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Hydrogel-droplet microfluidic platform for high-resolution imaging and sorting of early larval *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* is an important model organism in research on neuroscience and development because of its stereotyped anatomy, relevance to human biology, and ease of culture and genetic manipulation. The first larval stage (L1) is of particular interest in many biological problems, including post-embryonic developmental processes and developmental decision-making, such as dauer formation. However, L1's small size and high mobility make it difficult to manipulate; particularly in microfluidic chips, which have been used to great advantage in handling larger larvae and adult animals, small features are difficult to fabricate and these structures often get clogged easily, making the devices less robust. We have developed a microfluidic device to overcome these challenges and enable high-resolution imaging and sorting of early larval stage *C. elegans* via encapsulation in droplets of a thermosensitive hydrogel. To achieve precise handling of early larval stage worms, we demonstrated on-chip production, storage, and sorting of hydrogel droplets. We also demonstrated temporary immobilization of the worms within the droplets, allowing high-resolution imaging with minimal physiological perturbations. Because of the ability to array hydrogel droplets for handling a large number of L1 worms in a robust way, we envision that this platform will be widely applicable to screening in various developmental studies.

Introduction

The free-living nematode *Caenorhabditis elegans* (*C. elegans*) has been extensively used as a multicellular model organism for development studies.[1] In particular, during the first larval (L1) stage the animal undergoes significant post-embryonic developmental processes, which include a large number of cell migration and tissue specification events.[2] L1 arrest[3] and dauer diapause[4] are two important developmental decisions the animals make that are critical to a variety of physiological processes. Understanding the mechanisms underlying these complex processes, which are controlled by genetic networks and environmental cues, can be greatly facilitated by methods for imaging and screening L1 worms. For example, monitoring developmental events at whole-animal or subcellular resolution necessitates high-resolution imaging in both bright field and fluorescent modes (*e.g.* to track a cell or gene expression). Additionally, it is important to be able to recover and sort the worms after imaging to assess their physiology and development.

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Conventional imaging methods use agar pads and drugs for immobilization,[5, 6] resulting in a time-consuming and painstaking manual handling process. Recently, a variety of microfluidic techniques have been developed that considerably reduce the need for human intervention and increase throughput.[7] Trapping methods based on valves,[8-11] chambers,[12] and droplets[13, 14] have been successfully combined with immobilization techniques based on compression,[15] suction,[16] cooling,[8-11], CO_2,[15] tapered channels[17] and gel formation.[18, 19] However, most of these platforms, designed for adult animals, are unsuitable for imaging, manipulating, or screening early larval *C. elegans*. Downsizing chip designs from the adult to L1 requires several-fold decrease in size - from 1-mm long and 70-µm diameter adult animals to <250-µm long and 10-15-µm diameter L1 animals. This is challenging in microfluidics not just in terms of microfabrication (because small features need to be fabricated) but also in terms of device operation (in terms of robustness and not getting clogged during operation). For example, Chung *et al.*[20] demonstrated a screening platform for L1 based on valves and cooling. In this design, adapting the channel size to L1 worms presents challenges with respect to trapping efficiency and preventing clogging therefore affecting

the robustness of the device. Shi *et al.*[13] encapsulated L1 into droplets and stored them on chip, but recovery of the worms was not possible and, because it lacked immobilization capability, high-resolution imaging could not be performed. Thus, a different approach is necessary to overcome the challenges of the small size of L1s and fulfill the requirements for high-resolution imaging and sorting. This approach needs to be (1) robust and efficient for worm handling, (2) able to isolate individual worms, (3) able to perform reversible non-disturbing immobilization, (4) of high optical quality, and (5) able to sort.

Here we present a microfluidic device for high-resolution imaging and sorting that specifically addresses the handling of early larval *C. elegans*. The animals are isolated in droplets and temporarily immobilized for imaging using a reversible hydrogel. We show that high-resolution imaging of L1 is possible, as well as selective recovery using a flow-based sorter.

Experimental

Device fabrication

Soft lithography was used to fabricate devices in polydimethylsiloxane (PDMS, Dow Corning Sylgard 184, Midland, MI).[21] First, to fabricate the mold, a 50 µm layer was spin-coated on a wafer using SU8-2050 (MicroChem). The features on transparency masks were transferred to the SU8-coated wafer by standard UV photolithography. The wafer surface was treated with tridecafluoro 1,1,2,2-tetrahydrooctyl-1-trichlorosilane vapor (United Chemical Technologies, Inc, Bristol, PA) to facilitate release of PDMS from the mold. A mixture of PDMS (part A and B in a 10 : 1 ratio) was poured on the mold to form a ~5 mm thick top layer and the whole preparation was left for curing for 2 h at 75 °C. After peeling off the PDMS, the devices were cut into shape and access holes were punched using 19 gauge needles (McMaster-Carr, Elmhurst, IL). Next, the substrate layer was spin-coated on a cover glass slide to a thickness of 70 µm and partially cured on a hot-plate at 70 °C for 7 min. The two layers were thermally bonded at 75 °C for 2 h.

