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Long-term high-resolution imaging and culture of *C. elegans* in chip-gel hybrid microfluidic device for developmental studies

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Developmental studies in multicellular model organisms such as *Caernohabditis elegans* rely extensively on the ability to cultivate and image animals repeatedly at the cell or subcellular level. However, standard high-resolution imaging techniques require the use of anaesthetics for immobilization, and may have undesirable side effects on development. Thus such techniques are not ideal in allowing the same animals to grow and be imaged throughout development to observe specific developmental processes. In this paper, we present a microfluidic system designed to overcome these difficulties. The system allows for long-term culture of *C. elegans* starting at L1 larval stage and repeated high-resolution imaging at physiological temperatures without using anaesthetics. We use a commercially available biocompatible polymer, Pluronic F127 for immobilization; this polymer is capable of a reversible thermo-sensitive sol–gel transition within ~ 2 °C, which is well-controlled in the microfluidic chip. The gel phase is sufficient to immobilize the animals. While animals are not imaged, they are cultured in individual chambers in media containing nutrients required for development. We show here that this method facilitates time-lapse studies of single animals at high-resolution and lends itself to live imaging experiments on developmental processes and dynamic events.

Introduction

The nematode *Caernohabditis elegans* is an excellent genetic model system for studying cell and neurobiology, development, and the neural basis of behavior.[1–4] Studies of synaptogenesis and synaptic functions using *C. elegans* have made substantial contributions to the field, by identifying major genetic players in these processes.[5–11] Experimentally, observing transgenic animals with, for example, synaptic proteins fused with green fluorescent protein (GFP), requires high-magnification imaging. These markers are typically dim, the structures of interest are small (*e.g.* synapses), and animals have to be fully immobilized to prevent image distortion. In addition, if development is to be observed over time, animals have to be immobilized repeatedly, preferably at physiological conditions, to obtain a sequence of images delineating the events of interest, and allowed to develop normally between imaging cycles. The standard methods at present employ a variety of anaesthetics to biochemically achieve immobilization[12] or glue to mechanically immobilize animals.[13] However, anaesthetics impede natural cellular activity and both

methods inhibit natural development, affecting the phenotypes of interest and making them unsuitable for repeated use.

Recently, several methods of immobilization using microfluidics have been developed. Most of these utilize microfluidic chips for rapid imaging, phenotyping or sorting of animals. In one method, cooling to a temperature of ~4 °C was used to immobilize *C. elegans*.[14] Although cooling animals for ~2 s does not affect morphological phenotypes and allows animals to reproduce, it cannot be used where longitudinal studies of physiologically active processes with repeated imaging are of interest. In another method, the application of CO_2 in conjunction with compressive forces *via* membrane deflection was used to achieve immobilization,[15] which similarly affects development. Other methods of compressive immobilization have also been demonstrated, such as the application of suction channels in tandem with a membrane[16,17] or immobilization *via* tapered channel geometry.[18,19] However, while compressed, animals are deformed, which may cause anatomical features to shift during imaging and possibly alter the native developmental processes over time. Feeding/long-term culturing and recapturing the same animals for imaging would also be impractical. Hulme *et al.*[20] have successfully designed a device specifically for long-term culture and aging studies. An array of chambers is used to individually culture animals from L4 stage until death, with occasional imaging in a tapered channel. This design, however, cannot be used for the much smaller L1–L3 larval stages. Thus, a different approach is necessary to overcome the challenges of live and repeated imaging with the ability to individually culture and track animals, particularly from L1 stage onward.

Here, we present a hybrid microfluidic chip-gel system, which we developed in order to perform long-term high-resolution imaging of specific developmental processes at physiological conditions. We show the novel utility of the triblock copolymer Pluronic F127, as the immobilization agent, in combination with a microfluidic platform. The system allows us to culture, individually track and repeatedly image *C. elegans* at high resolution directly inside a microfluidic device while allowing for normal development for any stage of the animals.

Materials and methods

Device fabrication

We fabricated all microfluidic devices in poly(dimethylsiloxane) (Sylgard 184, Dow Corning) using well-established multilayer soft-lithography techniques.[21,22] Briefly, each of the two layers of the device was designed in AutoCAD 2008 (Autodesk); masks were printed by CAD Services (California). The masters were fabricated on a silicon wafer using SU8-2025 (MicroChem), and treated with tridecafluoro-(1,1,2,2-tetrahydrooctyl)-1-trichlorosilane silane (UCT Specialties, LLC). Each of the two layers were then molded into PDMS with the ratio of base polymer to cross-linker to toluene of 20 : 1 : 5 (flow layer) and 10 : 1 : 0 (flow and temperature control layer).

The 50-μm-thick flow and temperature control layer was partially cured at 70 °C for 20 min at a PDMS thickness of 3 mm. Next, the flow-layer master was spin-coated to a thickness of 45 μm and partially cured on a hot-plate at 65 °C for 15 min, giving a 30 μm membrane between the 15 μm thick flow channels and culture chambers and the top layer.

The two layers were aligned and thermally bonded at 70 °C for 2 h and irreversibly bonded to a glass substrate *via* oxygen plasma treatment for 20 s (PDC-32G plasma cleaner).

