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Engineering Approaches to Biomanipulation

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Key Words

intracytoplasmic sperm injection (ICSI), optical trap, dielectrophoresis, electrorotation, contact/noncontact laser, zona drilling, magnetic bead, magnetic tweezer, microrobot, PVDF, bilateral control, force feedback, transgenesis

Abstract

This article presents a review on the existing techniques for manipulating biological cells. Because biomanipulation involves a wide range of disciplines, from biology to engineering, we concentrate on some of the key methodologies that would result in an efficient biomanipulation system. Some of the key methodologies discussed in this article for cell manipulation relate to the use of magnetics, microelectromechanical systems (MEMS)-based approaches, optics, electric field, and mechanical techniques. Recent advances in engineering have allowed researchers worldwide to address the problems arising from conventional manipulation techniques. This paper assimilates significance and limitations of biomanipulation techniques described in the literature.

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Contents

INTRODUCTION 36
OPTIC AND ELECTRIC MICROMANIPULATION 38
MAGNETIC MICROMANIPULATION 41
MICROELECTROMECHANICAL SYSTEMS AND MECHANICAL
MICROMANIPULATION 43
DISCUSSION 46

INTRODUCTION

Single cell manipulation is a prevalent process in the field of molecular biology. The process plays an important role in intracytoplasmic sperm injection (ICSI), pronuclei DNA injection, gene therapy and other biomedical areas. ICSI is one of the assisted reproductive techniques to treat male-factor infertility and involves direct injection of a single immobilized spermatozoon into the cytoplasm of a mature oocyte. Injecting DNA into the pronuclei of an embryo produces a transgenic species. In gene therapy, a normal gene is inserted into the genome to replace an abnormal, disease-causing gene. The efforts to micromanipulate cells under the microscope date back to the last half of the nineteenth century (1, 2). Marshall A. Barber developed the principles of microinjection by creating the pipette method to isolate bacterial cells. A detailed history of the microinjection process can be found in Reference 3. Researchers in the field of biology have conducted experiments using conventional microinjection techniques to understand (*a*) that the nucleus plays a role in embryonic differentiation (4), (*b*) that pronuclei formation from nuclei of a species depends on the activation of egg cytoplasm (5), and (*c*) that surgically injecting the egg cytoplasm with a spermatozoa of the same species or different species aids in pronuclei development (6, 7). In vitro fertilization (IVF) is the first assisted reproductive technique to treat male-factor infertility and involves combining an egg cell with sperm cells in a laboratory dish. In this technique, the zona pellucida of the oocyte serves as a major barrier to sperm oocyte interaction. Therefore, in severe oligospermia, the sperm may not come in contact with the oocyte, resulting in low fertilization rates (8). Alternatives to IVF are partial zona dissection (PZD), subzonal insemination (SUZI), and intracytoplasmic sperm injection (ICSI). In PZD, a small hole is made in the zona (outer shell of the egg), to assist the sperm in reaching the egg membrane, whereas in SUZI, the sperm is introduced into the perivitelline space of the oocyte. PZD results in low fertilization rates (less than 27%) in male-factor infertility patients (9, 10), whereas SUZI achieves fertilization rates of approximately 30% and less than 8% poor pregnancy rates (11, 12). However, the results obtained by ICSI are promising. Palermo et al. (13) reported the first human pregnancies achieved by ICSI, with a fertilization rate of 66%. ICSI ensures high fertilization and pregnancy rates compared with IVF, PZD, or SUZI of oocytes (14–17). However, conventional ICSI involves (*a*) the risk of mechanical damage to the oocyte and (*b*) the possibility of injecting foreign substances

or contaminants into the oocyte, thus affecting the fertilization rate and viability of an oocyte.

