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A Review of Recent Advances in Robotic Cell Microinjection

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ABSTRACT Cell microinjection is a direct and effective way to transfer external materials into the cell. In the field of modern biomedicine, cell microinjection is very important, no matter in genetics, reproductive health, tumor therapy or others related research directions. Traditional manual cell microinjection has the disadvantages of low efficiency and low survival rate. In contrast, robotic cell microinjection can achieve precise and effective cell microinjection, and can be used in the injection of large quantities of cells. This paper reviews recent advances in robotic cell microinjection technologies. It summarizes the main approaches of key technologies and their advantages and disadvantages, such as cell identification, cell holding, precise positioning platform, cell injection strategy, sensors in cell microinjection, and cell modeling. The conclusion of the investigation is that the robotic cell microinjection has achieved remarkable results and still has great potential for further development. It is expected to be widely used in biomedicine and other fields to realize convenient, fast, and efficient microinjection operations of large number of individual cells.

INDEX TERMS Robotic cell microinjection, cell identification, cell holding, precise displacement platform, injection strategy, sensors in cell microinjection, cell modeling.

I. INTRODUCTION

With the development of science and technology, researcher begin to explore the mysteries of biology from the microscopic fields such as cells and DNA. According to people's needs and designs, the cell structure and contents are recombined at the cell level via cell fusion, chromosome transplantation, cell injection and other operational methods, so as to improve the biological structure and function. Such operation has been widely adopted in biomedicine, gene therapy, reproductive research, and other fields [1]–[3]. The process of transferring external materials into cells can be divided into three major approaches (see Table 1), i.e., biological, chemical, and physical methods [4]. Biological methods refers to the use of living carriers (usually, viruses) to transport substances into cells. Kotterman *et al.* [5] explored the clinical transformation by using viral vectors in gene therapy. This method is simple and convenient, but it often causes side effects on cells. Specifically, it cannot control the amount of substances transported, and also cannot achieve non-manual automatic control. Ramamoorth *et al.* [6] summarized some non-viral vectors currently used in cell gene therapy, whose dominant advantage is safety. Unfortunately, none of the currently available non-viral vectors fulfills ideal vector properties. Chemical methods use natural or synthetic agents to deliver substances into cells [7]. The main problem with this approach is that many large molecules cannot penetrate cell membranes. Similarly, there is no precise control over the amount of substance transferred.

In contrast, the physical method has some obvious advantages. Commonly used physical methods include microinjection, electroporation, ultrasonic, and gene gun [8]. As early as the 1980s, researchers used pulsed electric fields (PEF) to introduce genetic material into living cells or promote cell fusion, with some success. When a voltage is applied to one or more cells, the cell membrane exhibits permeability, which allows the substances to be introduced into the cell [9]. Electroporation is also a physical method of introducing material into a cell by exposing the cell to potential across the cell membrane, which can create permanent or transient

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TABLE 1. Classification of methods for transferring foreign substances into cells.

Type	Methods
Biological	Viral vector Non-viral vector
Chemical	DEAE-dextran Artificial lipids Proteins Other compounds (natural and synthetic)
Physical	Microinjection Electroporation Ultrasonic Gene gun Other physical methods

pores in the cell membrane [10]. However, electroporation has limited application at present, because researchers have not fully grasped its principle, which constrains the accurate control and application of this technology [11]. Ultrasonic is a method of trying to introduce substances into cells by using ultrasound to enhance the permeability of cells. However, currently, this method is still in the theoretical stage, because many factors have to be considered, such as signal frequency, acoustic pressure, input duration, and so on [12]. The gene gun is particle bombardment essentially. An accelerator is used to accelerate the DNA particles and bombard the target cells, so as to realize the purpose of introducing the DNA particles into the cells. As the name suggests, the substances delivered in this way are genes. At present, it is mainly used in the field of gene therapy, gene regulation, and gene immunity [13].

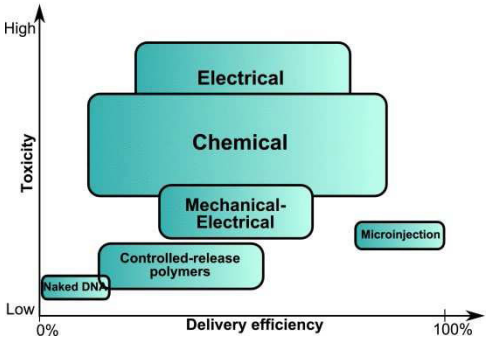


FIGURE 1. The comparison of available DNA intradelivery methods in terms of delivery efficiency vs. toxicity level [8].

Cell microinjection is one of the most widely used physical methods to transfer substances into cells. The idea is to use a fine needle to penetrate the cell membrane and inject material into the cell. As shown in Fig. 1, in comparison with other methods, microinjection has obvious advantages such as high delivery efficiency and low toxicity [8], [14]. The traditional method is manual cell microinjection. The manual method is simple and easy to operate, but its success rate largely depends on the professional level and proficiency of the operator. On the other hand, it is also affected by fatigue, working hours, and other factors. These issues mean that manual methods can only be used for small number of operations for single-cell injections [15]. With the development of automatic control technology and micro/nano-positioning platform, robotic cell microinjection method has been realized, which can meet the requirements of biological sciences, to complete fast and accurate microinjection tasks of a large number of cells [16], [17].

This paper is focused on the development progress of robotic cell microinjection technologies. An overview is given to show the technical challenges to be solved in cell microinjection system, including cell identification, cell holding, precise motion platform, injection strategy, sensors in cell microinjection, and cell modeling, which are presented in Sections II—VII. Section VIII summarizes the paper and presents the challenges and outlook for robotic cell microinjection process.