Solutions and materials

Fluids, such as culture medium and Pluronic F127 solution, were delivered to the device *via* plastic PE-60 tubing (Micro Medical Tubing, Scientific Commodities, Inc.) and metal pins, which were inserted into the PDMS device, by a pressure based delivery system.[14] The heating liquid was delivered from a hot bath, which was maintained at a constant temperature of 85 °C. For the heating liquid, the applied pressure not only determines the flow rate, but indirectly affects the temperature of the liquid reaching the device, the residence time inside the device, and the temperature of the culturing chambers. The optimal driving

pressure, which lead to a temperature change of ~2 °C for the environmental conditions in our laboratory, was 14 psi. The optimum pressure was determined by measuring temperature in the culture chambers using a novel temperature measurement technique.[23]

Solutions of Pluronic F127 (Sigma Aldrich) in water were prepared at concentrations of 23% and 25% w/v. The suspension was agitated at 4 °C until the polymer pellets were completely dissolved. Solutions of Pluronic F127 at 25% w/v with hydrocortisone were prepared by suspending 10 μL of hydrocortisone solution (6.06 μM in ethanol) (Sigma Aldrich) in 10 mL of the pre-mixed PF127 solution. Standard nematode buffer[24] solution containing OP50 bacteria was prepared by centrifuging OP50 bacteria suspended in LB medium, removing the supernatant, and re-suspending bacteria in M9 buffer at $OD_{600} \approx 0.9$. The solution was filtered through a 5 μm filter to remove clumps and debris.

Animal culture, assay, and microscopy

C. elegans were cultured at 20 °C on standard agarose plates with OP50 bacterial lawns, and were suspended in standard nematode buffer for experiments. We used the N2 strain for all experiments where fluorescence was not required. CZ456 (a strain that carries *juIs1* = P*unc*-25::*gfp*) [25] was used for all fluorescence studies.

Repeated immobilization experiments to assess animal viability were performed in Eppendorf tubes. Animals were age-synchronized by allowing them to hatch in M9 solution for a period of 24 h after embryo isolation. After 24 h, L1 animals were extracted from M9 and suspended in 25% w/v solution of Pluronic F127. Two different immobilization periods were tested; short cycles consisted of 60 s at 15 °C to trigger gel-to-liquid transition and 90 s at 21 °C for complete gelation, and long cycles consisted of 5 min at 15 °C and 10 min at 21 °C. Age-synchronized animals were divided into two groups, one exposed to a total of fifty short cycles and the other to five long cycles.

After the temperature cycling, 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 the same batch of animals, which were kept in M9 solution for the duration of the experiment and were placed on agar plates at the same time, and not exposed to the temperature cycling. After being placed on culture plates, animals were allowed to grow at 21 °C, and the time to reach egg-laying, pharyngeal pumping rate at young adult stage, and the number of progeny in the first 24 h of egg-laying was compared in all three groups.

To determine the effects on early development, unhatched embryos were suspended in 25% w/v solution of PF127, and PF127 with 60 nM hydrocortisone. Embryos were allowed to hatch, and were maintained in the solution for a period of 24 h. After 24 h, animals were processed in the same manner as during repeated immobilization experiments.

Fluorescent images were acquired using a Leica DM4500B microscope with a Hamamatsu C9100-13 EM CCD camera at 5× magnification to monitor the device operation and at 100× magnification for subcellular imaging. Images were acquired using Image-Pro (MediaCybernetics) and processed and pseudo-colored with ImageJ (NIH).

Results and discussion

Our device is capable of providing both nutrients and proper gas exchange to multiple animals cultured in individual chambers. These chambers are used to keep animals separated at all times, thus guaranteeing that development can be followed on a per-animal basis. Reversible and repeatable immobilization directly inside the culture chambers is facilitated by a solution of Pluronic F127 (PF127), which undergoes a thermo-reversible sol–gel transition. Thus, with efficient temperature control, cycling between a liquid and an immobilizing gel phase is easily achievable. The immobilization occurs anywhere in the chamber without positional requirements, with minimal environmental and physiological disturbances and physical deformation to the animals. This method of immobilization enables observation of physiological processes and events in an unprecedented manner.

Hybrid platform design

To successfully culture, immobilize, and image animals, all in a single platform, we designed a hybrid system composed of a microfluidic device and PF127 solution for repeated immobilization. The microfluidic device is a PDMS-based, two-layer device (Fig. 1). The flow layer contains all flow inlets and outlets and the individual culture chambers. The two inlets are used to load animals into the device and deliver culture medium (loading inlet) and to flow PF127 solution into the device (PF127 inlet). The inlets join to deliver solution and animals to the loading chamber. From here, animals enter the individual culture chambers, where they are trapped for the duration of the experiment.

