Cell Technology Employing Femtosecond Laser Pulses

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Practical advantages of using femtosecond laser pulses for manipulations in cell surgery were demonstrated. The use of femtosecond laser pulses enables precision punching of the zona pellucida of the embryo without damaging its cells. With the help of femtosecond laser tweezers/scalpel, auxillary laser hatching was performed and a technique of optical biopsy of mammalian embryo was developed, which enabled non-contact sampling of embryonic material for preimplantation diagnostics. Our findings suggest that about 90% embryos retained the ability to develop at least to the blastula stage after this manipulation.

Key Words: *femtosecond laser pulse; auxiliary laser hatching; laser micromanipulation;mammal embryo biopsy; cell microsurgery*

The development of laser technology opens new prospects for creating unique methods to influence precisely and modify biological objects at the organ, tissue, cellular and subcellular levels. During the last decades, technology of laser tweezers allowing transfer of viruses, individual cells, and their structures in three dimensions in field of optical microscope using sharply focused optical radiation became popular [2,3,7]. Along with optical micromanipulation, application of laser pulses (optical scalpel) also makes it possible to perform destructive [8] and constructive manipulations with the membrane [13] and structural and functional protein elements of the cell [14]; manipulations on individual chromosomes are also possible [4].

The peculiarities of interaction of femtosecond ($\sim 10^{-13}$ sec) laser pulses [5,9-12,14] with biological objects are intensively studied during recent years. The use of such pulses makes it possible to effectively localize the impact both in time and space. Being applied in biology and medicine, it provides high selectivity and precision of the influence on objects at the submicron level through realization of nonlinear absorption in the focus and reduces heat load on adjacent tissues. This allows the formation of clear boundaries of the modified region with a width of tens of nanometers [5,9,10]. Damage zone of biological polymers can be controlled much better than with lasers with pico- and nanosecond pulse duration [5,9].

Modern applied embryology uses rather crude mechanical methods when working with embryos. One of them, so-called assisted hatching, consists in dissection of the embryonic zona pellucida with a special tool. This procedure is quite difficult and insecure for the embryo. To simplify and increase the safety of working with the embryo, nanosecond laser pulses are used, which destroy zona pellucida in a controlled volume [6].

The aim of the work was to improve the methods of auxiliary laser hatching. The use of femtosecond laser pulses was expected to make this method more precise, convenient, and safe for the embryo. In case of successful realization of this task, it was planned to develop on this basis the technique for optical biopsy of mammalian embryos using optical tweezers.

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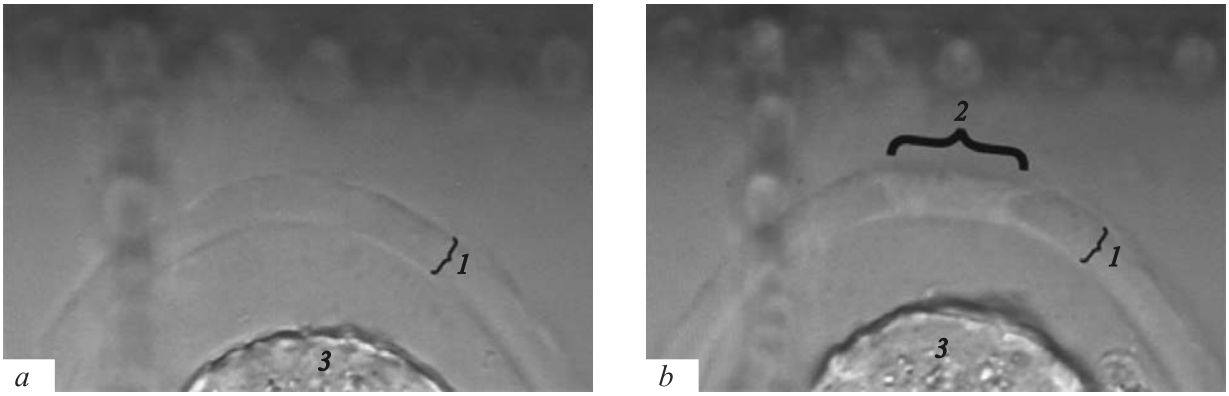

Fig. 1. Technique of auxiliary laser hatching with femtosecond laser pulses. *a*) fragment of mouse embryo before exposure; *b*) view of an embryo after exposure. *1*) zona pellucida, *2*) perforation of zona pellucida (hole size ~20 μ), 3) zygote. Transmission light microscopy of live embryos (×450).

