

A Microfluidic Device Integrating Electrical Impedance Spectroscopy Enables Label-Free Monitoring of *C. elegans* in Egg-Laying

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Summary:

Accurate characterization of reproductive phenotypes in *Caenorhabditis elegans* (*C. elegans*) is essential for investigating underlying physiological processes and hereditary mechanisms. However, monitoring of the egg-laying process, one of the central reproductive behaviors in *C. elegans*, has relied on counting expelled embryos, while overlooking organ-level dynamics within the animal, particularly those of the uterus. Here, we present a microfluidic device integrating a microelectrode array to perform electrical impedance spectroscopy measurements of *C. elegans* uterus. Both simulation and experimental results demonstrate that impedance signals at 40 MHz are correlated with the uterine state changes during egg-laying.

Keywords: Lab-on-a-Chip, Electrical impedance spectroscopy, *C. elegans*, Uterus, Egg-laying

Background

The reproductive system is characterized by tightly coordinated processes of organ patterning and morphogenesis across the lifespan, playing a fundamental role in species propagation and organismal health [1]. The nematode *Caenorhabditis elegans* (*C. elegans*) has been widely adopted as a model organism for studying neural regulation, inheritance mechanisms, and reproductive phenotypes [2]. In adult wild-type hermaphrodites, reproduction proceeds through self-fertilization followed by egg-laying [3]. Despite the central role of egg-laying in *C. elegans* reproduction, most existing approaches assess this process indirectly by quantifying expelled embryos, rather than probing the uterus itself [4]. In addition, imaging-based techniques often depend on transgenic strains expressing fluorescent reporters under tissue-specific promoters, which increases experimental complexity and limits accessibility [5]. In this work, we develop a microfluidic device incorporating a microelectrode array (MEA) to enable electrical impedance spectroscopy (EIS)-based monitoring of the *C. elegans* uterus during egg-laying.

System and Methods

The device was fabricated through a three-step microfabrication process. A Cr/Au microelectrode array was first patterned on a glass substrate, followed by deposition of a SiN_x passivation layer with defined openings for

impedance sensing. A polydimethylsiloxane (PDMS) layer containing a single microchannel and a pneumatic valve was subsequently bonded onto the substrate. The central sensing region of the device is illustrated in Fig. 1. The pneumatic valve, positioned at the outlet of the microchannel, was used to immobilize individual worms for EIS measurement. For EIS acquisition, a pair of microelectrodes was selected as the stimulus electrode (S) and recording electrode (R). A swept-frequency AC excitation was applied at the stimulus electrode, while the response current was collected at the recording electrode to obtain EIS signals.

Results

We first established a finite element model (FEM) of the microfluidic device and *C. elegans* to mimic EIS measurements during egg-laying. Four egg-laying states were defined by varying the number of embryos in the worm uterus (Fig. 2). Fig. 3a shows the raw EIS magnitudes simulated by the S - R electrode pair, ranging from 10 kHz to 100 MHz. Since the impedance variation caused by expelled embryos is small, we calculated the relative EIS signals, which demonstrate that EIS signals at high-frequency (> 40 MHz) can monitor uterine changes in *C. elegans* during egg-laying (Fig. 3b). To verify this conclusion, we performed EIS measurements in the microfluidic device during egg-laying. The results show that the post-laying EIS signals

increase most significantly at 40 MHz compared to those of the pre-laying state (Fig. 4).

References

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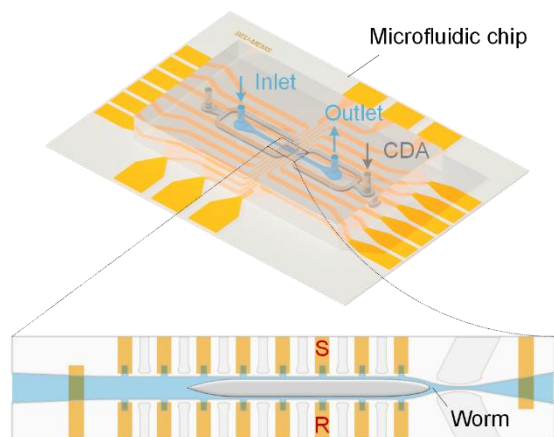


Fig. 1. The microfluidic device and its sensing region for measuring EIS signals of *C. elegans*.