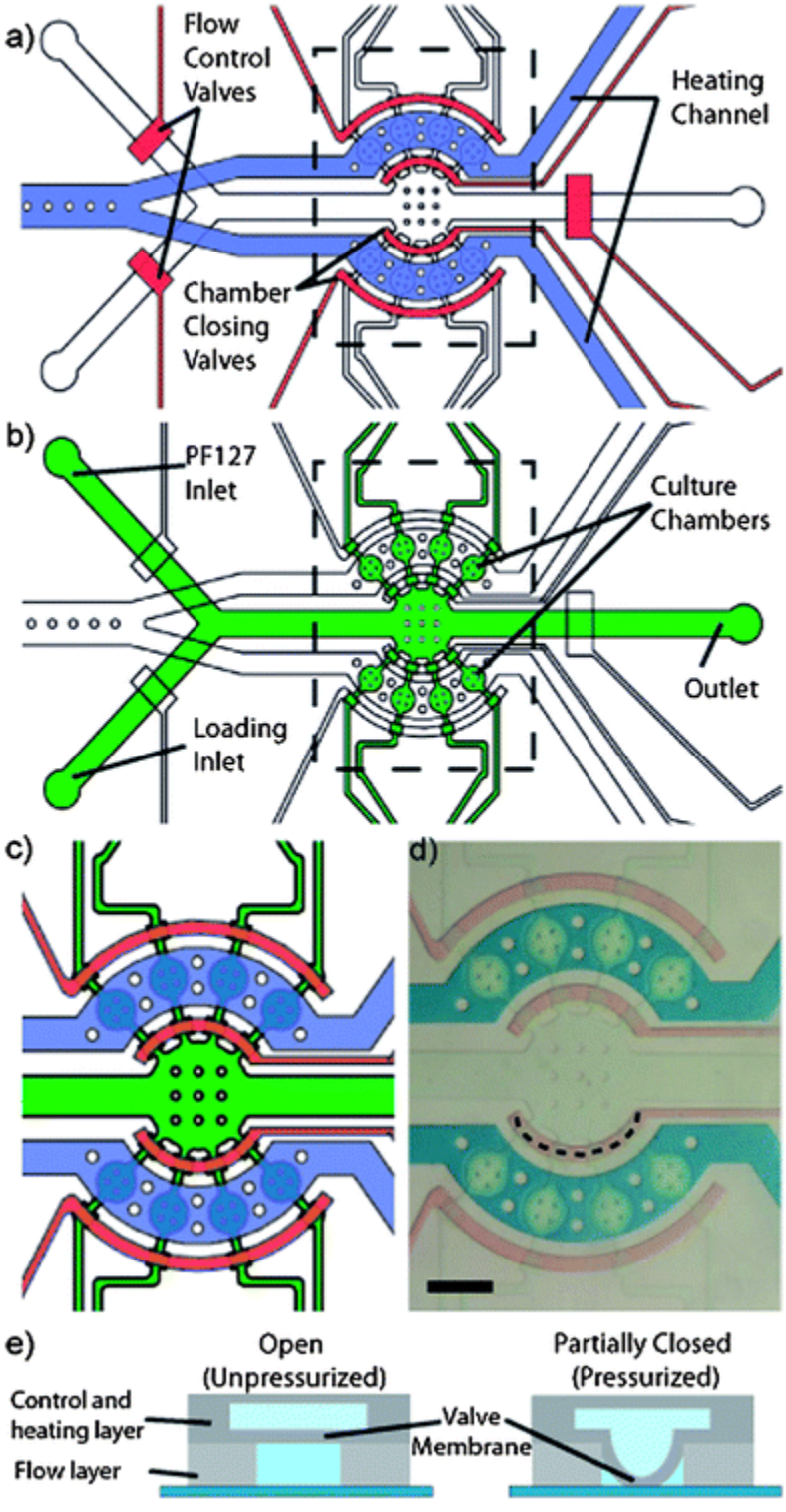

Fig. 1 Two-layer microfluidic device used for our hybrid platform. The flow control and heating layer (a) contains pneumatic valves (red) for flow control and trapping of animals inside chambers of the flow layer, as well as the channel used for flowing heating liquid (blue). The flow layer (b) contains the loading inlet (for animals and bacterial solution) and an inlet for the PF127 solution, 8 culturing chambers for individual culture of animals, and a waste outlet. Details of the chambers are depicted in (c); and a photograph of a dye-filled device is shown in (d); both correspond to the areas marked by dashed line rectangles in (a) and (b). The schematic in (e) represents the cross section of a partially closed valve, such as the one marked by a dashed line in (d). While unpressurized, the valve remains open. After pressurization, the valve membrane deflects into the flow layer, partially obstructing the channel. This prevents animals from escaping while allowing flow to continue. Scale bar represents 400 μm.

There are eight culture chambers per device; four on each side of the loading chamber. These two sets can be operated independently and thus the device can be operated with either four or eight active chambers. Large culture chambers were used for two reasons: (i) they allow animals to grow without mechanical constriction and move around freely in an aqueous environment, and (ii) the large cross-sectional area leads to the expansion of flow and thus a decrease in linear flow velocity and hence shear on the animals. Therefore, unlike in straight channels, sufficient exchange of media and nutrition occurs without animals having to constantly swim against a flow. The slowing of flow also leads to a longer residence time of bacteria in the chambers, which in turn allows animals to feed properly. Partially closed pneumatic valves at the inlet and outlet of the culture chambers function to trap animals for the duration of the experiment (Fig. 1e). When unpressurized, these valves remain open and permit flow through the corresponding channels. When pressure is applied, the membrane deflects down into the flow layer, increasing the resistance and reducing the flow,[14,22] as shown in Fig. 1e. Because of the rectangular cross section of the flow channels, these valves do not permit animals to escape while simultaneously allowing flow of liquids through the channels. Debris and wastes are flushed out *via* the flushing outlet.

