C.L.I.P – CONTINUOUS LIVE IMAGING PLATFORM FOR *C. elegans* AT PHYSIOLOGICAL CONDITIONS

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ABSTRACT

Studies of dynamic processes in *Caerhohabditis elegans* require the ability to image subcellular structures and thus immobilize animals continuously while providing them with nutrients for several hours. Standard techniques utilize glue or anesthetics, which alter physiology and prevent feeding. We present a platform to immobilize animals' bodies for several hours for continuous or frequent imaging, while simultaneously allowing feeding of the animals. This platform uses the thermo-reversible sol-gel transition of the polymer Pluronic F127 to immobilize animals in a microfluidic array of traps with positional guides, which immobilize only the body, and leave the head free to move and feed.

KEYWORDS

C. elegans, live imaging, continuous immobilization, physiological conditions

INTRODUCTION

C. elegans has been used as the model organism to study a plethora of functions of the nervous system, which aids in the understanding of processes such as synaptic development, neuronal regeneration, and genetic regulation of behavior [1-3]. The need for live and dynamic studies of these processes, where events can be observed uninterrupted as they are occurring, is becoming increasingly relevant to furthering our understanding. However, such studies are extremely difficult to perform in practice. Specifically, uninterrupted imaging requires continuous immobilization of the animals' bodies for high-resolution imaging at physiological conditions; the animals have to be allowed to feed while immobilized. We developed a platform, which combines the functionality of Pluronic F127 [4] on which we previously reported [5], together with a microfluidic device. The device is an array of channel traps with positional guides, or "muzzles". These muzzles position the animals such that the body remains in trapping channels to be immobilized, while heads protrude into a nutrition and stimulus delivery channel (Fig 1). The traps are then filled with Pluronic F127 solution, which gels and immobilizes the bodies, and heads are exposed to flow of nutrients. In this fashion, the bodies can be continuously imaged at physiological conditions, while the animals are free to feed throughout the experiment.

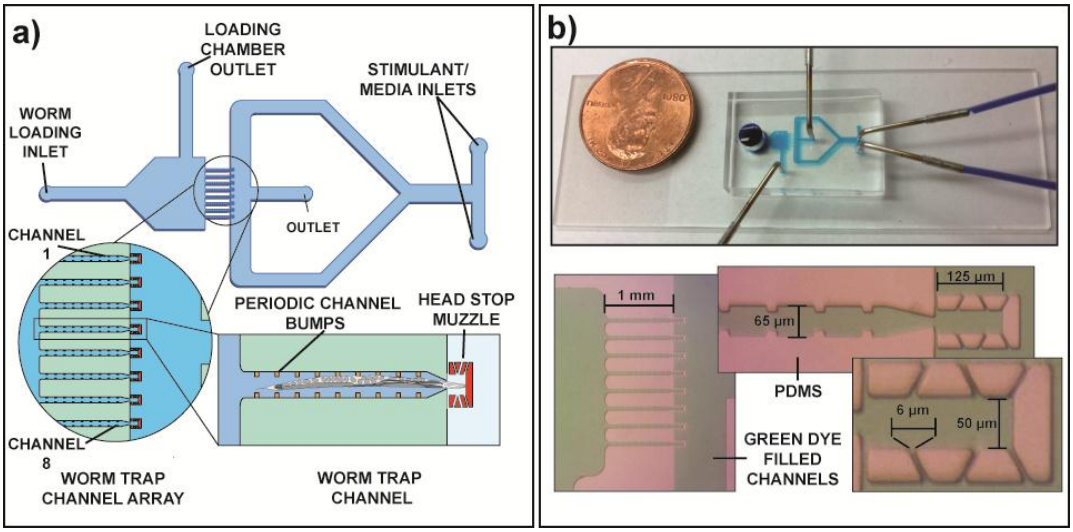

Figure 1: a) The device is an array of channels, each with a muzzle at its outlet, designed to trap and position animals so as to immobilize bodies, while leaving heads free to move. b) The dimensions and design elements are tailored to immobilize L4 through adult animals.