MATERIALS AND METHODS

The study was carried out using a femtosecond laser tweezers/scalpel described in detail previously [1]. Optical trapping and movement of embryo cells in the field of view of an inverted biological microscope were performed using cw infrared laser focused with a microobjective (×40, numerical aperture of 0.85). Femtosecond laser pulses (100 fsec) focused through the specified microscope objective were used as the optical scalpel. Power density of femtosecond pulses was set near the damage threshold of the zona pellucida, so that its destruction occurred only in the zone of laser exposure.

Isolated mouse embryos at the zygote stage were placed in an experimental tank with transparent bot-

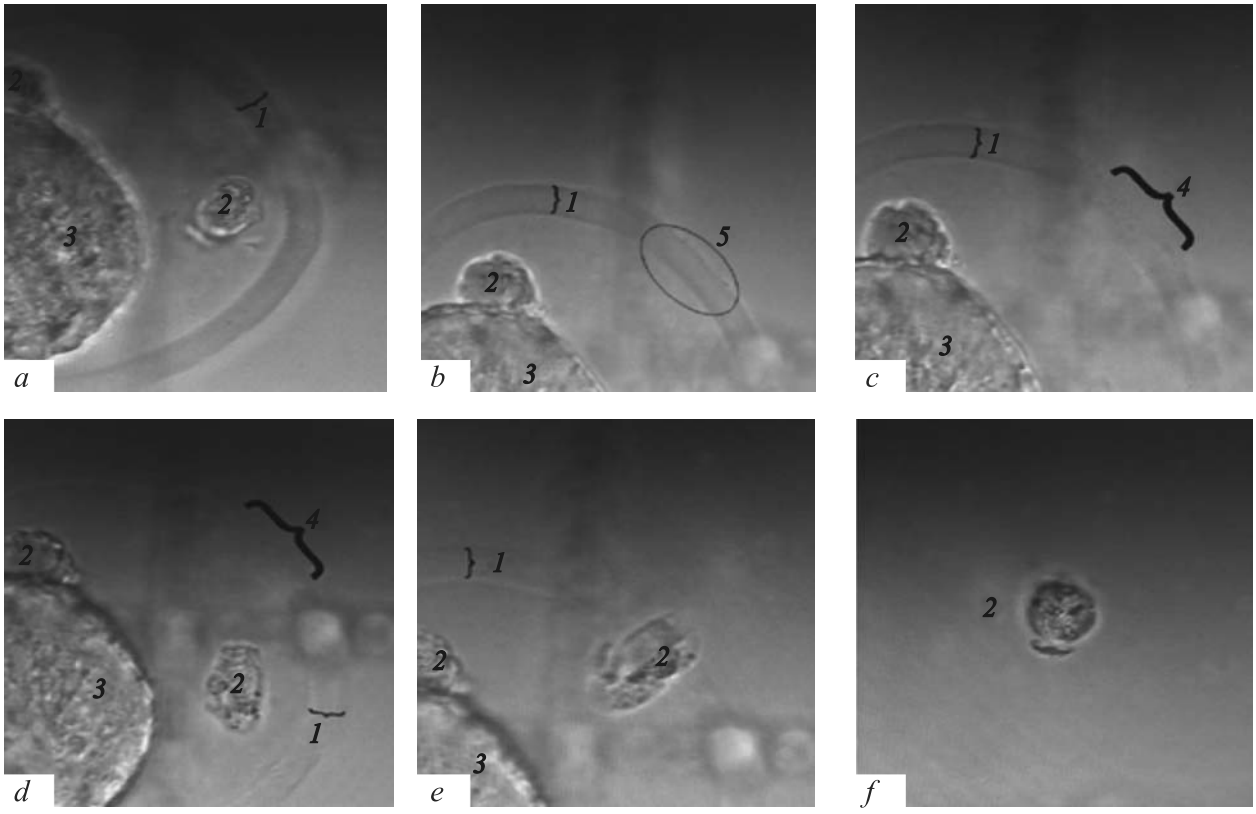

Fig. 2. Optical biopsy of a living mouse embryo: microscopy in transmitted light (×450). *a*) embryo before laser irradiation; *b*) aiming a femtosecond laser scalpel on the selected area of the zona pellucida; *c*) perforation of the shell to the desired volume; *d*) optical trapping of reducing body; *e*) removal of the polar body from the embryo through the perforations; *f*) free polar body. 1) zona pellucida, 2) polar body, 3) zygote, 4) perforation of zona pellucida, 5) supposed area of perforation of zona pellucida with femtosecond laser scalpel.

tom in Medicult flushing medium. Perforation of the zona pellucida in the chosen area was carried out by focusing the laser scalpel. The embryos after exposure and the control embryos were placed into a chamber with culture medium and incubated at 37°C and 5% CO_2 for 3 days. The result was compared to that of control embryos not exposed to laser. For evaluation of the effect of femtosecond laser pulses, we performed 3 experimental series; in each series, auxiliary laser hatching was performed in 20 embryos.

Embryo biopsy, another embryological technique important for preimplantation diagnostics, is also performed with mechanical tools. It could be carried out using optical technology combining two technological tools: optical tweezers and a femtosecond laser scalpel. First, holes of required size were formed in the zona pellucida similar to auxiliary femtosecond laser hatching and then polar body was removed through the hole with optical tweezers.

