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Laser - An idyllic boon for biotechnology

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ABSTRACT

In this review article we have out lined the applications of Laser in Biotechnology. The laser-based systems are the state of art technologies for distinct and noncontact manipulations in cellular or sub-cellular dimensions with nanometre accuracy and have become fundamental tools within entire community of life science research,. Laser-induced optical forces can be used to guide and deposit 100nm-10μm diameter particles with micrometer accuracy; this process is known as 'laser-guided direct writing'. Laser and its scrupulous application to various steps in IVF (*In-Vitro* Fertilization) process is a field of growing interest. The diversity of compact blue-green lasers in both the variety of technical approaches used to produce them and the wide range of applications for which they have been sought has made the field of compact blue green lasers interesting. Single cell manipulation in tissue is performed with the use of shockwave manipulation and laser trapping. The laser method is used to improve gene transfer for therapeutic purposes.

KEYWORDS

Laser;
LMPC;
IVF;
DNA sequencing;
Femtosecond laser.

INTRODUCTION

The first laser-assisted microdissection of tissue sections was published in 1976[1]. The dissectates were processed for quantitative, histochemical analysis. In 1995, single renal tubules were captured from freeze-dried sections for subsequent genetic analysis[2]. Optical forces can be used to manipulate thousands of particles simultaneously and to deposit them in a continuous stream onto surfaces with micrometer accuracy[3,4]. Hollow-core optical fibres can aid optical-force-mediated particle guidance, creating new opportunities for the laser guided direct writing of a wide variety of materials[3,5,6]. Any particulate material, including both biological and electronic materials, can be manipulated and deposited on surfaces with micrometer accuracy. Potential applications include biochip-array fabrication and three dimensional cell patterning for tissue engineering.

Laser technology has been used in Assisted Reproductive Technology since the 1980s[7]. Laser pulse has found wide application in IVF technology, particularly when efficient and precise manipulation is of paramount importance[8]. Laser beams travel through the objective lenses and only microscope stage movement is required to adjust embryo position[9-11]. In contrast, contact laser systems require direct contact between

the laser and embryo, usually with either glass or an optical fibre[12]. The first generation of lasers used in IVF are Argon fluoride (ArF), Xenon chloride (XeCl), Krypton fluoride (KrF), Nitrogen and Nd: Yttrium-Aluminum-Garnet (YAG) lasers. The next generation of lasers were designed to circumvent dangers of UV wavelength and cytotoxicity by emitting wavelengths in the infrared region (>800 nm)[7]. The first of the newer generation of lasers to be used in IVF was the 2.9 um pulsed Erbium: Yttrium-Aluminum-Garnet laser (Er: YAG)[13]. Currently, the 1.48 um diode wavelength Indium-Gallium-Arsenic-Phosphorus (InGaAsP) semiconductor laser is used in IVF. It is a non contact laser, has a safer wavelength and produces consistent results in the form of uniform, smooth edged tunnels[14].

The biocompatibility of polymer surfaces treated with UV-radiation in reactive atmosphere has been investigated for various different types of biological cells. Among those is Human Umbilical Vein Endothelial Cells (HUVEC). They play an important role in the avoidance of thrombosis, the immune response after injuries or the vascularisation of tissue[15]. The specialized topical meetings that sprang up in the early 1990s in response to interest in the field of compact blue green lasers such as the optical society of America's Topical Meeting On Compact Blue-Green lasers, held in 1992, 1993, and 1994 brought together researchers from such disparate field as submarine communications and DNA sequencing.

A new method has been proposed using nonlinear effects of femtosecond laser[16]. In comparison with force of a conventional laser trapping, the force of the shockwave is enough larger than that of the laser trapping, because the initial velocity of the particle, which is estimated to be over 3 mm/sec at *R0* of 6 μm, is extremely larger than the velocity when the particle is transported in water by the laser trapping[17]. The force of shockwave was applied to separate two daughter cells of fission yeast confirm the interaction between the shockwave and live cells[16].

