Lasers for gamete micromanipulation: basic concepts.

https://escholarship.org/uc/item/8cq3t64m

Journal of assisted reproduction and genetics, 10(2)

1058-0468

Tadir, Y
Neev, J
Ho, P
et al.

1993-02-01

10.1007/bf01207733

https://creativecommons.org/licenses/by/4.0/ 4.0

Peer reviewed
LASER

Lasers for Gamete Micromanipulation: Basic Concepts

INTRODUCTION

A brief literature search on the key words “micromanipulation (MM) and male factor infertility” may highlight the controversy regarding this issue. It has recently been suggested (1) that “so inconsistent are the reported results that at present it is pertinent to ask does microsurgical assisted fertilization (MAF) work at all”? The controversies are related mainly to patient selection needed for establishing prognostic criteria and the method of choice (2,3). Gamete manipulations require special equipment and expertise, while the preparation of disposable microneedles for MAF is time-consuming and, thus, expensive (4).

In an attempt to increase accuracy and simplicity, it has been suggested that the laser might offer several advantages. Since its first introduction for gamete manipulation in 1989 (5) several studies addressed basic questions on its potential role and discussed various methods (6–10). It is beyond the scope of this article to assess the important issues of effectiveness and safety of the laser for gamete manipulations. Several studies and clinical observations are being performed throughout the world and it will take some time until the controversies regarding the future role of MAF will be answered. However, in view of the increasing interest in lasers for gamete MM, some guidelines are needed. Strohmer and Feichtinger have recently presented in abstract form some biophysical criteria for laser MM (11). They suggested four basic requirements for the preferred approach: (i) heat deposition, (ii) DNA absorption, (iii) ablation threshold, and (iv) simplicity in equipment and training. One may suggest adding a few more prerequisites such as (v) absorption in water and proteins, (vi) a spot size smaller then the thickness of the zona pellucida (ZP), and (vii) precision of the entire unit.

In a previous study (12) we have discussed the influence of various physical parameters on the expected effects during gamete manipulations (i.e., cutting geometry, ablation beam size, pulse repetition rate and duration, and laser fluence, i.e., energy per unit area). It is the intent of this column to discuss some aspects related to the above-mentioned prerequisites in order to take full advantage of the laser as “light scalpels.”

Lasers, (an acronym for Light Amplification by Stimulated Emission of Radiation) are electromagnetic waves with unique properties. The beam is collimated, monochromatic, and coherent. Progress in physics and laser technology in recent years has resulted in the introduction of many lasers for biomedical studies. Although lasers differ from each other by the wavelengths, which are in the visible range (red, green, or blue), ultraviolet (UV), or in the infrared (IR) range (Fig. 1a), effects may also vary as a result of different application modes as will be discussed later.

HEAT DEPOSITION IN THE OOCYTE OR THE EMBRYO

Some heat will always be generated in the micromanipulated oocyte or embryo if the ablation WL is
absorbed by the components in the irradiated area. This is particularly true if the ablation mechanism is thermal in nature. To reduce the thermal deposition, one has to choose a WL which is not excessively absorbed. However, some absorption is necessary to achieve the wanted effect. By operating with a pulsed laser, unwanted thermal effects can be reduced to a minimum, provided that the pulse duration is short compared to the thermal relaxation time of the medium and the time between the pulses is sufficiently long. In this case the heat will be confined to the treated area, and heat will not diffuse to adjacent structures. Shortening of the pulse duration will not be sufficient if the laser pulse repetition rate (PRR) is too high. Here a pulse-to-pulse heat buildup will result in a significant temperature rise and, ultimately, in thermal damage (12). Finally, a high pulse energy will also result in greater deposition of energy per unit volume, and this can contribute (at high PRR) to greater thermal damage as well as possible mechanical disturbances. Thus, one must be careful to establish an upper limit to the fluence level used. Excimer lasers which emit light in the UV can produce precise incisions with very little heat deposition. This is due to the high-energy photons in UV light, which are capable of molecular bond breaking. Indeed, several other researchers (7,10) have utilized UV radiation because of their accurate nonthermic action.

