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The effect of femtosecond laser on DNA destruction assisted with SYTO fluorescent dye

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Abstract. Tightly-focused femtosecond laser radiation is widely used as a convenient tool for manipulating biological objects. The method of femtosecond laser surgery proved to be useful for the multiple applications, from eye correction to the subcellular structures destruction. Among other things, femtosecond laser surgery shown to be used as a tool for DNA destruction, which could be beneficial for assisted reproductive technologies in humans. In the context of the development of this method, the study of the DNA laser assisted destruction efficiency is of significant importance. In this work, DNA laser assisted destruction efficiency in presence of SYTO fluorescent dye was studied.

Keywords: DNA damage, femtosecond laser surgery

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Исследование разрушения ДНК при помощи фемтосекундного лазерного излучения в присутствии флуоресцентного красителя SYTO

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Аннотация. Узконаправленное, сфокусированное фемтосекундное лазерное излучение широко используется как удобный инструмент для манипуляции биологическими объектами. Метод фемтосекундной лазерной хирургии оказался полезным для множества приложений, от коррекции зрения до разрушения субклеточных структур. Среди прочего, фемтосекундная лазерная хирургия продемонстрирована в качестве инструмента для разрушения ДНК, что может быть полезным для вспомогательных репродуктивных технологий. В контексте разработки этого метода изучение эффективности разрушения ДНК лазером является необходимым этапом. В данной работе была исследована эффективность разрушения ДНК лазером в присутствии флуоресцентного красителя SYTO.

Ключевые слова: повреждение ДНК, фемтосекундная лазерная хирургия

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Introduction

Near-infrared femtosecond laser radiation has recently proved to be a low-invasive and high-precision tool for manipulating biological objects. Through the focusing of femtosecond laser pulses with the microscope objectives of a high numerical aperture, highly localized biomaterial destruction on a submicron resolution level could be achieved (so called nanosurgary technique). Nanosurgery can be applied to perform operations inside living cells without damaging their integrity – in particular, to destroy DNA, that is, to perform enucleation. This approach has specific practical applications, for example, the preparation of recipient cytoplast for assisted reproductive technologies in humans and animal cloning. We have previously shown that using a femtosecond laser, mouse and human oocytes can be successfully enucleated [1, 2]. We have previously shown that femtosecond laser enucleation of mouse oocytes can be effectively performed using the fluorescent dye Hoechst 33342. The fluorescence spectrum of this dye is in the blue region of the spectrum (max. 454 nm), and ultraviolet light (max. 342 nm) is required for its excitation. In this work, possibility of using another dye with visible excitation was considered.

Materials and Methods

Animals and oocyte collection. The study was carried out on C57BL6/CBA female mice aged 6–8 weeks (Mus musculus). The mice were induced to superovulate by the standard method of intraperitoneal (i.p.) injection of 10 IU pregnant mare's serum gonadotropin (A036A02, Intervet) followed by an i.p. injection of 10 IU human chorionic gonadotropin (hCG) (A038A01, Intervet) 48 h later. The mice were sacrificed by cervical dislocation 17 h after hCG injection. The oviducts were placed onto a Petri dish in a drop of M2 medium (M7167, Sigma-Aldrich) supplemented with 0.1% hyaluronidase (H4272, Sigma-Aldrich). The ampulla of the oviduct was disrupted with a pair of thin tweezers. The oocytes purified from cumulus were placed in M2 in four-well dishes (179830, Nunc). We used metaphase II oocytes in the experiments.

Fluorescence measurement. The oocytes were stained in M2 medium at a dye concentration of 1 μM for 30 minutes, then washed twice in a clean medium. The fluorescence of SYTO 9 was excited at wavelengths of 430–490 nm. The oocytes were stained with 5 μg/ml Hoechst 33342 dye (B2261, Sigma-Aldrich) for 15 minutes in M2 and then washed twice. Single-photon excitation of Hoechst was performed with the 405 nm laser wavelength. The fluorescence intensity of oocytes DNA was measured on a confocal microscope Zeiss LSM 980 (Carl Zeiss Microscopy, Jena, Germany), 20x Plan-Apochromat objective (NA = 0.8).

Femtosecond laser treatment. Enucleation was performed at a wavelength of 900 nm, femtosecond laser pulse duration was 100 fs, pulse energy 3 nJ, and bursts of 50 ns with a pulse repetition frequency of 3 MHz were selected from the initial pulse sequence of 80 MHz using a modulator built into the laser. The selected wavelength of femtosecond laser radiation is due to the fact that it corresponds to 450 nm during a two-photon absorption process, which in turn falls into the absorption spectrum of the fluorescent dye SYTO 9 (Fig. 1).

In the experiment, a metaphase plate was first visualized, then it was irradiated with femtosecond laser pulses until the fluorescence stopped. After that, a control staining with a spectrum-contrasting dye (Hoechst 33342) was performed and the samples were visualized on a confocal microscope in order to identify the remnants of the metaphase plate after enucleation.