Transgenic techniques have been in use for 20 years for the creation of knockout mice. These procedures are straightforward but technically challenging, and the transfection rate and survival rate are typically approximately 20% (18). Typical transgenic organisms are created by introducing modified genetic material mechanically, one cell at a time. This method is preferred because it introduces the gene of interest along with the desired regulatory sequences without introducing other potentially confounding elements. The method is tedious and technically challenging even with current mechanical assist devices. Alternatives to this approach for gene delivery include viral vectors, electroporation, and liposomal carriers (19). These techniques all have the benefit of being able to transfect multiple cells, yet their limitations do not make them viable alternatives for the creation of stable transgenic organisms. Viral vectors can carry large DNA or RNA molecules for introduction into the cell, and the gene of interest must be packaged within the basic viral genome as part of the vector creation process. Therefore, if a stable transgenic organism is created, it will have some of the viral genome integrated as well. The use of viral vectors also limits the maximum size of the delivered transgene, thereby limiting the amount of flanking DNA and regulatory elements introduced into the cell. The lack of these regions may reduce nuclear localization, chromosomal integration, and expression (20). In addition, although the infection rate with many viral vectors is very high, a true stable transfection indicating integration into the genome is not the norm. Electroporation is a viable way to introduce genetic material into cells, yet stable transfection is not reliably produced (21). In addition, the procedure is toxic to a percentage of the cells. Finally, introduction through the use of liposomes or other DNA carriers is less toxic but has the lowest infection rate (22). All three modalities share one other limitation, namely, once delivered to the cell, the genetic material may exist in the cytoplasm as an isolated plasmid or it may be endocytosed and remain within an intracytoplasmic vesicle, in either case preventing integration into the genome within the nucleus as a stable transfection. The direct injection method therefore remains the most reliable approach for creation of transgenic organisms. This method can introduce larger amounts of DNA to include regulatory elements as well as other agents, such as restriction enzymes to improve integration. However, current transgenic technology is labor intensive and has relatively low yield. To understand the roles of all the genes identified in the human genome, to overcome the risks in conventional ICSI and other single cell manipulation procedures, we would need to understand biomanipulation techniques that have the potential for the development of a high-throughput system.

Knockout mice: experimental mice created by disrupting or knocking-out the function of a specific gene

Electroporation: a technique for introducing foreign DNA into cells by the application of a high-voltage electric pulse

Early efforts have been made to automate the cell injection process. Capillary pressure microinjection (CPM) is one of the supporting technologies for injecting macromolecules into a single living cell. Injection in nuclei or cytoplasm is performed using an ejection system with pressure levels manipulated by a single button, which requires no learning time and the injection rate obtained can be as high as 70%–80% (23). A semiautomatic microinjection system has also been developed to increase the cell survival rate in CPM (24). The introduction of computer control in manipulating

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biological cells improves the efficiency of the process. A computer-controlled microrobotic system with three degrees of freedom (DOF) was developed for SUZI in mouse (25). The sperm injection was successfully completed without damaging any of the mouse ova. Subsequently, a piezo-driven pipette was used to perform ICSI in mouse (26), which demonstrated 80% survival rate of sperm-injected oocytes. Yanagida et al. (27) used a piezo micromanipulator to perform ICSI in humans and obtained superior results compared with conventional ICSI. Different control strategies have also been used to develop a visually servoed microrobotic system. For example, Sun & Nelson (28) developed an autonomous embryo pronuclei DNA injection system by implementing a hybrid visual servoing control scheme. In the sections below, we provide a comprehensive overview of the state of the art in biomanipulation. We cover a variety of approaches for biomanipulation, namely, optic and electric micromanipulation, magnetic micromanipulation, microelectromechanical systems (MEMS), and mechanical micromanipulation.

Radiometric forces: thermal forces caused by temperature gradients in the medium surrounding an object

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OPTIC AND ELECTRIC MICROMANIPULATION

Optic micromanipulation technique involves manipulating microscopic objects by optical forces. Ashkin (29) was the first to report the acceleration and trapping of micron-sized particles by the forces of radiation pressure from visible laser light. In this setup, the laser beam produces an axial force and a radial force on the particle. The axial force propels the particle along the axis of the beam and the radial force traps the particle on the beam axis.

The optical forces depend on the optical (refractive index and absorption) and geometric properties (shape, composition, and surface charge) of the particle. In particular, the axial force on the particle depends on the focal spot size of the laser beam (30). One of the conditions for optical trapping is that the refractive index of the microparticle (n_1) should be greater than the refractive index of the surrounding medium (n_2), i.e., $n_1 > n_2$. When $n_1 = n_2$, there is no force acting on the particle; when $n_1 < n_2$, the particle is pushed out of the beam, for example, air bubbles in glycerol (29). Thus, a major limitation in biomanipulation is that the cells should have refractive index contrast with the surrounding medium while using optical forces. Absorption can increase the temperature of the particle and generate radiometric forces, which are usually larger in magnitude than radiation pressure. In a typical optical micromanipulation setup, suspending relatively transparent particles in a relatively transparent media eliminates the radiometric forces. Ashkin et al. (31) was also the first to demonstrate the use of optical traps for biomanipulation. An individual bacterium was manipulated and reproduced within the infrared laser trap (32). An amoeba was also maneuvered successfully without any physical damage. Continuous-wave laser beams have harmful effects on single living cells. The effects include changes in membrane permeability and alterations in cloning efficiency (33). The photo damage of a living cell depends on the wavelength as well as the power of the light source. Infrared lasers have a less detrimental effect on cell viability compared with visible laser light because the cell absorption is lower in the infrared region (32). Apart from positioning, the laser beam also transports cells over certain distances with certain velocities