Materials

Solutions of Pluronic F127 (Sigma Aldrich) in water were prepared at concentrations of 25% w/v. The suspension was agitated at 4 °C until the polymer pellets were completely dissolved and then the solution was filtered through a 0.2 mm nylon membrane filter (MF-75, Nalgene) to remove particulates. To generate droplets, the Pluronic solution and fluorinated oil FC-70 (provided by 3M) were used as dispersed phases while silicone oil (Clearco Products Co) was used as the continuous phase.

Interfacial tension measurements

Interfacial tensions were measured using the pendent drop method on Advanced Digital Automated Goniometer (model 500, from Rame-Hart Instrument, NJ) using with data analysis software DROPimage (Advanced version 1.5.04).

Rheological measurements

Rheological measurements were performed using a rheometer (Brookfield DVT3HB-CJ0) with temperature-controlled bath. We used 2.5 mL of Pluronic solution in circular chamber and spindle (CPA-40Z). We scanned temperature by steps of 0.5 °C from 8 °C to 17 °C, waiting 3 min between consecutive acquisitions. Sample-to-sample differences in viscosity measurement was less than 5 %.

C. elegans strain and sample preparation

We used the transgenic strain CX6858: *tax-4(ks28)*; *kyIs342* [*pgcy-32::tax-4::GFP, punc-122::GFP*]. The strain was cultured at 22 °C on Nematode Growth Medium (NGM) plates seeded with OP50.[22] For all the assays, age-synchronized animals were prepared as follows: embryos were obtained by bleaching adults using a solution containing about 1% NaOCl and 0.1 M NaOH, washed and allowed to hatch on plate. After 20 h, L1 animals were washed and suspended in 25% w/v solution of Pluronic F127. For the developmental assay, after screening, animals were extracted into M9 solution, washed to remove the residual PF127, and placed on standard agar culture plates seeded with OP50 bacterial lawns. The control sample consisted of animals from the same batch, which were kept on agar plates all the time. After being placed on culture plates, animals were allowed to grow at 22 °C. Then, the time to reach egg laying, the pharyngeal pumping rate at young adult stage, and the number of progeny in the first 24 h of egg-laying were compared in the two groups.

Operation of the microfluidic device and microscopy

Flow was delivered by syringe pumps. Before connecting the chip to syringe pumps, the chip was primed with silicone oil. Then the aqueous solution and fluorocarbon oil were injected into the microfluidic device. Droplets were generated at 12 °C, high-resolution imaging was performed at 22 °C, and the recovery of worms was at 12 °C. Temperature was set up by bringing the chip into temperature controlled room (22 °C laboratory) or chamber (12 °C environmental chamber). Device operation was monitored using a dissecting scope (Zeiss Stemi SV11) with an Infinity camera at 2x magnification. Bright-field and fluorescence high-resolution imaging were acquired using epifluorescence microscopy (Leica DM4500DB microscope, 63x objective, and Hamamatsu C9100- EMCCD camera).

System design

We have developed an integrated platform that uses hydrogel droplets on microfluidic chip to perform high-resolution imaging and sorting of L1 worms (Fig. 1). To realize high-resolution imaging, it is necessary to immobilize the worms. The immobilization mechanism must be both non-disturbing physiologically, which eliminates cooling or the use of drugs, and reversible. Other techniques using tapered channels,