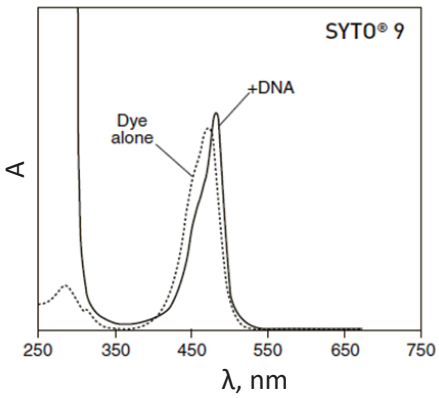

Fig. 1. The excitation and absorption spectrum of the dye SYTO 9 (S34854, Molecular probes)

Results and Discussion

As an alternative to the previous studies were we used Hoechst 33342 fluorescent dye, the vital fluorescent dye SYTO 9 (S34854, Molecular probes) was chosen, the absorption spectrum of which is shown in Fig. 1. Light with a wavelength of 400–500 is less phototoxic than UV, therefore, the use of this dye for enucleation may be less traumatic for the sample. We observed visible fluorescence from SYTO 9 dye during femtosecond laser treatment, which proves two-photon excitation of dye. Similar results were reported by Kwok et al on successful two-photon excitation of cyanine-based dyes [3].

When the metaphase plate of an oocyte stained with the vital fluorescent dye SYTO 9 is exposed to femtosecond laser radiation with a wavelength of 900 nm, two-photon processes occur, leading to the luminescence of the dye at the time of irradiation, as in the case of Hoechst. The femtosecond laser treatment was performed until this visible fluorescence stops – this could be due to photobleaching. Moreover, in case of SYTO 9 usage, DNA destruction practically does not occur – upon repeated staining, the metaphase plate is revealed almost unchanged, and the area of its detection after irradiation does not significantly differ from the area of detection before exposure (Fig. 2, *b*).

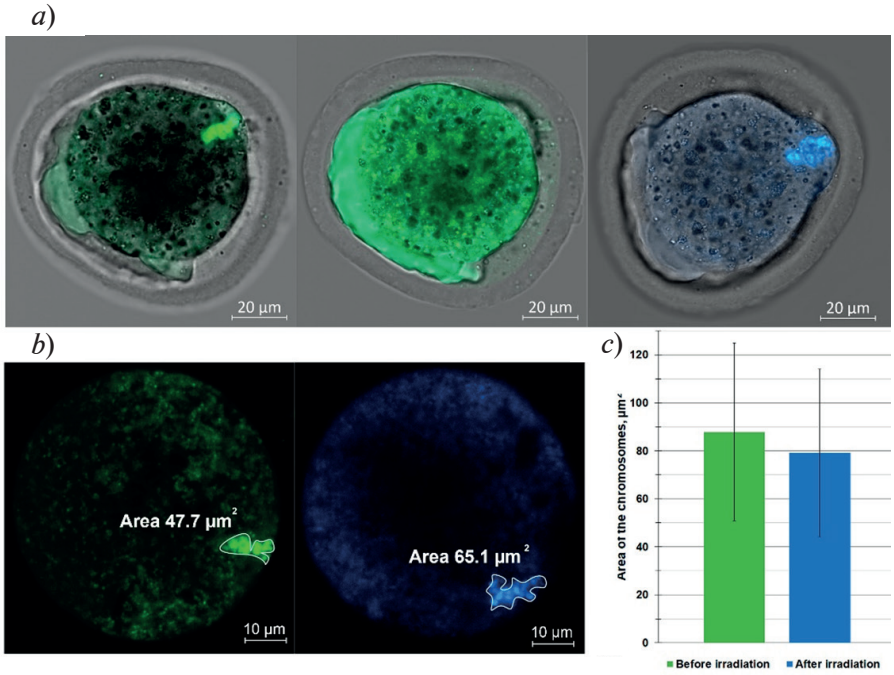

Fig. 2. In a row from left to right: oocyte before laser treatment, immediately after treatment, contrast staining with Hoechst 33342 (*a*); fluorescent image of oocyte SYTO before treatment, Hoechst 33342 after treatment (*b*); area of metaphase plate before and after treatment comparison (*c*)

The mechanism of DNA destruction mediated via fluorescent dye under femtosecond laser irradiation is not fully revealed yet. Mechanisms of DNA destruction by itself (without any additional reagents) under femtosecond laser irradiation are traditionally described through low-density plasma formation and resonant electron-molecule scattering [4, 5]. Additionally, reactive species produced upon the laser-induced lysis of water could be involved in DNA destruction and modification processes [6]. In our previous studies, we have shown that usage of fluorescent dye could decries amount of laser power density, required for DNA destruction [7] and for this reason we assume that fluorescent dye could be involved in processes of DNA destruction. In case of SYTO 9 usage, we think that the laser-induced lysis of water became less effective than in case of Hoechst 33342 dye usage, and so the production of reactive species declines. However, this hypothesis needs further investigation.

Conclusion

In this work, possibility of SYTO vital fluorescent dye usage for performing femtosecond laser-assisted metaphase plate enucleation of mouse oocyte was studied. We have shown that under femtosecond laser treatment photo bleaching of dye take place without significant DNA destruction. This result is quite interesting, considering that under similar laser parameters with only difference in wavelength (800 nm and 1033 nm) usage of Hoechst 33342 was efficient for DNA destruction. These results introduce new material for the model of femtosecond laser and biomaterial interaction.

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