II. CELL IDENTIFICATION

For cell microinjection, the first problem encountered by researchers is how to identify the cells. The material is injected into a probe with an inner diameter of 1–5 μm for the needle tip, and the needle is then inserted through the cell membrane into a cell with a diameter of 20–30 μm, or into the nucleus with a diameter of 5 μm [18]. The traditional method is to make use of multistage optical amplification system (such as, microscope) to enlarge the operating site for the operator to observe, and then conduct manual positioning [19]. This method is not efficient and accurate. In recent years, computer vision based control system has been applied in cell identification, providing new technical support for rapid and accurate identification of a large number of cells. Especially, since the beginning of the 21st century, with the significant improvement of computer power, new computing methods have been put forward constantly, the available data resources have been greatly increased, and new application modes have emerged [20].

Generally, after the grayscale conversion, noise filtering, and enhancement processing, the image data transferred into the computer memory by the image acquisition card should be used to identify each cell in order to conduct the cell injection operation, which requires image segmentation technique. Image edge detection is an important basis for image segmentation. It is an image processing technology to make the image's outline more prominent. Edge detection is usually carried out before feature extraction and contour extraction [21], [22]. Sobel edge detection is an efficient edge detection algorithm, which conducts the convolution operation on each point by using horizontal edge operator and vertical edge operator. The two operator templates work independently, and the maximum value of two convolution results is taken as the output value of this point [23]–[25]. There are some other common methods, such as the Canny algorithm [26], Hough transform [27], snake model [28], and so on.

The basis of image segmentation is the similarity and jump between pixels. ''Similarity'' means that the pixels in a cer-

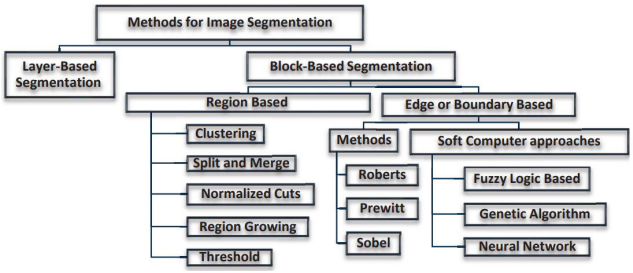


FIGURE 2. Methods of image segmentation [30].

tain area have some similar characteristics, such as grayscale. ''Jump'' refers to the discontinuity of characteristics, such as a sudden change in grayscale value. Image segmentation is a processing technique to divide an image into several meaningful regions. These regions do not overlap each other, and the grayscale value within each region is similar. The grayscale value between different regions is obviously different [29]. As shown in Fig. 2, Zaitoun and Aqel [30] conducted a survey on the current mainstream image segmentation technologies, among which the cell-oriented image segmentation technology is mainly threshold segmentation technology based on block segmentation. Threshold segmentation takes advantage of the grayscale difference between the object to be extracted and the background [30], [31]. This is, selecting an appropriate threshold, determining whether the pixel in the image belongs to the target area or background area by judging whether the grayscale attribute of each pixel in the image meets the threshold requirements, and thus generating binary image.

When using threshold segmentation method, choosing an appropriate threshold becomes the key step of proper segmentation. Firstly, the grayscale information of the image is statistically obtained from the grayscale distribution histogram of the image. As there is a clear difference of the grayscale between the object and background, there will be two obvious peaks in the histogram of grayscale distribution. Generally, the minimum value between the two peaks is selected as the optimal threshold [32]. After the cell threshold segmentation in the image is completed, the contour of the cell must be extracted so that the cell injection control system can accurately identify a single cell. Because majority of suspended cells are usually considered to have spherical or nearly spherical shape, they are often identified by Hough transform approach. This method has the advantages of fast calculation speed and high identification efficiency [33]. The recognition process of cell images is shown in Fig. 3 [34].

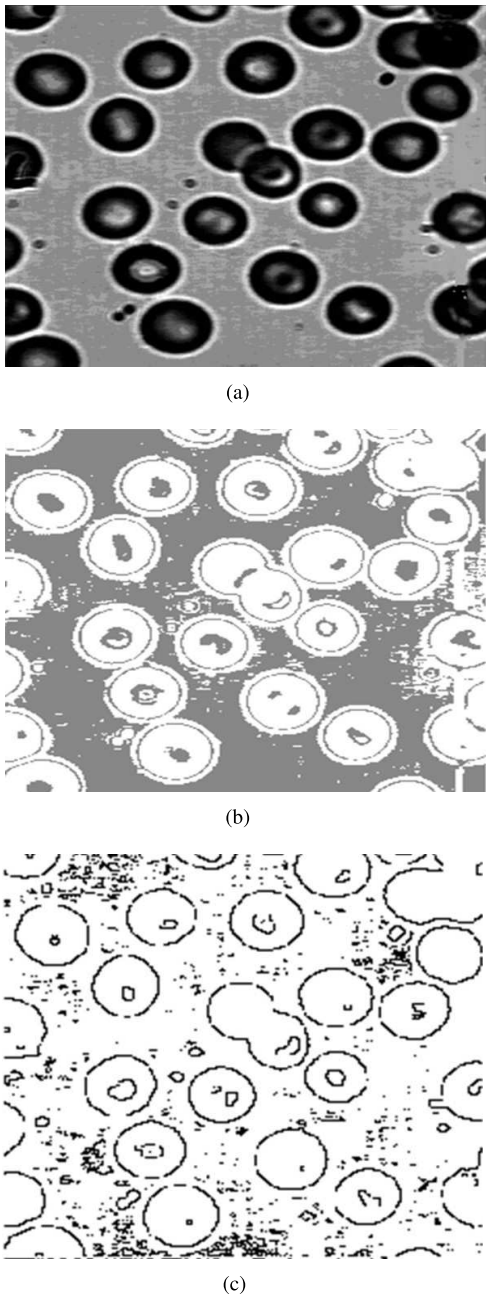


FIGURE 3. Image processing for cell identification [34]. (a) Grayscale image of cell; (b) threshold segmentation of cell image; (c) cell image after contour extraction.