(34). A model was proposed to quantify the axial force generated in a single-beam optical trap as it acts upon microparticles (30). However, the major limitations of this work include the following assumptions: (*a*) absorption of light by the particle is negligible, (*b*) momentum transfer is the same for both the reflected and transmitted beams, (*c*) the laser beam has a Gaussian intensity profile, and (*d*) the diffraction effects are neglected. Experiments were performed to measure the minimum power required to trap a particle and to measure the effective trapping range over which a particle could be caught and held within the trap. Experimental results were found to be in good agreement with model prediction. The application of laser trapping to cell biology was demonstrated by performing experiments involving chromosome movement in mitotic cells and the trapping of spermatozoa. However, the possibility of sperm damage from absorption and subsequent heating after exposure to the laser trap remained a concern. A detailed history and review of optical trapping can be found in Reference 35.

Gaussian beam: a beam whose electric field profile in a plane perpendicular to the beam axis can be described by a Gaussian function

In addition to optical trapping, dielectrophoresis (DEP) and electrorotation are two other noncontact manipulation techniques. DEP involves the manipulation of dielectric particles using nonuniform electric fields. Pohl (36) first investigated the phenomenon in 1951. Electrorotation involves manipulation of electrically polarizable objects by controlling the phase and magnitude of electric fields. A rotating electric field was used to distinguish between live and dead cells (37). Live cells exhibited two to three response peaks, whereas dead cells exhibited only one response peak when subjected to the field over a frequency of 500 to 700 Hz. Electrorotation was used to determine the dielectric parameters of individual cells (38). Dielectrophoretic forces can be analyzed using the finite element method (FEM) (39). DEP and electrorotation generated translational and rotational force, respectively, on living bacteria (40). The characteristics of the bacteria will play an important role in understanding its mechanism. A new technique, called opt-electrostatic micromanipulation, combines dielectrophoretic force and the optical pressure of the laser beam. The technique achieves more flexible micromanipulation of cells (41). *Escherichia coli* and *Schizosaccharomyces pombe* were optically trapped by the laser and subsequently oriented with a high-frequency electric field by controlling the switching frequency of the AC voltage (42). A peak of critical rotation frequency (PCRF) characterized the live and dead cells. The knowledge of PCRF can be utilized for the investigation of the dielectric properties of single cells. Another combination technique (43) involved a laser scanning manipulator for local position control of a target cell and DEP for exclusion of other cells around the target cell, which proved to be an efficient method of selective separation. Manipulation of cells using DEP and electrorotation involves applying an electric field to aqueous solutions. The temperature of the solution and the gas bubble formation (electrolytic process) in the solution increases with an increase in the conductivity of the solution. Therefore, applications of DEP and electrorotation are limited to an aqueous solution of conductivity between 10^{-6} and 10^{-4} S/m. The other alternative involves reducing the applied voltage by proper arrangement of electrodes because bubble generation by electrolysis will occur at approximately 2V potential in water. It has been reported that cell exposure to high-frequency electric fields does not cause any harmful effect to cell viability (44).

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Table 1 The fertilization rate of mouse oocytes increases by zona drilling

Group type	Number of oocytes	Sperm concentration (in sperm cells/ml)	Fertilization rate (in %)	
			Laser drilling	In vitro fertilization (IVF)
Group 1	89	10(5)	67	31
Group 2	94	10(6)	90	54

Chemical zona drilling: creating a hole in the zona pellucida of an oocyte/embryo by using acidic tyrode's solution

Laser light can be used to drill holes in the zona pellucida of an oocyte/embryo. Laser-assisted zona drilling increases the fertilization rate of an oocyte in vitro and the efficiency of embryo biopsy compared with conventional PZD (9, 10) as well as chemical zona drilling (45, 46). The laser light can be used in contact or noncontact mode to create a hole in the zona pellucida. In contact mode, the laser is guided through an optical fiber or glass pipette touching the oocyte/embryo. In noncontact mode, the laser is guided using an optical lens tangential to the oocyte/embryo. The size of the hole depends on the irradiation time. For example, the infrared 1.48 μm diode laser created a hole with a diameter of 5–10 μm in 10–15 ms (47). A larger diameter can be obtained by increasing the irradiation time. However, laser zona drilling may cause genetic defect to cells. Having minimal thermal effect of the laser on the cells and choosing a laser whose wavelength is sufficiently distant from the maximum absorption of DNA can prevent the defect. Palanker et al. (48) first reported laser-assisted zona drilling. A UV laser emitting at 193 nm was used in contact mode to obtain uniform, circular holes in the zona of mouse oocytes. The zona drilling of mouse oocytes increased the fertilization rate (as high as 90%) compared with conventional IVF at low sperm concentrations (49) (**Table 1**). However, one must be careful in selecting UV radiation for zona drilling because of its potential harmful mutagenic effect (50).