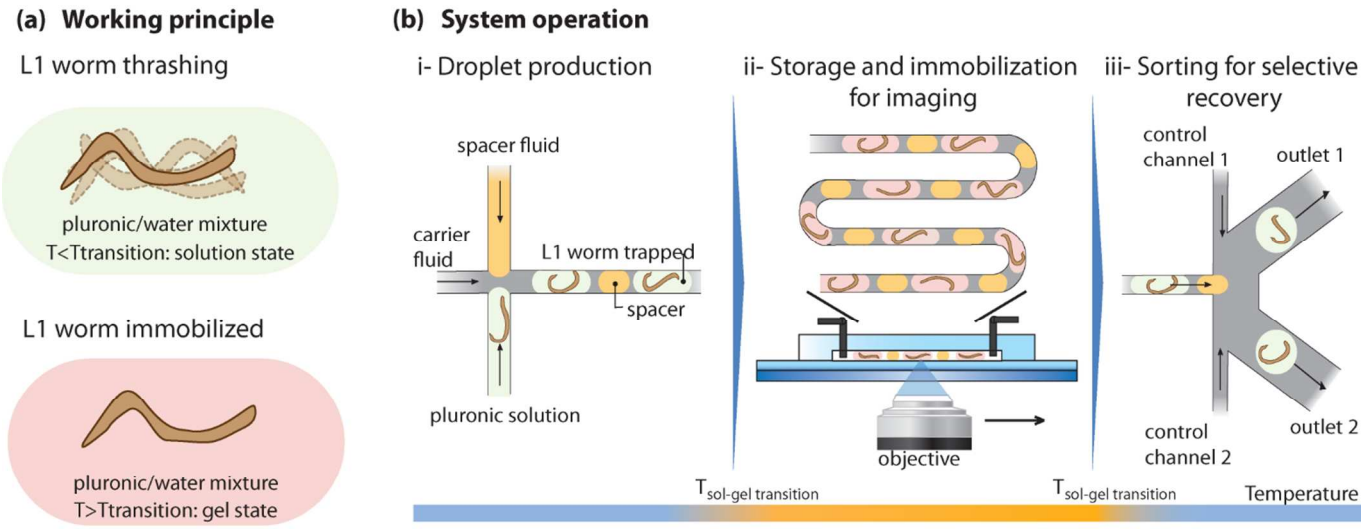

Figure 1 Design of an integrated system with microfluidics and Pluronic hydrogel to manipulate and image *C. elegans* larvae. (a) Working principle of the hydrogel droplet-based method. Worms are trapped in droplets, separated from each other. Using the reversible gelling property of Pluronic hydrogel, the worms can be temporarily immobilized for imaging. (b) Device operation modules. The device integrates three units that correspond to three operational steps cycling through the sol-gel transition temperature of the Pluronic mixture. (i) In liquid state, the Pluronic aqueous solution containing worms is injected in the device along with two other immiscible oils. Spatially confined droplets are produced at the junction with alternating spacer droplets, allowing for isolation of encapsulated animals. (ii) Once the storage channel is filled with droplets, the tubing connections are unplugged and the device is transferred to a microscope for high-resolution imaging, the droplets gel with the increase in temperature. Phenotyping and scoring are performed; the positions of droplets containing worms of interest are noted during this step. Having confined droplets in the channel, their positions remain constant through the whole process. (iii) The chip temperature is decreased, Pluronic droplets turn back to liquid phase, and the targeted worms are sorted.

suction, and valve-based compression would be challenging to implement due to the small size of L1 worms.[15-17] Hence, we opted for a method based on Pluronic F127, a thermosensitive hydrogel;[18, 19] full immobilization of the worms is achieved in a gentle manner and the sol-gel transition temperature is within the physiological temperature range of the animals. In addition, reversible immobilization is ensured by tuning the temperature since sol-gel transition in Pluronic F127 solutions is reversible (Fig. 1.a).

To be able to selectively recover the worms, we designed a microfluidic droplet device to precisely handle individual worms. Achieving this goal is challenging because L1s are small, typically 15 µm in diameter and up to 250 µm in length; animals of this larval stage are also fragile, flexible and very active. The worms can pass through 5 µm wide gaps, and sometimes clog small channels and obstruct flow. Hence systems such as valves and chambers cannot be used for reliable trapping. Droplets, in comparison, are fully enclosed spaces which separate the nematodes from each other entirely (Fig. 1a). In addition, the small size of the worm is no longer a problem in the droplets as handling animals is now translated into handling droplets, which is well controlled in microfluidics. To keep the system as simple as possible, we designed T-junctions to produce the hydrogel droplets, added spacers to keep them separated, and used two control side channels at the other end to sort the droplets (Fig. 1b).

A second goal of the system is to perform high-resolution imaging of multiple worms; this necessitates imaging one worm after another. Two strategies are possible: one could either stop a droplet within the field of view of the camera and switch temperature to immobilize and image (stop-and-flow scheme), or store all the droplets in a sequence-preserved manner and image them by moving the camera and using only one temperature cycle (completely stopped mode). In this work we opted for the non-moving mode to keep the system as simple as possible, which led to the design of the serpentine channel as shown in Fig. 1b. Furthermore, to improve robustness and user-friendliness, we designed a three-step procedure that is simple to operate and in a single-layer PDMS chip that has no active components (Fig. 2a). In addition, because of the use of hydrogel droplets, the channels can be much larger than the worm diameter, therefore avoiding clogging problems, while still fulfilling its requirements as an imaging and sorting platform. For the same reason, this chip could be used for L2-L3 animals as is, and older animals by scaling up the channel dimensions; here, for the purpose of this study we focused on first larval stage.