III. CELL HOLDING

The cells need to remain alive in the culture medium. The cells are required to be adjusted to proper position during the injection, and then remain stable. It is one of the prerequisites for ensuring that the cells can remain alive during and after the microinjection. Therefore, cell holding is an important part of the robotic cell microinjection system. Many methods have been proposed in the research. The commonly used methods can be divided into two categories in terms of contact and non-contact methods.

In the contact cell-fixation method, the researchers first used a micropipette with a certain vacuum suction. For example, Lu *et al.* [35] used a micro-suction micropipette with low sucking force to inhale a small part of cells to achieve the purpose of fixing cells. However, this method can only handle one cell at a time, and it requires high precision and suitable sucking force. Otherwise, the cell will be damaged. Current operations require the ability to process

cells in batches. Thus, many multicellular stationary platforms have been proposed. Lu *et al.* [36] proposed a fixing platform with multiple V-shaped grooves. However, if the puncture track of the injecting needle is not perpendicular to the fixing platform, the cells will move and the success rate of injection will be reduced. Researchers have also proposed to use multiple circular grooves as "cell trap" to hold cells in place. But this approach has some limitations [37]. Specifically, if the hole is too small, the cells will leave the culture, raising the risk of cell death. If the groove of the round hole is too large for allowing the cell to be wetted, the cell will move and affect the injection accuracy. Alternatively, as shown in Fig. 4, Liu and Sun [38] designed a circular groove, which is connected to a vacuum source for fixing cells. It not only ensured the stability of cells, but also improved the cell survival rate.

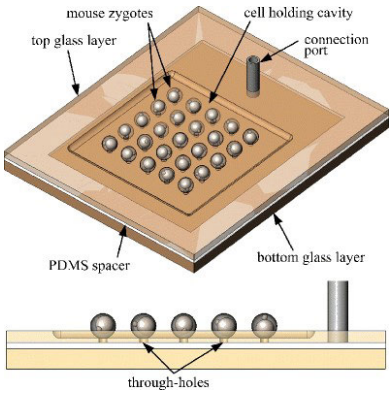


FIGURE 4. Illustration of the vacuum-based cell holding device [38].

Non-contact methods are mainly used to adjust the posture of cells. Microfluidics, dielectrophoresis, external magnetic field, and optical tweezer are popular approaches at present. Microfluidics method mainly uses microfluidic flow to generate forces for acting on cells, e.g., fixing cells and adjusting their postures. This method does not need to contact cells and cause little damage to cells, whereas it has the disadvantages of complex process and low efficiency [39]–[41]. For example, Shin *et al.* [42] designed a microfluidics system to control the rotation and postures of mouse embryonic cells through the hydrodynamics in microchannel, which is shown in Fig. 5. The method of dielectrophoresis uses electric field force to act on cells. This method has high accuracy and fast response, but the electric field will also affect the injection equipment, which should be considered [43]. Similarly, although the method of using magnetic force to control cells is convenient and highly accurate, it will also affect the working accuracy of other devices [44], [45]. Optical tweezer provides a method to manipulate cell. In particular, a laser beam is used to form a light trap, which exerts a force on the cell due to the refraction, reflection, and absorption of the light. It can move and rotate the cell by changing the focus of the laser beam [46], [47]. Zhou *et al.* [48] summarized the current application of laser capture technology in biology,

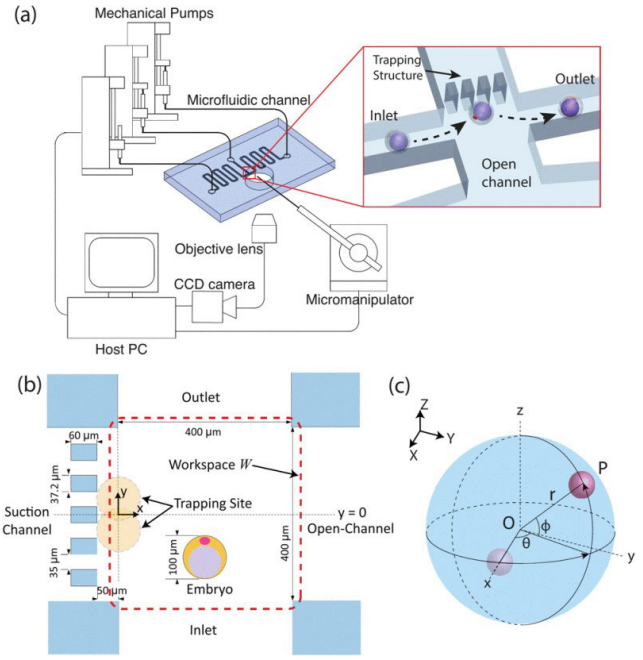


FIGURE 5. Illustration of (a) microchannel, (b) system configuration, and (c) coordinate description of the polar body orientation [42]. The positions of the polar body and cell are denoted by points P and O, respectively.

and compared the working principles and unique functions of various optical tweezers.

IV. PRECISE MOTION PLATFORM

When the cells to be injected are identified and immobilized, the syringe needle needs to be moved to a suitable position using a motion platform that can achieve accurate positioning for successful microinjection of the cells. During the whole injection process, precise motion control is required to ensure that the microinjection needles can move in and out of cells quickly without damaging the cell wall and cell structure. The average diameter of most animals is between 10 and 200 microns. Hence, cell microinjection requires precise motion platforms with displacement resolution at the micro- and nanometer level. The micro/nano-positioning platform is mainly composed of two parts, i.e., the actuator that provides the output displacement/force and the flexible mechanism that transmits the displacement/force.