Sometimes healthy preembryos are not able to hatch from their protective shell, i.e., zona pellucida after IVF. Erbium laser in contact mode was used to create a 20–30-μm-diameter hole in the zona pellucida of human embryo to improve embryo hatching after embryo transfer (51). The wavelength of the erbium-YAG laser is 2.9 μm, which is sufficiently distant from 268 nm, the absorption maximum of DNA (52). The same laser was used to create 14-μm-diameter holes in the zona pellucida of human oocytes, with a fertilization rate of 30% (53). A laser operating at wavelength 1.48 μm in noncontact mode is preferred over other laser systems in biomanipulation because water molecules absorb strongly at this wavelength and the cleavage of cellular material is mainly due to heat transfer from water. Therefore the laser system has no mechanical, thermal, or mutagenic effects on cells. Researchers reported the use of an infrared 1.48-μm-diode laser to achieve (*a*) a high fertilization rate of mouse oocytes (47), (*b*) an efficient biopsy of human embryos (54) and mouse oocytes (55), and (*c*) an efficient cryopreservation of single human spermatozoa (56). Apart from zona drilling of an oocyte, the other condition to achieve high fertilization in ICSI is immobilization of the spermatozoon prior to injection. Conventional immobilization technique involves mechanical breakage of the spermatozoon tail. Immobilization can also be achieved by treating the spermatozoon with a laser (57).

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The whole immobilization process, i.e., identification, aspiration, and injection of a potential spermatozoon, took less time compared with the conventional technique. The fertilization rate was the same for both techniques. The photodamage of the sperm cell from high optical intensities should be considered during the process. A laser is also used to decrease the thickness of the zona pellucida of embryos for assisted hatching. The process commonly known as laser zona thinning improved the implantation and pregnancy rate of human embryos compared with embryos with intact zona pellucida (58, 59). However, laser zona manipulation should be carefully evaluated before performing any clinical application (60).

Phagocytosis: intake of material into a cell by the formation of a membrane-bound sac

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MAGNETIC MICROMANIPULATION

The manipulation of micron-sized magnetic particles by an external magnetic field is referred as magnetic micromanipulation. The concept was first introduced in the field of molecular biology by Crick et al. (61). Biomanipulation using magnetic energy can be used to study intracellular properties, determine mechanical properties of an individual cell, and separate certain cells labeled with magnetic beads. The magnetic particles are introduced into the cell via phagocytosis, a natural process that does not involve forcible manipulation. Thus, the process does not cause physical damage to the cell. The magnetic particles do not affect the rate of growth of cell cultures. Therefore, introduction of these particles inside a living cell is a viable process. A constant force can be generated on a magnetic bead by two fields: a large uniform homogeneous magnetic field and a constant magnetic field gradient. Magnetic manipulation is preferred over optical manipulation when investigating intracellular properties. Optical forces are exerted on microscopic objects when they have refractive index contrast with the surrounding medium. Optical tweezers cannot always selectively operate in an intracellular environment because of the innumerable objects inside a cell. Moreover, in the field of biomanipulation, photodamage of cells from high optical intensities is a major limitation. Magnetic manipulators used for biomanipulation should have a compact size and be able to mount onto the stage of a microscope without intervening with the functionality of the microscope or video acquisition hardware. Two-pole magnetic tweezers (62) consist of two magnetic coils and were used to determine the intracellular properties of a mouse macrophage consisting of four 1.28-μm spherical superparamagnetic particles. Experimental results showed that the bead inside the macrophage did not relax to its original position upon turning off the magnetic field, and beads at distinct locations within the same cell responded differently to the magnetic force. Thus, the cytoplasm of a macrophage is viscoelastic and inhomogeneous in nature. Three-pole magnetic tweezers (63) achieve more flexibility in manipulating magnetic probes within a cell. The tweezers produce high forces in a controllable fashion. The achieved magnetic flux gradient was 8×10^3 T/m and the beads were moved either along a linear path or a triangular path (63). Beads with diameters of 1 μm and 0.35 μm were manipulated inside a cell and the force-displacement relationship of the beads indicated that the interior of a cell is viscoelastic. The magnetic particles can also be introduced into a living cell via endocytosis into preformed membrane compartments called magnetic

endosome (64). Initially, the endosome was circular in shape. Under the influence of an external magnetic field, the endosome became elliptical in shape. The deformation of the endosome was averaged over a large number of instantaneous shapes. The movement of magnetic endosomes guided by an external magnetic field within an individual cell could offer signatures of specific molecular mechanisms.

