A Review of Single-Cell Pose Adjustment and Puncture

Xiangyu Guo, Youchao Zhang, Daoyuan Jin, and Mingchuan Zhou*

1. Introduction

In 1665, Robert Hooke, a pioneer in discovering the hidden details of nature, first observed cells under a simple light microscope. Since then, human life has started to be recognized at a cellular level. A cell serves as the basic unit of organism structure and function, as well as the most basic living system. Studies on cell structure and function, growth and development, metabolism and reproduction, movement and communication, aging and death, heredity, and evolution have played a critical role in biology. Cell manipulation lays a basis for cell research. In particular, single-cell manipulation technology is one of the challenges in the field of bioengineering, medical engineering, agricultural engineering, chemical engineering, etc. Single-cell manipulation technology refers to cell pose adjustment, cell puncture, cell cutting, cell sorting, and others by driving individual cells in a direct contact or noncontact manner under an inverted high-power microscope. The single-cell manipulation ranges from the nanometer to the submillimeter, which covers nearly all sizes of cells. Therefore, single-cell manipulation has to rely on assisted tools due to the limitation of human hand tremor. As depicted in Figure 1, considerable important achievements have been made in cell biopsy, cellular 3D structure reconstruction, drug delivery, intracytoplasmic sperm injection (ICSI), preimplantation genetic diagnosis (PGD), nuclear transplantation, and assisted reproduction. Cell manipulation at the microscale differs from general particle manipulation. Even though with the assisted tool of micromanipulator and microscope, the cell manipulation is difficult due to cell viability and fragility. First, cells are intrinsic viability and should be kept to be viable in a certain medium. Cells have numerous organelles. These organelles play a significant role in cell growth and development, so they cannot be injured. There are some factors for cell viability (e.g., mechanical stress, transmembrane potential, joule heat, and shear force), which need to be taken care during manipulation. Second, cells are multilayer structures exhibiting different physical and chemical properties compared with particles. This multilayer structure can significantly affect the dielectric constant, compressibility, refractive index, and other parameters. In addition, different types of cells show differences in response to external physical fields. Therefore, cell type, structure, and cell operation conditions are difficulties affecting the effectiveness and efficiency of single-cell manipulation.

Cell pose adjustment and cell puncture are considered as two basic operations of a single cell. Single-cell pose adjustment can be used to achieve accurate 3D position and 3D posture adjustment, which can realize multiple views of cell observation, cell trapping, cell orientation, cell transportation, etc. If cells are damaged during manipulation, it can cause cell death. Thus, noninvasive or microinvasive technology should be investigated. Cell pose adjustment methods can be divided into direct contact and noncontact methods. Mechanical contact is recognized as a typical direct contact method. In 1904, Barber first proposed the principle of microinjection, and he published his technological
innovations to isolate yeast cells from bacteria, which were considered as the origin of mechanical contact methods. Through years of development and improvement, the equipment of mechanical contact has been commercialized with a micromanipulator and glass pipette to suck and push the cell for transportation and rotation, respectively. However, this method will deform the cell which can lead to mechanical damage. The noncontact manipulation method can make up for the shortcomings of the earlier method. Noncontact manipulation primarily consists of two methods. One is to apply external forces on the cell to make it move or rotate (e.g., electric field methods, optical field methods, and magnetic field methods, as presented in Figure 1). Early in 1981, Zimmermann et al. observed the rotation of yeast cells in an alternating current (AC) electric field due to the dielectrophoretic force (DEP) in a nonuniform electric field. When the applied electric field rotated, the cells were rotated. Electric field methods show plentiful advantages, including being label-free and nondestructive and not affecting the cell function under low-intensity alternating electric field. However, electric field methods can cause significant damage and affect cell development under high voltage. Compared with the electric field, the optical field (e.g., optical tweezers) can be flexible to manipulate cells. In 1986, Ashkin et al. three-dimensionally captured dielectric microspheres using a single-beam laser focused with a high numerical aperture. On that basis, the technology of optical tweezers emerged, which can clamp and control cell movement and rotation. However, these tweezers have an upper limit on cell size and only capture particles near the beams. In addition, the high energy of the beam can cause damage to the cell and affect cell viability. A rotational magnetic field is also introduced to rotate the cell. However, on the one hand, the cell orientation is significantly limited by the magnetic field alignment; on the other hand, the cell should be labeled with magnetic particles. The other type of noncontact methods is to make the liquid flow around the cell to generate microvortices that drive the cell to move or rotate (e.g., acoustic field methods and hydrodynamic drive methods). Compared with the optical field, acoustic field methods are more advantageous, which have lower energy and nonnegative effects on cell viability. Furthermore, acoustic field methods do not require magnetic or other special properties of materials.

Different from cell pose adjustment, cell puncture is an invasive cell manipulation. Cell puncture is of high significance for accurately injecting exogenous material into the target location inside cell or extracting specific material inside cell. Existing cell puncture methods can fall into ordinary glass needle puncture methods, piezoelectric-assisted puncture methods, and rotational oscillatory drill (Ros-drill) puncture methods. The cell puncture usually uses an ordinary glass needle made of borosilicate materials through capillary stretching. Early in 1931, Bois developed a device for batch drawing glass needles. The needle tip diameter range is 0.5–5, thus reducing the trauma on the cell during the puncture. In this research, the needle installed on the manipulator was pushed by a mechanical drive to puncture the cell. Compared with ordinary glass needle puncture methods, the piezoelectric-assisted puncture methods are characterized by a higher success rate. In 1995, the method was first applied by Kimura. Nearly 80% of sperm-injected oocytes survived, and 70% of them developed into blastocysts. The earlier result was better than the conventional puncture success rate with 30–40% obtained previously. The piezoelectric-assisted methods directly deliver piezoelectric pulses to the needle end, whereas there is transverse vibration which will increase the trauma on the cell membrane during puncture. To make up for this...
shortcoming, Ros-drill puncture method has been proposed. The method applies a rotational micromotor, thus generating high-frequency and small-amplitude vibrations at the injection needle tip.

Over the past few years, there have been increasing research advances in single-cell manipulation and numerous new methods and equipment have been proposed. In this review, the achievements of single-cell manipulation are systematically summarized from two typical methods, including single-cell pose adjustment and puncture. The rest of this review is organized as follows. In Section 2 and 3, the single-cell pose adjustment and cell puncture methods are summarized respectively, including the basic mechanisms, methods, applications, advantages, and disadvantages. In Section 4, the future trend of single-cell manipulation is discussed. This review is expected to provide a reference for academic research and industrial applications of single-cell manipulation technology.

2. Single-Cell Pose Adjustment Methods

Precise pose adjustment is important for cell sorting, cell surgery, precise delivery of substances into cells, cell characterization, etc. Single-cell pose adjustment refers to the adjustment of cells position and posture in 3D space through direct contact or noncontact methods. Single-cell pose adjustment methods have been transformed from simple manual operation to semiautomatic or automatic operation and from 2D to 3D pose adjustment. It improves the efficiency and the consistency of operation results. Existing cell pose adjustment methods can be classified into mechanical contact, electric field, optical field, magnetic field, acoustic field, and hydrodynamic drive methods.

2.1. Mechanical Contact Methods

In general, mechanical contact methods refer to the direct contact with cells by a microneedle installed in a manipulator, and the single-cell pose is adjusted using the friction between cell and glass pipette to achieve precise cell positioning, as presented in Figure 2a. Based on the visual servo system, the method generally has a success rate of more than 90%, and its rotation accuracy is $0.3^\circ$ significantly higher than the manual operation accuracy of $8.3^\circ$.

The method has been used to breed research in species (e.g., mouse oocytes and zebrafish embryonic cells). There are two typical manipulations during cell rotation. One rotational manipulation is in-plane rotation, where the cells are rotated in the focal plane (XY plane). The other one rotational manipulation is out-of-plane rotation, where the cells are rotated in the vertical plane (ZX plane), (in Figure 2a). Mechanical contact methods can be classified into four types, including single-

Figure 2. Schematic of the different mechanical contact methods. a) i: schematic of system setups. ii: in-plane rotation is that the cells are rotated in the focal plane (XY plane); out-of-plane rotation is that the cells rotate in the vertical plane (ZX plane). b) Manipulation based on single-pipette push. c) Manipulation model based on double-pipette grapple. d) Cell manipulation strategies based on double-pipette grapple. e) expelling, ii: out-of-plane focal plane (XY plane); out-of-plane rotation is that the cells rotate in the vertical plane (ZX plane). f) Device with cell delivery channel and manipulation cell, move the pipette and the cell holding device along the XZ plane. g) V-shaped electrothermal microdriver based on topology optimization and microgripper. Reproduced with permission. h) Zebrafish rotating device. i) V-shaped electrothermal microdriver based on topology optimization and microgripper. Reproduced with permission. i) V-shaped electrothermal microdriver based on topology optimization and microgripper. Reproduced with permission.
pipette push, double-pipette grapple, multi-pipette synergy, and relative motion between micropipette and substrate methods. In the single-pipette push operation method, a single holding needle is used to push the cell motion, as presented in Figure 2b. It enables cell reorientation in only one degree of freedom (DOF) with a rotation angle error of 5°,73] which has limited freedom adjustment. In contrast, the double-pipette grapple operation method can adjust cells’ pose more flexibly. One end of the cell is fixed by the holding pipette, and the other end is pushed by the injection pipette (Figure 2c). To the spindle of a mammalian cell, the cell should be rotated from 3 o’clock to the 6 or 12 o’clock position for injection. Wang et al.[41] manipulated mouse oocytes with the double-pipette grapple method under the visualservo system. The out-of-plane (ZX plane) and in-plane (XY plane) rotations were based on a trial-and-error method (Figure 2d). The success rate of cell rotation was higher than 94%, with an average error of reorientation 3.9°. However, the extrusion deformation of mouse oocytes was relatively significant and easy to cause the shedding of the polar body of mouse oocytes and hinder cell development. Therefore, in accordance with the minimum rotation force model, Dai et al.[76] reduced the cell deformation for the double-pipette grapple, with a maximum deformation of only 2.70 μm, while improving the success rate to 97.6%.

This double-pipette grapple method has a limitation that it should frequently switch the objective lens to find a single cell or a pipette. To solve this limitation, Wang et al.[69] proposed the multipipette synergy manipulation method, in which two pipettes were added to fixed-point transportation and collection oocytes, as presented in Figure 2e. The microscopic view is relatively fixed when manipulating. Compared with the global vision method, the method increases the efficiency of nuclear transfer by 25%. However, due to the inertia of the fluid, the above method cannot ensure that only one cell is spat out at a time. To solve the challenge of stability and efficient cell manipulation, Wang et al.[61] designed a device with a cell delivery channel for the manipulation batch of zebrafish embryo cells, as illustrated in Figure 2f. To be specific, the cell is held between pipette and substrate. When the pipette relatively moves to the matrix, the cell is derived to achieve in-plane and out-of-plane cell rotation. Thus, fully drawing upon the friction between cells, pipette, and substrate, the experimental results suggested that the system had a fast embryo orientation (13 s cell−1) with reposition error as low as 0.5°. Liu et al.[77] and Wang et al.[70] proposed a microfluidic groove-based cell pipeline operation, as illustrated in Figure 1. Cells were placed in an open microfluidic groove in advance, and the cells were operated continuously one by one by moving the stage. Compared with the device designed by Wang et al.[61] the above device is indicated to be simple and easy to process, whereas the cells should be placed into the microfluidic groove beforehand. So, the total time of cell manipulation will be longer. Different from the cells, zebrafish have surface pigments and complex physiological structures. There are challenges for posture recognition and adjustment during micro-injection. To address these challenges, Fan et al.[71,78] designed a rotation stage and a rotation actuator, as shown in Figure 2h. The former is used to adjust the horizontal posture of the zebrafish and the latter is used to rotate the zebrafish. Under the visual feedback system, the success rate of zebrafish body axis recognition exceeded 95% and the survival rate was 88%.

Over the past few years, as 3D printing technology and new materials have been leaping forward, numerous microgrippers with exquisite structures have been manufactured and applied in the single-cell operation. Through topology optimization, Zhang et al.[72,73] designed a five-link flexible microgripper driven by a V-shaped electrothermal microdriver, as presented in Figure 2i. The microgripper made of SU-8 photoresist exhibits biocompatibility and can generate significant displacement under low voltage. It is also capable of grasping, releasing, and moving individual cells. Furthermore, Power et al.[74] used two-photon polymerization technology to fabricate a monolithic

### Table 1. Summary of the development of cell pose adjustment by the mechanical contact methods.

| Authors[a] | Time | Method | Rotation DOF | Speed [s cell] | Success rate [%] | Rotation angle error [°] | Manipulation object | Sizes [μm] | Application |
|------------|------|--------|--------------|----------------|------------------|--------------------------|-------------------|-----------|-------------|
| Ajamiieh et al.[75] | 2018 | Single pipette push | 1 | – | – | 5 | Mouse embryos | 100 | Embryo biopsy |
| Wang et al.[41] | 2019 | Double pipette grapple | 2 | 11.2 | Out of plane 94, in plane 95 | 97.6 | Mouse embryos | 120 | Cell biopsy |
| Dai et al.[76] | 2019 | Double pipette grapple | 2 | 10–15 | – | 0.7 | Mouse embryos | 120 | Cell biopsy |
| Wang et al.[41] | 2016 | Multipipette synergy | 2 | 90 | – | 97 | Mouse embryos | 100 | Nuclear transplantation |
| Wang et al.[61] | 2016 | Relative motion between micropipette and substrate | 2 | 13 | 97.5 | 0.5 | Zebrafish embryos | 1200 | Cell orientation and position |
| Wang et al.[61] | 2015 | Relative motion between micropipette and substrate | 2 | 31 | x-axis 92.5, z-axis 97.5 | 0.3 | Zebrafish embryos | 1200 | Cell orientation and position |
| Wang et al.[69] | 2017 | Relative motion between micropipette and substrate | 2 | 12 | ≥96 | – | Porcine oocytes | 150 | Somatic cell nuclear transfer |
| Liu et al.[77] | 2020 | Relative motion between micropipette and substrate | 2 | 70 | 93.3 | – | Porcine oocytes | 150 | Somatic cell nuclear transfer |
| Fan et al.[71] | 2022 | Relative motion between micropipette and substrate | 2 | – | 95 | – | Zebrafish | – | Toxicology research and drug screening |

[a]DOF: degree of freedom.
force-sensitive 3D microgripper at the tip of an optical fiber (Figure 2i). This microgripper is capable of more precisely controlling the force of holding cells and shows great potential for cell manipulation in the future (Table 1).

