 50°C and injected the mixture solution into the nematode inlet. Because the volume of the solutions was very small, the temperature of the final mixture rapidly dropped

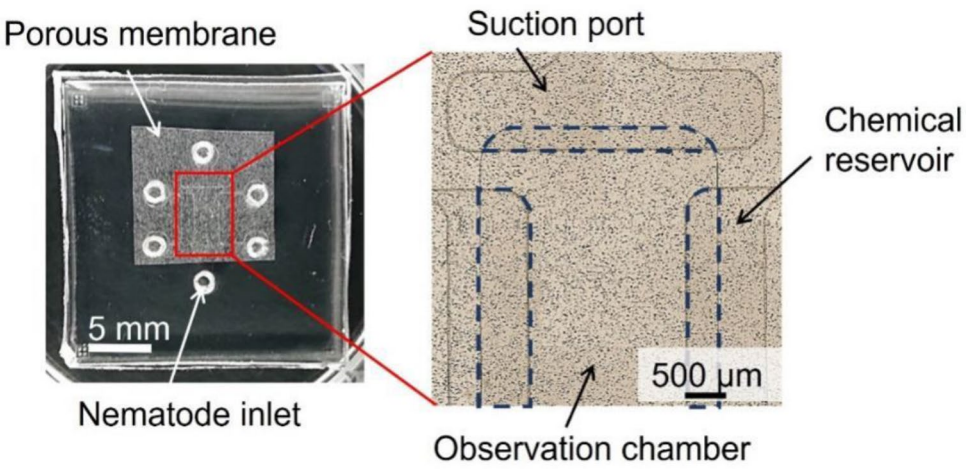

Fig. 2 Photography of the fabricated microfluidic device. The dashed-line area in the magnified view of device indicates overlapping area between the suction port, chemical reservoirs and the observation chamber

to below about 30°C. This temperature condition is physiologically acceptable for the common organisms including nematodes, thus the effect on the vital functions of the nematodes is considered negligible.

The mixture solution was then sucked into the chamber by aspirating through the suction port (Fig. 3a). During the suction, the nematodes are trapped in the observation chamber by filtering through the pore membrane. After introducing the nematodes, the microfluidic device was placed in a 4°C refrigerator for 15 min to allow the nematodes to move in the gel-filled chamber (Hida et al. 2015). As in the chemical gradient analysis, the microfluidic device was stored at RT for at least 10 min. As a control, we recorded the behavior of nematodes in the observation chamber for 5 min before injecting the chemical solution. After injecting the aqueous chemical solution into one of the chemical reservoirs, we immediately recorded the nematode mobility for 10 min under a microscope.

To quantify the chemotactic properties of nematodes, we defined the index number I_c calculated by measuring distribution number of nematodes in the observation chamber. We equally divided the observation chamber into two regions: the chemical region, which was upstream of the chemical diffusion, and the reference region, which was downstream of it as depicted in Fig. 3b. The time of injecting the chemical was set to $t = 0$. Based on the assumption that the number of nematodes in each chemical and reference region was equal before injecting chemicals, the chemotaxis index number I_c was defined as

$$I_c = \frac{N_t - N_0}{N_0} \tag{1}$$

, where N_0 and N_t indicate the number of nematodes in the chemical region at the initial time ($t = 0$) and at time t, respectively. From the Eq. (1), positive and negative values of I_c denote the attractant and repellent for the nematodes. In the chemotaxis assays, we used aqueous solution of chemical substances including KNO_3, putrescine and cadaverine as candidates of attractant/repellent for the RKNs. KNO_3 was previously defined as a repellent for *M. incognita* (Castro et al. 1991). Cadaverine and putrescine, which are polyamines founded in root exudates, are defined as attractant for the nematodes by using on-agar-plate assay (Oota et al. 2020). To quantitatively analyze the effect of the chemical concentration gradient, we used the chemical solutions with different concentrations (1 mM, 10 mM and 100 mM) in the chemotaxis assays. For each chemical condition, at least 30 nematodes were tested five times per experiment.