Electromagnet: a coil of wire wrapped around a magnetic material such as iron that produces a magnetic field when current flows through the wire

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A single DNA molecule is manipulated magnetically by attaching one end of the molecule to a magnetic bead and immobilizing the other end of the molecule by attaching it to a surface (preferably glass). Permanent magnets are used for manipulating DNA because they are portable and do not require power. The elasticity of a single linear DNA molecule was studied (65) by plotting the stretching force (6 fN to 20 pN) versus extension curves for the molecule. The DNA molecule was coiled in a controllable and reversible fashion by the rotation of small magnets. Magnetic tweezers composed of two sets of coaxial electromagnetic coils applied a constant force on a magnetic bead attached to a single DNA molecule (66). The force-extension curve of a single DNA molecule was obtained by tracking the centroid of the magnetic bead and measuring the applied magnetic force (0.1 pN). Larger forces on the order of 200 pN can be applied to a DNA molecule by a small permanent magnet (67). The force measurement was performed by three glass micropipettes, namely, a loading pipette, a force-measuring pipette, and a catching pipette. One end of the DNA molecule was labeled with a 3-μm-diameter paramagnetic particle and the other end was labeled with a nonmagnetic particle. The pipettes manipulated the DNA in the following manner: The loading pipette injected the DNA into the sample medium, and the catching pipette grabbed the nonparamagnetic particle attached to the DNA and transferred it to the force-measuring pipette (force constant: 137 pN/μm). The system was also able to measure forces as low as 0.2 pN. However, the primary limitation is that permanent magnets cannot control the movement of the bead precisely and magnetic coils have high coil resistance, which generates heat when applying high current. On the other hand, electromagnets offer excellent controllability during operation. Three-dimensional manipulation of a DNA molecule was achieved by six electromagnets and a ring trapper (68). The magnetic bead attached to the DNA molecule was manipulated linearly/angularly by the electromagnets and vertically (out of the plane) by the ring trapper. The measured force-extension relationship for a single DNA molecule was found to be different from the theoretical model. The theoretical model assumed that the molecule is a perfectly homogeneous cylinder rod and there are negligible electrostatic interactions between the molecules. A magnetic force transducer composed of two electromagnets was used to measure forces produced by an individual leukocyte during locomotion both in vivo and in vitro (69). A cell consisting of nickel magnetic particles was positioned between the two electromagnets and the force produced by the cell was measured in terms of the currents through the two electromagnets. Experiments showed that the extension of a lamellipod was always accompanied by an increase in force production. In in vitro experiments, the resolution of the transducer was 100 pN, but for in vivo experiments, the resolution was limited to 1 nN owing to mechanical noise in tissue. Forces in the range of 1.9 to 10.7 nN were generated by the cells. Such results may be helpful in determining the mechanisms driving locomotion in leukocytes and other nonmuscle

cells. An electromagnetic tweezer was used to apply focused and quantifiable mechanical stress to individual cells in culture (70). This technique examined cell mechanics of an individual cell, which can play a major role in quantifying the material properties of the integrin-cytoskeleton linkages. Experiments were performed on wild-type F9 embryonic carcinoma cells and cells from vinculin knock out mouse F9 Vin (−/−). The tensional forces applied to the transmembrane receptors were in the range of 10 pN to greater than 1 nN. Magnetic micromanipulation was also proposed to measure the elasticity of the zona pellucida of oocytes (71). The setup used a force-sensing manipulator to measure forces in the nN range using permanent magnets and diamagnetic material. Thus, magnetic energy is used to explore the mechanical properties of individual cells.

Specific cells carrying magnetic beads can be separated from other cells in a cell culture medium by applying an external magnetic field. MiniMACS magnetic separation method (MB42102, Miltenyi Biotec) isolated and purified mouse primodal germ cells (PGCs) from 10.5–13.5 days post cortium (d.p.c) (72). Cells sequentially stained with an antibody and superparamagnetic particles were separated on high-gradient magnetic columns. With this technique, a maximum of 90% of the PGCs are recovered and the cell viability is never lower than 90%. Yeast cells labeled with magnetic beads were trapped by a microelectromagnet matrix (73). The unlabelled cells were trapped by microposit matrix-generating electric fields. This setup allows the possibility of constructing an efficient microfluidic system for sorting cells. Various other magnetic tools for biomanipulation are the magnetic micromanipulator, the micromotor, and the microtweezer (74). The magnetic micromanipulator was fabricated by winding a 25-μm-diameter copper magnet wire around a 50-μm-diameter soft-ferromagnetic wire. High field gradient is achieved by etching the soft-ferromagnetic wire into a sharp probe. Experiments on 2.8-μm-diameter superparamagnetic beads demonstrated forces of 10 pN and submicron positioning control. The magnetic micromotor was developed by arranging three micromanipulator coils and tips into an equilateral triangle. This arrangement acted as the three-phase stator of the micromotor placed outside the fluid, and the rotor was a cylindrical nickel particle (40 μm long and 1 μm in diameter). The setup demonstrated one full rotation of the motor. The magnetic microtweezer was developed by manipulating magnetic microwires in aqueous media. However, the usage of magnetic wires for biomanipulation involves power consumption and a long period of manipulation can cause local heating, possibly damaging the cells (75).