2.2. Electric Field Methods

Electric field methods follow the principle of DEP. Particles are polarized in an uneven electric field and interact with the applied electric field to generate a resultant force to move to the area of the strong electric field. The force model is defined as 

\[ F_{DEP} = 2\pi \epsilon_0 \sigma R_c [K(W)] \times \nabla E^2 \]  

(1)

where \( \gamma \) denotes the radius of cell, \( \epsilon_m \) represents the medium permittivity, \( R_c [K(W)] \) is the real part of the Clausius–Mossotti (CM) factor, and \( \nabla E^2 \) is the gradient of electric field squared. CM is calculated as

\[ K(W) = \frac{\epsilon_p^2 - \epsilon_m^2}{\epsilon_p^2 + 2\epsilon_m^2} \]  

(2)

\[ \epsilon_m^* = \epsilon - j \frac{\sigma}{\omega} \]  

(3)

where \( \epsilon_p \) and \( \epsilon_m \) are the complex permittivity of the cell and medium, respectively, \( \sigma \) is the medium conductivity, \( \omega \) is the angular frequency of AC signal, and \( j \) is the imaginary unit. The sign of the real or imaginary part of the CM factor can be changed by selecting different frequencies of the input signal, so the DEP force and the current variable moment are in opposite directions. In a nonuniform electric field, as presented in Figure 3a, DEP forces are classified into two categories, positive-DEP (p-DEP) force and negative-DEP (n-DEP) force. Particles are polarized and interact with the applied electric field. If the applied electric field is more polarized than the medium, p-DEP will pull the particles to higher electric gradients. In contrast, n-DEP repels the particles away from the higher electric gradients. Similar to the DEP phenomenon, electrorotation (ER) draws upon an electric field to change the direction of particles’ circular motion.\(^{106}\) A rotating or AC field is applied to a set of electrodes around the cell to induce the ER to rotate the cell. The rotational torque acting on the cell is defined as

\[ T_{ER} = -4\pi \gamma \epsilon_m^* R_m [K(W)] \times |E|^2 \]  

(4)

The resultant angular velocity \( \Omega(\omega) \) of ER particles is calculated as

\[ \Omega(\omega) = -\frac{\epsilon_m^* R_m [K(W)] \times |E|^2}{2\eta} \]  

(5)

The speed of cells can reach 240 r min\(^{-1}\) in a rotating electric field.\(^{88}\) The cell movement is correlated with several factors (e.g., electrical properties, external electric field, and electrode shape). So cells with different dielectric properties can be manipulated by controlling the dielectric properties or the input voltage conditions.\(^{89–92}\) A wide variety of electrode operating devices from a single nonuniform electric field to multiple physical fields have been reported. In accordance with the form of the electrodes, the electric fields can be classified into planar-electrode electric field, 3D planar- and columnar-electrode electric field, and multipipette electrode electric field.

Planar electrodes have been used in cell rotation operation earlier.\(^{93}\) Planar electrode electric field not only captures and

---

**Figure 3.** Schematic of the different electric field methods. a) Schematic diagram of DEP principle, p-DEP attracts particles to the strong electric field region, while n-DEP repels particles away from the strong electric region. b) Electric field of four planar electrodes\(^{82}\). c) Control strategy of the four planar electrodes’ electric field\(^{79}\) input signal for embryo translation, and input signal for embryo orientation. d) A microfluidic chip based on planar electric field, integrated focusing area, DEP motion area, and ER area\(^{81}\). e) Electric field of polarized cells as electrodes\(^{84}\). f) 3D planar electrode electric field\(^{95}\), the numbers 1–8 represent the electrode number. g) 3D columnar electrode electric field, in-plane rotation, and out-of-plane rotation\(^{85}\). h) 3D model chamber device design arrangements with cylindrical electrodes with a height \( p = 500 \mu m \) and a diameter \( q = 150 \mu m \), three-electrode, four-electrode, six-electrode, and eight-electrode configurations\(^{86}\). i) Schematic of 4D ER near the pipette tip and\(^{87}\) three AC signals with 0°, 120°, and 240° phase differences are applied to the three electrodes, respectively. Reproduced with permission. Copyright 2015, IEEE. Reproduced with permission. Copyright 2020, IEEE. Reproduced with permission. Copyright 2018, John Wiley and Sons. Reproduced with permission. Copyright 2018, John Wiley and Sons. Reproduced with permission. Copyright 2014, Royal Society of Chemistry. Reproduced with permission. Copyright 2015, John Wiley and Sons. Reproduced with permission. Copyright 2018, Wiley.
controls cell rotation by generating a nonuniform electric field through AC voltage. Moreover, the rotation speed and equilibrium position of particles are controlled by adjusting the frequency of applied electric field. The particle is capable of rotating without doing any plane movement in the electric field. \cite{88} Based on DEP and ER electrostatic phenomena, Jiang et al.\cite{82} designed a rotational electric field to control suspended cells (Figure 3b). The electrodes were fabricated using PCB and micro electromechanical system (MEMS) fabrication techniques. When suspended cells were placed in the central region, cells are rotated under an electric rotating field. The electric field is generated by two sets of electrodes, one with a voltage shift of 90° relative to the other. To achieve flexible screening, sorting, and characterizing cells, Huang et al.\cite{79} developed a robot n-DEP tweezer (Figure 3c), that is, an inverted microchip with quadrupole electrodes, capable of easily manipulating mouse embryos on polyvinyl chloride (PVC) petri dish. n-DEP rejected the embryo to the center of the region, thus enabling longer-distance cell delivery. Electrical performance could be acquired according to the motion trajectory and rotational speed of individual cells, which could characterize the conductivity of the cell wall and the dielectric constant of the membrane. However, throughput was recognized as a problem. To increase the measurement throughput of single cell, Huang et al.\cite{83} developed a microfluidic chip based on a planar electric field (Figure 3d). This chip integrated the focusing area, the DEP motion area, and the electrorotation area. Yeast cells could pass through the microfluidic chip, and electrical parameters were calculated using translational and rotational motions. So, electrical properties of single yeast cells could be continuously characterized. Of course, it can also measure the mechanical properties of cells.\cite{94} The cells were polarized in the AC electric field and rotated polarized under the action of DEP. In general, it requires at least three flat electrodes to generate a rotating electric field. Huang et al.\cite{84} developed a novel microfluidic chip. The chip was composed of two flat electrodes, single-cell trap groove, and a polydimethyl siloxane (PDMS) channel (Figure 3e). The polarized cells captured in the groove as a third electrode combined with the two planar electrodes generated a rotating electric field. The electric field could be easily controlled by changing the parameters of the electric signal (e.g., voltage, angular frequency, phase shift). This method can eliminate the complexity of multielectrode manufacturing and simplify the control of electrical signals (Table 2).

Planar electrodes are easy to manufacture, whereas the electric field strength is not uniform. Especially, the electric field strength changes significantly in the vertical direction, which decays rapidly and negatively affects stable cell rotation. If electrode intervals less than 60 μm are prone to short circuits, high voltages may lead to several problems (e.g., hydroelectrolysis and Joule heat).\cite{35,95} To solve the mentioned problems, Huang et al.\cite{35} proposed a 3D electrode method and developed a microfluidic chip, as illustrated in Figure 3f. The rotation direction of the electric field can be achieved by switching the signal phase on the four sidewall electrodes. By changing the electrical signal of the electrode bottom and sidewall electrode phase shift, the electric field can rotate about Z-, X-, and Y-axis. The 3D electrode is capable of increasing the strength of electric field and improving the uniformity of electric field distribution. It also solves the sinking problem due to fluid and cell gravity. The above method can be directly applied to tomography and biophysical parameters for the cell research process. Moreover, Prateek et al.\cite{85} also attempted to design an open sky DEP chip (Figure 3g). The in-plane rotating electric field was generated by activating only the sidewall electrode, and the out-of-plane rotating electric field was established by activating two opposite sidewall electrodes and the two bottom electrodes. The maximum rotational speed could reach 140° s⁻¹. In this electric

**Table 2. Summary of the development of cell pose adjustment by the electric field method.**

| Authors         | Time  | Method       | Electrode type            | Rotation DOF | Speed [°/s] | Frequency [KHZ] | Manipulation object          | Sizes [μm] | Application                        |
|-----------------|-------|--------------|---------------------------|--------------|-------------|-----------------|-------------------------------|------------|-----------------------------------|
| Jiang et al.\cite{92} | 2015 | DEP, ER      | Planar circular electrode | 1            | 59.7        | 400             | Yeast cells                   | 6          | Cell surgery                      |
| Walid et al.\cite{98} | 2016 | Constant electric field | Interdigitated electrode | 1            | ≤240        | ≤1              | Barium titanate particles     | 25         | Particle rotation                 |
| Huang et al.\cite{79} | 2020 | Rotational electric field | Four flat circular electrodes | 3            | ≤1.17       | 0.5             | mouse embryos                | 100        | Assisted reproduction             |
| Huang et al.\cite{93} | 2021 | DEP, ER      | Eight planar electrodes   | 1            | In-plane 306, Out-of-plane 373 | 500, 1000 | Yeast cells                  | 6          | Cell rotation                     |
| Huang et al.\cite{94} | 2019 | Rotational electric field | Two parallel planar electrodes | 1            | ≤120        | 1000            | HeLa and HepaRG               | 8–20       | Cell rotation                     |
| Huang et al.\cite{95} | 2019 | Rotational electric field | Four sidewall electrodes | 3            | In-plane 30, Out-of-plane 60 | ≤790 | HeLa cell                     | 8–20       | Chromatography imaging, biophysical parameter acquisition |
| Huang et al.\cite{95} | 2018 | Rotational electric field | Four C-PDMS sidewall electrodes and one ITO electrode | 3            | 200–1200    | 100.100         | HeLa, C3H10 Blymphocytes and HepaRG | 8–14       | Cell imaging, cellular biological properties measurement |
| Prateek et al.\cite{96} | 2014 | Rotational electric field | Multiple columns electrodes | 2            | In-plane 17, Out-of-plane 140 | 10,10000 | Bovine oocyte and HepaRG     | 120         | Cell rotation                     |
| Chow et al.\cite{97}  | 2018 | DEP, ER      | Liquid metal electrodes | 1            | –           | 400–10 000      | HeLa and PANC-1 cells         | 20         | Cell rotation and transport       |
field, the cell should be ensured to enter the center of the chamber due to the limitation of electric field space. The earlier problem could be solved by the cylindrical electrode. Benhal et al.\[86\] analyzed the electric field characteristics of three-column electrodes, four-column electrodes, six-column electrodes, and eight-column electrodes arranged in a circular region using finite-element techniques (Figure 3b). They suggested increasing the number of electrodes within a fixed circular boundary. A larger region of constant rotating electric field will be created. So an individual cell could be induced to rotate simply by being introduced to a location in the chamber. Solid metals could be made into electrodes certainly, whereas some liquid metals exhibited high electrical conductivity and could also serve as electrodes. Chow et al.\[87\] proposed a liquid metal-based multifunctional pipette for 4D single-cell manipulation (Figure 3i), which filled the liquid metal gallium into the microtube as a microelectrode. They employed multiphase electrical signals to generate 3D dielectric electrophoresis traps and 1D electrical rotation to achieve cell posture adjustment, with the cell vitality of over 90%. The above method requires neither clean rooms and lithography steps to create 3D microelectrodes and microchannels, nor a sealed chip.