EXPERIMENTAL

Chip Fabrication. Microfluidic devices were fabricated in poly(dimethylsiloxane) (Sylgard 184, Down Corning) using soft-lithography techniques. The device was designed in AutoCAD (Autodesk); chrome masks were prepared by Elvesys (Paris, France); these were required due to small spacing between muzzle features. Masters were fabricated on silicon wafers using SU 2050 (MicroChem) photoresist with 45 micron tall features and treated with tridecafluoro-(1,1,2,2-tetrahydrooctyl)-1-tri-chlorosilane (UCT Specialties, LLC). Masters were molded into PDMS with the ratio of polymer to cross-linker of 5:1. The devices were bonded to glass slides or cover slips via plasma bonding.

Experimental Setup. The device has three inlets – two for media and nutrients, and one for loading animals, and two outlets – one to withdraw liquid (main outlet) and one to empty the loading chamber (flushing outlet). (Fig 1, Fig 2). All inlets and outlets are cut with 21 gauge pins except for the animal loading inlet, which is cut to a large ~4x5mm opening. Fluids are delivered and withdrawn via PE-60 tubing (Micro Medical Tubing, Scientific Commodities, Inc.) connected to

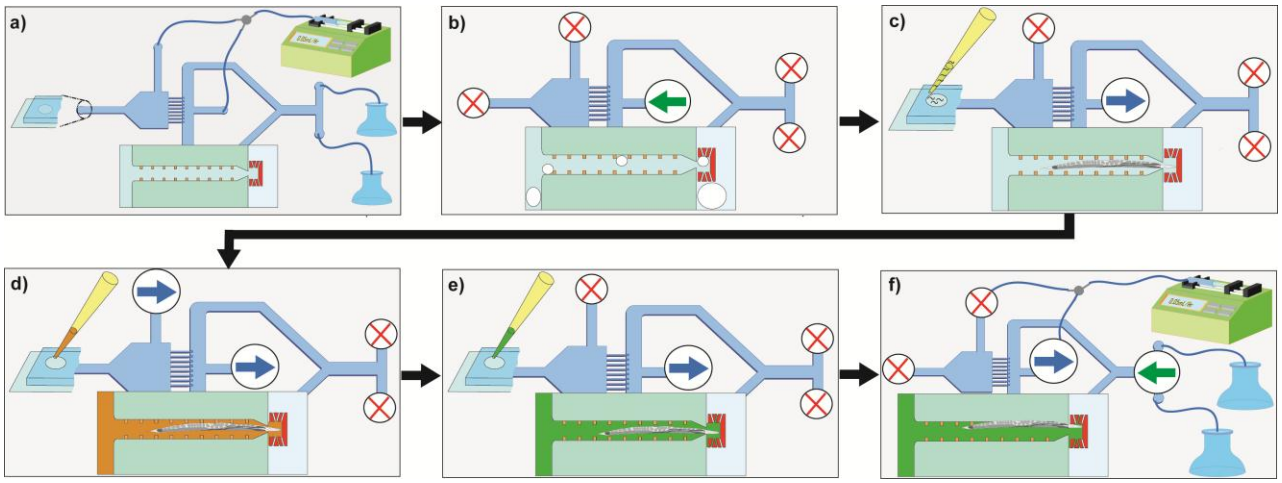

Figure 2: The setup procedure is designed to result in properly positioned and immobilized animals, so that they can feed throughout the experiment while being imaged. The procedure is very simple and only requires the researcher to load proper solutions and open or close inlets or outlets as specified in each step.

the device with metal pins. Fluid manipulation is done using a simple syringe pump. First, the device is pressurized via the outlet (Fig 2b). After all gas bubbles have been removed, negative pressure is applied to the main outlet, and animals are loaded into the animal loading inlet in 0.2% w/v Pluronic F127 solution. From here they are pulled into the traps (Fig 2c). Next, both outlets are opened and the device is flushed with 10% w/v PF127 solution to remove untrapped animals (Fig 2d). Then, chilled 25% w/v PF127 solution is dropped into the inlet and allowed to flow through the traps until the sol-gel transition occurs, which immobilizes the animals. Lastly, the media inlets are opened to allow flow of nutrients and stimulants to wash over the animals' heads. Proper positioning of animals and immobilization thus all occur without direct manipulation from the researcher, but are handled passively by the device. Nutrient solution consists of S. basal nematode buffer containing cholesterol and suspended OP50 *E. coli* bacteria. Flow of this solution is maintained throughout the experiment for constant nutrient delivery and gas exchange after loading.

