The use of optical chopper increases the efficiency of femtosecond laser-induced cell fusion

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Abstract. Artificial cell fusion is a widely used approach in cell biology and biomedicine. Femtosecond laser-induced cell fusion is considered to be a precise and low-invasive tool for the cell fusion. However, the percentage of somatic cell fusion remains not very high, and the use of polyethylene glycol is often required. In our research we propose the use of rotating optical chopper to produce ultra-short trains of femtosecond pulses (up to 1 ms) for decreasing the laser impact. It helps to control the appearance and size of gas-vapor bubbles, avoiding cell destruction. We achieved the cell fusion efficiency of 45% without the use of polyethylene glycol, but only in freshly thawed cells. Continuously cultured cells completely failed to fuse.

1. Introduction
Artificial cell fusion is a widely used approach in cell biology and biomedicine, e.g., for hybridoma production [1] or for tetraploid embryo obtainment [2]. Femtosecond (fs) laser-induced cell fusion is known as a precise and low-invasive tool for the embryo cell fusion. It allows fusing 2-cell embryos with 50% efficiency, but percentage of somatic cell fusion does not exceed 15-20% [3] or 37% according another data [4]. One research reports on 80% level of fusion [5], but in this work fusion medium was supplemented with polyethylene glycol, which is known as a fusogen. Relatively low percentage of somatic cell fusion can be explained by the appearance of the gas-vapor bubble, which occurs in the contact area of the cells due to the laser exposure. On the one hand, gas-vapor bubble is required to initiate cell fusion by destabilizing plasma membranes of the cells. On the other hand, gas-vapor bubble can impair plasma membranes of the cells and can be resulted in cell destruction [6]. So, the success of fusion depends on the cell size (consequently, on the cell-adhesion area) and on the gas-vapor bubble size. Therefore it is easier to fuse 2-cell embryos, which cells have size 30-50 μm (each blastomere) and natural cell-adhesion area, than to fuse suspended somatic cells, which are significantly smaller (10-20 μm) and have not any adhesion.

In this research we propose the use of optical chopper to improve the fusion technique. We used optical chopper to reduce the length of femtosecond pulse trains up to 1 ms. It allowed to rise up the pulse energy but to diminish heat accumulation, therefore to lower vapor-gas bubble size.

2. Materials and methods

2.1. Cell culture and sample preparation
Frozen THP-1 cells (human leukemia monocytic cell line) were thawed in a 37°C water bath and then diluted with pre-warmed RPMI 1640 medium (Gibco, 11875093) supplemented with 10% FBS (Gibco, 10099141). Cell suspension was centrifuged at approximately 200G for 5 minutes.
Supernatant was decanted and cell pellet was resuspended with M2 (Sigma, M7167) medium. During the experiments cells were kept in M2 medium on ice to avoid cell adhesion.

For the other set of experiments we used the same cells, but they were continuously cultured in RPMI 1640 medium supplemented with 10% FBS and 2 mmol/L L-glutamine in CO₂ incubator (37°C, 5% CO₂). Before experiments cells were washed in PBS (Sigma, D8662) and resuspended in M2.

2.2. Experimental setup for the cell fusion
In the experiments we used the Olympus IX71 microscope (objective 60×, NA = 0.7). Cell fusion was performed using a femtosecond laser Mai-Tai (Spectra Physics). Laser parameters: λ = 800 nm, pulse duration = 100 fs, pulse energy = 1.5 nJ, 80 MHz repetition rate. The diameter of the beam waist was 1.39 μm, peak power density for the pulse in the beam waist was 1.1·10¹² W·cm⁻². Optical chopper (Thorlabs MC2000B-EC with MC1F30 blade, only one left open) was set up with frequency 26 Hz to incise laser pulse trains with 1 ms duration. Mechanical shutter installed next the optical chopper controlled number of femtosecond pulse trains for one exposure. In our experiments exposure time was set to 120 ms, so one exposure composed of 3 femtosecond laser pulse trains with 1 ms duration. Fusion of 2-cell embryos has performed as described [7].

2.3. Cell adhesion and fusion protocol
For the fusion experiments suspended THP-1 cells were placed onto a cover glass in 50 μl M2 supplemented with 40 μg/ml lectin (Sigma, L8902) to provide cell-cell adhesion. Single cells were joined by an optical tweezer and adhered by the action of lectin. Femtosecond laser radiation was focused at the cell-adhesion area.