LIGHT ABSORPTION BY DNA AND PROTEINS

It is well-known that UV light may cause mutagenic damage and the sensitivity of this issue when dealing with genetic material of gametes is obvious. The first observation of UV effects on living systems dates back to 1877 when Dowens and Blunt reported that bacteria were inactivated by light. The next landmark was the finding by Gates in 1928 that the relative effectiveness of killing bacteria by different WL correlated with the absorption spectrum of nucleic acid. However, the first law of photobiology (Grotthus-Draper law) states that "light must be absorbed by a molecule before photochemistry can occur," and thus, one has to prove that absorption takes place through barriers such as the ZP, basal membrane, and cytoplasm, especially at low energy levels and at tangential superficial orientation. In addition, certain factors such as the choice of solvent, the pH, the concentration of a solution, and even the temperature may alter the absorption characteristics of the medium and the target (13,14). The absorption spectrum of DNA is illustrated in Fig. 1b. In general, there is a gradual reduction from the high absorption level at 150 nm toward a minimum at about 300 nm, with two peaks, at 180–200 and at 245–275 nm (13,14). Laufer et al. (15) have recently demonstrated the safety of the excimer 193 nm laser in the contact mode for zona...
Lasers for Gamete Micromanipulation

IVF - Micromanipulations "accuscale"

Fig. 2. Micromanipulation "accuscale." Various accessories used for conventional and laser during micromanipulations as compared to the size of gametes.

Pellicula drilling. The procedure enhanced fertilization rate at low sperm concentration and did not interfere with embryo development in a mouse model.

The absorption curve of various proteins (BSA) at pH 7 is tabulated in Fig. 1c. In large, the UV absorption spectrum of the polymer and polar macromolecules (such as protein and nucleic acids) is often not strictly the linear sum of the absorption of its component conjugated groups (13).

In the tangential laser approach toward the zona pellucida (ZP), energy deposition is oriented toward minimum exposure of any vital intra cellular material (12). Studies have been conducted (14,16) that demonstrate mutagenesis and cell toxicity when cells are exposed directly to an UV laser wavelength. However, it is clear from these studies that the least damaging wavelength was at 308 nm (which is also tested for DNA absorption; Fig. 1b). It would appear unlikely that mutagenesis would be a problem at the low fluence used in zona manipulations, especially when used in a tangential approach (when few, if any, photons would scatter through the membrane).

Fig. 3. Basic parameters of contact and noncontact application of lasers during gamete micromanipulations (contact, fiber delivery of the laser; noncontact, free beam delivered through the microscope optics and the fluid medium).
Table I. Some Commercially Available Lasers at Various Parameters that Have Been (or Might Be) Useful for Gamete Micromanipulations

| WL (nm) | Source | Pulse duration | Max. energy/pulse | PRR (Hz) | Application mode |
|---------|--------|----------------|-------------------|----------|------------------|
| 193     | ArF    | 15 ns          | 100 mJ            | 1-100    | Contact          |
| 248     | KrF    | 15 ns          | 100 mJ            | 1-100    | Noncontact       |
| 266     | Nd:YAG — 4th harmonic | 15 ns | 0.5 mJ | 1; 5; 10 | Noncontact |
| 266     | Nd:YAG — 4th harmonic | 15 ns | 10 μJ | 1; 5; 10 | Noncontact |
| 308     | XeCl   | 15 ns          | 100 mJ            | 1-100    | Noncontact       |
| 308     | XeCl   | 125 ns         | 100 mJ            | 1-50     | Noncontact       |
| 308     | XeCl   | 100-150 ns     | 25 μJ             | 1-2000   | Noncontact       |
| 337.1   | Nitrogen | 600 ps       | 1.4 mJ            | 3; 10; 20 | Noncontact     |
| 355     | Nd:YAG — 3rd harmonic | 15 ns | 10-20 mJ | 1; 5; 10 | Noncontact |
| 355     | Nd:YAG — 3rd harmonic | 70 ps | 10-100 μJ | 1; 5; 10 | Noncontact |
| 366     | Nitrogen pumped dye | 600 ps | 1.4 mJ | 3 | Noncontact |
| 532     | Freq.-doubled Nd:YAG | 15 ns | 50 mJ | 1; 5; 10 | Noncontact |
| 694     | Ruby   | 15 ns          | 2 J               | 1        | Noncontact       |
| 532     | Freq.-doubled Nd:YAG | 70 ps | 1 mJ | 1; 5; 10 | Noncontact |
| 700-1100| Ti. Saph. | CW or pulse |                | Noncontact | |
| 1064    | Nd:YAG | 15 ns          | 500 mJ            | 1; 5; 10 | Noncontact       |
| 1064    | Nd:YAG | 70 ps          | 5 mJ              | 1; 5; 10 | Noncontact       |
| 2120    | Ho:YAG | 250 μs         | 100 mJ            | 1-9      | Contact          |
| 2710    | Er:YSSG| 250 μs         | 100-500 mJ        | 1-9      | Contact          |
| 2940    | Er:YAG | 250 μs         | 100-500 mJ        | 1-9      | Contact          |