Laser microdissection and pressure catapulting (LMPC)

To start, the capture of a few hundred or some single cells from heterogeneous tissue samples was performed mechanically using scalpels for dissection and needles or forceps to lift the dissected areas from the object slide[18,19]. However, these needle-based microdissection methods are not convenient for routine work. There is also a danger of contamination due to direct contact of samples with mechanical tools. Laser pressure catapulting (LPC^{pat}) is a fascinating technology that allows precise capture of pure cell populations from morphologically preserved cells and tissue sections without any mechanical contact, solely by the force of the focused laser light[20,21]. With a single laser shot, the selected sample is ejected out of the object plane and transported over centimetre-wide distances into an appropriate collection vial. This process was named "laser pressure catapulting" (LPC^{pat})[20].

LMPC in immunobiology and immunopathology

Recently, Cytokine mRNA levels were quantified in single alveolar macrophages isolated by laser microdissection from normal and immunologically challenged mice[22]. It has been shown that multiple RNA and DNA sequences can be analyzed from microdissected cell samples containing as little as ten cells, demonstrating that laser microdissection gives the specificity necessary to study these complex interactions in detail[23]. Microdissection approaches have been used to study the cellular origin of lymph proliferative disorders. These studies have shown that laser-assisted microdissection can be a valuable tool in the diagnosis of these pathological conditions[24-27].

LMPC in oncology

LMPC can easily be applied to analyze expressed genes from a few cells or even from single cells, which were retrieved from archival, formalin-fixed, and paraffin-embedded tissue as published by various research groups[20,22,23,28,29]. After microdissection of cells from archival tissues followed by RT-PCR analysis, specifically RET/PTC1 transcription was detected in about 80% of thyroid papillary carcinomas or in variants thereof[28]. There is emerging evidence that epithelial tumour cells are able to disseminate to secondary organs at an early stage of primary tumour development[29]. In association with laser isolation, individual, disseminated tumour cells derived from bone marrow aspirates were analyzed at the genetic level[30]. In association with laser isolation, ISET (Isolation by Size of

Review

Epithelial Tumour Cells) allowed genetic analysis of individual CTCs (Circulating Tumour Cells)[31].

LMPC for life-cell isolation

Up till now the microdissection method has been limited to cells from fixed or frozen tissues. Capture of living cells using the cookie cutter method resulted in the destruction of the isolated Cells[32]. However, capture of living cells segregated from admixtures of cells would be highly desirable for different investigations[33]. A modification of the laser technique and a newly developed cell culture protocol now allows microdissection and "ejection" of living single cells or cell clusters with ongoing cultivation for potential treatment and analysis[34].

Laser-guided direct writing

By simply changing the laser-beam focus, it has been suggested that optical forces can be used to manipulate thousands of particles simultaneously and to deposit them in a continuous stream onto surfaces with micrometer accuracy[3,4]. Using a weakly focused laser beam (i.e. a low-numerical-aperture focusing lens), the guidance of living cells in an aqueous fluid demonstrated for cell sorting[35]. 'Laser-guided direct writing' has been demonstrated with a variety of organic and inorganic particles in both gas and liquid phases[4,36], and with living cells in culture medium[3]. Laser-guided direct writing has advantage over photolithography because it does not require corrosive chemicals. In initial studies with embryonic-chick spinal-cord cells, it has been found that individual cell (diameter 59 mm) could be guided by a 450 mW near-infrared laser beam and deposited in arbitrarily defined arrays onto a glass target surface[3]. The laser-guided direct writing system is fundamentally different from optical trapping in that it provides propulsion along the beam axis instead of trapping. However, by simultaneously coupling light into both ends of a hollow optical fibre, a trap can be set up inside the fibre[36].