The second layer of the device contains valves for flow control and heating conduits for temperature control. The temperature control channel is used for raising the temperature within the device by no more than a few degrees Celsius to trigger gelation of PF127 for immobilization of the animals. Precise temperature control is achieved by controlling the flow rate of the heating fluid and maintaining the heating fluid source temperature.

In combination with the microfluidic device, PF127 solution is used for the immobilization. PF127 is an amphiphilic block copolymer (PEO_{99}-PPO_{67}-PEO_{99}). At different temperatures, differential solubilities of PEO and PPO in water determine the polymer behavior.[26–30] At low temperature the Pluronic solution behaves like a viscous liquid. At temperatures higher than the critical gelation temperature, packed polymer micelles form a gel, which is capable of immobilizing animals. The solution-to-gel transition is reversible; the gel will disperse once the temperature is lowered below the lower critical gelation temperature.[26–30] The transition is concentration dependent, and at high enough concentration, the transition temperature is not only lowered to a range near *C. elegans* culture temperatures, but extremely sensitive to temperature changes (*i.e.* a very small temperature change can induce the phase transition). For instance, a change of 1 °C triggers the transition for concentrations greater than 22% w/v of PF127 in aqueous solution near room

temperature. Gelation of the polymer produces a 100 to 10000-fold increase in viscosity at shear rates commonly caused by animal movement (data not shown), and the storage modulus of the resulting gel is ~ 3000 Pa.[31] Thus by controlling a small change in temperature, we achieve immobilization of the animals near their cultivation temperature and allow physiological functions to continue unaltered.

Pluronic F127 having no negative effects on image quality

Low scattering and low autofluorescence are critical for imaging extremely small features, such as subcellular or synaptic expression patterns. To show that image quality does not degenerate by the presence of Pluronic solution, we imaged animals containing *juIs1*, the integrated P*unc-25*-SNB-1::GFP marker. The *unc-25* promoter-driven GFP is expressed in 26 GABAergic neurons and is localized in cell bodies, axonal branches, and synaptic regions.[25] The expression from this transgene is dim, photobleaches rapidly, and is difficult to observe even using standard methods; it is thus a good candidate to assess the imaging methodology.

We compared the quality of images obtained using a standard agar pad and sodium azide as the immobilizing agent with that of images obtained using 25% w/v PF127 gel inside our microfluidic device (Fig. 2). As can be seen in Fig. 2a–d, both methods yielded similar high quality images. It was possible to identify neuronal cell bodies and synapses (some ~1 μm) along the ventral and dorsal nerve cords, even in the presence of autofluorescence from the animal intestine. The PF127 gel itself did not exhibit any noticeable autofluorescence, and we did not observe more scattering from the polymer. Photobleaching was also comparable to that of the standard method.

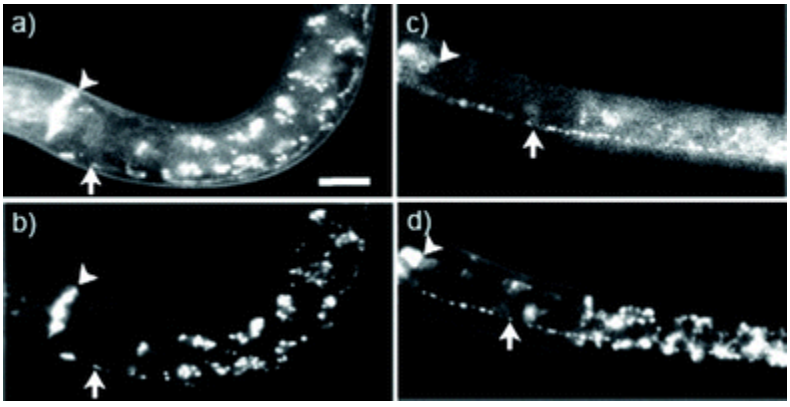

Fig. 2 Comparison of image quality between standard procedures (on agarose pads with anaesthetics) (a,b) and using PF127 gel in microfluidic devices (c,d). The imaged animals here carry *juIs1*, a very faint and easily photo-bleachable synaptic GFP marker. (a,c) Single image at a focal plane, (b,d) flattened *z*-stack (40 images, step size 0.5 μm). Scale bar represents 5 μm. Arrows point to GFP expression localized to synapses, arrowheads point to neuronal expression.

PF127 having no effect on long-term viability and development of *C. elegans*

PF127 is FDA approved for use in clinical applications,[32] is used in cell encapsulation techniques[31] and drug delivery applications,[33] and has been shown to exhibit a very low level of cytotoxicity.[32,33] Despite being generally accepted as biologically benign for cells and tissues, we performed tests to verify that this is also the case with *C. elegans*.

In some applications, embryos or younger larvae would be used for imaging. Since development continues long after hatching, we wanted to ensure that development is not adversely affected by the surfactant-like properties of the PF127 molecules. Therefore we evaluated the effect of exposure to the PF127 solution during the embryonic and L1 larval stages. In these experiments, we exposed the animals to 25% w/v PF127 solution, which is as high a concentration as we would potentially choose in experiments, and to PF127 solution containing hydrocortisone, which is a membrane stabilizing agent. We observed three indicators of development and physiology: (i) time to reach egg-laying, (ii) the pharyngeal pumping rate at young adult stage, and (iii) the number of progeny in the first 24 h of egg-laying. As can be seen in Fig. 3a–c, none of these parameters showed statistically significant differences between the PF127 or PF127 + HC and the control samples. This suggests that exposure to PF127 solution, even during early developmental stages, has no adverse effects on *C. elegans* development, and the polymer solution can therefore be used for immobilization during live imaging experiments and long-term developmental studies.