3 Results and discussion

3.1 Generation of the chemical gradient in microchamber

Figure 4 shows the results of chemical gradient analysis using fluorescein aqueous solution on the developed microfluidic device. We confirmed that no leakage was occurred between the each PDMS layer and the porous membrane because the edge of chemical reservoir was clearly observed as shown in Fig. 4(a). This result indicates that the bonding strength between the PDMS layers and the porous membrane was strong enough to the pressure applied when injecting the chemical solution with a micropipette. To investigate the spatial distribution of diffused fluorescein

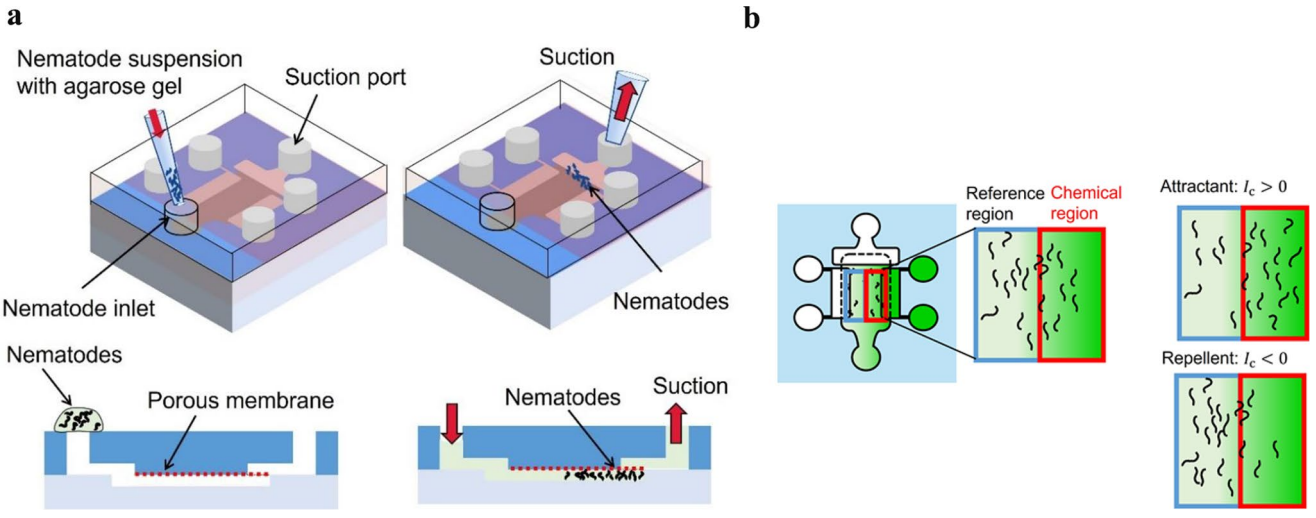

Fig. 3 Schematic illustration of the chemotaxis assay of the nematodes by using the microfluidic device. **a** Protocol for introducing the nematodes into the microfluidic device. Left: Injection of the agarose gel containing the nematodes to the device. Right: Suction for introducing the nematodes into the chamber. **b** Definition of the index number I_c for the chemotaxis assay of nematodes. The attractant and repellent properties were characterized by measuring the number of nematodes in the chemical region at time t