Results and discussion

Droplet production and manipulation

In order to handle individual worms through the device and to be able to immobilize them using Pluronic, the system needs to first produce Pluronic droplets to encapsulate worms. The main challenge with this is that Pluronic is a surfactant; the presence of Pluronic at very high concentrations (25% w/v) in the aqueous phase changes the surface energy of water and its wetting properties. To address this issue, we needed to select a material and an immiscible liquid that will preferentially wet the chip material. For the chip material, we chose to work with

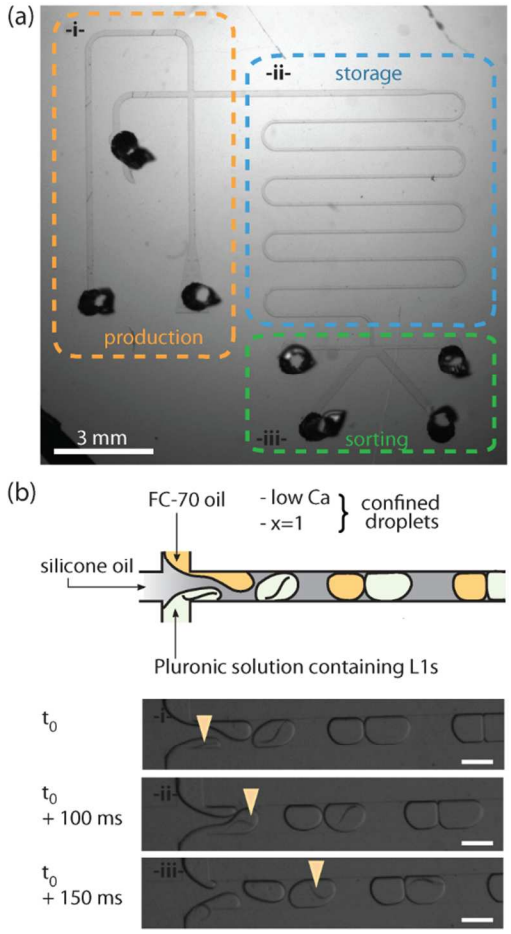

Figure 2 Microfluidic device for producing, storing, and sorting droplets for *C. elegans* imaging. (a) Overall micrograph of the microfluidic platform showing the integration of the three operational units: production, storing, and sorting. (b) Production of confined Pluronic droplets in alternation with FC-70 oil spacers and encapsulation of L1 worms. The generation of plugs and the use of spacers allow for maintaining the order of droplets during the operation of the platform. The sequence of images (i-iii) show smooth process of worm encapsulation (arrows point to the same worm through the sequence). Scale bar = 200 µm.

PDMS due to the ease of microfabrication, availability, low cost, and natural hydrophobicity. We tested glass-PDMS devices but the presence of a hydrophilic glass surface and the hydrophilic effect of plasma bonding on PDMS resulted in partial wetting of the channels with the aqueous phase. To overcome this problem, we thermally bonded PDMS chip to flat PDMS pieces to create a completely hydrophobic environment (Fig. 2a). For the immiscible phase, we tested various oils commonly used in droplet microfluidics (mineral oil, fluorinated oil FC-40, and silicone oil) with surfactants (perfluoroalcohol and SPAN 80). Despite the hydrophobic environment, mineral oil and fluorinated oil with perfluoroalcohol surfactant failed to produce regular droplets due to wetting competition. In contrast, silicone oil preferentially wetted the walls of the PDMS channels and we were able to produce Pluronic droplets (Fig. 2b).

For throughput purposes, it is necessary for a chip to encapsulate a large number of worms; this requires regular production of droplets. The formation of droplets depends on the viscosities of the liquids. However, due to the thermosensitive phase behavior of Pluronic F127, the apparent viscosity of the Pluronic solution changes with temperature. Fig. 3a shows the measured viscosity of 25 % Pluronic solution as a function of temperature. The sol-gel transition occurred above 17 °C and is characterized by a dramatic increase in viscosity (several 10,000 cP/°C at 17 °C). Fig. 3b shows a close-up of the viscosity in the 10-15 °C range and its associated rate. Temperature variations in the liquid state change at lower rates when further away from the sol-gel transition (160, 65, 17, and 0.7 cP/°C at 16, 14, 12, and 10 °C respectively). Too high a working temperature would make the system unstable. Temperature variations would disturb the droplet alternation pattern and potentially lead to clogging if the sol-gel transition is reached. On the other hand, too low temperatures will affect worm physiology. We worked at 12 °C ensuring relatively normal physiology of the worms and flow stability against perturbations provoked by small temperature fluctuations in the room. Although here we used an environmentally controlled chamber to perform this step, this step could be easily done in a temperature controlled water bath for example.

In order to sort the worms, droplets must remain in the same sequence from the imaging step to the sorting step. Changes in the droplet sequence can be caused by droplet coalescence or

