ELECTROPHYSIOLOGICAL ANALYSIS OF NEMATODE LARVAE WITH AN INTEGRATED MICROFLUIDIC PLATFORM

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ABSTRACT

A microfluidic platform for electrophysiological analysis of larval 2 (L2) stage *C. elegans* and juvenile 2 (J2) stage *G. pallida* is demonstrated. This device measures the electrophysiological activity associated with nematode feeding behavior. It has many advantages over the conventional microelectrode method, most notably easier manipulation and higher throughput. A microfluidic chip for smaller nematodes would be of great utility in analysis of feeding in this economically and medically important phylum and furthermore have potential applications in antiparasitic drug discovery and crop protection.

KEYWORDS: Microfluidic, Pharyngeal, Electrophysiology, Nematodes, Two-photon polymerization

INTRODUCTION

Caenorhabditis elegans (*C. elegans*) has been used as a model organism as its genome has been fully sequenced and its neuronal circuits have been mapped. However, owing to its small and continuously moving body it is a difficult organism to handle for conventional imaging and electrophysiological experiments. Microfluidic devices for electropharyngeal (EPG) analysis of *C. elegans* have been recently developed [1-3], and these have many advantages over conventional microelectrode methods, most notably much easier manipulation and higher throughput.

These microfluidic chips demonstrated trapping of adult worms, but there is a need to be able to trap and process smaller nematodes. Such a device would be of great utility as an analytical tool to probe this economically and medically important phylum and would also have potential in antiparasitic drug discovery. This paper describes a chip for trapping and electrophysiological analysis of larval 2 (L2) stage *C. elegans*, and juvenile 2 (J2) stage plant parasitic nematode *Globodera pallida (G. pallida)*. *G. pallida*, commonly known as white potato cyst nematode, is a plant pathogenic nematode. It is a pest of plants in the family *solanaceae*, primarily infesting potatoes and tomatoes, as well as a variety of other root crops [4]. Electrophysiological analysis of this nematode with microfluidic device provides a means to discover approaches for controlling the infection. The chip is based on our previous design [3] but with a much smaller trapping channel fabricated using high resolution two-photon lithography.

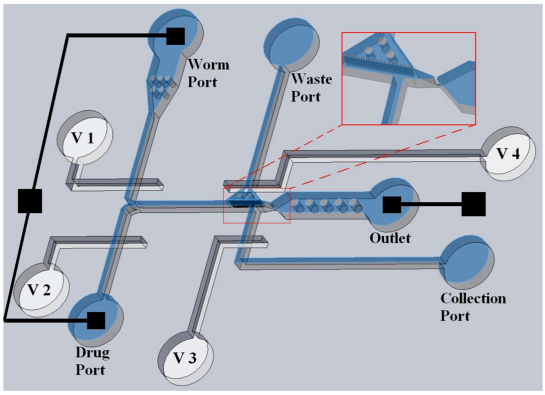


Figure 1: Overall layout of the microfluidic device.

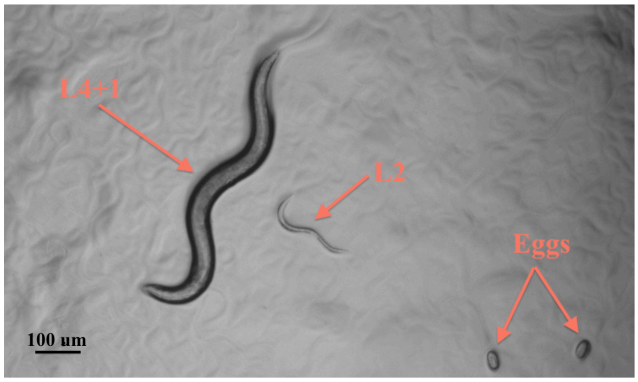


Figure 2: Comparison of the size between the young adult (L4+1) and L2 stage C. elegans.

PRINCIPLE

High quality electrophysiology data requires an optimized trapping channel with a size that captures the worm's head to produce a tight electrical seal. Without this seal, the electrophysiological recordings are of poor quality and cannot deliver key information about neural function or drug effects. Our previous device showed excellent recording from adult worms using devices with integrated electrodes [3]. It is shown schematically in Fig 1. The device traps a worm in a narrow constriction and is able to perform fast drug screening using valves for reagent delivery. Compared to young adult *C. elegans*, the L2 stage worm has a smaller body size (~400 µm in length, ~20 µm in diameter) (Fig. 2), which increases the difficulty of manipulation in conventional way. Owing to the restrictions of conventional photolithography, a miniature trapping channel was fabricated made from masters manufactured using two-photon polymerization (Fig. 3). This approach provides very small 3-D structures to be written directly in a negative photoresist. The ideal shape for a perfect seal around the worm's head is a cylinder, which is easier to fabricate by the two-photon laser than any other method. Compared to conventional photolithography, two-photon polymerization is quicker, cheaper, and has a higher quality, allowing custom-made devices to be made. The rest of the microfluidic chip was fabricated with the standard li-

thography using a patterned acetate film mask (Fig. 3). Precise control of the microscopic nematode was achieved within the miniaturized channels. The worm can be tightly captured in an aperture in the trapping channel leading to two electrically isolated compartments corresponding to the anterior and posterior of the worm. . This permits recording of EPG signals which correlate to the pharyngeal pumping of the L2 stage *C. elegans*.