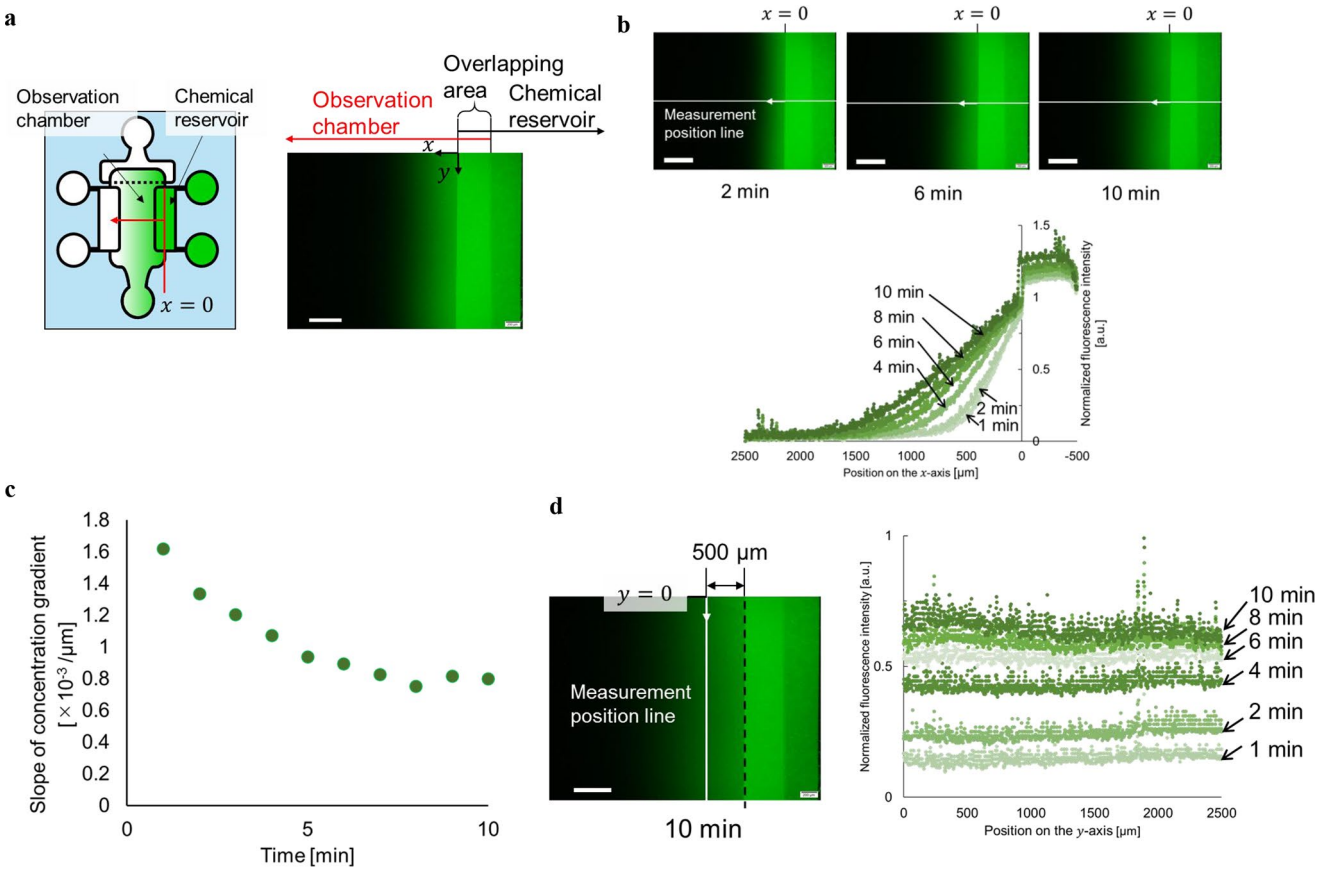

Fig. 4 Evaluation of the chemical concentration gradient in the observation chamber. **a** Fluorescence microscopy image of fluorescein introduced into the device (t=5 min, scale bar: 500 μm). **b** Quantification of the time-course fluorescent intensity in the observation chamber. **c** Change in concentration gradient value over time. **d** Evaluation of concentration distribution on the y-axis

substance, we defined an x-axis whose origin is at the edge of overlapping area between the chemical reservoir and the observation chamber and a y-axis that is perpendicular to the x-axis and whose origin is at the top of the microscope images as shown in Fig. 4(a). The measured fluorescence intensity was normalized with the averaged value of fluorescence intensity in the chemical reservoir at each time.

We evaluated the time course of the fluorescence intensity in the observation chamber on the x-axis. As shown in Fig. 4(b), the concentration gradient of fluorescein was formed in the chemical region (defined in Sect. 2.3) in approximately 5 min. Based on the measurement results of the fluorescence intensity, we calculated the concentration gradient value at each time by linear approximation using the least square method. As shown in Fig. 4(c), we verified that the concentration gradient value became almost constant after approximately 5 min. The molecular weight of the chemical substances used in the chemotaxis (potassium ion: 39.9 g mol^{-1}, nitrate ion: 62 g mol^{-1}, cadaverine: 102.18 g mol^{-1}, and putrescine: 88.15 g mol^{-1}) were smaller than that of fluorescein, thus we estimated that the concentration gradients in the observation chamber were formed in less than 5 min.