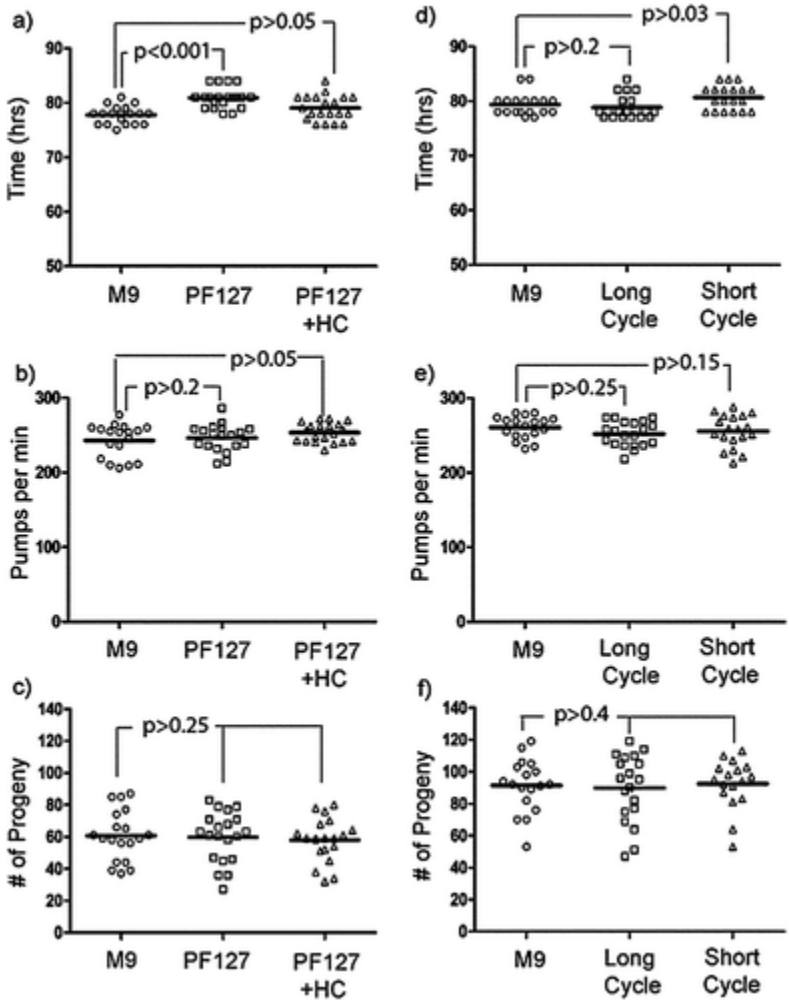

Fig. 3 Analysis of the effect on animal development by exposure to PF127 during early development (a–c) and repeated immobilization cycles (d–f). (a,d) mean time to reach egg-laying, (b,e) pharyngeal pumping rate at young adult and (c,f) number of progeny in the first 24 h of egg-laying. For all experiments, $n \geq 18$. In (a–c), embryos were exposed to nematode buffer M9, PF127 solution, and PF127 solution containing hydrocortisone (PF127 + HC) while hatching and during the L1 developmental stages. In (d–f), animals were

exposed to 50 short cycles (immobilization for 90 s) and 5 long cycles (immobilization for 10 min) as compared to no immobilization cycle (only in buffer M9).

To evaluate the effect of immobilization with the PF127 gel on animals, we performed a series of immobilization cycles. The conditions of these cycles corresponded to conditions similar to actual experiments on-chip: animals were suspended in PF127 solution, which was warmed and cooled to trigger the sol–gel–sol transitions as would happen during an imaging cycle. Besides visually inspecting the gross morphology of the animals, we recorded the same three indicators as before. As shown in Fig. 3d–f, for all three indicators, L1 animals exposed to short (90 s) or long (10 min) cycles showed no significant difference to the control population (no temperature cycling and no exposure to PF127). This suggests that the nematodes are unharmed by the small-range thermocycling and the presence of PF127 and proceed with normal development, which is critical for the applications in developmental studies.

Long-term culture inside a microfluidic device for early stages of development

The features of our method make it easy to use for long-term culture directly on the microfluidic device in addition to longitudinal live-imaging of animals throughout their developmental stages. As shown in Fig. 4, we tested the ability of our microfluidic system to maintain culture conditions optimal for normal development of *C. elegans*. We loaded animals into the device and trapped individual worms in the culturing and imaging chambers (Fig. 4a). To fill the culturing chambers, we first delivered animals to the loading chamber. We then opened valves controlling the inlet to the culturing chambers to load animals into the culture chamber. If multiple animals entered a chamber, the outlet valve was opened temporarily to allow the extra animals to be flushed out. Typically, the total time to load all eight chambers with a single animal each is less than 5 min.