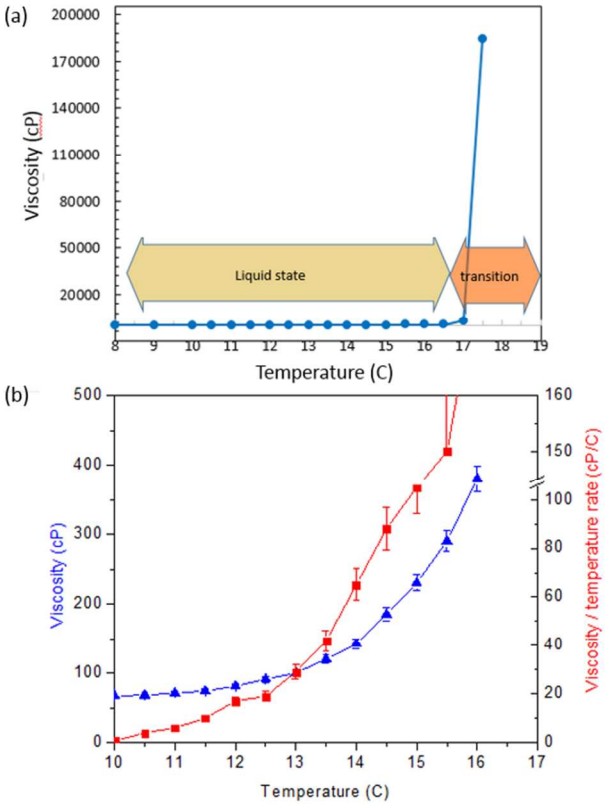

Figure 3 Variation of viscosity with temperature for 25% Pluronic solution. (a) Viscosity varies dramatically in the 16-19 °C range, corresponding to sol-gel transition of the Pluronic solution. (b) Variation of the viscosity within 10-16 °C (blue curve) and its associated rate (red curve). The solution is entirely liquid on 10-16 °C range but temperature dependence increases with temperature.

re-ordering and must be avoided. One challenge in this problem is that preventing coalescence is difficult because of the composition of the aqueous solution we use. In this case, Pluronic is a surfactant but, unlike the technique using surfactant to stabilize emulsions, PF 127 is dissolved in the discrete phase at 25% w/v, which is ten to a hundred times the concentration used in stabilizers. As a result, no stabilizing effect on the emulsion is observed. Pluronic droplets coalesce as soon as they make contact with each other under all experimental conditions. Adding another surfactant in the oil phase also did not improve the stability.

To avoid coalescence, we therefore introduced a third immiscible phase to serve as spacers to separate Pluronic droplets from each other. To be efficient, spacers must flow next to the Pluronic droplets without any engulfment. Assuming that the Capillary number (*Ca*) is small, engulfing is dominated by interfacial forces. Engulfing and non-engulfing correspond then to the presence of different liquid-liquid interfaces.[23] In our design, silicone oil must encapsulate both PF-127 droplets and spacer droplets, while PF-127 droplets must not encapsulate spacer droplets, and spacer droplets must not encapsulate PF-127 droplets. These requirements translate in three inequalities on the interfacial tensions that need to be satisfied: $\gamma_{PF127\text{-}spacer} > \gamma_{Si\text{-}PF127} + \gamma_{Si\text{-}spacer}$, $\gamma_{PF127\text{-}spacer} + \gamma_{Si\text{-}spacer} > \gamma_{Si\text{-}PF127}$, and $\gamma_{PF127\text{-}spacer} + \gamma_{Si\text{-}PF127} > \gamma_{Si\text{-}spacer}$. The first inequality renders that the net force along the three-phase contact line causes the replacement of the PF127-spacer interface by a thin layer of silicone oil, while in the two other inequalities both Si-PF127 and Si-spacer interfaces are maintained. We selected fluorocarbon FC-70 oil to serve as the second dispersed phase because the liquid-liquid interfacial tensions ($\gamma_{PF\text{-}127\text{-}FC70}$ = 18 mN/m ; $\gamma_{Si\text{-}FC70}$ = 8 mN/m; $\gamma_{Si\text{-}PF127}$ = 4 mN/m, measured in our laboratory) satisfy these three inequalities. Silicone oil wets the PDMS and the two emulsions water in silicone oil and fluorocarbon oil in silicone oil coexist without any engulfment

Furthermore, to make the use of spacer efficient, droplet re-ordering must be avoided. Droplet arrangement can be maintained by confining the droplets in the channel. To this end, we set the parameters the system to minimize the action of shear-stress that would lead to the break-up of the droplets before they can block the channel. The chip was operated at low capillary numbers so that viscous forces are negligible compared to interfacial forces (Ca~0.1 for Pluronic-in-silicone-oil emulsion, Ca~0.03 for FC-70-in-silicone-oil emulsion; Q=3 µL/min, μ_{Si} = 52 cP, $\mu_{FC\text{-}70}$ = 24 cP, $\mu_{Pluronic}$ = 80 cP at 12 °C). The channel geometry was set for a channel width ratio x= $w_{inPF\text{-}127}/w_{out}$=$w_{inFC\text{-}70}/w_{out}$ equal to one. In this squeezing regime, the discrete phases obstruct the channel before breaking into droplets due to the resulted increase in the dynamic pressure upstream (Fig. 2b). Fig. 2b illustrates a stable alternated production of Pluronic droplets and FC-70 droplets for the following flow rates: Q_{Si}=3µL/min, $Q_{FC\text{-}70}$=0.4 µL/min, and $Q_{PF\text{-}127}$=0.4 µL/min. We show that droplets were confined and spacers were reliably introduced between each Pluronic droplets (see suppl. video1), thus avoiding coalescence and maintaining order.