To quantitatively evaluate the in-plane concentration distribution in the observation chamber, we measured the fluorescence intensity in the y-axis at position x = 500 μm and confirmed that fluorescein uniformly diffused from the chemical reservoir along the x-axis as shown in Fig. 4(d).

This result suggests that the chemotaxis of the nematodes can be simply determined from its movement along the x-axis regardless of its position on the y-axis using this device.

According to the Stokes-Einstein equation (Bruus 2010), the diffusion time of a substance is proportional to the square of the distance. In previously reported PDMS microfluidic devices for nematode chemotaxis assay (Hida et al. 2014; Beeman et al. 2016), it took relatively long time (one hour or several hours) to generate the chemical concentration gradient in the analytical chamber because of millimeter-scale diffusion path. Furthermore, molecular adsorption onto the long PDMS diffusion path prevented the stable formation of a concentration gradient in the chamber (Meer et al. 2017). In our developed device, the thickness of porous

membrane corresponding to the diffusion path is less than fiftieth of that in previous reports (Hida et al. 2014; Beeman et al. 2016). Thus, we can significantly reduce not only the diffusion time to generate the chemical gradient but also the effect of molecular absorption onto the diffusion path without additional surface treatments in chemotaxis assays.

3.2 Nematode chemotaxis assay

Figure 5 shows the optical microscope image taken immediately after introducing the nematodes in the observation chamber by suction. The nematodes were efficiently introduced into the chamber without leaking the agarose gel. Before injecting the chemical solution, we counted the number of nematodes in the chemical region for 10 min. The obtained index number I_c maintained close to zero as shown in Fig. 6(a). This result indicates that the nematodes were uniformly distributed in the observation chamber without a chemical stimulus. Thus, we experimentally confirmed that the chemotaxis of the nematode can be determined from variation of I_c after injecting the chemicals. The time-course microscopic images of the nematodes' behavior after injection of the chemical solution (100 mM KNO_3 and 100 mM cadaverine) are shown in Fig. 6(b). When KNO_3 aqueous solution was injected, most of the nematodes gradually moved from the chemical area to the reference area, which indicates the repellency. In contrast, the nematodes gradually migrated toward the chemical region after injecting the cadaverine solution. These results suggest that we can visually determine the repellency or attraction based on the uneven distribution of nematodes in the observation chamber.

Figure 6(c) shows the time-course of I_c when injecting aqueous KNO_3 solution at concentrations of 1, 10, and 100 mM. The index number I_c decreased one minute after the injection of KNO_3 solution and turned negative value, which indicates the repellent for the nematodes.

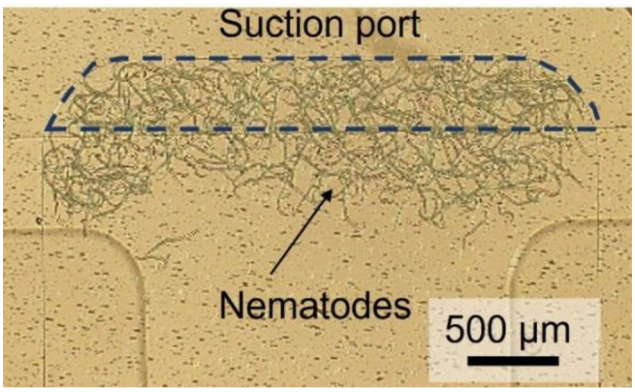

Fig. 5 Optical image of the nematodes introduced in the observation chamber. The dash-lined area indicates the overlapping area between the suction port and the observation chamber

In addition, the rate of decrease in I_c increased with increasing the concentration of KNO_3 solution, i.e., the magnitude of concentration gradient. These results suggest that the effective concentration gradient for chemotaxis can be quantitatively defined by using this method.