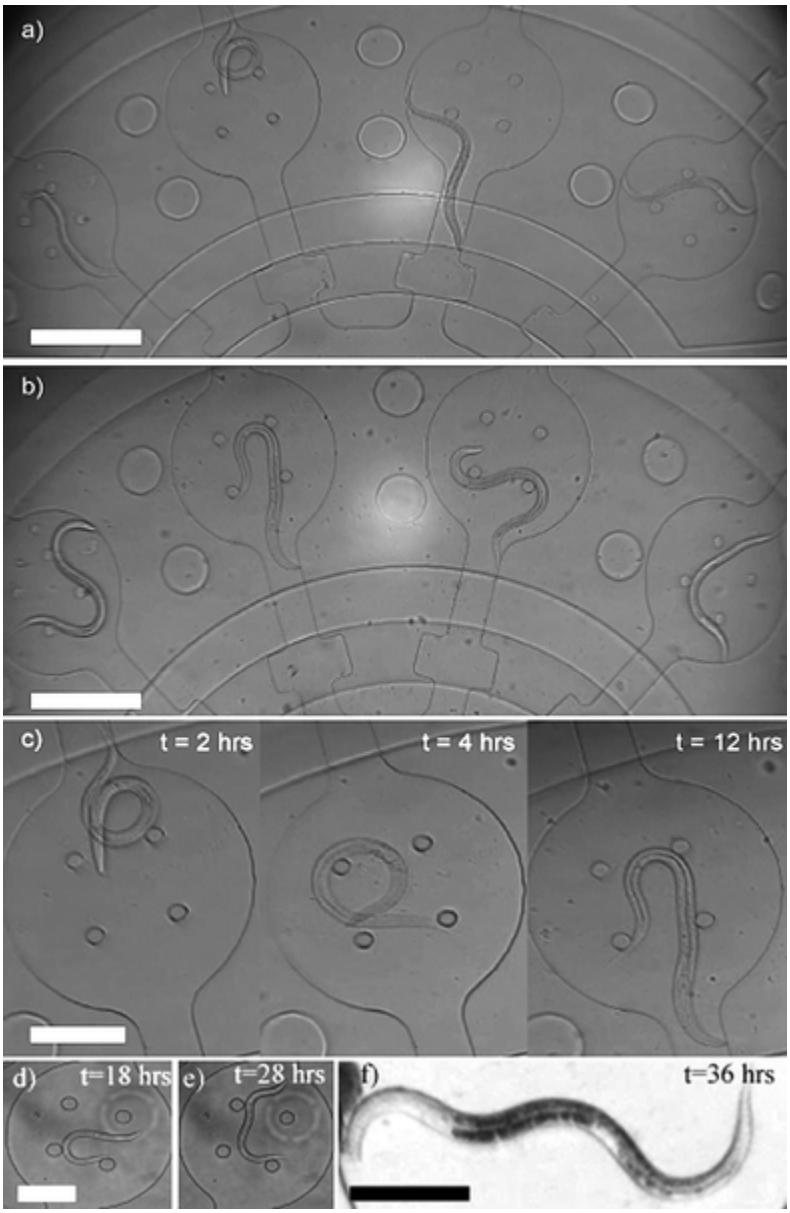

Fig. 4 To culture animals long-term, they were loaded into the device and trapped individually inside culture chambers (a), where they can remain for 12 h (b). Animals are provided with bacterial food, allowing them to grow and develop normally (c). To demonstrate the ability of the device to culture animals long term we maintained animals from early L1 to L3 stage (d–f). (d–e) show an animal during the L2 stage at 18 and 28 h after hatching and loading; (f) shows an L3 animal at 36 h past hatching. Scale bars represent 200 μm in (a–b) and 100 μm in (c–f).

Throughout the culture, animals were suspended in buffer with food and supplemental cholesterol at a concentration of 0.1 μg mL^{-1}.[24] Upon loading and trapping inside the channels, flow at 0.02 mL per hour continued to provide a steady supply of nutrients and gas exchange. Animals were maintained in this state for over 12 h (Fig. 4c), during which time they maintained normal locomotory behaviors and a pharyngeal pumping rate similar to that of animals on standard agar culturing plates (data not shown). Although the

device is intended for monitoring of specific developmental processes, we verified its ability to maintain culture conditions through the developmental stages it was designed for: L1–L3 larva. We loaded early L1 animals and maintained these animals for a period up to 36 h. Fig. 4d–e show animals during L2 stage and Fig. 4f L3 stage inside a culture chamber. In scaled up devices similar to the one reported here, we have also cultured animals for ~2 days until they became young adults (data not shown). We observed normal development (as seen by the increase in size of the animals and normal pharyngeal pumping) as well as molting in these individual chambers.