3. Results

3.1. Freshly-thawed THP-1 cells fused successfully
We joined cells by optical tweezers (figure 1, a, b), and lectin provided acceptable adhesion. None of 60 pairs we made has lost adhesion even if we pulled one of the cells within a pair by tweezers. Femtosecond laser were applied to the cell-adhesion area and resulted in a gas-vapor bubble (figure 1, c). Gas-vapor bubble broke plasma membranes of the cells and caused fusion immediately (figure 1, d, e). 27 of 60 pairs (45%) successfully fused forming a single cell with 2 nuclei (figure 1, f). Complete cell fusion took nearly 20 seconds since the moment of gas-vapor bubble appearance.

We have tried to perform THP-1 fusion without an optical chopper, as usually we worked with two-cell mouse embryos. Long-lasting pulse train resulted in a big gas-vapor bubble, which caused plasma membrane breakdown and cell destruction in 100% cases.

3.2. Continuously cultured THP-1 cells failed to fuse
The same manipulations as described above were performed with continuously cultured THP-1 cells. Firstly, it should be noted that these cells were different in their morphology from freshly thawed THP-1 cells (compare figure 1 and figure 2). Continuously cultured THP-1 cells had not visible nucleus unlike freshly thawed ones, and they had more transparent cytoplasm. Secondly, continuously cultured THP-1 cells completely failed to fuse. None of 50 pairs fused, and in 100% cases one or two cells were destroyed (figure 2). The destruction occurred immediately after the formation of the gas-vapor bubble (and even without visible gas-vapor bubble) and took nearly 10 seconds.

3.3. Gas-vapor bubble size with or without optical chopper
We compared the gas-vapor bubble size in the living cells, produced by two different modes of femtosecond laser: with or without optical chopper (figure 3). Bubbles, generated by 30 ms pulse train of fs pulses in 2-cell embryos, seem to be bigger at average and have more dispersion in their size than bubbles, produced with 1 ms pulse train in THP-1 cells. Nevertheless, analysis of variance (one-way ANOVA) has not revealed significant difference between samples (F-ratio = 2.77; p = 0.11).
Figure 1. Laser-induced fusion of freshly thawed THP-1 cells. Optical tweezers move a cell towards to another one (a) and join them (b). Gas-vapor bubble appears as a result of the laser impact (shown by an arrow, c) and induces cell fusion immediately (d, e). Presence of 2 nuclei within a one cell is confirmed by Hoechst 33342 staining (f).

Figure 2. Laser-induced fusion of continuously cultured THP-1 cells. Optical tweezers move a cell towards to another one (a, b) and join them (c). Gas-vapor bubble appears as a result of the laser impact (shown by an arrow, d) and induces cell destruction immediately (e, f).
4. Discussion

In this work we propose the use of optical chopper to increase the efficiency of somatic cell fusion. Before, we applied 1 nJ 30 ms experimental mode for successful fusion of embryo cells, which is described in our previous research [7]. In this mode total energy provided to the sample was 2.4 mJ. When this parameters were applied for fusing THP-1 cells, we observed destruction in 100% cases. By the optical chopper we managed to diminish the pulse train up to 1 ms. Next, by the shutter we controlled the number of 1 ms pulse trains per one exposure, and it was set up for 3 pulse trains. The use of optical chopper allowed us to raise the pulse energy (1.5 nJ), but to lower total energy (0.36 mJ). It is important, because the first moments of the laser absorption is of nonlinear nature. We know that 800 nm femtosecond radiation is able to induce carbonization of biomaterial by nonlinear processes [8]. Carbon dots, generated by the laser, switch over the absorption to linear mode, resulting in heating and boiling, which we observe as a gas-vapor bubble [9]. So, shortening of the exposure time reduces boiling, therefore, we produce smaller gas-vapor bubbles. It allows making disruption of plasma membrane more precisely. Therefore, by lowering of destruction we can increase the fusion rate. Albeit, the data on the bubble size are preliminary and require further investigation.

What else is interesting, is an effect of cell condition on the fusion ability. We have noted that freshly thawed and maintained THP-1 cells have different morphology and extremely different reply to the laser impact. While freshly thawed THP-1 kept their integrity even after several bubbles, continuously cultured THP-1 rapidly destroyed even without visible gas-vapor bubble. It seems that plasma membrane of freshly thawed cells is more flexible and plastic. We suppose that this phenomena can be explained by the nature of monocytes, which are able to differentiate into macrophages during cultivation [10].

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