2.3. Optical Field Methods

Optical field methods suggest that a highly focused laser beam is capable of forming optical trapping that applies trapping force and torque to particles of interest. It ranges in size from dozens of nanometers to dozens of micrometers. Cells are subjected to two forces in the light field, the gradient force and the scattering force.\[96-98\] as presented in Figure 4a). The gradient force is defined as $F_{\text{grad}}$ and the scattering force is defined as $F_{\text{scatt}}$.\[99,100\] Moreover, $F_{\text{scatt}}$ denotes the force formed by the incident light on the particle when it is transmitted and reflected on the particle surface, following the direction of light propagation. $F_{\text{scatt}}$ is calculated as

$$F_{\text{scatt}} = \frac{I_0 \sigma n_m}{c} \left( \frac{m^2 - 1}{m^2 + 2} \right)^2$$  (6)

where $I_0$ is the light intensity, $n_m$ is the refractive index of liquid medium, $c$ is the speed in vacuum, $m = \frac{n}{n_m}$ is the ratio of particle refractive index to medium refractive index, $a$ is particle radius, and $\lambda$ is optical wavelength. $F_{\text{grad}}$ is derived from the intensity of the optical field and is directed toward the center of the beam. $F_{\text{grad}}$ is calculated as

$$F_{\text{grad}} = \frac{2\pi a^2}{c} \left( \frac{m^2 - 1}{m^2 + 2} \right)^2 \nabla I_0$$  (7)

The net force of the particle in optical field is defined as $F_{\text{trap}}$. $F_{\text{trap}}$ is calculated as

Figure 4. Schematic of the different optical field methods. a) Schematic of the optical field principle. i: cells are subjected to two forces in the light field, $F_{\text{grad}}$ and $F_{\text{scatt}}$; ii: a dielectric sphere larger than the wavelength of light either reflects or refracts light (pink arrows) focused by a lens.\[101\] The change in direction of each ray corresponds to a change in momentum of the light and an equal and opposite change in bead momentum. Reflected rays of light lose forward momentum that is gained by the bead, resulting in a net force (Freflection, red arrow) and pushing the bead to the direction of light propagation. Refracted rays are deflected forward because of the high incidence angle of the light, thus generating momentum change and reactive force (Frefraction, green arrow) that pulls the bead toward the focus.\[101\] b) Working principle of double-optical fibers for multimodal manipulation of single cell.\[102\] i: optical trap, ii: optical stretch, iii: optical translation, iv: optical revolution, v: in-plane spin rotation, vi: out-of-plane spin rotation. c) Optical robot with three spherical handles. Group 1 refers to a 3D reversal around the Z-axis; group 2 represents 3D control of a four microbead square, and the “Radial” operating mode of the gripper; group 3 is the travel of one microbead around all the others locked in rotation.\[103\] d) Two microrobots that are driven to push and rotate a filamentous cell.\[104\] e) Gripper formation method.\[105\] f) Indirect cell pushing method.\[106,107\] g) Schematic of the microgripper using thermo-responsive gel actuators.\[108\] h) The particle is driven by optical tweezer to produce microvortices, the intermediate particle represents cell.\[109\] Reproduced (Adapted) with permission.\[101\] Copyright 2021, Springer Nature. Reproduced with permission.\[102\] Copyright 2021, AIP. Reproduced with permission.\[103\] Copyright 2019, IEEE. Reproduced with permission.\[104\] Copyright 2019, Optical Society of America. Reproduced with permission.\[105\] Copyright 2014, IEEE. Reproduced (Adapted) with permission.\[106\] Copyright 2013, IEEE. Reproduced (Adapted) with permission.\[107\] Copyright 2014, SAGE Publications. Reproduced with permission.\[108\] Copyright 2022, MDPI. Reproduced with permission.\[109\] Copyright 2020, John Wiley and Sons.
A dielectric sphere larger than the wavelength of light either reflects or refracts light (pink arrows) focused by a lens. The change in direction of each ray indicates a change in momentum of the light and an equal and opposite change in bead momentum. Reflected rays of light lose forward momentum gained by the bead, thus resulting in a net force (Freflection, red arrow) that pushes the bead along the direction of light propagation. Refracted rays are deflected forward because of the high incidence angle of the light, leading to the generation of momentum change and reactive force (Frefraction, green arrow) that pulls the bead to the focus. The stiffness and magnitude of the optical trapping forces can be adjusted by the intensity of the laser beams, and the trapping forces change in a range from femto-Newton to nano-Newton, thus making them well suited to capture micrometer-sized particles with different sizes and weights. The optical beam operates on the cell like a pair of tweezers, so they are termed optical tweezers. Optical tweezers are classified as single-beam optical tweezers, holographic optical tweezers, optic-fiber optical tweezers, planar waveguide optical tweezers, photonic crystal optical tweezers, and plasma optical tweezers. Single-beam optical tweezers can only capture and manipulate one particle at a time. While researchers generally seek to control multiple particles simultaneously and use multibeam coupling to generate multiple optical traps. However, this method has a lack of flexibility, and the system is complex. Holographic optical tweezers are capable of splitting a single beam into multiple independent beams to capture and manipulate multiple particles. In contrast, optical fiber optic tweezers can improve the integration of conventional optical tweezer system. The manipulation methods of optical tweezers can be classified into direct contact and indirect contact methods. Direct contact methods are single cell directly exposed to the light beam. Xie et al. developed several direct cell manipulation algorithms to improve the robustness of the operating system and the effectiveness of the directional control. They also achieved 3D rotation of leukocytes using dual beams generated by holographic optical tweezers. Huang et al. proposed a dual-fiber microfluidic chip for multimodal manipulation of single cell (Figure 4b), and dual-fiber relative position in space was configured to generate different misalignment. If there is a certain offset between the two optical fibers, multimodal manipulation of single cell can be achieved, including spin rotation or orbital revolution with in-plane or out-of-plane rotation. Therefore, the approach provides flexibility and capability to observe the cells from different angles. On that basis, Hu et al. studied the trajectory of particles in a four-beam fiber. Compared with two-beam optical tweezers, four-beam optical tweezers are capable of generating two or more vortices with more controllable variables, thus contributing to the measurement of unknown parameters (e.g., torque, viscosity, and angular momentum). It can be employed to explore the biophysical properties of various cells. The direct optical trapping cell method is simple and fast to operate. However, the direct exposure of cells to intense optical beams leads to a 67% cellular photodamage rate. Accordingly, numerous authors have proposed different ways to reduce the photodamage, including lower laser power or the application of gel particles. Lower laser power leads to a weaker trapping force. Cells can be wrapped in thermosensitive hydrogels. Hydrogels can be converted from sol to gel or gel to sol through local heating or cooling of microheaters, which are mostly suitable for cell fixation.

At present, indirect control methods are generally adopted, which can be classified as microrobot method, gripper formation method, and indirect push method. Gerena et al. designed a microrobot with three spherical handles to achieve 3D rotation and position movement of cells under the constraint of three optical beams (Figure 4c). In addition, Hu et al. proposed a novel idea, in which two collaborative optical traps are adopted to automatically control the micromachine (Figure 4d) to translate and rotate the filamentous cells, and the moving speed can reach 2 μm s⁻¹. Compared with the microrobot with three spherical handles, the above simplified structure can reduce the space occupation of the microrobot under the microscope field of view. It is convenient for cooperative control of multiple microrobots.

To achieve collision-free transport of yeast cells, Chowdhury et al. used holographic optical tweezers in a feedback closed-loop system with four-clamp structures, including 2, 3, 4, and 6 silica particles (Figure 4e), thus reducing light damage by 90%. Based on the gripper formation, Chowdhury et al. and Thakur et al. placed an intermediate bead (Figure 4f) between the target cell and the optical manipulation bead to solve the problem of the remaining 10% light damage. Impacted by fluid viscosity, laser power, and other factors, the mentioned strategy is still challenging to achieve the position and direction control of 3D elements. Soft materials can also be used for single-cell manipulation. Kodera et al. proposed a microgripper actuated by soft thermoresponsive hydrogels (Figure 4g). The gripper is driven by soft actuators using local heating from laser irradiation to manipulate live cells, which does not require complex control system. It can hold live cells of different stiffness and size. Micromotors hold exciting prospects in biomedical applications, whereas there have been rate few reports on micromotors with high safety, exible controllability, and full bio compatibility. Cellular micromotors based on optical tweezers have been proposed to generate dynamic scanning optical traps following a given circular trajectory, which are capable of capturing and driving microparticles or individual cell, as presented in Figure 4h. Impacted by shear stress or torque, the cells rotate controllably in the microvortex, and the rotation rate and direction can be adjusted by changing the scanning frequency and direction of the dynamic optical trap. The above method shows promising applications in targeted drug delivery, biological microenvironment monitoring and sensing, and biomedical therapy (Table 3).

### 2.4. Magnetic Field Methods

Magnetic field methods are adopted to drive tiny objects to move by magnets or external magnetic fields. Changes of the magnitude and direction of the magnetic field allow flexible control of the particle attitude(Figure 5a). The magnetic force acts at a long distance and can be applied without direct contact with the cells, thus significantly reducing the potential danger that could reduce cell viability. As depicted in Figure 5b, two typical magnetic phenomena exist in microfluidic systems, positive
magnetophoresis, and negative magnetophoresis. When a magnetic field is applied, the diamagnetic cell, labeled with magnetic beads, is moved to the position of maximum magnetic field intensity. The magnetic manipulation technique is termed positive magnetophoresis. As opposed to that mentioned, when the magnetization intensity of the cell is less than its surrounding medium, the cell is moved to the position with the minimum value of magnetic field intensity. The magnetic manipulation technique is termed positive magnetophoresis. The label-free negative magnetophoresis is capable of reducing the time and cost of cell labeling. Superparamagnetic salt solutions and ferromagnetic fluids are commonly used medium, which has high biocompatibility. The above supermagnetic particles themselves have no magnetism. When an external magnetic field is applied to obtain magnetism, the magnetic force $F_{mag}$ is calculated as follows:  

$$ F_{mag} = \frac{\Delta \chi V_p}{\mu_0 |\nabla B|} $$  

Table 3. Summary of the development of cell pose adjustment by the optical field methods.

| Authors       | Time   | Method          | Rotation DOF | Speed          | Number of beams | Damage [%] | Manipulation object | Sizes [µm] | Application               |
|---------------|--------|-----------------|--------------|----------------|-----------------|------------|---------------------|------------|--------------------------|
| Xie et al.    | 2018   | Direct rotation | 3            | --             | 2               | 67         | Leukemia cells      | 20         | Cell surgery             |
| Xie et al.    | 2018   | Direct rotation | 2            | 8.59 s⁻¹       | 2               | 67         | Yeast cells         | 6          | Single cell surgery      |
| Gerena et al. | 2019   | Micro robot     | 3            | Max 1100 µs⁻¹  | 3               | 33         | Red blood cell      | 6–9        | Cell manipulation        |
| Hu et al.     | 2021   | Micro robot     | 2            | Movement speed 2 µm s⁻¹ | 1             | 33         | Anabaena sp         | 20         | Cellular transport and rotation |
| Chowdhury et al. | 2014   | Gripper formation | 1          | 7–10 µm s⁻¹    | 2–6             | 33         | Yeast cells         | 5–8        | Cellular transport and rotation |
| Chowdhury et al. | 2013 | Indirect push | 1            | --             | 3               | 0          | Dictyostelium discoideum cell | --         | Cellular transport and rotation |
| Thakur et al. | 2014   | Indirect push   | 1            | --             | 2               | 0          | Yeast cells         | 4–7        | Cellular transport and rotation |
| Kodera et al. | 2022   | Microgripper    | --           | --             | 1               | --         | Madin–Darby canine kidney cells | 14.7      | Cell manipulation |
| Zou et al.    | 2020   | Micro swirls    | 1            | --             | 1               | --         | --                  | --         | Cell manipulation |

Figure 5. Schematic of the different magnetic field methods. a) Schematic of a rotating cell placed inside a magnetic coil: two identical sinusoidal signals, phase shifted by 90°, pass through two pairs of coils, applying a magnetic field that does not coinciding with the magnetic moment of the cell, thus generating a torque that drives the cell to rotate. b) Positive magnetophoresis and negative magnetophoresis. c) Acoustic levitation to levitate magnetic microrobots, transportation, and rotation. d) Two mode-chopstick-style two-finger microhand Contact mode: grasping, active release; noncontact mode: trapping/transportation, rotation. e) Annelid-worm-like microswimmer: Z-shaped magnetic tweezer system that includes four magnetic coils in the X–Y plane. f) Multifunctional superparamagnetic/catalytic microrobot (PM/Pt microrobot). g) The microfluidic platform was designed with “T” and “I” shapes. h) Double-tailed flexible microrobots: i) spherical magnetic particles; ii) swinging flexible nanomotor; iii) the cork-screw microswimmers; iv) nanowires. i) Microfluidic device based on magnetic tweezer: 3D magnetic tweezer system that includes four magnetic coils in the X–Y plane and a vertical coil along the Z-direction. The magnetic beads approaching the cells in two modes of motion are shown in the central platform. j) Schematic diagram of the movement of the rotating mode and the magnetic gradient translation mode at constant magnetic flux density. k) Cell deformation of magnetic beads under the action of squeezing pressure. Reproduced with permission. Copyright 2016, SAGE Publications. Reproduced with permission. Copyright 2021, IEEE. Reproduced with permission. Copyright 2018, John Wiley and Sons. Reproduced with permission. Copyright 2018, John Wiley and Sons. Reproduced with permission. Copyright 2021, American Chemical Society. Reproduced with permission. Copyright 2012, American Chemical Society. Reproduced with permission. Copyright 2021, IEEE. Reproduced with permission. Copyright 2021, American Chemical Society.
where $\Delta x$ is the difference between particle and fluid magnetic permeability, $V_p$ denotes the particle volume, $\mu_0$ represents the magnetic permeability of vacuum, and $B$ expresses the magnetic field intensity. If the inertial force and other forces of particles are not considered, the magnetic force and the drag force $F_D$ are balanced. The resultant force is calculated as follows

$$F_{\text{mag}} + F_D = 0 \quad (10)$$

the magnetophoresis velocity of particles is calculated as follows

$$v_{\text{mag}} = \frac{F_{\text{mag}}}{F_D} = \frac{2a^2 \Delta x B V B}{9\eta a} \quad (11)$$

where $\eta$ is the dynamic viscosity of fluid and $a$ denotes the particle radius. It is known that the speed of cell movement is correlated with the size of magnetic particles and the strength of magnetic field. Magnetic field methods can primarily classified into direct contact and indirect contact methods.