MICROELECTROMECHANICAL SYSTEMS AND MECHANICAL MICROMANIPULATION

MEMS technology is an important tool to manipulate a single cell or an array of cells. The technology fabricates devices with dimensions in the same order of magnitude as individual cells and allows single cell characterization. MEMS devices used for biomanipulation should be able to operate in an aqueous solution without affecting the viability of cells. An electrochemically activated microrobot transported a 100 μm glass bead over a distance of approximately 200 to 250 μm in an aqueous media (76).

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The robot was fabricated from a conducting polymer, polypyrrole (PPy), in a bilayer configuration with gold. Apart from manipulating glass beads, the microrobot was not tested on cells. An electrothermally activated polymer (SU-8) microgripper was fabricated to manipulate a single He La cell (diameter ~10 μm) in solution (77). The high coefficient of thermal expansion of SU-8 allows in plane activation of the gripper at low voltages (less than 2V) and average temperature changes (less than 32°C). Voltages greater than 2V result in bubble formation owing to electrolysis of water, and high temperature changes may affect the viability of cells. The chemical etching-based process, one of the microfabrication processes, was used to fabricate single cell trapper and sharpened microinjector (78). A model was developed for the etching of cell trapper and the experiments demonstrated successful injection of a fluorescent dye in *Brassica oleracea* (cabbage) protoplasts (diameter ~50 μm).

SU-8: A type of photoresist that becomes relatively insoluble to developer when exposed to light (negative photoresist)

Very large-scale integration (VLSI): the process of placing thousands of electronic components on a single chip

Piezo material: a material that undergoes mechanical strain with the application of an external voltage and vice versa

The limitations of the model are that the etching process is one-dimensional axisymmetric and that the etchant has steady-state concentration distribution. MEMS devices are also used to obtain characteristics of single cells. Thermally actuated cantilever array integrated with microfluidic channels was proposed for individual cell characterization (79). The device consisted of three cantilevers on the flow channel. The middle cantilever was used to immobilize an individual cell and measure its impedance. The other two cantilevers were actuated to open and close the flow of cells. However, the setup was not tested on cells and no investigation was carried out on the viability of cells in a high-temperature environment. A cell clinic was proposed to perform impedance measurements on a single cell (80). The clinic consisted of a microvial that can be closed with a lid activated by two PPy hinges. The microvial was fabricated with an SU8-negative photoresist and has two gold electrodes for impedance measurements. Experiments were performed on *Xenopus leavis* melanophores, but there was no demonstration of automated placement of an individual cell in each microvial. The layout of the cell clinic was improved by integrating each vial with bioamplifiers to form a lab on a chip (81). The cell clinic prototypes were fabricated on top of custom very large-scale integration (VLSI) circuitry designed to record signals from cells within individual vials. Extracellular signals obtained from bovine aortic smooth muscle cells were in the micro- to millivolt range. Hence, the lab on a chip offers the following merits over the conventional cell biology studies carried out in petri dish: (*a*) ease of use, (*b*) low consumption of reagent and samples, (*c*) faster analysis, and (*d*) high reproducibility. Apart from analyzing single cells, MEMS has the advantage of treating an array of cells, thus reducing the time of operation (82, 83). The functionality of MEMS devices depends on the size of the cells to be manipulated, hence a single MEMS device can be operated specifically on cells of certain size.

Mechanical micromanipulation, commonly referred as contact manipulation, is widely used in ICSI, pronuclei DNA injection, gene therapy, and other biomedical areas. The drawbacks associated with conventional cell manipulation techniques, such as low success rate owing to poor reproducibility and human contamination, have motivated researchers to automate the biomanipulation process. Piezo materials are ideal for actuating micromanipulators because they provide high positional accuracy, high control bandwidth, and smart structure design (84–87). A two-fingered

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microhand was developed to manipulate a microscopic object by simulating a chopstick manipulation (88). The microhand is made up of piezoelectric actuators and has a lower module to provide global motion, whereas the upper module provides local relative motion of the two finger tips. Apart from transporting, the microhand can also control the orientation of a micro-object. Experiments on human white blood cells (diameter ~10 μm) demonstrated successful actuation of the microhand. A piezo-driven micropipette was used to perform ICSI in mouse (26). A resolution of 0.5 μm was achieved by piezoelectric actuation. The pipette punctured the cell membrane with minimal distortion of the cell (oocyte). Experiments showed that 80% of sperm-injected oocytes survived, and 70% of them developed into blastocysts using the piezo-driven micropipette. By the conventional method, only 16% of the oocytes survived. Direct nuclear injection using a piezo drill has been shown to be an efficient method for nuclear transfer between horse and cattle oocytes (89).

