ADDRESSABLE LIGHT-INDUCED HEAT KNOCKDOWN (aLINK) FOR *CAENORHABDITIS (C.) ELEGANS* IMMOBILIZATION

Han-Sheng Chuang[1,2], Wen-Tai Chiu[1], Cheng-Shi Chen[3]

[1]*Department of Biomedical Engineering, National Cheng Kung University, Taiwan,*
[2]*Medical Device Innovation Center, National Cheng Kung University, Taiwan,*
[3]*Institute of Biochemistry and Molecular Biology, National Cheng Kung University, Taiwan*

ABSTRACT

Caenorhabditis (C.) elegans is an intriguing model animal. Often times, researchers may need to silence the squirming worms and brought them to various measurements. Here we present a method to rapidly silence the tiny animal by sublethal light-induced heat in a special microfluidic chip. The worm's neural functions are temporarily shut down when a threshold temperature is reached. The neural functions can be resumed once the heat is removed. A safe operating range as to inducing heat knockdown was investigated. The finding showed that well-controlled heat knockdown is autonomous, reversible, and potential to worm immobilization.

KEYWORDS

Heat Knockdown, *C. elegans*, Immobilization, Microfluidics, Optoelectric Effect

INTRODUCTION

Study of *C. elegans* grows rapidly in broad research fields due to its unique advantages, such as simplicity, fully sequenced genome, optical transparency, short life cycle, etc. Although the great progress in biology, little is achieved in engineering. In the cases that the worms need to be measured precisely or undergo a surgery, one can only constrain the worms by limited measures, such as microstructures [1] or paralysis with anesthetic [2], which may result in physical or neural damages. Prior research work showed that *C. elegans* is able to perceive and respond to a small temperature change (<0.1°C) [3]. Since *C. elegans* is a well thermo-regulated animal, temperature plays an important role in the worm's physiological activity. To the present, prior studies have reported that the worm has several responses to different heat stimulations, including thermotaxis[4], heat avoidance[5], and heat shock responses[6]. However, a sublethal temperature, *a.k.a.* knockdown temperature (KT), has rarely received attention yet has been found able to prompt a widespread shutdown in the worm's neural system. Even though under such a treatment, the worm's cells and neurons remain intact. As a result, the worm can continue its life after the heat is removed. The short heat impact on the worms is hence regarded tentative, unharmful, and reversible.

Considering that the knockdown temperature can silence *C. elegans* temporarily, we then developed an optoelectric microchip (aLINK, Fig. 1) using laser (20 mW, 640 nm) mediated heat to immobilize the worms. The microchip is composed of two indium-tin-oxide (ITO) glass plates. One of which is coated with a photoconductive layer (a-Si). An aqueous droplet is sandwiched between an ITO glass plate and a photoconductive ITO plate. When the liquid is irradiated by a laser beam, the liquid medium (NGM buffer) will be heated by Joule heating as well as laser energy simultaneously. Consequently, the liquid temperature is increased and approaches the threshold (KT) in a very short time. An analysis as to the equivalent circuit of the system showed that the major temperature increase takes placed only within the irradiated spot. Compared to pure laser heating or electrical heating, aLINK enables rapid heating as well as addressable maneuverability. An adult worm immobilized by the technique was successfully used to demonstrate the removal of a fraction of lipofuscin granules utilizing femtosecond laser ablation.

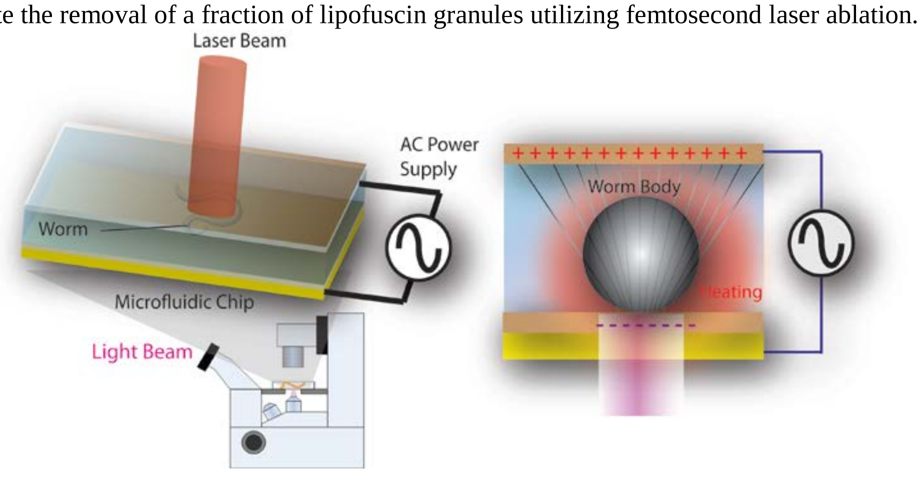

Figure 1. Schematic of the setup (left) and the electric field in an aLINK microchip under irradiation of a laser beam (right).