* Actual energy used for micromanipulations can be attenuated to a minute fraction.
* Pulse repetition rate.
* Tunable, continuous wave (CW).

WATER ABSORPTION

Some of the potential advantages of the laser as a micromanipulating tool are its simplicity, accuracy, small effective spot size (in the range of 0.5–1 μm; Fig. 2), and maneuverability without any mechanical handling. Noncontact laser manipulations using a free beam delivered through the microscope objective are conditioned by the absorption curve in water (Fig. 1d). Wave lengths in the IR range, longer than 2000 nm (with peaks at 2900 and 6000 nm), are highly absorbed by water. This means that a free laser beam at conventional parameters will not cause any effect to oocytes "shielded" by any fluid medium, water, or oil (Fig. 3). Moreover, lasers in WL longer than 2500 are not transmissible via conventional silica fibers and some of the other fibers (such as zirconium fluoride) are toxic and expensive. For that reason, laser micromanipulations at WL ranging in this spectrum should be delivered via flexible fibers that will come in close contact with the ZP. Similarly, a nonthermic excimer laser in the far UV is also highly absorbed by water and, thus, needs to be delivered through a hollow glass pipette in contact with the target area (7,15). The micropipettes or conically sculpted fibers should be resterilized and mechanically manipulated and the oocyte should be fixed with a vacuum (holding) pipette in the same way as conventional MM. On the other hand, laser "free" beams that are not absorbed by water can be delivered to the ZP (or to subcellular organelles) through the microscope optics and fine targeting with an X-Y-Z motorized microscope stage (Fig. 3). The effective spot size can be manipulated and reduced by the optical system to the smallest spot conditioned by the WL. Basic differences between contact and noncontact laser MM are summarized in Fig. 3.

Basic studies in laser–gamete interaction are being carried out in order to determine the potential use, advantages, disadvantages, indications, hazards, and cost effectiveness of this modality. Few laser systems at various physical parameters have been tested and many more are available on the market (Table I). Some of the assumptions described in this article are based on theoretical principles and the laws of physics. One may argue that biomedicine is not among the exact sciences, and thus, careful studies of laser effects on gametes should guide us in the delivery of this technology to clinical practice. Conventional alternatives should serve as a reference in order to determine potential superiority.
REFERENCES