As shown in Table 2, Zhao *et al.* [17] summarized the advantages and disadvantages of current popular actuators. Among them, piezoelectric ceramic actuator has the advantages of high stiffness, large blocking force, fast response speed, and being applicable to high frequency. It has become the main actuator applied to the micro/nano-positioning platform [49], [50]. The piezoelectric actuator utilizes the inverse piezoelectric effect of piezoelectric ceramic materials to produce force or displacement output by using electric energy input. However, piezoelectric actuators also have significant drawbacks, i.e., hysteresis, creep, and vibration [51]. It presents a challenge for the precise motion control of piezoelectric actuator. Hysteresis is a very complex nonlinear

TABLE 2. Comparison of the main types of actuators [17].

Type	Operational Principles	Performance Features	Precision
Direct current motor	Electromagnetic effect	Fast response but large force and displacement	Submicron
Piezoelectric ceramics	Piezoelectric effect	Applicable in a wide range of frequencies but insensitive to temperature No magnetic field influence but exhibits hysteresis	Sub-nanometer
Electrostrictive ceramic	Electrically induced effect	Fast response but small force and displacement	Sub-nanometer
Shape memory alloy	Metal phase change	Slow response and small force and displacement	Nano
Magnetostrictive material	Magnetic effect	Good reliability, simple driving mode but exhibits hysteresis, low precision, poor response, and a tendency to overheat	Sub-nanometer
Giant magnetostrictive material	Magnetic effect	Fast response but large force and displacement	Sub-nanometer
Magnetic effect	Piezoelectric effect Ultrasonic oscillation	Fast response speed but large force and displacement	10 nm (linear) Seconds (rotary)

phenomenon. The output displacement of the piezoelectric actuator is related to not only the current input voltage, but also the historical state of the system. For the same input signal, due to different historical state and the change of input frequency, the output signal is not the same [52].

The control methods for producing accurate displacement output of piezoelectric ceramic actuator can be divided into two main categories [53]–[55]. The first category is to build a hysteresis model, and then, the inverse model according to the hysteresis model. The inverse model is used as the feedforward compensation for the nonlinearity caused by hysteresis. Common hysteresis models are shown in Fig. 6. Then, other problems, such as creep and vibration, can be regarded as the disturbance of the system, and some closed-loop control methods (such as, fuzzy-PID controller [54]) can be used to realize the control. On the other hand, the second category of methods considers the nonlinearities of piezoelectric ceramic actuators, such as hysteresis, creep, and vibration, as a lumped disturbance of the system. And then, feedback controllers are designed to achieve accurate control. Recently, the commonly used feedback control methods include sliding mode control, neural network control, fuzzy control, or combined control with several controllers [56]–[60]. With the development of computer technology, more and more intelligent control methods will be developed.

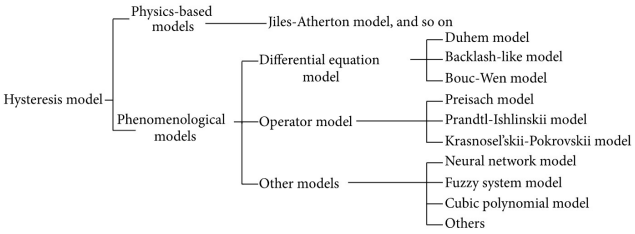


FIGURE 6. The classification of hysteresis models [42].

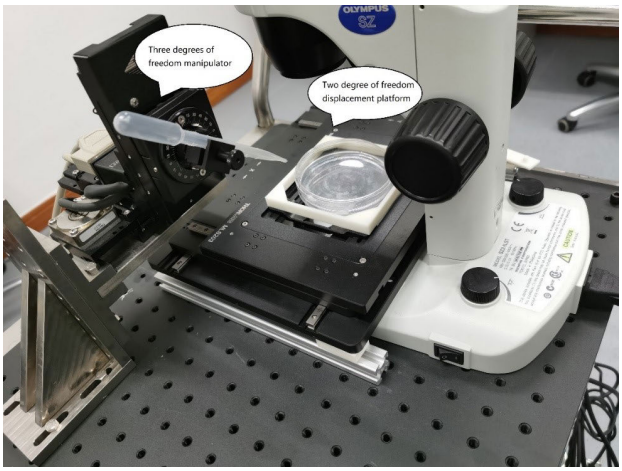


FIGURE 7. Prototype of XYZ microinjection manipulator with XY motion platform.

As a transmission structure, flexure hinge can transfer the driver's output force and displacement. At the same time, according to the design of flexure hinge, the output displacement can be enlarged and the motion direction can be adjusted to form a precise platform. According to different mobility of the flexure hinge, displacement platforms with 1, 2, 3, and 6 degrees-of-freedom (DOF) have been constructed [49], [61]–[63]. In cell microinjection platform, the XY motion platform with two DOF is mainly used to regulate the petri dish position, and the XYZ motion platform with three DOF is dominantly adopted to control the injection needle, as shown in Fig. 7. Alternatively, with an additional motion in injecting direction (T), the XYZT injection needle can achieve four DOF, as shown in Fig. 8.

V. CELL INJECTION STRATEGY

In robotic cell microinjection, how to quickly and effectively complete the puncture and injection while ensuring the survival of the cells is an important key technology [64]. The cell wall is a thick, tough, and slightly elastic structure outside the cell membrane. It is divided into mucous complexes, and some species also have a protective capsule made of polysaccharides outside the cell wall. Membrane is mainly composed of phospholipid elastic semi-permeable membrane. Its thickness is generally of 7–8 nm. For animal cells, the outer membrane is in contact with the environment. Its main function is to selectively exchange substances, absorb nutrients, expel metabolic wastes, secrete and transport proteins [65], [66].