Worm trapping and handling

To fulfil its purposes in precise handling of worms, the droplet platform must be robust. Because the worms are very active, there was a potential for them to disturb the encapsulation process or escape the droplets during the operation of the platform. To address this problem, we designed a 200 µm wide channel at the junction, in the same order of the length of L1 worms. The sequence of photographs in Fig. 2b shows different positions of worms as they go through the junction leading to their encapsulation. The animals did not have any significant influence on droplet production, all of them got encapsulated, and the alternating pattern of spacers and Pluronic droplets was not disturbed. In addition, to ensure an efficient trapping, we adjusted the flow rates to produce droplets with a diameter equivalent to worm body length. Breaking of the oil/aqueous interface and partial escapes occurred only in the case of

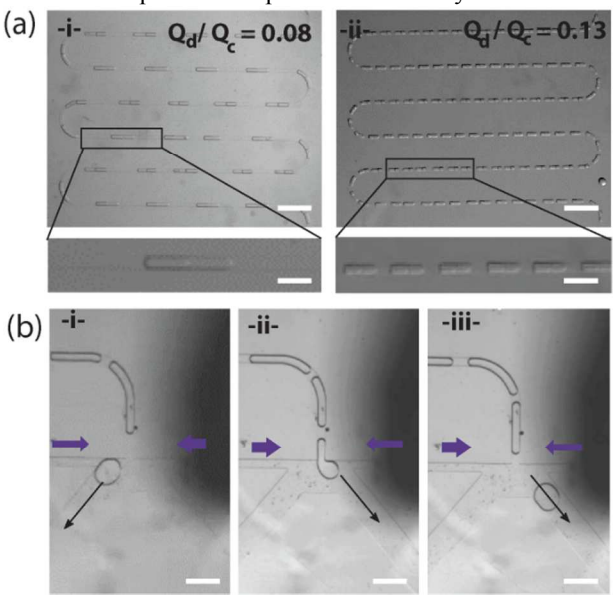

Figure 4 Droplet trafficking in the microfluidic platform. (a) Filling the storage channel. For different continuous and discrete phase flow rates (Q_c and Q_d respectively), the storage capacity varies from 80 droplets (i) to 250 (ii). Scale bars are 400 µm. (b) Pictures illustrating the sorting ability using flow-based control channels: (i) flow in control channel 2 orients droplet towards outlet 1, then (ii-iii) increased flow in control channel 1 orients droplets toward outlet 2. Scale bars are 200 µm.

droplet length being at least a third smaller than the worm body. Having droplets longer than the worm body length prevented this situation from happening, which is how we operated our chips. Once the worms were encapsulated, the Pluronic-oil interface resisted to worm thrashing, indicating an efficient trapping system. This allows for precise handling of the worms. Fig. 4a shows on-chip storage of worms (see suppl. video2) and Fig. 4b sorting by changing the flow rates in the side control channels (see suppl. video3).

For the platform to outperform or at least match the throughput of manual methods, the droplet system needs to be able to process several tens worms within an hour. The throughput of our current system is mainly determined by two parameters: encapsulation rate, which is the percentage of droplets containing a single worm (as opposed to droplets

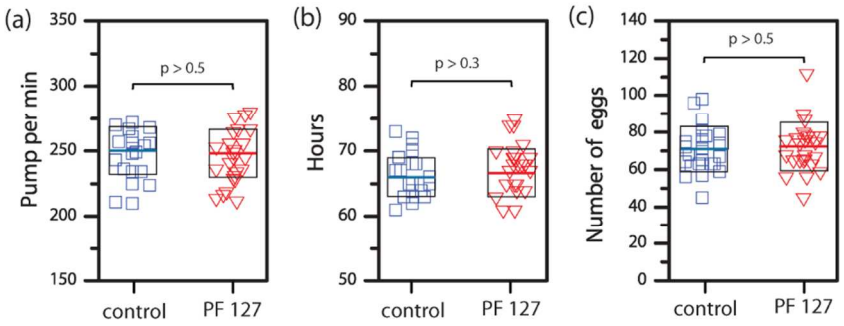

Figure 5 Comparison of developmental indicators after Pluronic and microfluidic manipulation. (a) pharyngeal pumping rate; (b) time to lay first egg; (c) number of eggs laid in one hour. (a-c) no statiscally significant differences are shown between control group and animals processed through the chip.

containing several worms or none), and the chip capacity, which is the number of droplets it can store. Worm encapsulation is difficult to control deterministically because of the shape and activity of the worms. The only experimentally controllable variable that could affect the worm distribution in droplets is the worm concentration in the initial solution. Therefore we optimized the initial concentration of worms in Pluronic solution and measured the single-worm encapsulation rate to be within 30-40%. Considering chip capacity that can be pushed to 250 droplets by adjusting the flow rates (e.g. Fig. 4a), 50 worms can be analysed in one batch on the current design. Additionally, if necessary, this number could be increased to several hundred by processing multiple devices in parallel or increasing the length of the serpentine channel.