In magnetic field contact methods, a magnetic micromanipulator is used to manipulate cells directly. These methods are similar to mechanical contact methods, whereas the difference is the driving field. Feng et al.\cite{119} adopted acoustic levitation to levitate magnetic microrobots on a glass substrate, which drove a pair of microrobots with permanent magnets, as illustrated in Figure 5c. The microrobots hold the cells and generate relative movement for enabling the transportation and 3D rotation of bovine oocytes. The average rotation speed was obtained as $3 \text{rad s}^{-1}$, the positioning accuracy was less than $1 \mu m$, and a force of $65 \mu N$ could be exerted. However, the height of cell suspension might reduce the operational effectiveness of this method. Thus, Liu et al.\cite{120} proposed a multifunctional magnetic manipulation system, consisting of two mode-chopstick-style two-finger microhand (Figure 5d). In this system, the microtubes containing ferromagnetic beads are driven by a magnetic field to directly hold cells, and the vibration of the micropipette tip can be exploited to release the micro-objects adhering to the micropipette tip. Furthermore, high-frequency circular vibration of micropipettes induces rotational flow to achieve noncontact cell manipulation. The method is expected to serve as an universal tool.

There are three methods of indirect contact by the magnetic fields. One is to achieve rotation by attaching magnetic beads to the cell.\cite{119,120,124} After being labeled with a magnetic substance, the cells can be manipulated by changing the direction of the magnetic field. Although the labeled magnets are made of biocompatible materials, the above materials are nearly nondegradable,\cite{130,131} and the potential effect remains unknown. Thus, the indirect push cell method has been proposed. Liu et al.\cite{121} proposed an annelid-worm-like microswimmer (Figure 5e). These bioinspired microswimmers were prepared by sputtering a Ni/Fe alloy via a shadow mask on a prestrained superelastic PDMS substrate. The microswimmer could be propelled efficiently in an oscillating magnetic field, which had a maximum speed up to $100 \mu m s^{-1}$. The speed and direction of the microswimmer can be efficiently controlled by regulating the magnetic field input parameters. The method can be adopted to transport single cell to a specified location. Over the past few years, the self-propulsion of micromachines acquiring energy from the surrounding environment and then turning it into motion have been at the forefront of the research of smart materials. Villa et al.\cite{8} designed a multifunctional superparamagnetic/catalytic microrobot (PM/Pt microrobot) (Figure 5f). The microrobot was made of superparamagnetic polymer particles, which contained iron oxide on the inside and a catalytic platinum (Pt) layer covering the external toluene-sulfonated surface. The Pt layer catalyzed the decomposition of hydrogen peroxide, which propelled the microrobot. Moreover, the magnetic part was allowed to operate by a magnetic field. Impacted by the magnetic field, they formed chain-like spherical structures, thus enabling large cancer cells of 10–50 $\mu m$ to be transported effectively. The precise manipulation of individual cells is essential for single-cell analysis. In order to solve the problem of attraction tendency and cluster formation between particles, Nassab et al.\cite{122} presented a microfluidic platform designed with “T”- and “I”-shaped magnetic traces as shown in Figure 5g. This approach has three advantages. First, all particle motions are synchronized with the external rotating field to

Table 4. Summary of the development of cell pose adjustment by the magnetic field methods.

| Authors | Time | Method | Rotation DOF | Manipulation object | Sizes [\mu m] | Application |
|---------|------|--------|--------------|---------------------|--------------|-------------|
| Sakar et al.\cite{113} | 2010 | External magnetic field drive | – | T. pyriformis cell | 25 x 50 | Cell separation |
| Feng et al.\cite{119} | 2016 | Acoustic levitation technology and permanent magnet movement | 3 | Bovine oocyte cell | 120 | Single-cell characteristic |
| Liu et al.\cite{120} | 2021 | Magnetic fields drive ferromagnetic beads microstraws containing | 1 | Mouse egg cell | 100 | Cell transportation |
| Blümler et al.\cite{128} | 2021 | permanent magnet rotation | 2 | HUVEC NHDF | 15 | Cell transportation |
| Villa et al.\cite{8} | 2018 | PM/Pt microrobot | – | Breast cancer cell | 10-50 | Drug delivery |
| Zhu et al.\cite{114} | 2018 | Nonuniform magnetic field drives magnetic nanorods | – | PC3 cells, A549 cell | – | Single-cell manipulation |
| Petit et al.\cite{123} | 2011 | Magnetic microdrivers rotate and move microvirobots | 1 | Polystyrene microspheres | 6 | Cell transportation |
| Nassab et al.\cite{122} | 2021 | Magnetized cells in a triaxial time-varying magnetic field | – | Acute monocytic leukemia cell | – | Cell transportation |
| Ji et al.\cite{125} | 2021 | Oscillating magnetic field | 1 | Neutrophils | 5 | Cell transportation |
| Tang et al.\cite{127} | 2021 | Oscillating magnetic field | 1 | Hela cells and C2C12 cells | – | Cell characteristic |

\( ^a \)HUVECs: human umbilical vein endothelial cells. NHDF: normal human dermal fibroblasts.
achieve precise control over individual particles. Second, single-particle and single-cell transport in a controlled fashion are achieved for many of them in parallel, without the need for complex control systems to send signals to individual particles. In addition to moving the particles along a straight track, it also demonstrated the use of a 60° bend to transport them.

The other method is the vortex induced by rotating magnetic field for cell rotation, which can be manipulated by the vortex induced by magnetic actuator. For instance, fluid capture of micro-objects is combined with flagellar swimming motion of soft microrobots. Single-tailed or double-tailed flexible microrobots (Figure 5h)\cite{123,132} achieved low-Reynolds-number swimming by drawing upon the flexibility of flagellar propulsion and magnetic steering via the dipole moment of iron oxide nanoparticles. Robotic sperms could swim at an average speed of 125 μm s^{-1} and could change this speed by adjusting the frequency of the external magnetic field. Furthermore, there are also spherical magnetic particles\cite{124} swinging flexible nanomotors,\cite{125} the cork-screw microswimmers\cite{28} and nanowires\cite{124,126} generating moving microvortices to capture and manipulate cells in the rotating magnetic field, as presented in Figure 5i. In addition, magnetic tweezers can also be used for

![Sound source](image)

**Figure 6.** Schematic of the different acoustic field methods. a) Schematic of acoustic field working mechanism. b) Schematic of acoustic levitation device.\cite{138} i–iii) The particles can be translated along 3D paths at up to 25 cm s^{-1} using different arrangements and without moving the array. iii–v) The traps are sufficiently strong enough to hold the spheres and counteract gravity from any direction. vi) Asymmetric objects, such as ellipsoidal particles, can be controllably rotated at up to 128 r min^{-1}. c) Schematic diagram of acoustic field synthesis device.\cite{139} d) Schematic of 3D acoustic tweezers. Configuration of the planar surface acoustic wave (SAW) generators, used to generate volumetric nodes, surrounding the microfluidic experimental area. The inset indicates a single particle within a 3D trapping node, which is independently manipulated along the x-, y-, or z-axis.\cite{140} e) Perspective schematic of the integrated acoustofluidic device.\cite{141} f) Design and working concept of the SAW-based C. elegans rotation device.\cite{142} g) Microstructure-based rotational flow, symmetrical,\cite{143} and asymmetrical\cite{144} microstructures. h) Schematic of an ultrasonic microfluidic device with an array of microstructures embedded in the microfluidic cavity,\cite{145} which vibrates in circular and linear vibration modes to generate two different types of ultrasonic microfluidic flow patterns. i) Acoustically driven microbubbles.\cite{146} j) Experimental setup for noncontact manipulation using an acoustically driven microbubble, ii: the rotation of side view in the xz plane, iii: the rotation of side view in the xy-plane. j) Versatile bubble-based acoustofluidic device.\cite{147} j–iv) Presentation of multiple acoustic streaming patterns induced from the oscillation of a bottom microbubble for (i) transportation, (ii) in-plane rotation, (iii) out-of-plane rotation, and (iv) circular revolution manipulation. (v) Sketch of the experimental setup. (vi) Optical images visualizing clockwise revolving manipulation around an oscillating bubble of multiple 20 μm particles, a single 50 μm particle, and a single E. gracilis cell. k) i: Schematic of the microswimmers.\cite{148} ii: Schematic of the acoustofluidic chamber. The microswimmers with and without the Au layer sink to the bottom or float to the top of the chamber, respectively. iii: Illustration of the pushing mode and the pulling mode. i) and ii are the structure diagrams of the miniature swimmer.\cite{149} i)–ii: Two modes of motion for the miniature swimmer. Reproduced with permission.\cite{138} Copyright 2015, Springer Nature. Reproduced with permission.\cite{139} Copyright 2018, MDPI. Reproduced with permission.\cite{140} Copyright 2016, PANS. Reproduced with permission.\cite{141} Copyright 2021, IEEE. Reproduced with permission.\cite{142} Copyright 2019, Royal Society of Chemistry. Reproduced with permission.\cite{143} Copyright 2016, Wiley-VCH. Reproduced with permission.\cite{144} Copyright 2019, IEEE. Reproduced with permission.\cite{145} Copyright 2020, Royal Society of Chemistry. Reproduced with permission.\cite{146} Copyright 2021, AIP. Reproduced with permission.\cite{147} Copyright 2021, Royal Society of Chemistry. Reproduced with permission.\cite{148} Copyright 2019, AAAS. Reproduced with permission.\cite{149} Copyright 2021, Royal Society of Chemistry.
the characterization of single cells. Tang et al.\cite{127} integrated magnetic tweezers in a microfluidic chip, as shown in Figure 5f. The cells were squeezed by magnetic tweezers controlled by magnetic particles. The mechanical properties of the cells were evaluated based on the mechanical model and the deformation of the cells. This research provides a reference for efficient measurement and characterization of cell biomechanical features in future (Table 4).

2.5. Acoustic Field Methods

Acoustic waves, also known as acoustic tweezers, are a type of mechanical wave with energy. Acoustic waves use two opposite sound sources to generate acoustic waves to capture and push the cell, as illustrated in Figure 6a. In addition, acoustic field can also be used in vivo.\cite{115} When there is an acoustic field in the fluid, the average pressure of the particles is not zero. The particles are capable of moving directionally since the particles are subjected to acoustic radiation force.\cite{136} When the trough is reached, the cells stop moving. The acoustic radiation force $F_{\text{rad}}$ is defined as\cite{94,117}

$$F_{\text{rad}} = -\varphi \left( \frac{\pi P_0 V_p \beta_l}{2 \lambda} \right) \sin(2kx)$$

(12)

$$\varphi = \frac{5p_f - 2p_l}{2p_f + p_l} \frac{\beta_p}{\beta_l}$$

(13)

where $P_0$ denotes the acoustic pressure amplitude, $V_p$ represents the volume of the particle, $\lambda$ expresses the acoustic wavelength, $V_p$ denotes the wave numbers, $\rho$ and $\beta$ are compressibility of the particle and compressibility of the medium, respectively. Subscripts $f$ and $p$ represent fluid and particle, respectively. $\varphi$ is contrast factor, which is determined by the properties of the particle and medium. The wavelength of acoustic wave can be changed to regulate the moving distance of particles.\cite{124,126} Moreover, frequency, driving voltage, and distance from the tip of the microstructure to the center of the oocyte will have an effect on acoustic wave performance. Acoustic waves are capable of manipulating objects of sizes ranging from submicrometer to microscale.\cite{118,122,150} According to the operating principle, acoustic waves can be divided into bulk acoustic waves (BAWs), SAWs (e.g., traveling and standing waves), and acoustic flow tweezers (AFTs) (Figure 6a). Among these, the first two groups are direct manipulation strategies induced by external acoustic radiation forces, while the last group is an indirect manipulation strategy with fluid flow.

BAWs exploit high-frequency vibrations to induce ultrasonic vibration of solid structures, while generating stable counter-rotating microvortex to rotate cells, microorganisms, and others around the surrounding fluid. BAWs represent the interaction of the reflected wave with the original wave on the reflecting surface, which are classified into ultrasonic traveling waves (UTWs) and ultrasonic standing waves (USWs) operation.\cite{151,152,153} The above technique has been generally used for cell separation or focusing, whereas it has been rarely used for cell translation and rotation. Marzo et al.\cite{118} proposed a single-sided array structure (Figure 6b) to deliver and rotate suspended particles at speeds up to 128 rpm. Deng et al.\cite{119} proposed and employed an acoustic field synthesis technique (Figure 6c) to achieve ultrasonic rotation and delivery of nonspherical microparticles. BAW based has some limitations and can only be operated in a specific position, static flow, or one direction (clockwise or counterclockwise) for rotation. SAWs are excited on the surface of piezoelectric crystals using interdigital transducers (Figure 6d).\cite{140} SAW-I tweezers work on a bounded fluid reservoir and couple the input acoustic waves from one or more sources into the fluid over a shared substrate.\cite{114} Dual-chip devices allow acoustic wave generator reuse. Thus far, the experiment of dual-chip operation has been limited to simple naked superlattice. As reported by Qian et al.,\cite{141} adding a thin-film structure to a dual-chip device (Figure 6e) could significantly affect standing wave and particle manipulation performance. Compared with direct SAW devices and bare silicon on a dual chip, the SAW generators could be reutilized. Although the particle motion speed decreased by over 90%, it could still be sufficient for biological applications in cell processing with the bioactivity of higher than 87%. Zhang et al.\cite{112} developed a SAW device for caenornabditis elegans (Figure 6f), with a rotation accuracy of 4° and bioactivity greater than 99.2%.