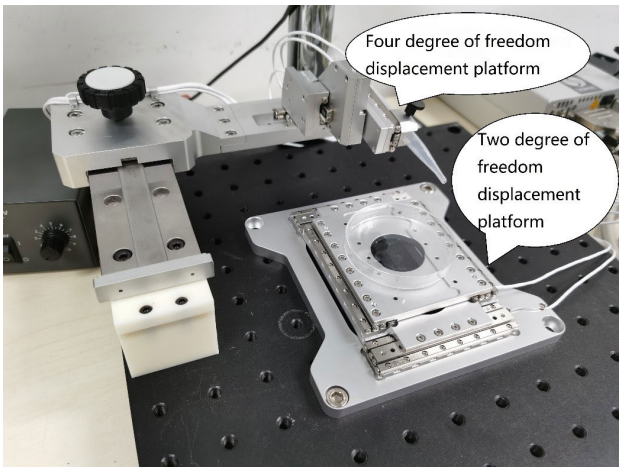


FIGURE 8. Prototype of XYZT microinjection manipulator with XY motion platform.

The needle used for cell microinjection is usually glass microneedle. The microneedles for cell injection are made of borosilicate materials by capillary stretching. For normal cell injection, the pore diameter of the tip is 0.5–4 μm, the pore diameter of the tip is 4–5 μm in cytoplasmic single sperm injection, and the pore diameter of the tip is around 40 μm in sperm stem cell transplantation. At the same time, in order to complete the injection action, the microinjection needle is required to have sufficient strength. The capillary tubes used for drawing microneedles require good strength and toughness when they are drawn to the required size of the microneedles. According to the glass forming theory, the viscosity value of the glass liquid determines the temperature range of the glass forming, and the curing speed governs the duration of each operating procedure in the glass forming. An improper control of the drawing process will lead to the bending of the needle tube, resulting in gas lines, streaks, stones, crystallization, and other phenomena at the point of the needle tube. The heating temperature, heating time, stretching speed, sequence, and time interval between heating and stretching are all key process parameters [67]–[70]. There are some well-known manufacturers in the world, including Sutter Instrument Company and Nikon Corporation.

To inject a substance into a cell, the needle must penetrate the cell walls and membranes with sufficient speed and force. For instance, to penetrate the cell safely, the tip of the needle must travel at 700 μm per second [71]. The force required for puncturing the cells is also different at different times. For example, Kim *et al.* [72] reported the difference between zebrafish embryonic cells at different times. The average force required to puncture the chorionic membrane in the blastocyst stage was 1.3 times that in the preincubation stage. The elastic modulus of chorionic membrane in blastocyst stage was 1.66 times that in early incubation stage. Therefore, reasonable puncture strategies should be designed to improve the success rate of microinjection for different cells.

Based on vibration or non-vibration method, there are three commonly used puncture strategies, as shown in Fig. 9 [17].

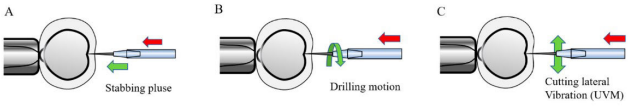


FIGURE 9. Schematic diagram of (a) piezo-driven pipette puncture [73]; (b) drilling puncture [74], [75]; (c) lateral vibration puncture [76].

For example, Kimura and Yanagimachi [73] successfully achieved intracytoplasmic sperm injection by using piezoelectric actuators instead of traditional mechanically driven micropipette (see Fig. 9(a)). After injection, the survival rate of oocyte reached up to 80%. Due to the use of piezoelectric actuators, this method has high intensity and good resolution and is easy to control. However, there is an obvious problem with this method. That is, if the injection direction is longitudinal, the piezo-driven micropipette will generate lateral vibration during the movement, which is easy to hurt or even kill cells. In this case, the work [73] used a high-density liquid (mercury) to reduce the vibration. Mercury has the advantage of high density and low cost. The load effect of mercury significantly reduced the natural frequency of shaking and effectively suppressed the lateral vibration of the needle. However, the disadvantage is that mercury may contaminate cells. In the literature [78], [79], researchers supported that mercury column can effectively reduce the vibration through simulation and experiment. Fig. 9(b) shows a drill puncture approach called the ''Ros-Drill'' (rotationally oscillating drill). Instead of direct puncture, micro-motor is used to rotate the microinjection needle during puncture without mercury, so as to reduce the damage to cells [74]. Johnson *et al.* [75] designed a new type of piezoelectric drill device, which uses a unique flexible guidance mechanism and pulse sequence to greatly reduce the lateral vibration of the microinjection needle and significantly reduce the cell deformation when penetrating the zona pellucida (ZP) of cells. Fig. 9(c) shows another puncture method. Huang *et al.* [76] used this vibration approach to generate an ''ultrasonic cutting'' method for cell puncture, which is called ultrasonic vibration microdissection (UVM). This approach achieved a success rate of 96%. Huang *et al.* [77] designed a new piezoelectric driving injection device, as shown in Fig. 10. This piezoelectric-drive injector design focuses on piezoelectric oscillating power on the injector pipette, eliminating vibration effects on

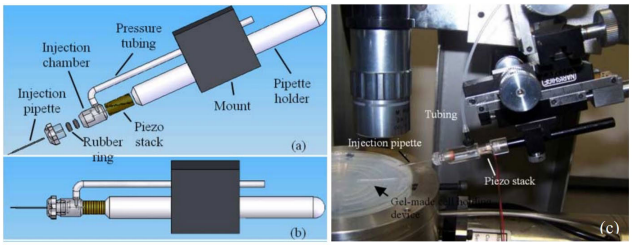


FIGURE 10. A new design of piezo-driven cell injector [77]. (a) Exploded drawing; (b) assembly drawing; (c) prototype of the piezo-driven cell injector.