Laser pulse application in IVF (*In-Vitro* Fertilization)

Studies using laser pulses have determined the extent to which the zona hardens during the period from oocyte to blastocyst and further identify which embryos may need assistance with sperm entry or hatching. Zona hardness is greater during in vitro culture as compared with in vivo growth[37]. Identical laser pulses create larger holes ranging from 13-17 um in the zona at earlier stages (oocyte, zygote) as compared to more advanced stages of development (morula, blastocyst) where holes are smaller at 10-13 um[37,38]. Also, larger holes were created in blastocysts cultured in vivo when compared with in vitro grown blastocysts, suggesting zona hardening during culture[14,37]. Polar body can provide helpful information by reflecting the maternal genetic material contained in that egg[39,40]. Abnormal oocytes with genetic defects can be selectively excluded[39]. When polar body biopsy is performed using lasers, a pulse is directed at the region of zona pellucida nearest the polar body. Two pulses of 14 ms are given by a 1.48 um non contact laser, creating an opening of approximately 14-20 um[41]. The material is then extracted with a blunt capillary, avoiding potential damage to the oocyte with a sharp instrument, and the entire procedure is completed in just a few minutes[41]. A similar procedure has been described using a nitrogen 337 nm laser and Nd: YAG laser[39].

Blastomere biopsy becomes relevant at a later stage in development, after fertilization. Until the introduction of laser assisted opening of the zona, blastomere biopsy was performed by zona drilling with an acid tyrodes solution[42]. Laser pulses are utilized to create a hole in the zona pellucida, through which a blastomere is removed[8]. Analysis of laser pulse length in generating a hole for blastomere extraction showed longer pulse duration (0.604 ms vs. 1.010 ms) produced larger hole sizes (10.5 nm vs. 16.5 nm, respectively)[8]. Studies comparing embryos after laser assisted biopsy to untreated embryos showed no adverse effects of treatment and similar hatching and development rates[43]. ICSI (Intra Cytoplasmic Sperm Injection) is performed by aspirating a sperm into a sharp glass needle (5 μm in diameter), perforating the oocyte's zona and depositing the sperm into the ooplasm[44]. Laser assisted zona drilling prior to ICSI can be used to increase the likelihood of successful fertilization[45]. This may be done with a 193 nm Argon fluoride (ArF) laser, which was shown to drill very precise holes without undesired damage to the zona pellucid[45]. A 1.48 um diode laser can also be used to assist with ICSI[46,47]. The ICSI injection pipette is introduced through this channel to deliver

the previously immobilized sperm[46-48]. To ensure even less traumatic manipulation, sperm may be injected into the oocyte through a laser drilled hole using optical tweezers to achieve fertilization[39,49].

Typically during ICSI, the sperm tail is positioned under the glass microcapillary injection needle. The needle is brought down and across the tail causing it to break and immobilizing the sperm[44,50-52]. Low level laser pulse can be used to immobilize sperm, without affecting viability[10,41,47,53]. Fertilization rates would be expected to be correspondingly higher if better sperm are selected for the injection[53]. Laser-assisted zona pellucida thinning prior to ICSI resulted in decreased oocyte degeneration rates, better blastocyst hatching rates and improved pregnancy rates after day 3 embryo transfer[54]. Laser assisted hatching is generally well-accepted in IVF labs, allowing improved standardization between operators[55,56].