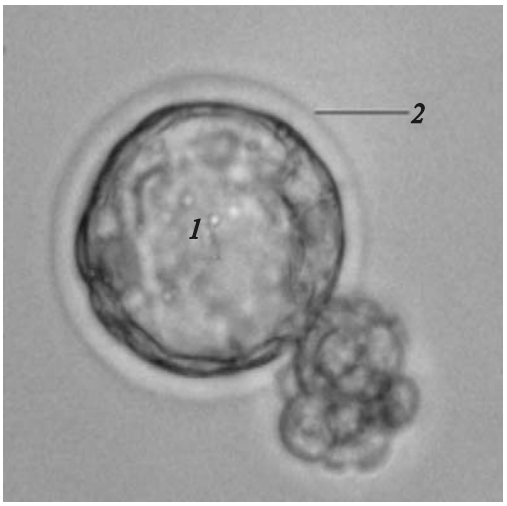

Fig. 3. Typical development of mouse embryo observed on days 4-5 after surgery: microscopy in transmitted light (×200). 1) developing blastocyst leaving zona pellucida through the perforation previously formed with a femtosecond optical scalpel, *2*) zona pellucida.

RESULTS

Figure 1 demonstrates the technique of laser-assisted hatching using femtosecond laser pulses. Repeated exposure of the zona pellucida to laser pulses led to the formation of perforations of the required size. The technique was highly accurate and precise, because the impact on the zona pellucida in the immediate vicinity (~5 μ) of the embryo cells did not lead to their destruction.

Examination of cultured embryos confirmed adequate development of ~90% embryos after laser hatching procedure in all three experimental series. No growth retardation and congenital malformations were observed compared to embryos of the control subgroups.

This success allowed us to come to the second task of this work, namely, the development of techniques for optical biopsy of mammalian embryos. Figure 2 shows the algorithm of developed technology.

The embryos subjected to optical biopsy and auxiliary laser hatching successfully developed, at least until the blastocyst stage (Fig. 3). Optical biopsy was also performed in three series (20 mouse embryos per group). In each series, ~85% embryos successfully developed, no growth retardation and congenital malformations were observed.

The results of our study suggest that the use of femtosecond laser pulses in both cases allowed precision punching of the zona pellucida of the embryo. These laser pulses were safe for further development of the embryos and the used femtosecond laser scalpel was a convenient and precise tool for various embryological techniques. The combination of femtosecond laser scalpel and optical tweezers allowed us to develop a technique of optical non-contact biopsy of mammalian embryo, which could be very promising for the research work in the field of preimplantation diagnostics.

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