Calibration of micromanipulators has also been proposed to increase the positional accuracy (90, 91). Apart from significant research carried out in the area of micromanipulators, some micromanipulators are commercially available, for example, the DC3-K motorized micromanipulator (manufactured by Stoelting Inc.) offers a highest resolution of 500 nm, the MP-285 nanomanipulator (manufactured by Sutter Inc.) offers a highest resolution of 40 nm, and the HS6-3 micromanipulator (manufactured by WPI Inc.) offers a highest resolution of 10 nm.

Even though there have been considerable efforts to automate manipulation of biological cells, vision has been the only sensing modality. Recently, there have been efforts aimed at sensing the interaction forces to improve the reliability of biomanipulation tasks (92, 93). Force sensing, in addition to vision, would make the manipulation process repeatable and accurate. A few researchers have proposed the concept of bilateral control, which involves a master-slave setup. The master manipulator (in the macro world) gives position command to the slave manipulator (in the micro world) and the force sensed by the slave manipulator is communicated to the master manipulator, which allows dexterous manipulation of cells. The bilateral control system takes into account the scaling effect in the micro/macro world and maintains a stable, transparent system. A typical schematic of the master-slave teleoperation setup (94) is shown in **Figure 1**. A nanomanipulation system was developed to provide force feedback from biological samples and carbon nanotubes (95). In this setup, the user does

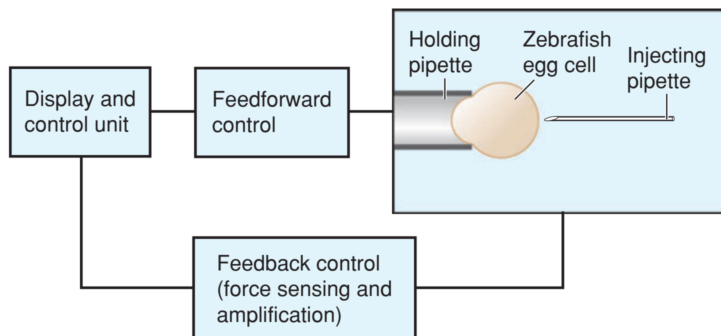

Figure 1
Schematic of master-slave teleoperated cell injection system with force feedback capability.

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not feel the actual forces from the sample, but instead feels a surface representation that is simultaneously reconstructed during the scan.

To measure real manipulating forces, MEMS force sensors have been developed that offer the advantage of miniaturization. A MEMS force transducer was developed by integrating 3-D microstructures and signal processing electronics onto a single chip 2 mm^3 in size (96). The average maximal force measured with the device is 7 ± 2.38 μN. By oscillating the heart cells at frequencies ranging from 10 Hz to 1800 Hz, it was demonstrated that the fully activated cell is approximately 15–20 times stiffer than a relaxed cell. A 2-DOF capacitive force sensor (97) is capable of measuring forces up to 490 μN with a resolution of 0.01 μN in the x direction, and up to 900 μN with a resolution of 0.24 μN in the y direction. The force sensor was used to characterize the mechanical properties of mouse oocytes and embryos (98). A piezoelectric force sensor was used to measure the injection force of zebrafish embryos at various developmental stages (99). The sensor played a critical role in characterizing the mechanical properties of zebrafish embryo chorion (100). Using a piezoelectric force sensor, it was shown that the use of combined vision and force feedback leads to a higher success rate in a cell injection task compared with using vision feedback alone (94, 101). However, the primary limitation is that the outcome of a cell injection task is not related to successful injection itself, but rather the successful integration of the genetic material into the genome within the nucleus as a stable transfection.

DISCUSSION

There are several promising approaches for biomanipulation of single cells. In this review, we have covered some of the most common approaches for single cell manipulation, namely, optic and electric micromanipulation, magnetic micromanipulation, MEMS, and mechanical micromanipulation. Each of the above techniques has their own advantages and disadvantages. Optic and magnetic techniques offer the ability to manipulate single cells without contact; however, high optical intensities and long periods of manipulation using magnetic wires can cause local heating, possibly damaging the cells. Moreover, cells should have a refractive index contrast with the surrounding medium to be manipulated optically. Individual cells are characterized using MEMS devices whose dimensions are in the same order of magnitude as cells. Effective manipulation of individual cells has been developed through the use of a bilateral teleoperation framework whereby individual cells can be grasped in place and injected with a genetic material by the operator through a vision and force feedback interface. Although there are several challenges in micromanipulation, some of the primary challenges are accurate nucleus fixation and genetic material delivery within the nucleus for effective transgenesis and the ability to perform successful high-throughput transgenesis.