AFTs are driven by ultrasonic transducers adhering to microfluidic devices, thus exerting acoustic flow effects to drive cells. For instance, acoustic flow effects arise from the vibration of the sharp structure or the bubble surface. Ozcelik et al.\cite{113} and Song et al.\cite{114} designed symmetrical and asymmetrical sharp-edge microstructures (Figure 6g), respectively. Both structures can induce rotational flow under external acoustic field oscillations to achieve positional adjustment of HeLa cells, nematodes, and porcine oocytes. Acoustic waves are capable of activating microbubbles to generate radiative forces and microvortices in aqueous media. 3D rotation of plant pollen is achieved by adjusting the frequency and voltage of the transducer, followed by capturing 3D reconstructed images of plant pollen.\cite{154} In addition, focused SAW-induced acoustic flow can be adopted for single-cell manipulation. Ma et al.\cite{145} proposed the structure as illustrated in Figure 6i, which vibrated under the circular and linear vibration modes to generate two different types of ultrasonic microfluidic flow patterns, including circular and quadruple vortex. However, the predesigned structure and the enclosed environment might limit the flexibility of target selection. Thus, Li et al.\cite{146} proposed an novel acousto-fluidic hybrid manipulation method to regulate cell manipulation in an open environment (Figure 6h). Acoustic waves drove micropipette containing microbubbles to generate radial forces and microflows in an aqueous medium for capturing and rotating micro-objects.

Fixed design and drive models make it difficult to switch multiple functions in a single device. It is also difficult to adapt to different sizes, shapes, and numbers of cells. To address this problem, Zhang et al.\cite{147} proposed a versatile bubble-based acoustofluidic device (Figure 6j), which is used to manipulate cells in a biocompatible manner. Transmission, capture, 3D rotation and circular rotation patterns can be generated in the microchannel by simply changing the applied frequency. It is not required to change the number of piezoelectric transducers or the design of the bubble array. The device is suitable for cells from micrometers to several hundred micrometers. In the acoustic field, the cells are usually manipulated at the acoustic pressure node. Ren et al.\cite{148} proposed a bubble microswimmer based on
acoustic dynamics (Figure 6k), which can select individual cells from a large number of cells. In the megahertz acoustic field, the microswimmers is subjected to two main forces: the secondary Bjerknes force and a locally generated acoustic streaming propulsive force. The acoustic field provides the momentum for the self-rotating and translational motion of the microswimmers, while the magnetic field adjusts its direction of motion. Acoustic and magnetic fields have become the two most popular energy sources for remote control of miniature swimmers in biomedical applications. Compared with the magnetic field, the control of acoustic field is easier. Luo et al.[149] designed a miniature swimmer remotely controlled and powered by ultrasound waves (Figure 6l). The speed of the swimmer is linearly related to the square of the ultrasonic wave amplitude. The swimmer can make a translational motion or a rotational motion by changing the frequency of ultrasound. Only a single ultrasonic transducer can be used to control the movement of the swimmer, greatly simplifying the control system (Table 5).

2.6. Hydrodynamic Drive Methods

Hydrodynamic drive methods are powered by liquid to drive the controlled object for the positional adjustment. It can be achieved to control the liquid flow rate and flow direction in the corresponding channel via micropump, microvalve, and microstructure for adjusting single-cell position. The above method will not cause mechanical damage to the cells, or exert unknowable biological effects on the object, and it has been recognized as the most environment-friendly manipulation method thus far. There have been two main regions of fluid flow, including laminar region and turbulent region.\[156\] The regulation of fluid motion under microscopic conditions is fundamentally different from that under macroscopic conditions, in which the Reynolds number satisfies $R_e \leq 2000$ at the microscopic scale, and the fluid in the pipe refers to a regular laminar flow.\[156\]

$$R_e = \frac{d v p}{\mu}$$

where $d$ denotes the diameter of the circular pipe, $v$ expresses the fluid speed, $\rho$ represents the fluid density, $d$ is the pipe diameter, and $\mu$ represents the fluid viscosity. Hydrodynamic drive methods can be classified into the method based on the internal microstructure, that based on the micropipette fluid spray, and that based on vibration-induced vortex.

### Table 5. Summary of the development of cell pose adjustment by acoustic field approaches.

| Authors     | Time     | Method                              | Rotation DOF | Speed          | Manipulation object | Activity [%] | Sizes [µm] | Application               |
|-------------|----------|-------------------------------------|--------------|----------------|--------------------|--------------|------------|---------------------------|
| Marzo et al.[138] | 2015     | Single-sided array acoustic suspension | 1           | 0–16,000       | Polystyrene particle | –            | 600–3100   | Particle manipulation    |
| Deng et al.[139] | 2017     | Acoustic field synthesis            | 1           | –              | Silicon rod        | –            | Length 90, width 15     | Cell rotation            |
| Qian et al.[143] | 2021     | Surface acoustic wave               | 2           | 24.24 µ        | Tumor cell         | Above 87     | 1200       | Biomedical sample processing |
| Zhang et al.[142] | 2019     | Surface acoustic Wave               | 2           | Max 706 rpm    | Cryptobacterium hidradenum | Above 99.2   | 10–20       | Biological research     |
| Ozcelik et al.[143] | 2016     | Oscillatory symmetric micro-structure generates circular flow | 3           | 14000 rpm      | HeLa cells, Cryptobacterium hidradenum | –            | 20,1200     | Targeted drug delivery  |
| Song et al.[144] | 2019     | Oscillating asymmetric micro-structure generates circular flow | 3           | 370–500 rpm    | Porcine oocytes    | No obvious   | 120         | Cell rotation            |
| Läubli et al.[155] | 2018     | Oscillating solid structure generates circular flow | 3           | 0–3000 rpm    | Arabidopsis oocytes | –            | 26.7 ± 2.4, 20.0 ± 1.3 | Cellular 3D image reconstruction |
| Li et al.[146] | 2021     | Acoustically driven micropipette with microbubbles | 2           | –              | Particle           | –            | 160         | Cell rotation            |
| Ma et al.[145] | 2020     | Circular and linear vibration       | 2           | –              | MCF-7 cell         | –            | 15          | Cell manipulation        |
| Zhang et al.[142] | 2021     | Acoustically oscillating bottom bubbles | 2           | –              | E.gracilis cell, DU145 cell | –            | Length 40.5–53.4  | Cell manipulation        |
| Ren et al.[148] | 2019     | Acoustically powered bubble-based microswimmers | –           | –              | HeLa cell          | –            | –           | Cell manipulation        |
| Luo et al.[149] | 2021     | Oscillating air bubbles generate streaming flown | –           | –              | –                  | –            | –           | Cell manipulation        |
The method based on internal microstructure is to manipulate the cells in a microfluidic chip with the predesigned microstructure and controlled pressure difference for parallel or serial operations (e.g., cell trapping, directional movement, and extraction). Tanyeri et al. designed a microfluidic device with a cross-slot channel geometry (Figure 7a) generating hydrodynamic traps to capture individual particle. This device can provide high-resolution limitation of single-micrometer and nanoparticles in low-viscosity aqueous solution using stagnation flow. The hydrodynamic trap follows the principle of active feedback control of a stagnation point flow field. It guides particles to the trap center and keeps the centroid position of the trapped particles in the trap center in 2D space. As revealed by the experimental results, trapping of submicrometer-sized particles (100 nm) and confinement of micrometer-sized particles with high resolution (within 1 μm) for extended time scales (>10 min) were revealed. Based on the previous research, Harada et al. proposed and demonstrated a shear flow-based mechanism. The mechanism can control the orientation and position of the target object (Figure 7b), so as to address the undesired rotation. The freedom of the cell is limited in the enclosed space to a certain extent.

Methods based on micropipette fluid spray are performed in open space. Micropipette spraying fluid enables the fluid to rotate directionally to form a vortex. The center is the stationary point, and the stationary point of the vortex can be employed to capture individual cells of various shapes. The position and intensity of the vortex can be adjusted to determine the position and speed of particle movement, and the cell can be rotated flexibly. Stable cell rotation requires control of the flow rate in the pipette to generate laminar fluid (Figure 7c), to achieve out-of-plane and in-plane rotation with a cell rotation accuracy of 1.9° under a visual feedback system. On that basis, Zhang et al. systematically explored the vortex phenomenon formed by dual-pipette and three-pipette spray fluids (Figure 7d), which arose from the directional motion formed by the fluid. Furthermore, the cells of different shapes and sizes, ranging from a few micrometers to several hundred micrometers in size, could be captured using the vortex stationary points. In addition, these researchers investigated the correlation between cell controlled performance and vortex factors and conducted the directional quantitative adjustment of cell position and attitude.

Methods based on vibration-induced vortex are primarily induced by mechanical and piezoelectric vibrations. It can generate multiple directions of high-frequency vibration-induced horizontal and vertical flow field-manipulated cells. Liu et al. developed a cantilever structure based on resonance (Figure 7e) to generate flow field-driven cells for extending the linear vibration of a single piezoelectric actuator to the 2D circular vibration of a micropipette. Circular vibration can only be caused at a certain resonant frequency, and the direction of circular vibration of the micropipette remains difficult to control. In addition, Kim et al. proposed a robotic arm driven by three piezoelectric actuators. The research established the correlation between

Figure 7. Hydrodynamic drive methods to cell manipulation. a) Microfluidic device exhibiting cross-slotted channel geometry. b) A shear flow-based mechanism. c) A standard suction-holding needle generating layers of fluid. d) Double-tube and triple-tube spray of fluid to form vortices. e) Schematic of the multifunctional manipulation using whirling flow generated by vibration of a single-piezo actuator. f) Mechanism of the multifunctional micromanipulation, immobilization and transportation in Y- and X-axis, rotation. g) Multiaxial noncontact in situ manipulation by the steady streaming around a pair of oscillating cylinders. Induced local rotational flow on a microcolumnar microchip: Local whirling flow around a single micropillar induced by circular vibration, ii: transport and rotation, iii: circular vibration in xy plane, and iv: circular vibration in z direction. Reproduced with permission.

Adv. Intell. Syst. 2022, 4, 2200096 © 2022 The Authors. Advanced Intelligent Systems published by Wiley-VCH GmbH
The radius and the velocity toward the root of the end effector. It can also be used for the manipulation of multiple targets. To achieve single-cell in situ triaxial rotation, Fuchiwaki et al. [156] employed two micropipette vibrations (Figure 7f) to generate steady-state flow for vertical and horizontal rotations with the rotational resolutions of 0.05° and 0.11°, respectively. The method enables multiaxis, noncontact, in situ, and compact micromanipulation, which confirms that the conditions for vertical and horizontal rotation are determined by two dimensionless numbers, including $R_c$ and $a/r_c$ (where $a$ is half of the

the radius and the velocity toward the root of the end effector. It can also be used for the manipulation of multiple targets. To achieve single-cell in situ triaxial rotation, Fuchiwaki et al. [156] employed two micropipette vibrations (Figure 7f) to generate steady-state flow for vertical and horizontal rotations with the rotational resolutions of 0.05° and 0.11°, respectively. The method enables multiaxis, noncontact, in situ, and compact micromanipulation, which confirms that the conditions for vertical and horizontal rotation are determined by two dimensionless numbers, including $R_c$ and $a/r_c$ (where $a$ is half of the

$\xi$ is dielectric constant, $\eta$ is electrical conductivity, $\chi$ is magnetic permeability, and $\sigma$ is refractive index.