1. Gordon JW: Current unresolved controversies in micromanipulation-assisted fertilization. J Assist Reprod Genet 1992; 9:184–186
2. Cohen J, Talansty BE, Adler A, Alikani M, Rosenwaks Z: Controversies and opinions in clinical microsurgical fertilization. J Assist Reprod Genet 1992;9:94–96
3. Ng SC, Lion SL, Bongo TA, Montag M, Sathananthan AH, Ratnam SS: Micromanipulation and possible new approaches to help the severe male factor patient. J Assist Reprod Genet 1992;9:91–96
4. Gordon JW, Zentner B, Garisi G J, Chin A, Boyle PM, Laufer N: Development of rapid and reliable microneedles for subzonal sperm insertion that does not require use of a microforge or needle grinder. Presented at the American Fertility Society 48th Annual Meeting. Fertil Steril program supplement P-160, New Orleans, LA, Nov 2–5, 1992
5. Tadir Y, Wright WH, Berns MW: Cell micromanipulation with laser beams. In G.I.R.T.: From Basics to Clinics, GL Capitanio, RH Asch, L De Cecco, S Croce (eds). New York, Raven Press, 1989, pp 359–368
6. Tadir Y, Wright WH, Vafa O, Liaw LH, Asch R, Berns MW: Micromanipulation of gametes using laser microbeams. Hum Reprod 1991;6:1011–1016
7. Palanker D, Ohad S, Lewis A, Simon A, Shenkar J, Penchas S, Laufer N: Technique for cellular microsurgery using the 193 nm Excimer laser. Laser Surg Med 1991;11:580–586
8. Feichtinger W, Strohmeier H, Fuhrberg P, Radijojvic K, Antoniori S, Pepe G, Versaci C: Photoablation of oocyte zona pellucida by erbium: YAG laser for in-vitro fertilization in severe male infertility. Lancet 1992;339:811
9. Neev J, Tadir Y, Asch R, Whalen WE, Ord T, Liaw L, Ho PT, Berns MW: Laser zona drilling and partial zona dissection: A comparative study. Proc Int Soc Photo-Opt Instrum Eng (SPIE) 1992;1650:11
10. Blanchet BB, Russel JB, Fincher CR, Portman M: Laser micromanipulation in the mouse embryo: a novel approach to zona drilling. Fertil Steril 1992;57:1337–1347
11. Strohmer H, Feichtinger W: Application of laser for micromanipulation: relevance of biophysical criteria. Presented at the American Fertility Society 48th Annual Meeting. Fertil Steril program supplement P-89, New Orleans, Nov 2–5, 1992
12. Neev J, Tadir Y, Ho P, Asch RH, Ord T, Berns MW: Microscope-delivered UV laser zona dissection: Principles and practices. J Assist Reprod Genet (in press)
13. Smith KC, Hanawalt PC: Introduction: Basic principles. In Molecular Photobiology. New York, Academic Press, 1969, pp 1–21
14. Kochevar IE: Cytotoxicity and mutagenicity of excimer laser radiation. Laser Surg Med 1989;9:440–445
15. Laufer N, Palanker D, Shufaro Y, Selran A, Simon A, Lewis A: The efficacy and safety of zona pellucida drilling by a 193 nm excimer laser. Fertil Steril 1993;59:889–895
16. Rasmussen RE, Hammer-Wilson M, Berns MW: Mutation and sister chromatid exchange induction in Chinese hamster ovary (CHO) cells by pulsed excimer laser radiation at 193 nm and 308 nm and continuous radiation at 264 nm. Photochem Photobiol 1989;49:413–418
17. Laws-King A, Trounson A, Sathananthan H, Kola I: Fertilization of human oocytes by microinjection of a single spermatozoon under the zona pellucida. Fertil Steril 1987;48:637–642
18. Tadir Y, Schiwee MC, Neev Y, Ho P, Ord T, Balmaceda JP, Asch RH, Berns MW: Spontaneous and induced zona pellucida hardness: Measurements using enzyme assay and a non-contact laser micromanipulator. Pacific Coast Fertility Society, Fertil Steril program supplement O-21, California, April 14–18, 1993

Y. Tadir1,3
J. Neev2
P. Ho2
M. W. Berns2

Beckman Laser Institute and Medical Clinic
Department of Obstetrics and Gynecology
University of California, Irvine
Irvine, California 92715

1 Department of Obstetrics and Gynecology, University of California, Irvine, Irvine, California 92715.
2 Department of Surgery.
3 To whom correspondence should be addressed at Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Science Road, Irvine, California 92715.