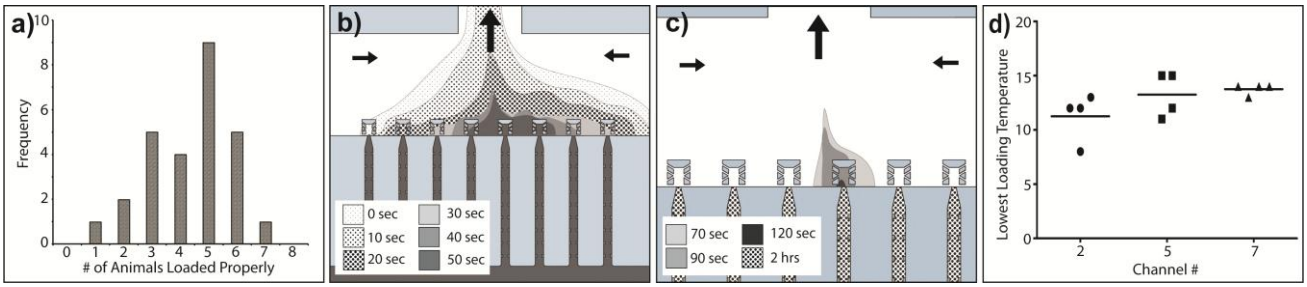

Figure 3: a) The loading efficiency is sufficient for both continuous and frequently intermittent imaging requirements. b) Temperature measurements on-chip shows that the animals are never exposed to temperatures outside their physiological range during the loading procedure. c) and d) The gel forms throughout the device where the solution is present and is eroded rapidly in the main channel and inside the muzzles while being stable in the trapping channels.

RESULTS

The main advantages of the design of the platform are the broad applications and the ease of transferring the technology to other laboratories. We focused on simple fabrication, minimal external peripheral requirements, and a simple experimental setup. By following the setup procedure, the microfluidic device is capable of trapping and immobilizing animals within the channels and leaving their heads free to move without active manipulation from the researcher. The positioning occurs passively via geometry and hydrodynamic properties of the device. Manual manipulation only includes loading the proper solutions and opening the appropriate inlets or outlets accordingly.

Proper loading depends on efficiently loading animals into the channels, on limiting the gel to the trapping channels only, and on not exposing the animals to temperatures outside their physiological range. The average number of animals trapped in the device after each loading is ~4 (Fig 3a). This is sufficient when continuous or intermittent imaging is required, as the number of animals that can be imaged is limited by imaging time and stage movement.

After loading of animals and of the chilled PF127 solution, the sol-gel transition occurs everywhere the solution is present, which includes the muzzles around the animals' heads. However, after opening of flow of media, the gel in the muzzles is rapidly eroded, while remaining stable inside the channels (Fig 3b and 3c). Also, loading the solution chilled, which is necessary to ensure that the solution surrounds the animals completely before forming a gel, never exposes the animals to temperatures outside their physiological range. The average lowest temperature as the solution enters the trapping channels is 13 ºC, after which the solution keeps warming up further (Fig 3d).