Table 6. Summary of the development of cell pose adjustment by the hydrodynamic drive methods.

| Authors | Time | Method | Rotation DOF | Speed | Manipulation object | Sizes [µm] | Application |
|---------|------|--------|--------------|-------|---------------------|------------|-------------|
| Harada et al. [160] | 2020 | Based on shear flow mechanism | 1 | 0.2 rad s | Janus particle | 50 | Cell manipulation |
| Leung et al. [64] | 2012 | Single-tube spray fluid | 3 | 22.8 cell s⁻¹ | Mouse oocytes | 50 | Polar body position |
| Zhang et al. [161] | 2017 | Double microtube-induced cyclonic flow | 1 | 0.0147 m s | Shrimp egg, particle | 100-550 | Cell rotation |
| Zhang et al. [162] | 2018 | Double microtube-induced cyclonic flow | 1 | – | Shrimp egg, particle | 100-500 | Cell rotation |
| Ou et al. [160] | 2021 | Three-microtube-induced cyclonic flow | 1 | 35 mm s | Cell | 270 | Cell manipulation |
| Liu et al. [165] | 2017 | Vibration-induced micro fabrication | 1 | 0.24–5.08 rad s | Mouse egg cell | 100 | Biological manipulation |
| Kim et al. [163] | 2017 | Vibration-induced flow caused by circular vibration of a single pipette | 1 | 0–120 rad s | Microspheres | 9.6 | Cell rotation |
| Hayakawa et al. [164] | 2015 | Microcolumn type microchips performing circular vibration | 2 | FP velocities 63.7 ± 4.0° s⁻¹ | Mouse oocytes | 100 | Cell position and posture adjustment |
| Fuchiwaki et al. [156] | 2018 | Vibration-induced flow mechanism | 3 | 34.8° s⁻¹, 188 s⁻¹ | Mouse oocytes | 100 | Cell observations and manipulation |

**Table 7. Summary of the different cell pose adjustment methods.**

| Methods | Mechanism | Parameters | Complexity | Sizes [µm] | Resolution [µm] | Advantages | Disadvantages |
|---------|-----------|------------|------------|------------|----------------|------------|---------------|
| Mechanical contact | Mechanical contact | $D$ | Low | 100–1200 | Higher (0.01 ≈ 1) | Higher repositioning accuracy, stable operation, label-free | Cell deformation, higher risk of cell mechanical damage, high precision mechanical motion system, complex operation system |
| Electric field | DEP Force | $D$, $η$ | Medium | 0.001–120 | Higher (0.1 ≈ 1) | Low cost, high efficiency, label-free, higher rotational accuracy, easy to integrate | Constrained by electric field distribution, potential electrical damage, low conductivity buffer when the liquid flow rate increases, lower efficiency and accuracy |
| Optical field | Trapping | $D$, $ξ$, $δ$ | Medium, High | 4–20 | Higher (0.0001 ≈ 0.001) | High rotation accuracy, label-free, single-cell, low drift | Optical damage, expensive instrumentation, weak capture low throughput, bulky optical system with opaque |
| Magnetic field | Magnetic gradient | $D$, $χ$ | High | 5–120 | Higher (0.0001 ≈ 0.01) | Greater drive power, reliability and efficiency | Constrained by the magnetic field distribution, requires pretreatment of cells, labeling, force hysteresis |
| Acoustic field | Axial acoustic forces | $D$, $ρ$, $β$ | Low | 10–3100 | Low (1 ≈ 10) | Label-free | Stimulation of cells, required piezoelectric substrates for chip fabrication, limited precise cell rotation |
| Hydrodynamic drive | Hydrodynamic inertial forces | $D$ | Low | $10–50$ | Low (1 ≈ 10) | Easy to integrate manufacturing | Rotational accuracy, complex and cumbersome external control equipment, poor flexibility, difficult to control specific single-cell |

$D$ is diameter, $ρ$ is density, $β$ is compressibility, $ξ$ is dielectric constant, $σ$ is electrical conductivity, $χ$ is magnetic permeability, and $η$ is refractive index.
oscillation amplitude and \( r_c \) is the radius of the cylinder. Hayakawa et al.\[164] proposed a method enabling 3D cell rotation on an open-chip structure (Figure 7g). When circular vibrations were applied to a microchip exhibiting a micropillar pattern, a highly localized vortex flow would be induced around the micropillar. The direction and speed of the above flow could be controlled by adjusting the direction and amplitude of the applied vibration. Due to the simplicity of the system setup and the open architecture, this method is expected to be a user-friendly technique for cell manipulation on a chip. In addition, a particle manipulation strategy based on cooling thermal convection is also used in microfluidic chips.\[166] The moving direction of particles transfers from the \( y \)-axis to the \( z \)-axis under the control of temperature (Table 6).

2.7. Summary

In this chapter, the six methods of single-cell pose adjustment are reviewed, and the advantages and disadvantages of the respective method are listed in Table 7. To better compare the application range of the different methods, Figure 8 with a scale ruler is used to represent it.

The mechanical contact methods are a classical cell manipulation style, which has a high orientation accuracy, low operation difficulty, and large contact forces. However, excessive contact forces can lead to large cell deformation and may damage important organelles inside the cell. In addition, the existence of bonding forces in mechanical contact makes it difficult to effectively release microobjects.\[170] The accuracy of the mechanical contact methods requires accurate movement of the end effector, which also has high requirements for the mechanical system and the control system.\[18] It is mainly used for single-cell manipulation above 100 \( \mu \)m. Noncontact manipulation avoids the shortcomings of direct mechanical contact methods, which may be the reason why numerous biologists prefer it. Electric field, optical field, and magnetic field methods use external force to actively control the cells with high precision. It has been shown that the electric field methods can cause electrical damage to the cells,\[164] and the movement of the cells is related to the electrical properties of the medium they are in, the applied electric field, the shape of the electrodes, etc. Accordingly, cells with different dielectric properties can be manipulated by controlling the dielectric properties or the input voltage conditions, which can be used for cell dielectric property characterization and imaging.

The optical field approaches driven indirectly by particles can reduce a lot of photodamages, but it is difficult to eliminate entirely. It is mainly used to rotate cells of 4–20 \( \mu \)m and difficult to rotate such large cells as 100 \( \mu \)m. The magnetic field methods usually require superparamagnetic material to label the cells introducing the difficult of separation, and the orientation of the magnetic field limits the cell rotation. Magnetic fields can penetrate human tissues and thus may be promising for targeted drug delivery or cell therapy.\[171,172] The hydrodynamic drive methods can be operated not only in single cell, but also multiple cells. However, once the object or task is changed, the microfluidic device should be redesigned, and the enclosed space limits single-cell puncture operations, which is undesired in universality. The acoustic field methods are different from the above methods. Although the accuracy is not as high as other methods, compared with the optical field, it has lower energy and does not destroy cell viability. Compared with the magnetic field, it does not require the material to have magnetism or other special properties.\[159,160,173] Even under the same conditions, different cell shapes and sizes can affect the effectiveness of the operation. Thus it is important to choose the operation method according to the characteristics of the application scenario.

3. Cell Puncture Methods

Different from the cell pose adjustment, the cell puncture is an invasive cell manipulation. Cell puncture is to use a common glass needle to penetrate the cell wall and insert it into the cell.

Figure 8. The application range of the different methods.
It can be used for foreign substance introduction, sperm injection, nuclear transfer, protein, peptide and genetic substance injection, drug delivery, etc.\textsuperscript{[37,174–176]} Cell puncture methods can be classified into ordinary glass needle puncture, piezoelectric-assisted puncture, Ros-drill puncture, and other methods.

3.1. Ordinary Glass Needle Puncture Methods

Ordinary glass needle puncture methods are based on external pressure operation, as presented in Figure 9a. When puncturing mammalian oocytes, microneedle puncture will generate great deformation due to the existence of the zona pellucida. So, the polar body of oocyte may be damaged, which leads to the failure of oocyte fertilization and embryo development. The ordinary glass needle puncture method is a challenging manual task with a reported survival rate of 30–40\% for mouse oocyte.\textsuperscript{[176]} The manual operation needs experienced skill to define parameters of the incision point, insertion depth, and puncture speed. Those parameters will affect the efficiency and effectiveness of the operation.\textsuperscript{[177]} To achieve batch puncture or injection of cells, two kinds of method are proposed to combine glass needles and microfluidic chips, as shown in Figure 9b–e. One is to move cells onto a stationary microneedle,\textsuperscript{[178]} and the other is to move the needle into an immobilized cell\textsuperscript{[179–181]} both of which can achieve pipelined cell puncture. The former controls the cell movement with the help of microvalves and micropumps. The latter is more frequently used in cell injection and drosophila research. As depicted in Figure 9e, the integrated microneedle is injected in a completely closed channel.\textsuperscript{[181]} The actuation of the needle is realized by the compliant deformation of the external actuator to the channel structure. Reagent transport is achieved using electroosmosis, which provides nonpulsating flow and accurate electrical dose control. The voltage for injection is between 5 and 25 V.

3.2. Piezoelectric-Assisted Puncture Methods

To puncture the cells more safely, the moving speed of the needle should be kept at $700 \mu \text{ms}^{-1}$\textsuperscript{[176]} during puncture, whereas the normal glass needle puncture methods are difficult to achieve. Figure 10a depicts the schematic. The piezoelectric-assisted puncture methods apply a pulse signal to the piezoelectric element, which is rapidly deformed to drive the tip of the injection pipette to move accurately and rapidly for the complete puncture with minimal cell deformation. Kimura et al.\textsuperscript{[60]} first used this method for cell injection and achieved a survival rate of 80\% in mouse oocyte injection experiments, which was significantly higher than manual injection. Impacted by the piezoelectric devices placed behind the injection needle device, the needle tip laterally vibrates, thus damaging the oocyte membrane. The amplitude of needle tip vibration can be reduced by adding mercury\textsuperscript{[182]} or fluorine\textsuperscript{[183]} damping fluid to the needle tip. The former can be toxic and pollute cells, whereas the latter is not desirable for damping, so the researchers have proposed to modify the structure of the piezoelectric injection needle. To solve the above problem, Ru et al.\textsuperscript{[184]} proposed two improvement methods.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9}
\caption{Ordinary glass needle puncture methods. a) Schematic of glass needle puncture of mammalian oocytes. b) Schematic diagram of fluid-driven cell impingement on needle puncture.\textsuperscript{[178]} 1: Cell to be injected is moved by the fluid stream to a fixed microneedle, valve 1 (V1) is open while valve 2 (V2) is closed. 2: The cell impinges on the needle and is pierced. Microinjection is performed (turning the cell from green to red). 3: V2 is open and V1 is closed, thus causing the fluid stream and the volume displaced by valve V1 operation to lift the cell off the needle and carry it via channel B to a collection reservoir. c) Schematic of mechanical arm-driven microneedle injection of drosophila larvae at specific sites.\textsuperscript{[179]} d) Microfluidic device for high-speed microinjection of C. elegans.\textsuperscript{[180]} e) Schematic of deformation of the substrate driving microneedle injection of cells.\textsuperscript{[181]} Reproduced with permission.\textsuperscript{[178]} Copyright 2008, Royal Society of Chemistry. Reproduced with permission.\textsuperscript{[179]} Copyright 2020, Royal Society of Chemistry. Reproduced with permission.\textsuperscript{[180]} Copyright 2016, AIP. Reproduced with permission.\textsuperscript{[181]} Copyright 2009, Royal Society of Chemistry.}
\end{figure}
Piezoelectric-assisted puncture methods. a) Schematic of piezoelectric-assisted injection needle structure. b) Schematic of a piezoelectric injection needle with a modified structure, the first option placing the piezoelectric actuator close to the tip of the injection tube and the second option combining the piezoelectric actuator and the microinjection by means of a connector. c) Schematic of an eccentric piezoelectric-assisted injection needle. d) Two-point flexible way fixed syringe needle schematic. e) Schematic of a bent injection needle with a triangular flexible mechanism. f) Schematic of a large stroke microinjector. Reproduced with permission. Copyright 2016, MDPI. Reproduced with permission. Copyright 2019, IEEE. Reproduced with permission. Copyright 2014, IEEE. Reproduced with permission. Copyright 2018, IEEE. Reproduced with permission. Copyright 2017, Taylor & Francis.

To be specific, the first method is to place the piezoelectric actuator close to the tip of the injection needle, and the other method is to combine the piezoelectric actuator and the connector for the microinjection. Accordingly, the kinetic energy is directly transferred to the injection needle, which is more compact and reduces its lateral vibration. The second method increases the lateral vibrations caused by coaxiality errors and installation errors in the manufacturing of the injection needle. Both of the above structures can decrease the lateral vibration to 1 μm, which is less than the conventional method. However, the efficiency of the first structure (80.1%) is obviously lower than that of the second structure (91.5%) since the placement of the piezoelectric driver behind the operating needle interferes with the installation of the injector in the standard holder.

Piezoelectric-assisted punctures primarily adopt concentric structures, and piezoelectric drivers are placed directly behind microustubes, thus disturbing the standard setting. In addition, an eccentric structure is developed, and the piezoelectric actuator is placed next to the microustube bracket. In the above devices, the piezoelectric actuator is connected to the inertial mass through a spring to drive the inertial mass to vibrate. However, the vibration is difficult to keep along the axial direction, due to the lack of lateral restraint. Accordingly, Dai et al. developed an eccentric piezoelectric-assisted injection needle structure (Figure 10c), in which the central beam was placed behind a piezoelectric actuator, multiple pairs of bending beams were connected to the central beam, and a flexible beam was used to guide the movement of the central beam in the penetration direction. The lateral vibration was only 0.08 μm, and the cellular deformation with the piezoelectric actuator was 5.68 ± 2.74 μm, significantly lower than that of the cellular without the piezoelectric actuator of 54.29 ± 10.21 μm, with a success rate of 95.0% for cell membrane puncture. The piezoelectric driver was installed next to a standard micropipette holder and does not interfere with the standard clinical setup. The device both constrains lateral polarization and removes the need for damping fluid. In addition, Ge et al. suggested that the piezoelectric actuator was located behind the pipette and the single-point flexible linkage method would lead to a large vibration amplitude of the injection needle due to unstable connection of the mechanism. So he proposed a two-point flexible way to fix the injection needle, as presented in Figure 9), the lateral dynamics would be significantly reduced, and the method solved the defect of unstable micropipette fixation from the source. Piezoelectric injection needles use straight needles, which may be the reason for large lateral vibration. Thus, Yan et al. designed a curved injection needle.
needle based on a triangular flexible mechanism, which can significantly reduce the lateral vibration during cell puncture and play as an axial guiding role (Figure 10e). According to finite-element analysis, at 22 kHz, the minimum value of lateral vibration was 0.31 μm. To compensate for the small travel of the present piezoelectric injection needle position, Wang et al. developed a large-stroke microinjector (Figure 10f) by increasing the displacement of the injection needle to 103 μm with both coarse and micromovements via a single-layer bridge mechanism flexible structure, so as to compensate for the small travel of the present piezoelectric injection needle position. In addition, Tsui et al. experimentally investigated the effects of two key injection parameters, including injection speed and pipette diameter. They concluded that the deformation increased from low-to-medium injection speed, while it decreased from medium-to-high injection speed.