Pluronic droplet method having no measurable effect on long-term viability and development of *C. elegans*

For the platform to be useful for developmental assays, the method must have no adverse effect on the development of the worms. PF-127 has previously been used for cell encapsulation,[24, 25] drug delivery,[26] pharmaceutical applications[27], and with nematodes.[18, 19, 28] In all of these studies, Pluronic showed no adverse effect on the organism involved. In our application, because the worms remain in Pluronic for a relatively large amount of time, we studied the development of worms after handling in the device. We monitored three indicators relevant to the development of worms: the time to reach egglaying, the pharyngeal pumping rate at young adult stage, and the number of progeny in the first 24 h of egg-laying. We compared two populations of worms from the same original culture plate. The control was a population of worms directly from the same original culture plate, while the experimental group was manipulated in the droplet-chip system in mock imaging experiments. Both experimental and control groups were allowed to grow on plates from L1 until we assayed the behaviour and physiology later. Fig. 5 shows the measurements for the three sets of indicators: pharyngeal pumping rate, time to egg laying and egg-laying rate. There are no statistical significant differences between the on-chip group and the plate control group, which suggests that our method based on Pluronic droplets has no discernable adverse effect on the worm development and therefore can be used for developmental assays.

High-resolution imaging of L1 worms

One major aim of our platform is detecting morphological phenotypes for developmental assays. To do so using high-resolution imaging requires full immobilization of the worms because movement blurs the images. The immobilization efficiency is related to the gel stiffness, which can be tuned by changing the concentration of Pluronic F127 in solution. In a previous work,[18] we optimized this concentration to 25% to ensure a good quality of immobilization while having a sol-gel transition temperature within the physiological range of the worms. Using this mixture to immobilize nematodes, we demonstrated a shift in position less than 0.5 µm over 10 s, which is enough for obtaining at least two z-stacks of 40 images each.[18] In our present system, worms were trapped in gel for a longer time, as it takes several minutes to image all the worms on chip.

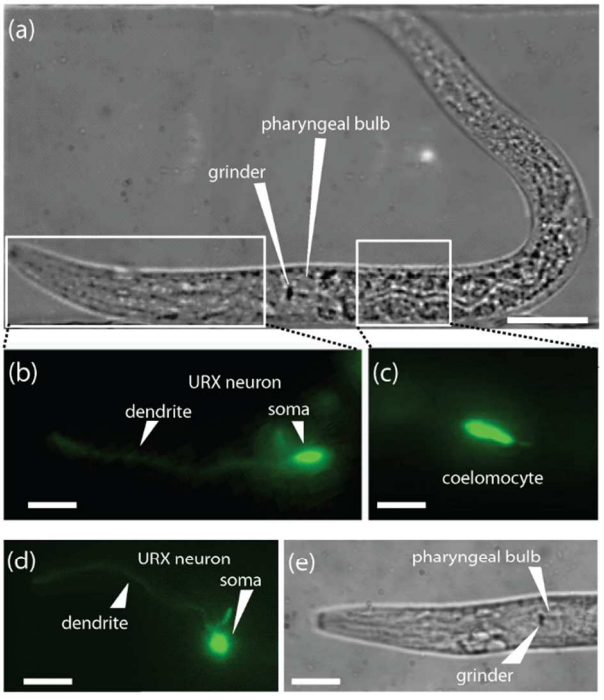

Figure 6 High-resolution imaging of L1 animals using microfluidic platform and conventional immobilization technique. (a) Bright-field high-resolution imaging of L1 worm in device. (b,c) On-chip fluorescence images of a coelomocyte and URX neuron in the head respectively using 63x objective. (d-e) Bright field and fluorescence images using conventional immobilization technique (drugs and agar pad). (a-e) show similar imaging quality between the two methods. Scale bars 25 µm in (a), 12 µm in (b-e).

To demonstrate the high-resolution imaging capability of our device, we imaged using a 63x oil objective a strain carrying a reporter transgene *kyIs342*. Fig. 6 shows bright-field images of L1 worm (Fig. 6a) and fluorescence images of green fluorescent protein (GFP) expressed in coelomocytes and URX sensory neurons (Fig. 6b-c). We also compared the image quality obtained with the microfluidic platform to the one using conventional methods, as the use of Pluronic, the presence of silicone oil film and the PDMS layer may introduce optical aberrations. Fig. 6(d-e) shows bright field and fluorescence images of a worm from the same strain imaged using an agar pad and anesthetized by tetramisole. Both images show similar details (with clearly visible soma and dendrites) when compared with Fig. 6(a,c). In bright field images, it is possible to distinguish the second pharyngeal bulb and the grinder and fluorescent images allow for the observation of subcellular features such as URX dendrite stretching from the cell body to the tip of the nose. Overall, the comparison between the two methods shows no significant difference, suggesting that there is no degradation in image quality.

