Femtosecond laser surgery on a chip for nerve regeneration

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Summary

Merging microfluidics and femtosecond laser nanosurgery has recently enlarged the specificities and the speed of laser surgeries and given a tremendous momentum in experimental biology, especially for fast genome-wide screenings of various biological processes, and more specifically nerve regeneration in animal models.

Introduction

Nanosurgery using femtosecond (fs) laser pulses permits precise ablation of cellular and subcellular structures with minimal collateral damage and without compromising the cellular viability. Its nano-scale precision and minimal invasiveness allow for studying the cellular organisation from interactions of subcellular organelles to biological pathways. Fs-laser nanosurgery is versatile enough to operate subcellular structures either in cultured cells or living organisms.

In the same way that recent advances in microfabrication techniques have impacted the electronics industry, microfluidics promises a great impact in fields ranging from analytical chemistry to biology and medicine. Microfluidic devices can increase throughput and decrease cost by densely integrating complex assays and analytical measurements in a chip format. Among the potential advantages of microfluidic devices, the use of parallel sample processing and/or automation make them ideally suited for high-throughput screening applications, such as DNA microarrays, enzymatic reactors, cell or larvae sorting, and stimuli testing.

The merging of these two technologies, nanosurgery and microfluidics, makes large scale screenings with nanoscale manipulations possible and renders drug and gene screenings on biological phenomena such as nerve regeneration readily available and time saving.

Discussion

The nematode *Caenorhabditis elegans* (*C. elegans*) is an ideal organism for studying one of the present challenges in neuroscience: nerve regeneration and degeneration [1]. In addition to the injury model of fs-laser ablation, a thorough understanding of these highly dynamic processes requires experimental conditions that minimally affect the animal: no chemicals for immobilisation, a reduced number of manipulations, and a reconstituted environment suitable for the animal's development. We developed a microfluidic device, the 'nanoaxotomy' chip that fulfils all these criteria.

The chip adopts a two-layer configuration for serial trapping of worms [2]. The bottom layer against the glass slide houses the worms in liquid and the upper layer contains the pressurized air that controls the mechanical trap. In the trapping area, a thin PDMS membrane in between these two layers is deflected downward, pressing the animal against the glass when pressure is applied in the upper microchannel (Fig. 1). Using this chip, we performed *in vivo* nanoaxotomy and subsequent time-lapse imaging of regrowing axons in the absence of anaesthetics, with the same precision and accuracy we previously achieved on agar pad with paralysing

chemicals. The nanoaxotomy chip, minimises the time during which the worms are immobilised and possibly improving their well-being. Notably, we observed that without anaesthetics, axons of both motor and touch neurons can regrow much faster. The severed processes of the touch neurons reconnect to their distal stumps within 1-2 hours whereas those of the motor neurons regrow all the way to their target within 6-8 hours. Most recently, we made a progress towards automation of the axotomy chip, to enable high-throughput genetic and pharmacological screenings.

The advantages of this microfluidic chip over the immobilisation techniques previously used in studies of *C. elegans*, such as anaesthesia on agar pads or glue, are: (i) no anaesthetics may interfere with the physiological processes of the worms, (ii) the adaptive deflection of the membrane may allow the immobilisation of any size of worms during and after development, (iii) the worms recover in a short period after surgery, permitting immediate study of the post-axotomy functionality, (iv) the design of the chip is simple enough to be adapted to other organisms or many other kinds of experiments, including ablation, irradiation, stimulation or simply observation, widening the possibilities of high-throughput biological investigations.

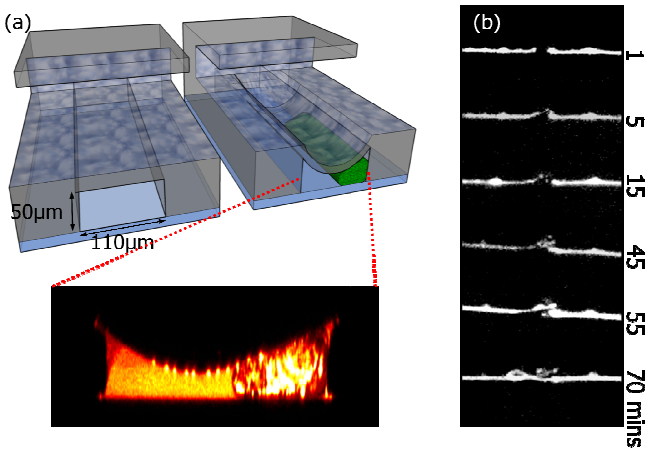

Fig. 1: (a) Conceptual 3D sectional renderings of the bilayer trap channels without and with an immobilized worm. Cross-sectional two-photon image of a trapped worm at 105 kPa. (b) Time-lapse imaging of axonal recovery on-a-chip. Fluorescence images of an ALM neuron at 1, 5, 15, 45, 55 and 70 minutes after axotomy. Distal ends are on the left side of the pictures, proximal ones on the right. At 70 minutes, the proximal end regrew and reconnected to the distal end a bit further past the distal stump.

Conclusion

Fs-laser nanosurgery undergoes a tremendous research momentum due to the need of understanding biological phenomena using small model organisms. The submicron precision of the fs-laser pulses does indeed guarantee minimally invasive surgeries. Combining laser nanosurgery with microfluidic devices permits the user to control both the environment of the sample and its immobilisation – the latter being particularly imperative because of the accuracy requirement of nanosurgery, and it allows rapid high throughput screening at low costs. Our approaches integrating these two technologies are devoted to studying nerve regeneration in the nematode *C. elegans*. But applications of nanosurgery microchips are endless when one considers how far both technologies have progressed and how diverse their uses in biology and medicine are. All it takes is integration…and imagination.

References

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