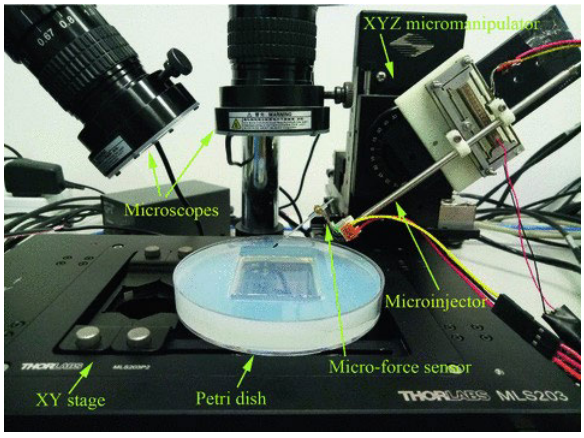
other components of the micromanipulator. The experimental results obtained by injecting zebrafish embryo cells were satisfactory. Wang and Xu [80] designed a force-assisted piezoelectric-actuation micro-injection system. Cell penetration and microtubule's relative position were measured in real time using specific sensors. During the injection, displacement control and force control were separated from each other, and the injection success rate of zebrafish embryo cells was increased up to 86%.

Through the combination of micro/nano-positioning platform and puncture strategy, the microinjection needle can successfully reach the expected position. The next step is to inject the substance. Animal cells are about 20 μm in diameter and nuclei are about 5 μm in diameter. The resolution of injected substances is on the 10^{-8} microliter scale. The ratio of length to diameter of microneedles for injection is very large. The traditional method is to inject the material into cells by hand or by oil pressure. Due to the difficulty of force control, in many cases, the amount of excessive liquid medicine is too large and the cells will be squeezed out of shape. Otherwise, the pressure of small liquid medicine does not come out. These methods have a certain degree of damage to the cell, and then have an impact on the cell's mechanism and function, affecting the direct results of many bioengineering experiments [81]–[83].

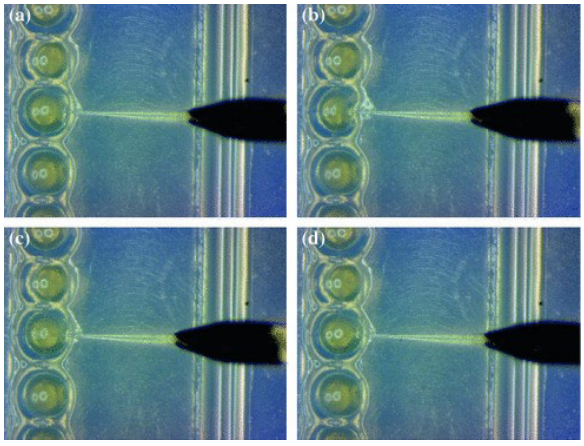
Commercial cell injectors with pneumatic devices have been provided (e.g., by Sutter Instrument Company and Nikon Corporation) to achieve quantitative injection of cells under manual adjustment and microscopic observation. This kind of equipment is very dependent on the skill level of the operator, and has problems such as large size and complex structure. Another approach is to use silicon lithography to make microfluidic components, such as microvalves and pumps, and then to build microfluidics systems for quantitative injection of cells [84], [85]. However, this method does not produce enough pressure to push the injection material beyond the critical injection state of the tip when the hole diameter is less than 4 μm. The application is not widespread, and the cost is high [86]. Precise single-cell microinjection can be achieved by controlling dosage according to injection pressure and time [87]–[89]. Now researchers have devised a way to inject tiny amounts of piezoceramics with tiny changes in volume, taking advantage of the micro/nano-meter motion property. The injection amount can be controlled by the driving voltage's amplitude and frequency as well as driving time of the piezoelectric ceramic actuator, which has good reliability and repeatability [90], [91].

VI. SENSORS IN CELL MICROINJECTION

In automated cell microinjection platform, two types of sensors are mainly involved, i.e., vision sensor and force sensor. Current injection platforms use microscopes as visual sensors to collect images. The target coordinates are obtained by image processing, and the injection tip is moved to the required position by the visual servo system with real-time guiding platform [93]–[95]. However, one microscope



(a)



(b)

FIGURE 11. (a) Experimental setup for a custom-built cell microinjection system with dual cameras; (b) photograph sequences of the cell piercing process for zebrafish embryos [92].

can only capture the images on a flat surface, some "depth of field" during injection cannot be accurately obtained [96], [97]. Thus, researchers have integrated microforce sensors to monitor the injection process and to improve the success rate of injection [92], [98]–[101]. For example, Nan *et al.* [98] combined the pyramid template matching algorithm with Kalman filter, guided the microinjection location of cells with a visual sensor, and added a force sensor to determine the exact time of penetration into cells, thus achieving multi-cell automatic injection. As shown in Fig. 11(a), Xu [92] built a complete system for cell microinjection with microforce injector under visual servo control. Fig. 11(b) shows the whole process of cell injection of zebrafish embryo using this system.