Conclusion

We have reported here a platform that allows for high-resolution imaging and sorting of early larval stage *C. elegans*. Using droplet microfluidics, we were able to handle first larval stage worms in a precise and robust manner. In addition, using a reversible thermosensitive hydrogel enabled temporary immobilization of the target for imaging. No anaesthetics were used allowing for minimal physiological perturbations. This strategy leads to a simple and robust system well suited for developmental assays. Further work could expand the applications of this platform. Behavioural studies may be performed by taking advantage of the liquid state of Pluronic solution prior to or after sol-gel transition. In this context, droplets could be exploited also to modulate the chemical environment of the worms. We envision this platform to be used for applications such as laser surgery, especially for cell ablation or neuro-regeneration studies. Furthermore, by scaling up the dimensions of the channels, this platform could be used with worms at any developmental stage or with other microorganisms. We envision that this platform will enable numerous post-embryonic developmental studies in *C. elegans* involving early processes, such as cell migration, tissue specification, and developmental decision-making leading to L1 arrest and dauer stage.

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1 W. B. Wood, *The Nematode Caenorhabditis elegans* (Cold Spring Harbor Lab. Press, New York, USA, 1988).
2 Z. F. Altun and D. H. Hall (2009). "WormAtlas: Introduction."
3 L. R. Baugh, Genetics **194**, (2013).
4 P. J. Hu Dauer. WormBook. T. C. e. R. Community, WormBook.
5 C. I. Bargmann and L. Avery, Methods in Cell Biology, Vol 48 **48**, (1995).
6 E. Kim, L. Sun, C. V. Gabel and C. Fang-Yen, Plos One **8**, (2013).
7 A. San-Miguel and L. H. (2013). "Microfluidics as a tool for C. elegans research." WormBook ed. The C. elegans Research Community, WormBook. from http://www.wormbook.org. .
8 K. H. Chung, M. M. Crane and H. Lu, Nature Methods **5**, 637 (2008).
9 M. M. Crane, J. N. Stirman, C. Y. Ou, P. T. Kurshan, J. M. Rehg, K. Shen and H. Lu, Nature Methods **9**, 977 (2012).
10 H. Lee, M. M. Crane, Y. Zhang and H. Lu, Integrative Biology **5**, (2013).
11 I. D. Caceres, N. Valmas, M. A. Hilliard and H. Lu, Plos One **7**, e35037
(2012).
12 K. Chung, M. Zhan, J. Srinivasan, P. W. Sternberg, E. Gong, F. C. Schroeder and H. Lu, Lab on a Chip **11**, 3689 (2011).
13 W. W. Shi, J. H. Qin, N. N. Ye and B. C. Lin, Lab on a Chip **8**, 1432 (2008).
14 W. W. Shi, H. Wen, Y. Lu, Y. Shi, B. C. Lin and J. H. Qin, Lab on a Chip **10**, 2855 (2010).
15 T. V. Chokshi, A. Ben-Yakar and N. Chronis, Lab on a Chip **9**, 151 (2009).
16 C. B. Rohde, F. Zeng, R. Gonzalez-Rubio, M. Angel and M. F. Yanik, Proceedings of the National Academy of Sciences of the United States
of America **104**, 13891 (2007).
17 S. E. Hulme, S. S. Shevkoplyas, J. Apfeld, W. Fontana and G. M. Whitesides, Lab on a Chip **7**, 1515 (2007).
18 J. Krajniak and H. Lu, Lab on a Chip **10**, 1862 (2010).
19 J. Krajniak, Y. Hao, H. Y. Mak and H. Lu, Lab on a Chip **13**, (2013).
20 K. Chung and H. Lu, Lab on a Chip **9**, 2764 (2009).
21 D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides,
Analytical Chemistry **70**, (1998).
22 S. Brenner, Genetics **77**, (1974).
23 D. L. L. Chen, L. Li, S. Reyes, D. N. Adamson and R. F. Ismagilov, Langmuir **23**, (2007).
24 H. Lee and T. G. Park, Journal of Biomedical Materials Research Part
A **88A**, (2009).
25 S. F. Khattak, S. R. Bhatia and S. C. Roberts, Tissue Engineering **11**, (2005).
26 C. Y. Gong, S. A. Shi, P. W. Dong, X. L. Zheng, S. Z. Fu, G. Guo, J. L. Yang, Y. Q. Wei and Z. Y. Qian, Bmc Biotechnology **9**, (2009).
27 M. M., *Amphiphilic Block Copolymers: Self-Assembly and Applications* (Elsevier, New York, 2000,
28 C. L. Wang, G. Bruening and V. M. Williamson, Journal of Chemical Ecology **35**, (2009).