Repeated image cycles inside the microfluidic device

To demonstrate how our system can be used to track features and events in a physiologically active animal, we used our chip-gel hybrid platform to culture worms long-term and perform repeated imaging. For this purpose, animals were loaded into the individual culture chambers suspended in buffer with food through the loading inlet; the buffer was continuously perfused through the device while animals were not being imaged (Fig. 5a). During an imaging cycle, flow of the buffer was stopped and was replaced by PF127 solution (Fig. 5b). Once the solutions were completely exchanged, flow of a warm fluid through the heating channel above the imaging chamber was turned on. This flow was driven at a pressure of 14 psi from a hot-bath maintained at 85 °C. This driving pressure was previously determined to raise the temperature in the culture chambers by ~2 °C in our laboratory environment, which was maintained at a temperature of 19 °C, which is also the culturing temperature for the animals. The 2 °C temperature change led to the gelation of the solution and subsequent immobilization of animals inside the chamber (Fig. 5c). During an imaging cycle, the flow layer of the device was also temporarily pressurized to prevent deflections of the PDMS membrane separating the two device layers, which may result in the compression of the animals. The time required for onset of gelation using this method is ~45–60 s after initialization of the heating cycle. After the imaging sequence is complete, flow of heating fluid was turned off and the device cooled to ambient temperature; the cool-off time plus the phase transition of the gel is approximately 30 s. This frequency is sufficient for almost all developmental studies where the imaging frequency is ~10 min. Once the temperature of the Pluronic F127 solution is decreased below its gelation temperature, it becomes a liquid and can be easily flushed out of the device and exchanged for buffer solution until the next cycle (Fig. 5d). The liquid driving pressure in the temperature control chamber is always set equal to the driving pressure in the flow layer to prevent deflection of the culturing chamber membrane and thus avoiding the mechanical flattening of the animals. Using this method, we can repeatedly image individual animals without direct manipulation by simply exchanging the suspension liquid inside the device and raising the temperature within the device by 2 °C. The method is also age-independent and works well for animals from L1 through adulthood. Additionally, this method does not require active manoeuvre of the animals (*e.g.* positioning) during imaging. In contrast to some other methods (*e.g.* the tapered-channel geometry), animals of all ages can be immobilized anywhere in the chamber at any time using our technique.

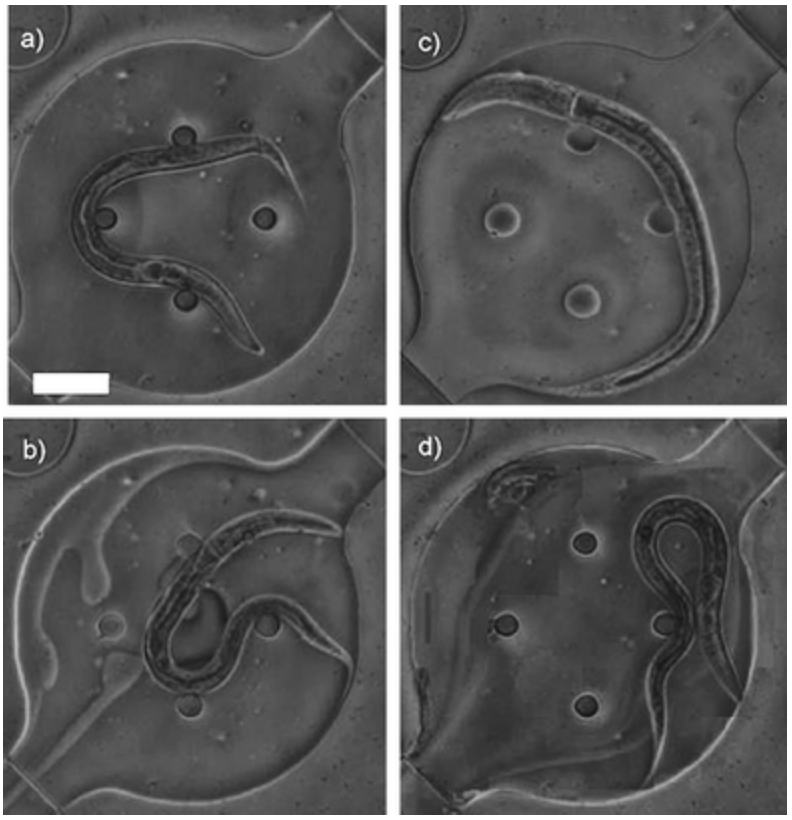

Fig. 5 Images during device operation: (a) while being cultured on device, animals are suspended in a solution of M9 containing OP50 bacteria; (b) during an imaging cycle, the M9 buffer is first replaced with PF127 solution; (c) the device is then warmed to trigger the sol–gel transition and thus immobilize the worm; (d) after the cycle is complete, the device is allowed to cool off and the PF127 solution is flushed out. Scale bar represents 60 μm.

Extent of immobilization with PF127 solution

To further assess the efficacy of PF127 in immobilizing animals for imaging, we characterized the extent of immobilization after a sol–gel transition has occurred (Fig. 6). We tested animals in 23% w/v Pluronic F127 solution. Animals were loaded into the device at a temperature below the critical gelation temperature and trapped in individual chambers while suspended in the PF127 solution. After temperature in the device equilibrated to 22 °C, which is above the sol–gel transition temperature of the polymer at this concentration, a sequence of images (100× magnification, 2 s apart) was taken. The sequence was then analyzed to determine whether individual features (*e.g.* synapses) shifted during imaging. False-coloring the images from different time points with different colors and overlaying images (Fig. 6c,d) illustrate the movement and we can also quantify positions of objects (*e.g.* synapses) of interest over time. Animals remained immobilized for a period of at least 10 s or more; within this time period the shift in position was less than 0.5 μm. This is enough time for obtaining at least two *z*-stacks of 40 images each, sufficient for most applications. Longer than 10 s, some but not all animals may struggle against the constraint of the gel, which may lead to a slight shift in position or a slight rotation of the animal body; however, the moment is gradual and for applications that require longer-term observation, such movement artifacts can be easily removed by other means (*e.g.* software correction).