Compact blue-green lasers in DNA sequencing

Blue-green lasers have uses in the field of biotechnology[10]. The objective of DNA sequencing is to determine the sequence of the nucleotides that encode genetic information in the molecular structure of DNA. Such sequences are important for diagnosing genetic disorders and also important in the field of forensic science. In a technique for DNA sequencing, the genetic code is determined by creating a series of replicas of a particular section of the DNA molecule. Each replica starts at the same point in the sequence of the DNA molecule, but differs in length from other replicas by one nucleotide. Fluorophores determine the terminating nucleotide of each replica by "tagging" the replica with one of four fluorophores, each fluorophore determine the presence of one of the four nucleotides. The fluorophores are chosen in a manner that their emission peaks are sufficiently separated to be easily resolved. Four common fluorescent molecules used for this purpose are Fluorescein, NBD, Texas Red, Tetramethyl rhodamine together with their absorption and emission spectra[57]. Sorting of the replicas by length can be done by forcing them to diffuse through an electrophoresis cell. Excitation from a blue green laser excites emission from the fluorophore as each replica reaches the end of the gel. By analyzing the fluorescent emission through a filter wheel, the fluorophore and corresponding nucleotide are identified[10]. Several commercial products are available that use argon-ion lasers as the source of blue-green light as a result of a great deal of development has gone into DNA sequencers based on this technology.

Non-destructive manipulation of single live plant cell by laser induced micro shockwave

Some kind of single cell manipulation for both plant and animal cells have been demonstrated by using laser trapping and laser ablation[16,58-61]. However, usage of the laser trapping is limited, because the optical pressure is not so strong[17]. To overcome this problem, a new method has been proposed using nonlinear effects of femtosecond laser[16]. When an intense femtosecond laser is focused on a transparent organic/biological material, various nonlinear photophysical and photochemical dynamic processes, such as, shockwave, cavitation bubble, plasma, and so on, are induced as a result of high efficient multiphoton absorption, mutual interactions between excited states, and/or ionization[62-65]. The force of the shockwave is considered to become much larger than that of the conventional laser trapping. It is supported that single cell manipulation in tissue is performed by combining the shockwave manipulation with laser trapping.

Laser enhanced gene transfer

Much interest has been shown in the use of lasers for non-viral targeted gene transfer, since the spatial characteristics of laser light are quite well defined[66]. A small hole made in a cell membrane by pulse laser irradiation to help a gene contained in a medium to be transferred into the cytoplasm through the hole[67]. This hole disappears immediately with the application of laser irradiation of the appropriate power[67]. Femtosecond laser treatment represents a novel and attractive method for in vivo gene delivery because the lasers are convenient to operate, relatively non-invasive, and have been shown to significantly enhance gene transfection efficiency without detectable tissue damage in mice[68,69]. They have been applied toward in vitro genetic modification of cells and have recently been found to improve intradermal and intramuscular delivery of DNA in mice[68,69]. Such a laser technology can be used to deliver DNA encoding antitumor genes in-

Review

cluding proapoptotic, immunostimulatory, and/or antiangiogenic factors to effect eradication of tumours through apoptosis, immune-mediated mechanisms, and nutrient deprivation. In future studies, it will be important to explore the employment of laser treatment in combination with gene therapy using plasmid DNA encoding these factors for the control of disease in preclinical models.

CONCLUSIONS AND PROSPECTIVE FOR THE FUTURE

In modern biotechnology, the LMPC technology is an indispensable for functional genomics and proteomics studies. LMPC in combination with MALDI (Matrix Assisted Laser Desorption Ionization) allows protein fingerprinting in order to differentiate between various cell types, normal, premalignant, or malignant cells. In botany, single chloroplast can be isolated to visualize interior of pollen grains and also for plant systematic studies. Laser-guided direct writing is an emerging technology for the high throughput deposition of particles with micrometer accuracy. Lasers are useful in IVF technology with which to perform delicate procedures such as blastomere biopsy, drilling through the zona pellucida, sperm manipulation and cellular microsurgery. Laser technology may make performance of these tasks easier and faster. As the use of laser technology in reproductive medicine spread among a large area, long term studies will be needed to evaluate DNA abnormalities that may not clear until later in life. Laser light scattering has proved a valuable technique for characterisation of macromolecules in solution. In the future, light scattering based assays will offer a faster and cheaper alternative to established screening methods. A new femtosecond laser-based DNA delivery strategy will aid gene therapy with plasmid DNA and DNA vaccination for the treatment of human disease.

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Review

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