An excitation and propagation of calcium wave at *A253* human cell by fs-pulsed laser irradiation

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Introduction

A photo-induced *intra*-cellular calcium wave excitation has been reported by femto-second pulsed laser irradiation to the human origin *HeLa* cell [1]. In this article, we report the excitation and the *inter*-cellular propagation of the calcium wave among *A253* cells, which was obtained from a human submandibular duct cell line and cultivated *in vitro*. At the stationary state, combinations of the positive and negative feedbacks by calcium stores and ion channels achieve the homeostatic concentration. The focused laser irradiation induces the localized increase of the calcium concentration supplied form the stores; a cell nucleus, endoplasmic reticulums and mitochondrias, and excites the traveling calcium wave. At this stage, although we have not identified which calcium store is the source in the cell, this is the first report that demonstrates the inter-cellular wave propagation among the living cells induced by the f-sec laser irradiation.

Experimental

A253 used has been cultured for about 5 days before the experiments in modified *McCOY'S 5A* medium under a CO_2 partial pressure of 0.05 atm and at the constant temperature of 37^{o}C till the number of the cell increased up to 90% of the confluent. The observation and fs-laser irradiation was conducted through the optics of a confocal microscope (Leica TCS-NT) equipped with Kr-Ar ion laser and Ti-sapphire laser (Spectra Physics Tunami,710nm, 82MHz, 40fs), for observation and excitation light sources. The period of the irradiation was 10 ms, controlled by a mechanical shutter. Calcium concentration was imaged by the probe dye, *Fluo-4AM*. To observe the intra-structure of the cell, mitochondria were stained specifically by *Rhod-2 AM* and *MitoTracker Green FM*.

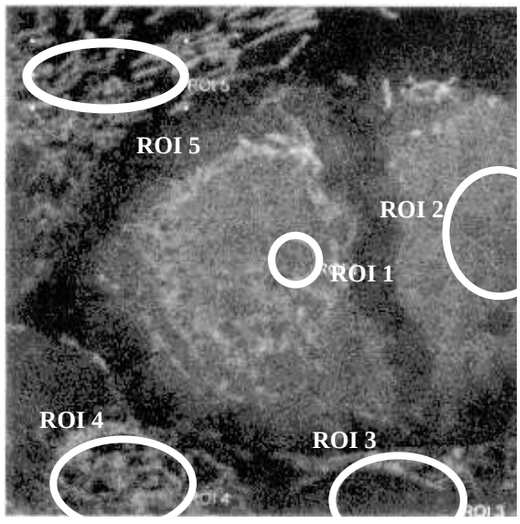

Fig. 1
Fluorescence image of A253 human cells stained by *Fluo-4AM*. The diameter of this cell was 20 μm. The fs-pulse laser beam was focused on an area the region of interest (ROI) 1.

Result and Discussion

On the organelle stained by *Rhod-2AM*, which *MitoTracker Green FM* did not stain *i.e.*, is not presumably mitochondria, the fs-pulse laser beam was focused and the time course of the spatial image of *Fluo-4AM* emission was obtained [Fig. 1 ROI 1]. The emission intensity of *Fluo-4AM* is proportional to the concentration of the calcium. The time evolution of the emission image demonstrates the excitation of the calcium wave in the targeted cell and also the propagation towards the cells surrounding the irradiated one. The time courses of the emission intensity at a few positions are shown in Figure 2. The rises of the calcium concentration were observed at each positions after time delays of 3-10 seconds.

Those observations suggest that the irradiation triggered the calcium release from a store and the traveling calcium wave reaching at the outermost sphere cell membrane successively induces the secondary release of a transmitter to neighboring cells to increase the calcium concentration. These chain reactions sustain the *inter*-cellular wave propagation. Thus, the spatially focused fs-pulse laser irradiation affects in wider area. The careful observation revealed that the *inter*-cellular spatial gap could not inhibit the wave propagation. These excitation and propagation of the wave was only obtained by the fs-laser irradiation. Although keeping the constant total fluencies, the laser irradiation with longer duration of n-sec and/or under off mode locked Ti-sapphire could never induce the wave. Only the short pulse having an extremely high peak power can specifically excite the calcium wave. Because of the ultra-short duration of the laser pulse, the cell can survive without any destruction of the biological functions even in the experience of the penetration of the high-energy pulse. The photo-triggering of the calcium waves recovered again after certain deactivated period. Conclusively, the fs-pulse laser has a potential to induce some specific biological activity without leaving fatal damage to the cell and tissue.

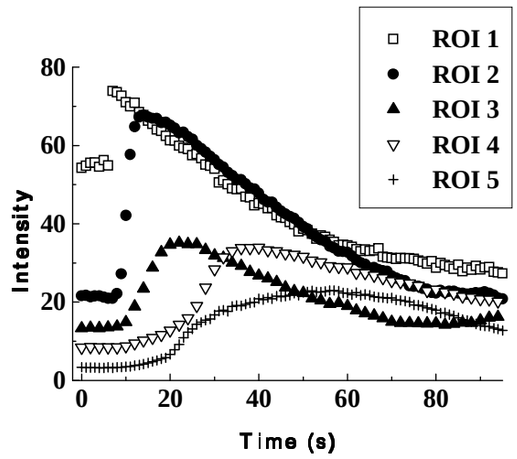

Fig. 2
Fs-pulse laser irradiated ROI 1 at 6s. All points started increasing fluorescence with time delay of 3-10s, which indicates the propagation velocity is slower than that governed by the simple diffusion of calcium ion.

[1] N. I. Smith, K. Fujita, T. Kaneko, K. Katoh, O. Nakamura, S. Kawata, T. Takamatsu, *Appl. Phys. Lett.*, **79** (2001) 1208-1210