Figure 6(d) shows the time-course of I_c when injecting aqueous cadaverine solution at concentration of 1, 10 and 100 mM. The index number I_c increased 3 min after the injecting and turned positive value, which indicates the attractant for the nematodes. As in the assay with KNO_3 solution, the attractant effect for the nematodes was enhanced with increasing concentration. Meanwhile, the onset time of the change in I_c in assay with cadaverine was slightly delayed compared to the assay with KNO_3. This is because the diffusion coefficient of cadaverine is lower than that of KNO_3 due to the difference in molecular weight.

To investigate the attractive effect of different substances, we performed the chemotaxis assay using cadaverine and putrescine at a concentration of 100 mM. Figure 6(e) shows the time-course of I_c when injecting cadaverine and putrescine. The increase in I_c in the cadaverine assay was greater than that of the putrescine assay. The diffusion coefficient of the cadaverine is expected to be relatively small compared with putrescine due to the difference in molecular weight. Nevertheless, the large increase in index number I_c when cadaverine was used suggests that cadaverine has a more effective attractant for *M. incognita* under similar concentration gradient condition.

3.3 Variation in nematode mobility

We analyzed the effect of chemicals on movement speed of nematodes by tracking individual nematodes in the observation chamber. The movement speed of each nematode was calculated by the displacement of nematode over each 10-second interval based on the time-course microscope images. Each experimental condition was repeated 20 times, with over 30 nematodes analyzed in each test. The statistical significance was determined based on Student's t-test. Figure 7 shows the results of measuring the movement speed of nematodes before and after the injection of KNO_3 (100 mM) and putrescine (100 mM). As shown in Fig. 7(a), the average movement speed of nematode was gradually decreased after injecting KNO_3 solution. Before injecting KNO_3 solution, the average movement speed of nematode was 3.71 µm s^{-1}, which decreased to 3.51 µm s^{-1} within 1–5 min and to 3.26 µm s^{-1} within 5–10 min after the injection. This result indicates that KNO_3 effectively inhibits nematode activity. Assuming the decrease in movement speed mobility of the nematode is due to KNO_3 toxicity, the repellency might help the nematodes avoid harmful chemical conditions including KNO_3. In contrast, no significant