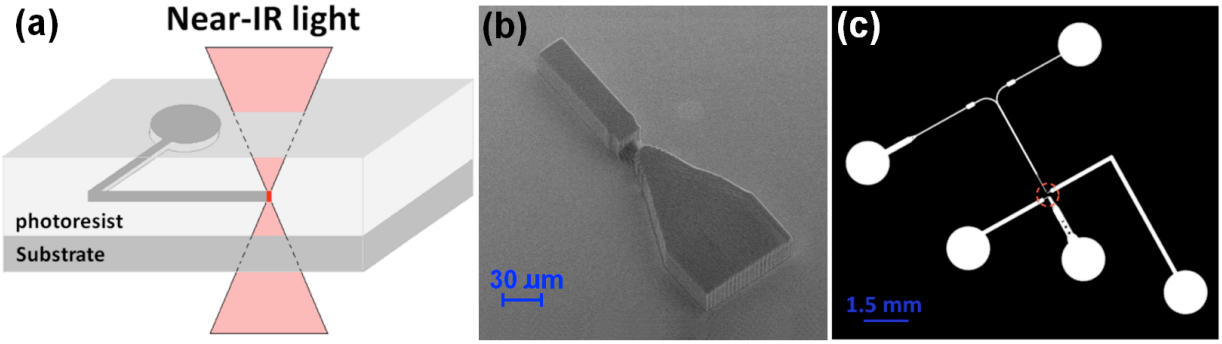


Figure 3: Illustration of two-photon polymerization (a) and the fabricated trapping channel (b). Also shown is the mask (c) used to make the flow channels using standard photolithography.

EXPERIMENTAL

L2 stage worms were obtained by placing adult *C. elegans* on a plate 29 hours prior to the experiment. Dent's saline was pre-loaded into the chip to remove air bubbles and provide a liquid environment. The operating procedure is as follows: Valves 2, 3 and 4 (V2, V3, and V4 in Fig. 1) are actuated to close the bypass channels. The nematode is pumped into the chip from the worm port with positive pressure, which pushes it into the trapping region. EPG signals are then captured using the integrated electrodes connected to an amplifier. The signals are correlated to the pharyngeal pumping of the animal and either observed in real time or recorded for *post hoc* analysis. Drugs are applied from the drug port by closing valve 1 (V1 in Fig. 1) and opening valve 2 and 4. The change in worm behavior, as well as the EPG waveform, due to the drug are observed and recorded. Finally valve 2 and 4 are closed and valve 1 and 3 opened to unload the worm from the device. This worm could be collected after EPG recording e.g. for genotyping by PCR; the device is then ready to receive the next worm.

RESULTS AND DISCUSSION

After several different size channels were made, the best size for trapping and recording L2 stage nematode was determined. No obvious difference between the waveforms obtained from young adult and L2 worms was seen except for the amplitude, which is greater for an adult compared to larvae. All functional spikes correlated to muscles and neurons were observed from the EPG waveform (Fig. 4).

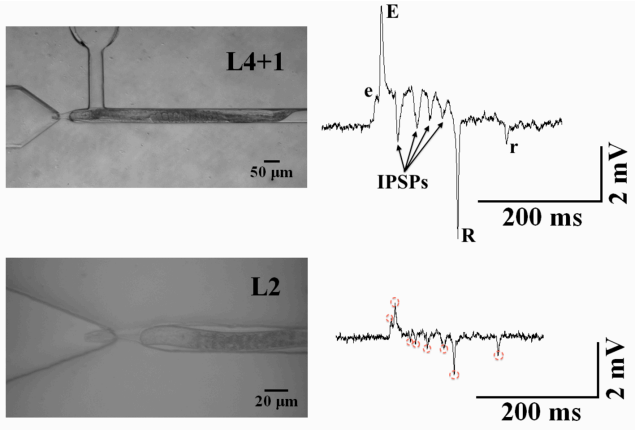


Figure 4: Comparison of the EPG waveforms collected from young adult (L4+1) and L2 C. elegans on-chip.