EXPERIMENT & RESULTS

The experimental setup is illustrated in Fig. 1. A continuous wave (CW) red laser (50 mW, 640 nm) was utilized to heat the medium in a microfluidic chip. A drop of 3 μL nematode growth medium (NGM) buffer was sandwiched between two ITO glass plates with a photoconductive layer. A high frequency electric field (350 kHz, 0.25 V_{pp}) was applied constantly. When the laser illuminated the entire worm, the local temperature of the medium was rapidly increased to the knockdown temperature due to Joule heating, laser heating and heat absorption on the photoconductor. In addition to the advantage of addressable maneuverability, a comparison (Fig. 2A) between laser only heating and the optoelectric method shows that the proposed technique stands out in the heating efficiency. Fig. 2B proves that the microfluidic chip has better heating capacity than normal ITO glass chips. Within a limited time frame, the medium temperature in the microfluidic chip can easily reach the knockdown zone while others remain under the threshold. The range of knockdown zone was carefully measured under a well temperature-regulated environment (Fig. 2C). It generally took less than 10 seconds to reach the threshold temperature and immobilize the worm utilizing the chip. It is believed that neural functions are shut down or disturbed at the moment when the heat remains high, but recover once the heat is removed. Since the cells and neurons are tentatively nulled yet damaged during the process, the worm can return to its normal life without any problem. Without external stimulation, the recovery takes time for different individuals. To aid the worm to wake up faster, low-wavelength laser irradiation (447 nm) can be used strategically to reactivate the worm's motor neurons. It should be noted that the light avoidant behavior deals with a different mechanism. The neurons are depolarized by the violet-blue light, hence promoting the kinematic motion.