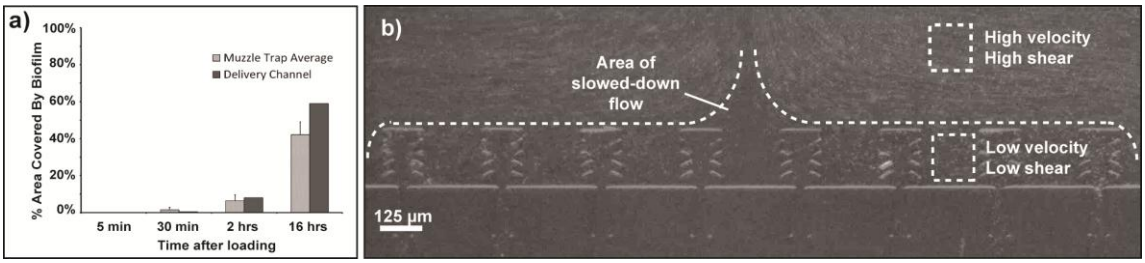

Figure 4: a) The flow of bacteria through the device causes minimal formation of biofilm in the muzzles and bulk of the delivery channel over the duration of a typical experiment. b) The flow around the muzzles (visualized with fluorescent particles) is slowed compared to the bulk of the channel, thus decreasing shear and improving feeding by increasing bacterial residence time.

Following the setup, animals are fed bacteria suspended in culture media at an OD_{600} of 0.8. We verified that the flow of bacteria through the device does not lead to excessive formation of bio-films (Fig 4a), which would affect the animals negatively. We also qualitatively observed the flow in around and in the muzzle area, as compared to the bulk of the channel (Fig 4b). The flow here, visualized by tracking fluorescent particles, is significantly slowed, which decreases shear and increases bacterial residence time, allowing for proper feeding. Immobilized and feeding animals' bodies may shift mildly over the period of several hours, but their bodies remain completely immobilized over a period of several 10s of seconds. The immobilization is sufficient to image subcellular objects such as fat droplets labeled with a fluorescent marker (Fig 5a). In figure 5b, we overlaid an image of the same animal from three time points 20s apart. As the animal is feeding and freely moving its head around, the body remains perfectly immobilized, even while being exposed to blue light as it would be while being imaged in standard experiments.

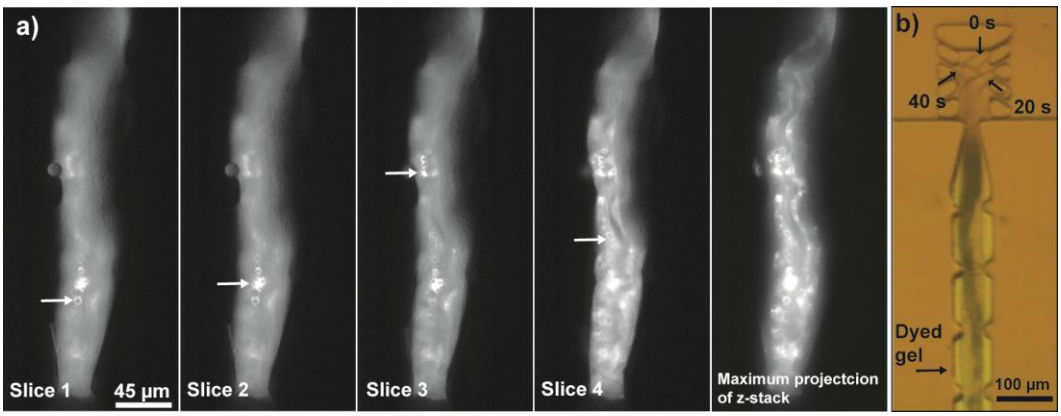

Figure 5: a) We were able to image fat droplets stored inside intestinal cells, which were labeled with a fluorescent marker. Images were obtained by combining 82 individual slices (four example slices are shown) spaced 0.5µm apart into a flattened z-stack, while the trapped animals were feeding. b) The three-image overlay shows that while animals are immobilized in the gel, their bodies remain perfectly immobilized, despite their heads being free to move and feed.

CONCLUSIONS

We present a platform, which traps animals in individual channels bodies using the functionality of PF127 solution, while leaving their heads free to move and feed. We have demonstrated the ability of the system to efficiently load animals at physiological conditions, and maintain these conditions via nutrient delivery, while keeping their bodies immobilized for imaging.

ACKNOWLEDGMENTS

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