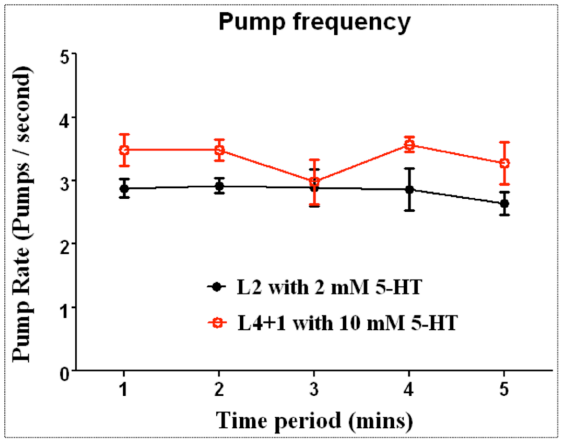
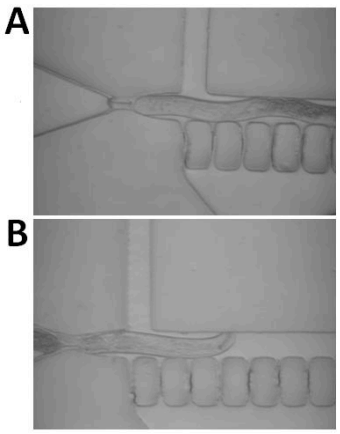


Figure 5: Comparison of the pump frequency collected from L4+1(3.43±0.15) and L2 (2.84±0.05) C. elegans on-chip. (Mean±s.e.m; n=5)

5-HT (serotonin) is a known activator of *C. elegans* feeding behavior. It was observed that the pump frequency of L4+1 *C. elegans* exposed to 10 mM 5-HT is the same as that of the L2 stage worm exposed to concentration of 2 mM 5-HT (Fig. 5) suggesting that the larvae may be more sensitive to this neuro-hormonal stimulus. Further pharmacological studies are required to investigate this.

The modified microfluidic chip was also used to observe the behavior of the plant parasitic nematode *G. pallida* second-stage juveniles (J2), which has similar size to L2 *C. elegans*. 2 mM 5-HT was applied to stimulate the stylet

thrusting mimicking the invading behavior of this organism. By repeating the procedure applied to the L2 stage *C. elegans*, the plant nematode was captured by the head, from which weak EPG signals were seen (Fig. 6A). Interestingly, high quality EPG recording was observed by changing the trapping position from the head to the middle of the body (Fig. 6B). A single EPG waveform obtained from the plant nematode is characterized by a large positive spike with several smaller peaks. It differs from the EPG signal collected from *C. elegans* or that demonstrated in a previous report for *G. rostochiensis*, a close relative of *G. pallida* [5]. Observation of the stylet movement showed a strong correlation between this and the EPG. As shown in Fig. 7, thrusting of the stylet triggers a positive spike, followed by several peaks during the time the stylet is kept extended. Withdrawal of the style is seen as a return of the voltage spike back to the baseline.



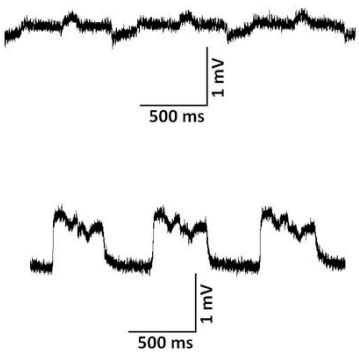


Figure 6: Comparison of the EPG waveforms obtained from the head (A) and from the body (B).

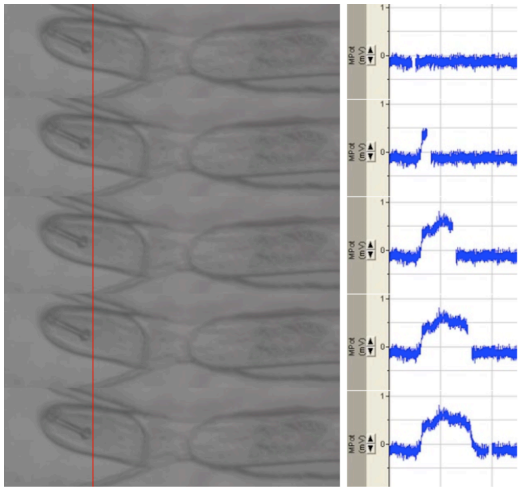


Figure 7: Correlation between the stylet thrust and the EPG waveforms.

CONCLUSION

We have demonstrated a microfluidic chip for trapping small nematodes that is fabricated using two-photon polymerization technology. EPG signals were obtained from the second stage larvae (L2) *C. elegans* from the pharyngeal pumping activity. Second stage juveniles (J2) *G. pallida* nematodes were also recorded and the EPG signals correlated to stylet movement. As demonstrated with L2 stage *C. elegans*, the design of the chip can be readily modified to accommodate other developmental stages of the organism. It provides a way for discrete analysis of age-dependent pharyngeal function. This microfluidic device could be used to trap other parasitic species of nematode which are too small to study with conventional approaches, and enable high throughput analysis of these species, which are of great relevance to human and animal disease.

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