SUMMARY POINTS

1. Marshall A. Barber is the inventor of the pipette method. It is widely used for manipulating biological cells.

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2. Large amounts of DNA can be introduced into a cell by the direct injection method, the most reliable approach to create transgenic organisms compared with viral vectors, electroporation, and liposomal carriers.
3. Optical forces are exerted on cells under the strict condition that the cells have refractive index contrast with the surrounding medium.
4. Noncontact lasers are preferred over contact lasers in drilling a hole in the zona pellucida of an oocyte/embryo.
5. Magnetic manipulation is an efficient technique to characterize intracellular properties.
6. MEMS devices have the ability to manipulate cells effectively, as the devices dimensions are on the same order of magnitude as individual cells.
7. Piezoactuating micromanipulators increase the efficiency of micromanipulation tasks, such as ICSI, compared with conventional cell manipulation techniques.
8. The addition of force feedback to an automated micromanipulation system increases the success rate of cell injection tasks.

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101. First article to conduct human factor studies and show that the use of force feedback leads to higher success rates in cell injection tasks compared with using vision feedback alone.

Annual Review of Biomedical Engineering

Volume 9, 2007

Contents

Annu. Rev. Biomed. Eng. 2007.9:35-53. Downloaded from arjournals.annualreviews.org
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Cell Mechanics: Integrating Cell Responses to Mechanical Stimuli
Paul A. Janmey and Christopher A. McCulloch 1

Engineering Approaches to Biomanipulation
Jaydev P. Desai, Anand Pillarisetti, and Ari D. Brooks 35

Forensic Injury Biomechanics
Wilson C. Hayes, Mark S. Erickson, and Erik D. Power 55

Genetic Engineering for Skeletal Regenerative Medicine
Charles A. Gersbach, Jennifer E. Phillips, and Andrés J. García 87

The Structure and Function of the Endothelial Glycocalyx Layer
Sheldon Weinbaum, John M. Tarbell, and Edward R. Damiano 121

Fluid-Structure Interaction Analyses of Stented Abdominal Aortic Aneurysms
C. Kleinstreuer, Z. Li, and M.A. Farber 169

Analysis of Time-Series Gene Expression Data: Methods, Challenges, and Opportunities
I.P. Androulakis, E. Yang, and R.R. Almon 205

Interstitial Flow and Its Effects in Soft Tissues
Melody A. Swartz and Mark E. Fleury 229

Nanotechnology Applications in Cancer
Shuming Nie, Yun Xing, Gloria J. Kim, and Jonathan W. Simons 257

SNP Genotyping: Technologies and Biomedical Applications
Sobin Kim and Ashish Misra 289

Current State of Imaging Protein-Protein Interactions In Vivo with Genetically Encoded Reporters
Victor Villalobos, Snehal Naik, and David Piwnica-Worms 321

Magnetic Resonance–Compatible Robotic and Mechatronics Systems for Image-Guided Interventions and Rehabilitation: A Review Study
Nikolaos V. Tsekos, Azadeh Khanicheh, Eftychios Christoforou, and Constantinos Mavroidis 351

SQUID-Detected Magnetic Resonance Imaging in Microtesla Fields
John Clarke, Michael Hatridge, and Michael Mößle 389

Ultrasound Microbubble Contrast Agents: Fundamentals and Application to Gene and Drug Delivery
Katherine Ferrara, Rachel Pollard, and Mark Borden 415

Acoustic Detection of Coronary Artery Disease
John Semmlow and Ketaki Rahalkar 449

Computational Anthropomorphic Models of the Human Anatomy: The Path to Realistic Monte Carlo Modeling in Radiological Sciences
Habib Zaidi and Xie George Xu 471

Breast CT
Stephen J. Glick 501

Noninvasive Human Brain Stimulation
Timothy Wagner, Antoni Valero-Cabre, and Alvaro Pascual-Leone 527

Design of Health Care Technologies for the Developing World
Robert A. Malkin 567

Indexes

Cumulative Index of Contributing Authors, Volumes 1–9 589

Cumulative Index of Chapter Titles, Volumes 1–9 593

Errata

An online log of corrections to *Annual Review of Biomedical Engineering* chapters (if any, 1977 to the present) may be found at http://bioeng.annualreviews.org/

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