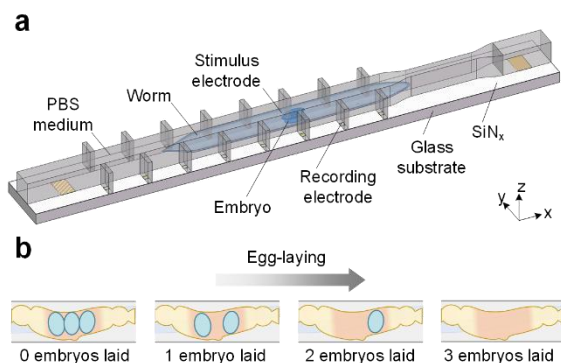


Fig. 2. Modeling and simulation of *C. elegans* egg-laying. (a) FEMs of the sensing region of microfluidic sensing region. (b) Four worm models at different egg-laying states were established by adjusting the embryos in the uterus.

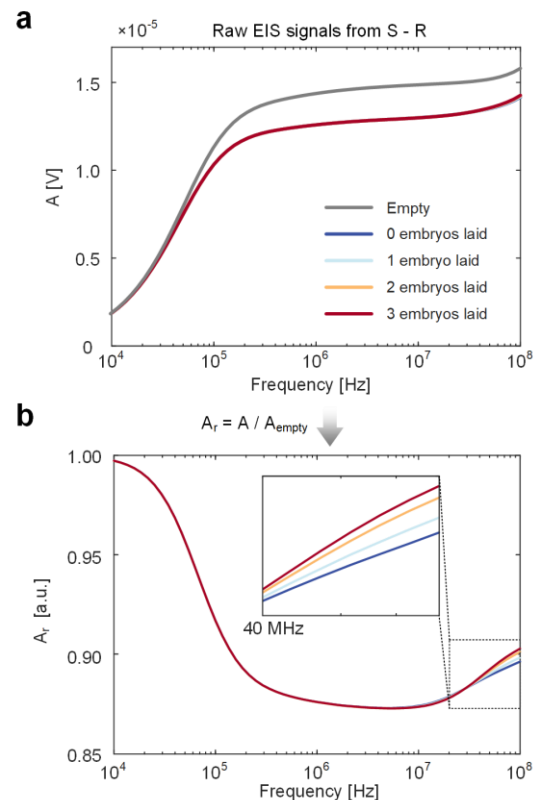


Fig. 3. Simulated EIS signals during egg-laying. (a) Raw EIS signals of the empty microchannel and four states during egg-laying, simulated by the selected electrode pair. (b) Relative EIS signals of the four egg-laying states.

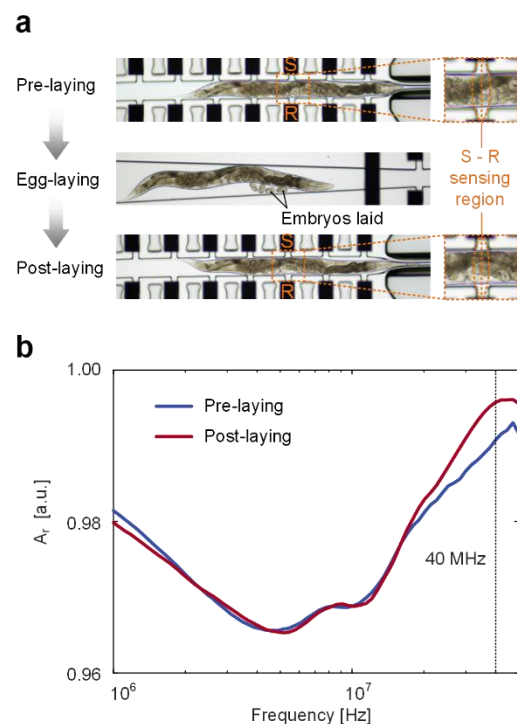


Fig. 4. Monitoring an egg-laying process in the microfluidic device. (a) The EIS measurements were performed in pre-laying and post-laying. (b) The relative EIS signals measured by S-R electrode pair.