In cell microinjection, a highly sensitive force sensor is required, as cell loading force is generally in the order of μN or less. There are many kinds of microforce sensors for cell microinjection platforms. The first method uses the direct piezoelectric effect of the piezoelectric material to transform the force into a deformation of the sensor, and then produces an electrical signal output, through which the force can be

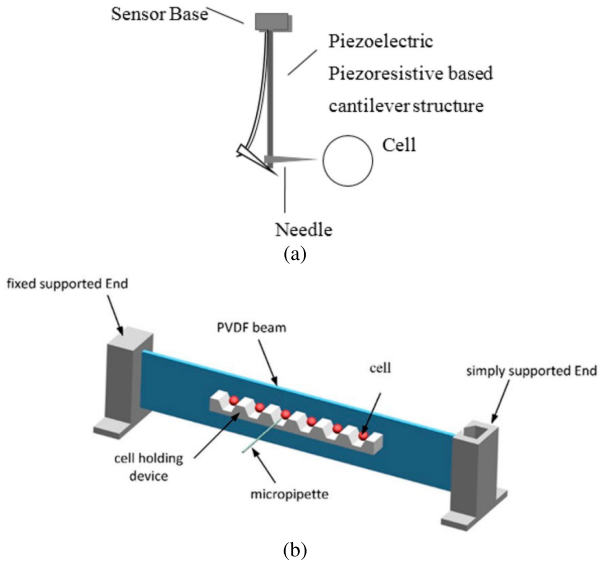


FIGURE 12. **Piezoelectric microforce sensors [102]. (a) Probe based microforce sensor; (b) PVDF beam based microforce sensor.**

obtained. As shown in Fig. 12(a), piezoelectric materials are used to construct a flexible beam structure for transforming the force exerted on the needle into the deformation. Xie *et al.* [102] proposed a cell microforce transducer based on piezoelectric polyvinylidene fluoride (PVDF) thin film support beams (see Fig. 12(b)). In addition, many microforce sensors have been proposed using PVDF materials, even in 3D form [103]–[105]. However, PVDF microforce sensor also has obvious shortcomings. For example, PVDF deformation may cause damage to cells. In addition, the vibration of injection needles and the movement of cells will also affect the measurement accuracy [106]. In 1996, NASA introduced a new piezoelectric material called macrofiber composites (MFCs). It is compact in structure, flexible in motion, and produces a larger output of electrical signals with the same input force. MFCs are rarely used in cell microinjection alone. Recently, Xu [106] proposed a new type of microforce sensor combined with PVDF and MFC, which was verified by experiments. It is able to smoothly monitor the process of cell microinjection and stably measure the puncture force of 27 mN. The structure also serves as a cell holding device in the system.

The second method adopts piezoresistive force sensor. As the input force changes, so does the resistance of the piezoresistive force sensor. Resistance is generally measured by Wheatstone bridge method. In the literature, Lu *et al.* [36] designed a system for batch operation of cell microinjection, as shown in Fig. 13(a). The microforce sensor is a commercial piezoresistive force sensor (model AE801, from Sensor One Technologies Corporation). Fig. 13(b) shows the improved structure of the microinjection needle with piezoresistive force sensor. Such microforce sensors are the most cost-effective, but errors may occur during assembly, and factors such as temperature also affect the coefficient between pressure and resistance [107].

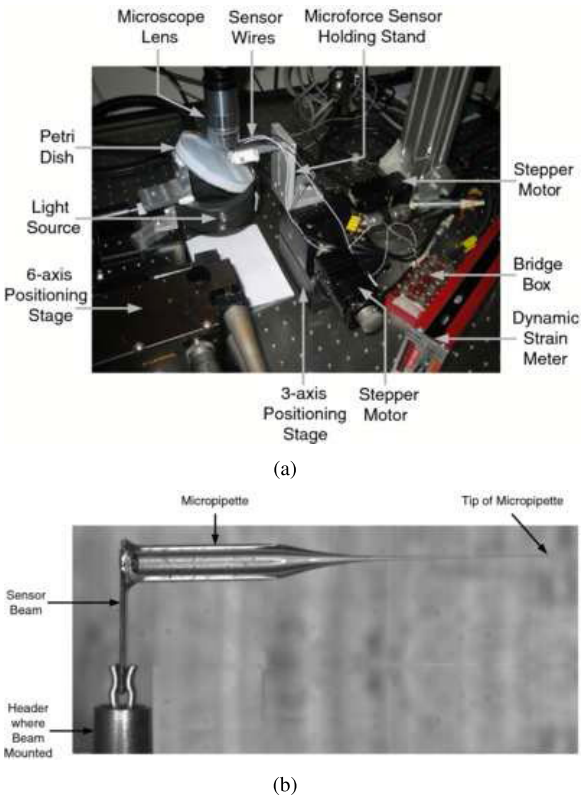


FIGURE 13. **(a) The micromanipulation system for batch microinjection [36]; (b) the modified piezoresistive microforce sensor with a micropipette [36].**

Capacitive force sensors based on microelectromechanical systems (MEMS) are also widely used in cell microinjection technology [108], [109]. Small change in force can be converted into the change in capacitance of the capacitive force transducer. MEMS capacitive force can sensitively detect the change of force ranging from pN to mN level [15]. It can be used as an ideal microforce sensor for cell microinjection. In theory, the value of capacitance is proportional to the area of the capacitor plate, and inversely proportional to the distance of the capacitor plate. Therefore, capacitive force transducers can be divided based on the two operating principles. The first one is the sensor in transverse mode, which changes the capacitance by changing the distance between the plates. On the other hand, the sensor in lateral mode (with comb-drive movement) changes the capacitance value by changing the overlapping area of the capacitor plates. In practice, the two methods can be combined to obtain a higher degree of design freedom. Capacitive force sensors with different DOF are summarized in reference [15] (see Fig. 14). At the same time, this kind of sensor has the characteristics of wide measurement range, high sensitivity, and low unit cost. However, capacitive force sensors have some limitations, i.e., they tend to be fragile, have a short service life, and are difficult to be equipped with microinjection needles.

