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# Monitoring of intercellular messengers released from neuron networks cultured in a microchip

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**Abstract**

A cellular biochemistry analysis system was integrated on a quartz glass microchip with a microchamber for cell culture followed by a microchannel for detecting with a thermal lens microscope (TLM). Nerve cells from rat hippocampus were successfully cultured to form neural networks in the microchip. An aqueous solution of glutamate, which is known as a neurotransmitter, was introduced to stimulate the cultured neuron to release a retrograde messenger, arachidonate which is considered to be critical for neuronal plasticity, especially for long-term potentiation (LTP). After the introduction, the solution that flowed through the culture chamber was analyzed using the UV-TLM (excitation wavelength, 244 nm). The measured signal intensity was dependent on glutamate solution concentration, and the neurons were considered to release the retrograde messenger according to the glutamate concentration. This system is suitable for time-course monitoring of ultra trace amounts of chemicals released from very small amount of cultured cells.


*Keywords:* Microchip; Cell culture; Neuron network; Thermal lens microscope; Cell response monitoring

## 1. Introduction

Microchip-based systems have desirable characteristics, such as lower consumptions of reagents and samples, smaller space requirements, and shorter analysis time. Applications of these systems, widely known as micro total analysis systems (μ-TAS) [1] or lab-on-a-chip [2], have been spreading rapidly. We have demonstrated many applications, including flow-injection analyses, solvent extractions, and microreactors [3,4]. In these systems, full advantage was taken of the scale merits of the microspace, i.e. a short diffusion distance, a large specific interface area, and a rapid and efficient reaction. Moreover, by combination of the micro unit operations connected with pressure-driven flow, a complex chemical system, i.e. continuous flow chemical processing (CFCP), can be constructed. We have reported that CFCP has a great potential to realize various chemical systems consisting of reaction, extraction and analysis, easily and efficiently, without troublesome mechanical operations. The major advantages of these microchip-based systems accrue especially to biochemical applications. We have demonstrated rapid and sensitive immunoassay systems for protein analyses [5–8].

Microchip techniques also appear to provide some advantages for cellular biochemical analysis systems, because the scale of the liquid microspace inside a microchip is fitted to the size of the cells. For microchambers fabricated on microchips, rapid and secure exchange of media or reagents will be achieved by simple operations under continuous

*Abbreviation:* TLM, thermal lens microscope

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medium flows. Moreover, if cell culture chamber and analysis channels are connected, analyses of the medium become possible without troublesome handling operations and extensive dilution. Therefore, mechanical operations or handling procedures of the assay are greatly simplified by integration into microchips.

For further miniaturization, we think that integration of cell culture as well as other chemical operations such as mixing, reaction, extraction, and detection on microchips is possible. Very recently, some researches about integration of cell culture and analyses into a microchip were reported [9–14].

In neurochemistry, it was reported that the quantity of neurotransmitters (in this case, arachidonate) released from each nerve cell can be estimated at about the zeptomole level, which corresponds to less than nano molar values [15]. It is difficult to monitor such levels of released materials because dilution by the medium occurs immediately. In order to avoid such dilution and realize real-time monitoring of compounds released from nerve cells, integration of a cell culture chamber and a detection channel seems to be useful.

Arachidonate and its metabolites are candidates for retrograde messengers, which are considered to play an important role in long-term potentiation (LTP), i.e. one of a neuronal plasticity, which interprets a mechanism of memory and learning. Other compounds such as platelet-activating factor, nitric oxide, and carbon monoxide, are also considered as candidate retrograde messengers. However, as mentioned above, the amount of neurotransmitters released from synapses are very small and their sensitive detection outside the cultured cell is limited due to diffusion. Radioisotopes or fluorescence tags are usually used for highly sensitive detection as they are incorporated into the cultured cells [15]; but some uncertainty still remains regarding incorporation rate and effect of pre-labeled materials on response to stimulation. If released materials can be detected without pre-labeling, this will offer the distinct advantage that direct verification of a chemical mechanism in nerve cells is possible.

We propose integration of a cellular biochemical analysis system on a glass microchip, which enables us to culture hippocampal nerve cells obtained from rat embryo, to get glutamate to act on a pre-synapse, and to detect retrograde messengers released from the post-synapse on the microchip. In this paper, we investigated time-course monitoring of arachidonate and its metabolites released from cells by using a microchip-based system. A thermal lens microscope (TLM) with UV laser was used to detect the released arachidonate directly.

## 2. Experimental

### 2.1. Fabrication of microchips

Microchips used for cell culture and analysis were made by laminating three quartz plates as shown in Fig. 1. The fabrication method of the cellular biochemistry chips was almost the same as the previously reported one [5].

The chips were composed of three quartz glass plates, which were the cover, middle, and bottom plates, having thickness of 170 μm, 100 μm, and 1 mm, respectively. For the cell culture chip, the cover plate had three holes with diameters of 2 mm and 0.5 mm. The middle plate was also pierced to make the channel part, which had a culture chamber followed by a flow channel for optical detection. The culture chamber was 1 cm long, 1 mm wide and 100 μm deep, and the detection channel was 4 cm long, 250 μm wide and 100 μm deep. The bonding surfaces of the three plates were polished to an optically smooth and flat (λ/10) finish, and the three plates were laminated together in an oven at ca. 1150 °C without any adhesives.

### 2.2. Primary neuron culture

Hippocampal nerve cells were obtained from rat embryos. The nerve cells were introduced into the culture chamber after the channel was treated as follows. First, the chamber was washed with 0.1 M NaOH, 0.1 M HCl, and water, successively, for 1 h each in an ultrasonic bath. Next, the chip was autoclaved at 120 °C for 15 min. The culture chamber

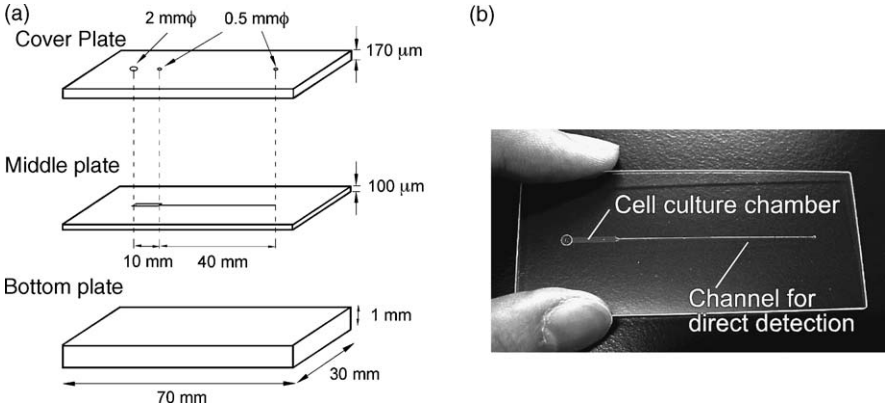


Fig. 1. Components of a cellular biochemical analysis microchip (a) and its photograph (b).