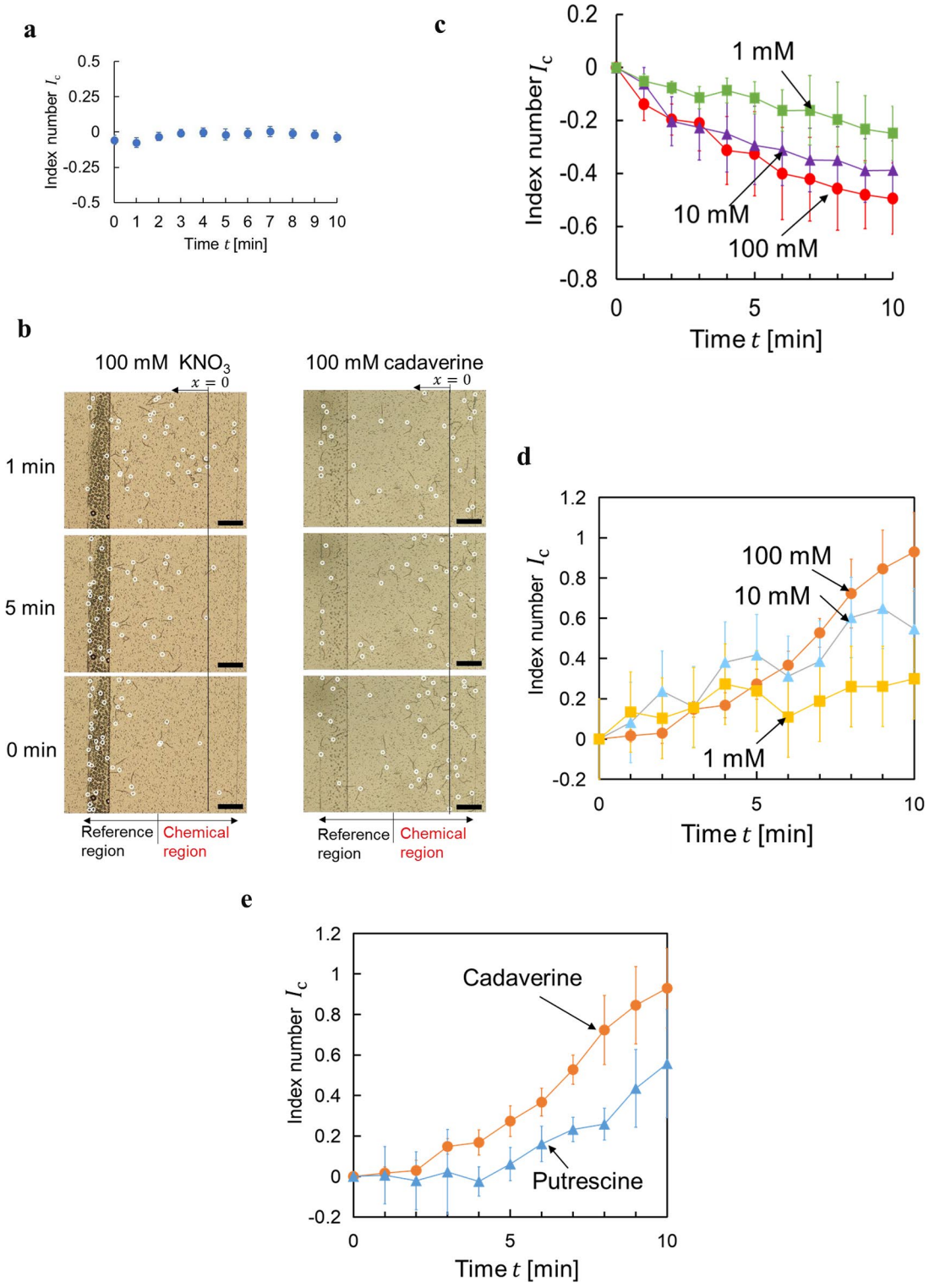

Fig. 6 Time-dependent index number I_c in the chemotaxis assay of *Meloidogyne incognita*. **a** Before chemical injection (Sample number $N > 20$). **b** Optical images of the nematode behavior after injecting the chemical solution 100 mM KNO_3 (left column) and 100 mM cadaverine (right column). Each white circle indicates the individual nematode head. **c** Injecting KNO_3 solution (1, 10 and 100 mM, each $N = 5$). **d** Cadaverine solution (1, 10 and 100 mM, each $N = 5$). **e** Cadaverine and putrescine solution at 100 mM (each $N = 5$)