3.3. Ros-Drill Puncture Methods

Ros-drill puncture methods adopt a micromotor rotating motion to make the tip of the injection tube produce high-frequency and small-amplitude rotation oscillation for puncture, as presented in Figure 11a. So, the above method is distinguished from the piezoelectric-assisted puncture method. Ergenc et al. designed a Ros-drill injection needle, as presented in Figure 11b,c. The injection needle holding end is placed in a precision bearing, which is embedded in the shell. Moreover, a coupling links the injection holder to a micromotor (marked as a DC brushed motor), which transmits the angular motion from the motor to the front end of the needle. To avoid resonance, pure harmonic signals are used, and all three types of injection needles (flat, serrated pipette, inclined, and spiked human-ICSI pipette) can be adopted. The cleavage rates range from 70 to 90%, the volume of solution delivered to the cells was controlled by flow rate in the pipette, thus enabling the puncture flow rate in the pipette, thus enabling the puncture.

3.4. Other Methods

Besides the above methods, there are some cell puncture methods based on other physical principles, which are applied to specific scenes (e.g., ultrasound puncture, nanopuncture, electroproporation, and DEP methods). The gene gun was developed by professor Sanford and his team at Cornell University. As shown in Figure 12a, the external DNA is coated with gold powder or tungsten powder to form microparticles. Under the action of gas-accelerating tube, it shoots at high speed to plant tissues or cells. The particles penetrate cell wall and enter the cell. Sailaja et al. used the gene gun for the genetic transformation of castor. Transformation frequency in terms of plants grown to maturity and showing the presence of the introduced genes was 1.4%. In addition, Ukherjee et al. proposed a single-cell electroporation method based on nanopipettes, which showed superior cell viability and efficiency compared with traditional methods. The automated system uses deep convolutional networks to identify cell locations.

The diameter of yeast cells is smaller than that of ordinary injection needles, leading to more difficult to directly puncture. Shen et al. proposed a nanoinjection system under a scanning electron microscope (Figure 12b), in which the micropull method was adopted to prepare nanotubes and control probes (150 nm in diameter) through focused ion beam (FIB) etching. The volume of solution delivered to the cells was controlled by regulating the flow rate in the pipette, thus enabling the puncture of yeast cells and the observation of the puncture in real time. Over the past few years, nanoinjection methods based on an
atomic force microscope (AFM) cantilever nanoprobe have been proposed. In these methods, a smaller nanosized injector is adopted to penetrate the cell and deliver the material into the cell.\textsuperscript{144} Nanoprobes are operated in a similar way to the glass pipette employed for single-cell injection. However, there have been rare reports on automated nanoinjection techniques.

Electroporation is the application of an electric field to a cell to increase the permeability of the cell membrane, thus allowing for chemical, drug, or DNA entry into the cell. Conventional devices generate the electric field using two parallel and close electrode plates, which can lead to problems (e.g., bubbles, edge effects, and transfection efficiency below 50%). So, these devices can reduce cell viability. To solve the above problem, Kim et al.\textsuperscript{153} proposed a novel electroporation method using a capillary pipette and a linear electrode (Figure 12c). There is an anode within the capillary pipette and a cathode outside the capillary; the anode and cathode are separate and electrically connected via the electrolyte in the buffer. Impacted by the small surface area of the electrodes, the capillary electroporation system creates a uniform electric field and reduces cell death during electroporation. The transfection rate is no less than 80% in general cell lines (e.g., HeLa and COS-7), while being no less than 50% in difficult-to-prepare cell lines.

The dielectric electrophoresis method is employed to generate an inhomogeneous electric field by applying an alternating current to a pair of electrode plates. Accordingly, the surrounding tiny objects are attracted, and the alternating current is capable of breaking and releasing the tiny objects. Based on the mentioned principle, Nadappuram et al.\textsuperscript{196} designed nanoscale tweezers (Figure 12d) for single-cell biopsies with a microtubule of nearly 50 nm at the tip and a layer of 10–20 nm on each side in the middle. This nanotweezer was filled with carbon for electrodes inside the nanopipette, and electrodes were placed at the end of the microtubule wire. When the microtubule is pierced into the cell, the organelle will be grasped and released by adjusting the on/off of alternating current.

3.5. Summary

This section introduces three main puncture methods, including ordinary glass needle puncture, piezoelectric-assisted puncture, and Ros-drill puncture methods. The ordinary glass needle puncture methods have been more extensively applied in practice with low-cost and easy fabrication. However, an experienced operator is required, and the cell deformation is larger than other methods. The piezoelectric-assisted injection method and Ros-drill injection methods are characterized by significantly higher efficiency, cell penetration rate, and oocyte division rate compared with the conventional injection method. The piezoelectric-assisted injection methods have high resolution and are easy to control, thus producing axial vibration and lateral vibration, which play a dominant role in cell penetration. However, larger lateral vibration can destroy cells, especially the resonance phenomenon, which is dominant in ICSI experiments and clinical activities. Compared with the piezoelectric-assisted injection method, Ros-drill injection methods have no lateral vibration, whereas their adjustable range of frequency and amplitude is relatively small, thus reducing control precision. Other injection methods basically aim at specific targets or application scenarios (e.g., nanoinjection for 3–6 μm cells). Table 8 summarizes the advantages and disadvantages of each approach in recent years.

### Table 8. Summary of different cell puncture methods.

| Method                        | Mechanism                       | Advantages                                         | Disadvantages                        | Application               |
|-------------------------------|---------------------------------|----------------------------------------------------|--------------------------------------|---------------------------|
| Ordinary glass needle puncture | External pressure               | Simple equipment, low cost                         | Larger cell deformation              | Cell injection            |
| Piezoelectric-assisted puncture | Piezoelectric effect            | Small-cell vibrations, damping fluid               | Transverse and axial deformation     | Cell injection            |
| Ros-drill puncture            | Rotational oscillation          | No lateral vibration, small-cell deformation       | Small adjustable range of frequency and amplitude | Cell injection            |
| Gene gun                      | High-speed particle bombardment | Easy operation                                     | Low transform efficiency             | Plant breeding            |
| Nanopunctur                   | Nanopipette injection           | Very thin needle holes                              | High manufacture price of nanoinjection needle and easy to break | Cell puncture             |
| Electroporation               | Changed cell permeability       | Faster operation, higher transfection rate, and higher cell viability | High voltage pulses lead to very high cell death rate | Exogenous material introduction |
| DEP                           | Dielectric effect               | Label-free, easy-to-integrate manufacturing, easy to operate | Heat generation is harmful to cellular activity, electrical damage | Organelle Sampling |

4. Discussion

This article reviews the recent research progress on single-cell pose adjustment and puncture, which are two typical cell manipulations with one no-invasive and the other one invasive. Single-cell pose adjustment can be realized by mechanical contact method, electric field, optical field, magnetic field, acoustic field, and hydrodynamic method. Based on the comparison of the above single-cell pose adjustment methods, we can conclude as follows. 1) Mechanical contact methods can achieve cell trap
and cell reorientation, while excessive force can cause cell deformation and may lead to mechanical damage, which is not beneficial to cell development. 2) Electrical field can be used to measure the dielectric properties of cell and sort the cell. However, high voltage can lead to electrical damage for cells and is bad for cell viability. 3) Optical field is more suitable at the scenario of precise pose adjustment (resolution of 0.1–1 nm). The side effect is that the cell can have light damage under direct high-strength laser and is limited in light-sensitive application. In order to reduce light damage, laser indirect methods can be adopted; 4) Magnetic field does not cause additional damage for cell but requires superparamagnetic material to label the cells. Although these materials are biocompatible, they are difficult to degrade or separate; 5) Hydrodynamic drive is a green manipulation approach for cells. However, cell movement and rotation need to rely on the internal microstructure, which can be designed according to the purpose of the experiment and the type of cell; and 6) Acoustic field has good biocompatibility for cell but the resolution of cell operations is poorer (1–10 μm) than other methods. From the above analysis, each method has its own advantages and limitations. So, the ideal method should be selected according to cell type and application purpose. Of course, a variety of methods can be coupled to make up for each other’s shortcomings and improve the performance.

For single-cell puncture, ordinary glass needle puncture methods, piezoelectric-assisted puncture methods, and Ros-drill puncture methods are summarized based on different driving approaches. 1) Ordinary glass needle puncture methods are common methods of cell puncture, while excessive force can lead to large deformation during puncture, which is harmful to cell viability; 2) Piezoelectric-assisted puncture methods are more likely to make the cell puncture successful but the lateral vibration is adverse to cell. Larger lateral vibrations may rupture cells and even cause cell death. In order to reduce lateral vibration, piezoelectric actuators can be placed directly behind injection needle. However, piezoelectric ceramic actuators show some shortcomings (e.g., small output range, hysteresis, and creep). Some scholars have studied to compensate for piezoelectric ceramic actuators to improve control performance to some extent, whereas the above problems have not been fundamentally solved; and 3) Ros-drill puncture methods take needle tip rotation movement to puncture cells, which requires high straightness of needle manufacture and coaxiality of assembly. Other puncture methods are suitable for some other application. A glass needle is usually used for cell puncture, which is made of quartz glass stretched under high heat. It is fragile because of thin needle diameter. There is a risk of rupture when the cells are punctured. This is obviously not good for cell puncture. The development of new materials, especially flexible ones, makes it possible to develop new injection needles while keeping micrometer diameter of the needle tip. Combined with the bionic design, a flexible and rigid coupling injection needle could be designed to improve the lifespan and safety of the needle significantly.

The future development of single-cell manipulation relies on better manipulation hands, smarter perception, and more intelligent brain. The scenario of single-cell manipulation will also not limit in vitro, but trend in vivo with a more unstructured environment. Multiple modes of operating platforms would be better manipulation hands for cell manipulation with higher efficiency and more functions. Each approach has its limits and advantages. The noncontact operation methods can operate the cells at a distance, while the contact operation method can realize the cell puncture easier. Multiple methods are promising to integrate on the microfluidic chip, which can realize complementary advantages for manipulation, contributing to the higher efficiency and multimaniupulations at a single shot.

Perception is an important basis for precise single-cell manipulation. Currently cell manipulation is under a 2D microscopic view which misses depth perception. To achieve 3D imaging, multiview microscopy,[198] confocal fluorescence microscope,[199] and photoacoustic microscope[200,201] have shown potential to enhance the perception for single-cell manipulation. Besides the visual perception, tactile perception and electrochemical sensors have shown positive effects in information perception. Force sensors can precisely sense the contact force of the cell, and electrochemical sensors can characterize the intracellular chemical substance. The multisensor information fusion can be used to achieve better cell perception by overcoming the drawbacks of incomplete or unreliable information from individual sensors.

Intelligent manipulation plays an important role in autonomous single-cell manipulation. The success of the manipulation task depends to a large extent on the quality of recognition and control model employed. However, in a high-noise, complex, dynamic, and unpredictable working environment, it is a challenge to establish an accurate recognition and control model, due to the fact that it requires a lot of priori knowledge. In recent years, with the development of artificial intelligence technology, especially convolutional neural network (CNN)[202] and deep reinforcement learning (DRL)[203] researchers have shown great interest in combining CNN and DRL algorithms with the recognition and control of single-cell manipulation.[204]

The scenario of single-cell manipulation is not only in vitro, but also in vivo. Wireless power supply for microrobots and the microrobots of biodegradable materials are promising for single-cell manipulation in vivo. Microrobots are increasingly used in single-cell manipulation. Single-cell manipulation is performed in mainly in vitro scenario at present, which is a structured environment. However, manipulation in vivo is difficult. One challenge is the remote power supply and control of the microrobot. Traditional microrobots need to be equipped with energy storage devices, but they are too bulky and limited to provide power for long periods of time. Magnetic, acoustic, and optical fields can enable remote power transmission, which can achieve free motion and control of the robot. However how to improve the efficiency of energy transmission is a pressing issue. Another challenge is the degradability of materials. Biocompatible materials are already a common material in biomedical applications, but degradable materials design is still an open challenge. It is essential to develop degradable materials for in vitro single-cell manipulation, which does not require exclusion from the living organisms.

5. Conclusion

Single-cell manipulation technology has been supposed as an essential method of cell research, with wide applications in biological engineering, medical engineering, agricultural
engineering, and other precise manipulation fields. Cell pose adjustment and cell puncture have been reported as two basic operations of single-cell manipulation. In this review, the major methods and applications of single-cell pose adjustment and puncture are systematically summarized, and the advantages and disadvantages are analyzed. Some potential directions for future research are proposed in accordance with the above analysis. Single-cell manipulation technology has been considered as an interdisciplinary research field in microscale, involving different subjects (e.g., mechanical engineering, material science, electrical engineering, and biomedicine). High efficiency, high precision, high throughput, low cost, and low damage have been the hot topics of single-cell manipulation. We hope that this review can provide a reference for academic research and industrial applications of single-cell manipulation.

