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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

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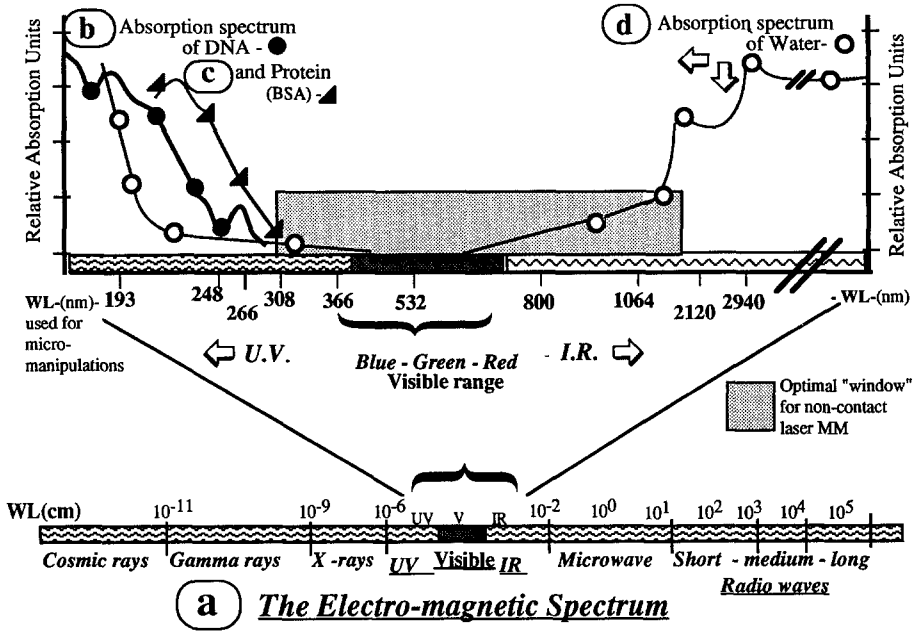

Fig. 1. The electromagnetic spectrum and absorption curves of laser beams by DNA, proteins, and water.

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

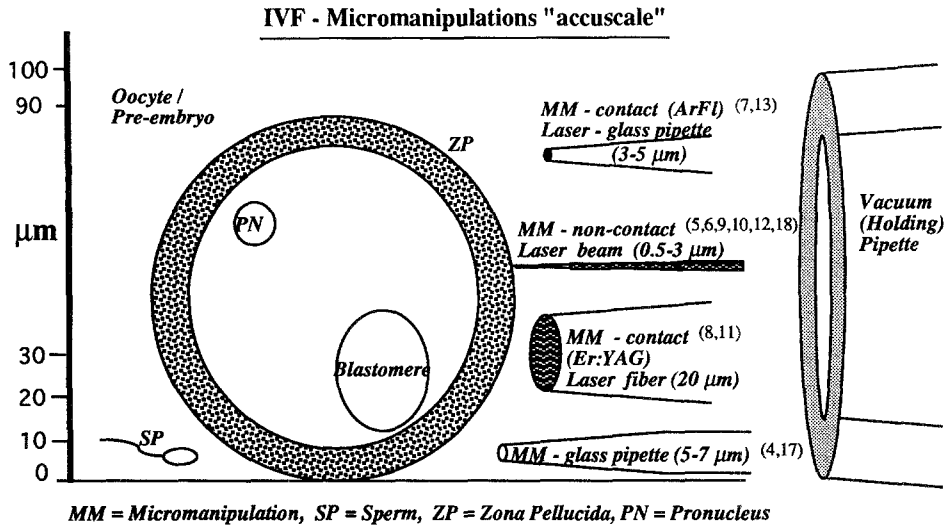

Fig. 2. Micromanipulation "accuscale." Various accessories used for conventional and laser during micromanipulations as compared to the size of gametes.

pellucida 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).

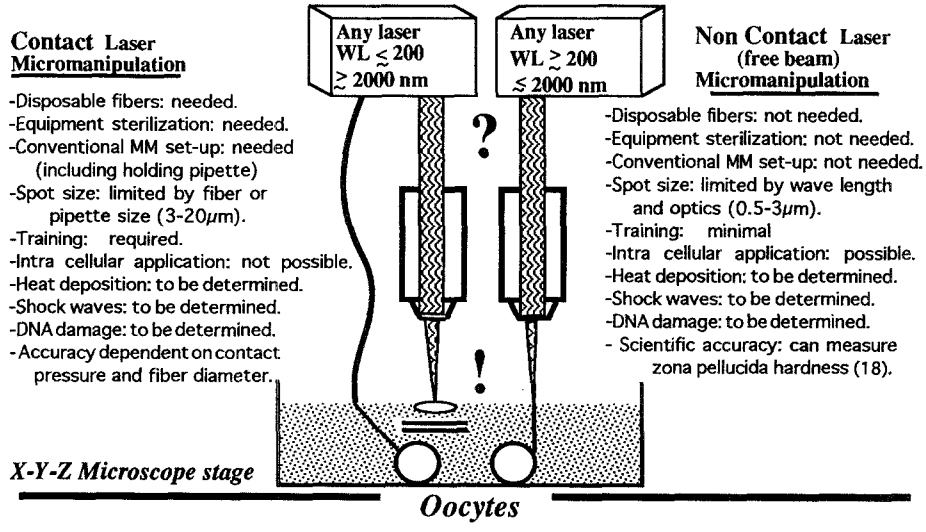

Fig. 3. Basic parameters of contact and noncontact application of lasers during gamete micromanipulations (contact, fiber delivery of the laser; noncontact, free beam delivered through the microscope optics and the fluid medium).

Table I. Some Commercially Available Lasers at Various Parameters that Have Been (or Might Be) Useful for Gamete Micromanipulations

WL (mm)	Source	Pulse duration	Max. energy/pulse[a]	PRR[b] (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[c] 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

[a] Actual energy used for micromanipulations can be attenuated to a minute fraction.
[b] Pulse repetition rate.
[c] 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.

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