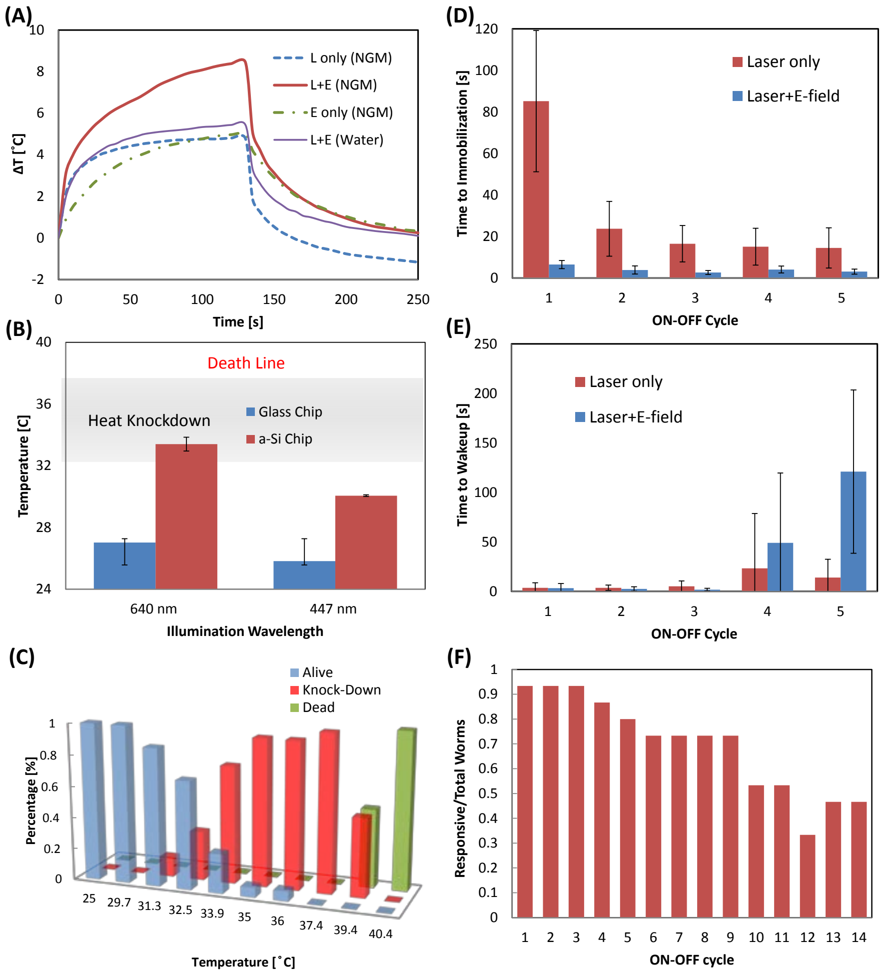

Figure 2. (A) Graph of different heating modes with an aLINK microchip. (B) Heating effects of an ITO glass chip and an optoelectric chip with different light wavelengths. (C) Distribution of knockdown temperature (n=15). (D) Time to immobilization (n=5), and (E) time to wakeup in different heating modes (n=5). (F) Evaluation of responsive worms v.s. cycle (n=18).

A complete operating cycle may include a combinatory use of a red laser (immobilization) and an optional violet laser (wakeup). It has been found that laser only and laser with an electric field treatments may have different effects. Once the laser irradiation was removed, the worm usually resumed its activity in seconds to minutes. However, the immobilization and recovery times vary primarily depending on operating conditions. The time required to immobilize the worm is short with a laser plus an electric field yet long with a laser only treatment. Both cases the time shortens as the on-off cycle increases (Fig. 2D). In contrast, the worm needs a long time to wake up spontaneously after more operating cycles (Fig. 2E). Our observation showed that most worms remained immobile for over two minutes after five cycles. Especially, the worm appeared to take longer time to recover with the laser only treatment. This is very likely attributed to a long accumulated time under a high temperature. In addition, our experiment indicated that over 50% of the overall worms remained active or responsive to the stimulation before 14 cycles (Fig. 2F). Although the immobilization is reversible, the worm needs more recovery time after the on-off cycle increases. A over operation may cause permanent damages in neurons. For a safe operation, the maximum heating temperature should be less than 37°C and the continuous on-off cycle should be no more than three. For a practical demonstration, a laser ablation was successfully performed to remove the lipofuscin granule in a live worm immobilized by the proposed technique (Fig. 3).

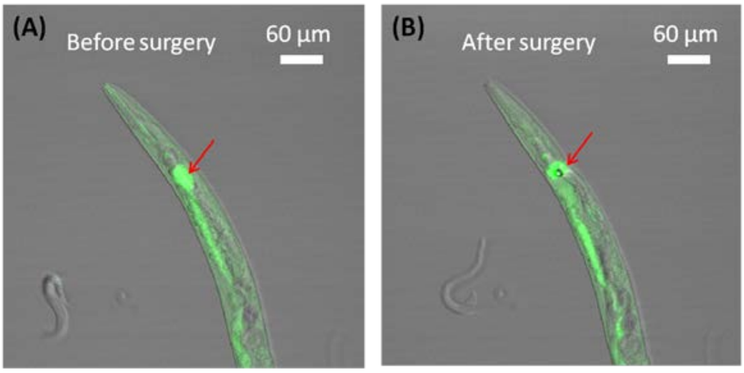

Figure 3. View of laser ablation in an adult worm. The arrow indicates where the lipofuscin granule is removed. (A) Before the surgery; (B) after the surgery.

CONCLUSION

This paper presents a technique using an innovatively optoelectric microchip to immobilize the nematode *C. elegans*. The immobilization is mainly due to heat knockdown, a sublethal temperature, created by a combinatory effect of laser irradiation and electric field. Several treatment conditions were conducted and compared. It was found that NGM buffer in an optoelectric microchip imposed irreversible, unharmful, and rapid immobilizing effect on the worm. The photoconductive layer enabled rapid heating to take place within the irradiation spot. As a result, addressable maneuvering can be achieved. The technique was actually applied to the surgical removal of lipofuscin granule in an aged adult worm accompanying with a femotosecond laser. The success of the treatment is believed beneficial to some applications that need non-contact *C. elegans* immobilization.

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CONTACT

Han-Sheng Chuang +886-6-2757575 ext 63433 or oswaldchuang@mail.ncku.edu.tw