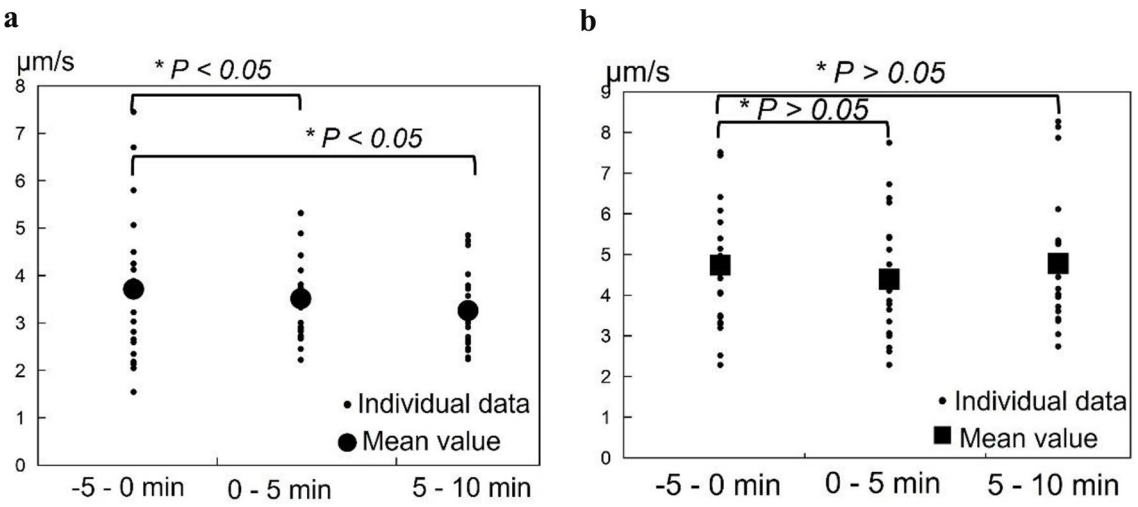

Fig. 7 Movement speed of the nematodes before and after chemical injection. **a** KNO_3 (100 mM). **b** putrescine (100 mM)

difference in speed of nematode was observed before and after injecting putrescine as shown in Fig. 7(b). Putrescine has been reported to be present in root exudates of tomatoes and soybeans (Oota et al. 2020). These results suggest that *M. incognita* can explore their host plants by sensing the ingredient of root exudate without impairing their motility.

3.4 Discussion

The purpose of our research was to develop a method for quantitatively and efficiently analyze the chemotaxis properties of the RKNs. In this study, we developed a PDMS microfluidic device integrated with a porous membrane to easily manipulate the nematodes and rapidly generate concentration gradients for chemotaxis assays. Using the developed method, we quantitatively defined the chemotactic properties of chemical substants to *M. incognita* at different concentrations: KNO_3 acts as a repellent, meanwhile cadaverine and putrescine act as an attractant. We also revealed a positive correlation between the magnitude of the concentration gradient of chemical substances and their attractant/repellent effect. Tsai et al. reported a threshold concentration that is attractive to *M. incognita* using on-agar-plate chemotaxis assay with a kind of galactose (Tsai et al. 2021). Our microfluidic-based method will contribute to effectively identifying new chemical/biological substances and their concentration condition that act as attraction or repellent for the plant-parasitic nematodes including *M. incognita*. In chemotaxis assays, the nematode quickly responded to the formation of a concentration gradient in the chamber. This result suggests that this device could be applied in a new chemical sensing devices (Ozasa et al. 2013; Krakos et al. 2022) using the nematodes as a sensing indicator.

This study will provide valuable insights not only for fundamental research relating to study of plant-parasitic mechanism of the nematodes but also for improving agricultural productivity by developing new management method of the nematodes using environmental-friendly substances. However, additional comprehensive studies in chemotaxis are needed because the results regarding chemical conditions that affect the nematode behavior are currently limited. Using this analytical method, we will investigate more effective chemical/biochemical conditions for chemotaxis, furthermore, explore a new substance that inhibits parasitic behavior of the plant-parasitic nematodes.

4 Conclusion

In this study, we developed a simple and effective method for chemotaxis assay of the plant-parasitic nematodes M. incognita using a microfluidic device integrated with a porous membrane. Using this method, we quantitatively the chemotactic effect of the target substances and their concentration for *M. incognita*. However, this study has several limitations. The chemotaxis was evaluated using only three chemicals, thus various types of chemicals with different concentration need to be investigated to enhance the generalizability of the findings for chemotaxis. When using plant-derived proteins for chemotaxis, it may be necessary to perform surface treatment on PDMS chips to prevent the adsorption of substances. In addition, the experiments were conducted using *M. incognita*, a representative root-knot nematode. For other harmful nematode species with different body sizes, it may be necessary to optimize the microchannel dimensions to better accommodate the target organisms. This analytical method may contribute to improving crop yields in the future through the development of new, environmentally friendly management method of plant-parasitic nematodes including *M. incognita*.

Acknowledgements The authors thank Editage (www.editage.jp) for English language editing. This work was supported by JSPS KAKENHI Grant Numbers JP24K08220.

Author contributions H.H. and S.S. initiated the study and I.K. directed the project. S.S. prepared the plant-parasitic nematode Meloidogyne incognita by culture. J.L. and H.H. performed the chemotaxis assay of nematodes. J.L. and H.H. wrote the main manuscript text and prepared all figures and table. All authors reviewed the manuscript.

Funding Open Access funding provided by Kobe University.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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