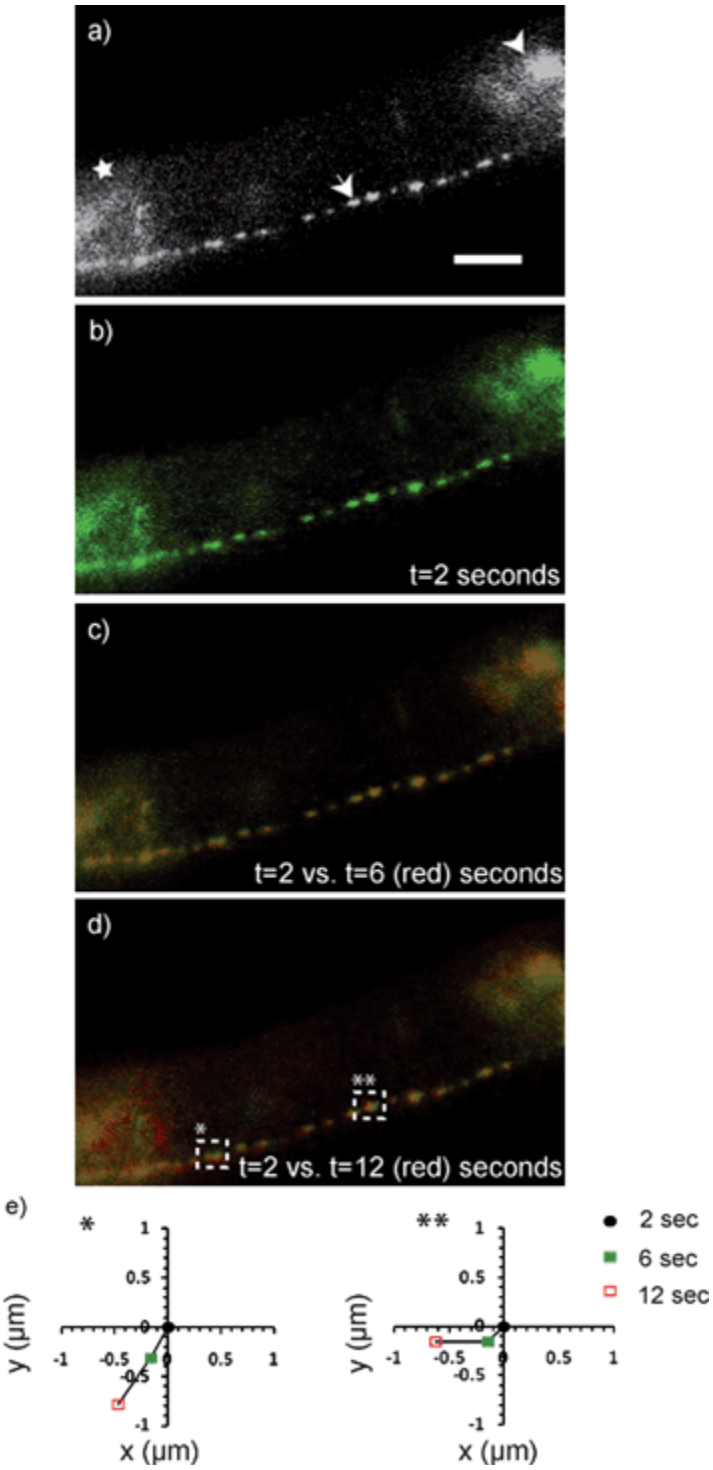

Fig. 6 Verifications of the extent of immobilization by the PF127 gel inside the microfluidic device by imaging animals. The single z-plane greyscale images (a) were processed and false-colored green (b). The arrow points to synapses labeled by the presence of SNB-1::GFP, arrowhead points to neuronal localization, and star denotes intestinal fluorescence. Images at later times from the same z-plane were false colored red. The green colored image was then merged with the red colored images taken at 6 s (c) and 12 s (d), showing complete overlap and hence

suggesting that there is no movement of the sample. Dashed-line squares in (d) show the synapses selected for positional tracking in (e). The *x*,*y* position of these synapses was tracked and graphed over time (2, 6, and 12 s) to show negligible movement. Scale bar represents 5 μm.

It is important to point out, that even though the immobilization is ultimately mechanical, no deformation of the animal body occurs. This is because the formation of the gel from the liquid solution is relatively uniform around the animal body. Since the formation of the stiff gel is uniform, there is no directional imbalance in the compressive force that would lead to deformation of body shape (*e.g.* flattening). Thus, in this method, there is no distortion of features, and it is ideal to image subcellular features over time in many developmental studies.

Conclusions

In this work, we have demonstrated the ability of our hybrid chip-gel platform to perform long-term culture with individually trackable animals (from L1 and on), and repeated live imaging at high resolution at physiological conditions. We verified that repeated immobilization for various durations and exposure to the immobilizing agent—Pluonic F127—during early larval stages has no discernable effect on animal development. Our platform can be easily expanded into larger arrays to facilitate more rapid acquisition of data; more importantly, in contrast to currently available methods, this technology is advantageous in gaining new insights on developmental and dynamic processes such as vesicle transport, synaptic re-arrangement, and growth cone development.

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