Acknowledgements

The authors thank the National Natural Science Foundation of China (Grant No. 32101626) and ZJU 100 Yang Talent Program (Grant No. 2019M650419).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

cell pose adjustments, cell punctures, cell rotations, single-cell manipulations

Received: April 19, 2022
Revised: June 7, 2022
Published online: July 24, 2022

[1] J. Bailey, Inventive Geniuses who Changed the World, Springer, Berlin 2022, pp. 117–152.
[2] M. S. Adil, S. P. Narayanan, P. R. Somanath, Tissue Barriers 2021, 9, 1848212.
[3] N. Ghassemi, A. Poulhazan, F. Deligey, F. Mentink-Vigier, I. Marcotte, T. Wang, Chem. Rev. 2021.
[4] M. Levin, Biochem. Biophys. Res. Commun. 2021, 564, 114.
[5] F. Soto, J. Wang, R. Ahmed, U. Demirici, Adv. Sci. 2020, 7, 2002203.
[6] Y. Yalikun, Y. Kanda, K. Morishima, Micromachines 2016, 7, 140.
[7] S. Tasoglu, U. A. Gurkan, S. Wang, U. Demirici, Chem. Soc. Rev. 2013, 42, 5788.
[8] K. Villa, L. Krejčová, F. Novotný, Z. Heger, Z. Sofer, M. Pumera, Adv. Funct. Mater. 2018, 28, 1804343.
[9] H. Gong, L. Li, J. Qiu, Y. Yao, Y. Liu, M. Cui, Q. Zhao, X. Zhao, M. Sun, IEEE Rob. Autom. Lett. 2021, 6, 7909.
[10] Y. Hu, J. Ma, Y. Zhang, J. Li, Y. Hu, J. Wen, Mech. Syst. Sig. Process. 2021, 157, 107743.
[11] W. Shang, D. Li, H. Lu, T. Fukuda, Y. Shen, Appl. Phys. Lett. 2017, 110, 043701.
[12] D. Schraivogel, T. M. Kuhn, B. Rauscher, M. Rodriguez-Martinez, M. Paulsen, K. Owsley, A. Middlebrook, C. Fischer, B. Ramasz, D. Orozco, M. Dees, S. Cuylen-Haering, E. Diebold, L. M. Steinmetz, Science 2022, 375, 315.
[13] R. H. Pritchard, A. A. Zhukov, J. N. Fullerton, A. J. Want, F. Hussain, M. F. la Cour, M. E. Bashtanov, R. D. Gold, A. Hailes, E. Banham-Hall, S. S. Rogers, Lab Chip 2019, 19, 2456.
[14] T. Tang, Y. Hosokawa, T. Hayakawa, Y. Tanaka, W. Li, M. Li, Y. Yalikun, Engineering 2021, 10, 110.
[15] X. Sha, H. Sun, Y. Zhao, W. Li, W. J. Li, Micromachines 2019, 10, 843.
[16] C. Dai, L. Xin, Z. Zhang, G. Shan, T. Wang, K. Zhang, X. Wang, L-T. Chu, C. Ru, Y. Sun, IEEE Rob. Autom. Lett. 2019, 5, 339.
[17] M. J. Gerber, M. Pettenkofer, J.-P. Hubuschman, Eye 2020, 34, 1554.
[18] A. Shakoor, M. Xie, T. Luo, J. Hou, Y. Shen, J. K. Mills, D. Sun, IEEE Trans. Biomed. Eng. 2018, 66, 2210.
[19] R. Memeo, P. Paik, F. Sala, M. Castriotta, C. Guercio, T. Vaccari, R. Osellame, A. Bassi, F. Bragheri, J. Biophotonics 2021, 14, e202000396.
[20] K. Sermon, A. Van Steirteghem, I. Liebaers, Lancet 2004, 363, 1633.
[21] H. Zhang, L. Su, H. Wei, Y. Yu, X. Zhang, in 2019 IEEE Intere. Conf. on Mechatronics and Automation (ICMA). IEEE, Piscataway, NJ 2019, pp. 656–661.
[22] K. H. Campbell, I. Wilmut, Anim. Breed. 2021, 47–.
[23] N. Nitta, T. Sugimura, A. Isozaki, H. Mikami, K. Hiraki, S. Sakuma, T. lino, F. Arai, T. Endo, Y. Fujiwaki, H. Fukuzawa, M. Hase, T. Hayakawa, K. Hiramatsu, Y. Hoshino, M. Inaba, T. Ito, H. Karakawa, Y. Kasai, K. Kokui, S. Lee, C. Lei, M. Li, T. Maeno, S. Matusaka, D. Murakami, A. Nakagawa, Y. Oguchi, M. Oikawa, T. Ota, et al., Cell 2018, 175, 266.
[24] N. Emami, S. Sedaei, F. Ferdousi, Visual Inf. 2021, 5, 1.
[25] O. Otto, P. Rosendahl, A. Mietke, S. Golferer, C. Herold, D. Kraue, S. Girardo, S. Pagliara, E. Expenyong, A. Jacobi, M. Wobus, N. Töpfner, U. F. Keyser, J. Mansfeld, E. Fischer-Friederich, J. Guck, Nat. Methods 2015, 12, 199.
[26] T. Iino, K. Okano, S. W. Lee, T. Yamakawa, H. Hagiura, Z.-Y. Hong, T. Maeno, Y. Kasai, S. Sakuma, T. Hayakawa, F. Arai, Y. Ozeki, K. Goda, Y. Hosokawa, Lab Chip 2019, 19, 2669.
[27] A. Paris, D. Decanini, G. Hwang, Microelectron. Eng. 2021, 235, 111466.
[28] Z. Zheng, Z. Li, T. Xu, L. Chen, F. Fang, D. Wang, P. Dai, Q. Wang, X. Wu, X. Yan, Appl. Mater. Today 2021, 22, 100962.
[29] Y. Koike, H. Wada, Y. Yokoyama, T. Hayakawa, in 2020 IEEE 33rd Inter. Conf. on Micro Electro Mechanical Systems (MEMS). IEEE, Piscataway, NJ 2020, pp. 1094–1097.
[30] J. Goldman, Annu. Rev. Biomed. Eng. 2006, 8, 425.
[31] C. C. Cheah, X. Li, X. Yan, D. Sun, IEEE Trans. Rob. 2013, 30, 68.
[32] K. Kolesnik, M. Xu, P. V. Lee, V. Rajagopal, D. J. Collins, Lab Chip 2021.
[33] S.-Y. Yu, T.-Y. Zhang, Y.-L. Liu, J. Song, D.-M. Han, W.-W. Zhao, D. Jiang, J.-J. Xu, H.-Y. Chen, Anal. Chem. 2021, 93, 6831.
[34] V. Korzh, U. Strähle, Differentiation 2002, 70, 221.
[35] L. Huang, P. Zhao, W. Wang, Lab Chip 2018, 18, 2359.
[36] M. Xie, A. Shakoor, Y. Shen, J. K. Mills, D. Sun, IEEE Trans. Biomed. Eng. 2018, 66, 199.
[37] X. Wang, C. Ho, Y. Tsatskis, J. Law, Z. Zhang, M. Zhu, C. Dai, F. Wang, M. Tan, S. Hopyan, H. Mcneill, Y. Sun, Sci. Rob. 2019, 4, 28.
[38] U. Zimmermann, J. Vienken, G. Piliwat, Z. Naturforsch. C 1981, 36, 173.
[39] D. S. Gray, J. L. Tan, J. Goldman, C. S. Chen, Biosens. Bioelectron. 2004, 19, 1765.
[40] A. Ashkin, M. Dziedzic, J. E. Bjorkholm, S. Chu, Opt. Lett. 1986, 11, 288.
[41] Z. Wang, Y. Hu, J. Wei, W. T. Latt, IEEE Trans. Biomed. Eng. 2019, 67, 2389.
[42] H. Xin, N. Zhao, Y. Wang, X. Zhao, T. Pan, Y. Shi, B. Li, Nano Lett. 2020, 20, 7177.
Y. Sriphutkiat, Y. Zhou, M. S. Sakar, E. B. Steager, D. H. Kim, M. J. Kim, G. J. Pappas, A. Thakur, S. Chowdhury, P. X. Tang, X. Liu, P. Li, D. Liu, M. Kojima, Q. Huang, T. Arai, I. S. Khalil, A. Klingner, Y. Hamed, Y. S. Hassan, S. Misra, S. Kodera, T. Watanabe, Y. Yokoyama, T. Hayakawa, M. Ogiue-Ikeda, S. Ueno, L. V. Panina, A. Gurevich, A. Beklemisheva, A. Omelyanchik, K. Levada, V. Rodionova, Cells 2022, 11, 950.

L. V. Panina, A. Gurevich, A. Beklemisheva, A. Omelyanchik, K. Levada, V. Rodionova, Cells 2022, 11, 950.

L. Feng, P. Di, F. Arai, Int. J. Rob. Res. 2016, 35, 1445.

S. Liu, X. Liu, P. Li, X. Tang, M. Kojima, Q. Huang, T. Arai, IEEE Rob. Autom. Lett. 2021.

Y. Liu, D. Ge, J. Cong, H.-G. Piao, X. Huang, Y. Xu, G. Lu, L. Pan, M. Liu, Small 2018, 14, 1704546.

R. Abedini-Nassab, S. Bahrami, Lab Chip 2021, 21, 1998.

I. S. Khalil, A. Klingner, Y. Hamed, Y. S. Hassan, S. Misra, IEEE Trans. Rob. 2020, 36, 1320.

Q. Zhou, T. Pettit, H. Choi, B. J. Nelson, L. Zhang, Adv. Funct. Mater. 2017, 27, 1604571.

F. Ji, T. Li, S. Yu, Z. Wu, L. Zhang, ACS Nano 2021, 15, 5118.

T. Pettit, L. Zhang, K. E. Peyer, B. E. Kratochvil, B. J. Nelson, Nano Lett. 2012, 12, 156.

X. Tang, X. Liu, P. Li, D. Liu, M. Kojima, Q. Huang, T. Arai, IEEE Rob. Autom. Lett. 2021, 6, 2978.

P. Blümmer, R. P. Friedrich, J. Pereira, O. Baun, C. Alexiou, V. Mailänder, NanoTech., Sci. Appl. 2021, 14, 91.

H. Itoh, A. Takahashi, K. Adachi, H. Noji, R. Yasuda, M. Yoshida, K. Kinosa, Nature 2004, 427, 465.

S. Kim, F. Qiu, S. Kim, A. Ghanbari, C. Moon, L. Zhang, B. J. Nelson, H. Choi, Adv. Mater. 2013, 25, 5863.

T. Wei, J. Liu, D. Li, S. Chen, Y. Zhang, J. Li, L. Fan, Z. Guan, C.-M. Lo, L. Wang, K. Man, D. Sun, Small 2020, 16, 1906908.

I. S. Khalil, A. Fatih Tabak, A. Klingner, M. Sitti, Appl. Phys. Lett. 2016, 109, 031701.

M. S. Sakar, E. B. Steager, D. H. Kim, M. J. Kim, G. J. Pappas, V. Kumar, Appl. Phys. Lett. 2010, 96, 043705.

L. Zhu, W. Huang, F. Yang, L. Yin, S. Liang, W. Zhao, L. Mao, X. Yu, R. Qiao, Y. Zhao, Adv. BioSyst. 2019, 3, 1800246.

V. M. Jooss, J. S. Bolten, J. Huwyler, D. Ahmed, Sci. Adv. 2022, 8, eabm2785.

S. Yang, Z. Tian, Z. Wang, J. Rufo, P. Li, J. Mai, J. Xia, H. Bachman, P.-H. Huang, M. Wu, C. Chen, L. P. Lee, T. J. Huang, Nat. Mater. 2022, 21, 540.

Y. Sriphutkiat, Y. Zhou, Sens. Actuators, A 2021, 332, 113072.

A. Marzo, S. A. Seab, B. W. Drinkwater, D. R. Sahoo, B. Long, S. Subramanian, Nat. Commun. 2015, 6, 1.

S. Deng, K. Jia, E. Wu, X. Hu, Z. Fan, K. Yang, Appl. Sci. 2018, 8, 73.
Xiangyu Guo received his M.Eng. degree in agricultural mechanization engineering from the Chinese Academy of Agricultural Sciences, Nanjing, China, in 2021. He is currently working toward his Ph.D. in agricultural electrification and automation in the Zhejiang University, Zhejiang, China. His research interests focus on the microrobotic manipulation, biomicrofluidic chips, and computer vision.

Youchao Zhang received his B.Eng. degree in agricultural engineering in 2021 from Zhejiang University, Hangzhou, China, where he is currently working toward his Ph.D. in biosystem engineering. His current research interests include micromanipulation and microrobots, deep learning, machine vision, and robots.
**Daoyuan Jin** is an undergraduate majoring in agriculture engineering from Zhejiang University, China. He is currently focusing on robotics and computer vision and is preparing to apply to a graduate school in a relevant field.

**Mingchuan Zhou** received his Ph.D. in computer science from the Technical University of Munich, Germany, 2020. He was the visiting scholar with the Laboratory for Computational Sensing and Robotics, Johns Hopkins University, USA, 2019. He was a joint postdoc with the Helmholtz Center Munich and Technical University of Munich from 2019 to 2021. He is currently an assistant professor leading the micronano robotic manipulation lab in Zhejiang University. His research interests include micronano robotic manipulation, agricultural robotics, medical robotics, and autonomous systems.