As a typical non-contact force sensor, optical microforce sensor exhibits more applications in recent years. The sensor

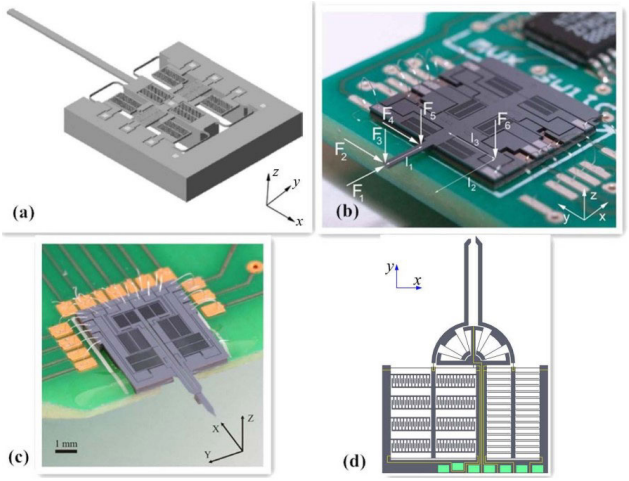


FIGURE 14. Structures of typical capacitive force transducers [15]. (a) Two-axis force sensor, (b) three-axis force sensor, (c) six-axis force sensor, and (d) single-capacitance force sensor for two-axis force sensing.

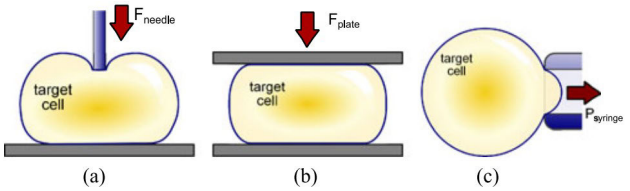


FIGURE 15. Three ways of applying force on cells. (a) Point force, (b) plane force, and (c) micropipette suction force [8].

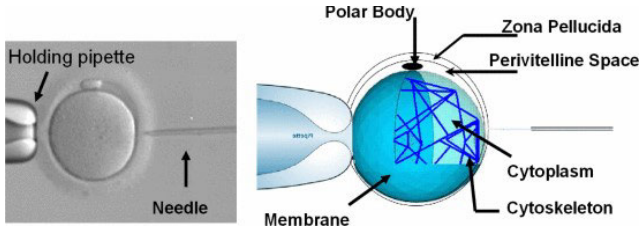


FIGURE 16. Biological oocyte injection and its multilayer mechanical model [131].

has high sensitivity, anti-electromagnetic interference, small size, and is not subject to the problem of too small operating space [110]–[114]. In some fields, it has been regarded as an alternative to electric force sensors. The current main microforce sensor methods are summarized in the literature [115]. However, optical force sensors are easily affected by the reflection and refraction of liquid media (such as in petri dish). At the same time, cell absorption of light energy and other reactions may cause cell damage [116]. At present, it has been presented that near-infrared light can be used to reduce this effect [117]. In conclusion, the application of optical microforce sensor in cell microinjection platform is not widespread, and more development is needed in the future.

VII. CELL MODELING

In the process of puncture, the force of the microinjection needle exerted on the cells will inevitably cause the deformation of the cells. In order to guarantee the success rate of injection and prevent cell damage, a quantitative relationship between applied force and deformation can be established [118]–[120]. However, the physical property of living cells is typically non-linear over time and it cannot be described as simple objects of control. In the past year, researchers have been working to build reliable cell models for microinjection by squeezing cells and other methods, combined with various sensors [121]–[123]. Different methods of applying force, different mathematical parameters and assumptions were used to obtain different cell models. As shown in Fig. 15, there are three classic mainstream methods, i.e., point force, plane force, and micropipette force, which are conducive to the traversal test.

Plane force method is mainly used to squeeze cells with parallel plates to measure the young's modulus, cell rupture strength, and elastic compression modulus of cells. This method is adopted to obtain the stress of cells under large deformation [124], [125]. The micropipette force method is to apply a force in a negative direction to the cell through the suction of the micropipette. This method can test how much the cells deform under greater force [126]–[128]. The above two methods are not commonly used in the modeling of cell microinjection, because the first method is more similar to the action in cell microinjection by applying pressure on the cell point. Kuznetsova *et al.* [129] used an atomic force microscope to simulate "point forces" acting on cells to test their elasticity and to obtain specification data such as Young's modulus. Tan *et al.* [119], [130] proposed a cell microinjection model based on membrane theory. PVDF polymer film was used as a microforce sensor to measure the changes during microinjection of cells, and the force-deformation relationship under different slant caused by different elastic modulus in the model was verified, indicating that the slant played a leading role in the mechanical properties of the injection unit. Ladjal *et al.* [131] presented a multilayer mechanical model of biological oocytes for microinjection in their study, as shown in Fig. 16. Then, the three-dimensional finite element tensegrity model of biological oocyte was established (see Fig. 17). Through qualitative and quantitative experiments, the model is applied to the simulation and practice of cell microinjection.

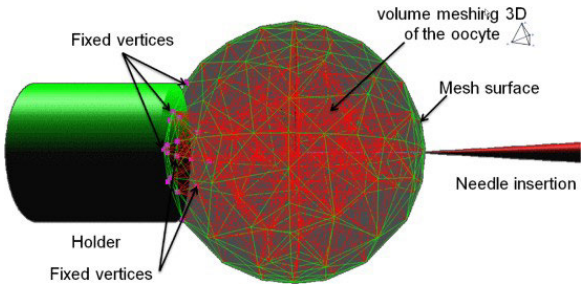


FIGURE 17. Finite element model for mouse oocyte microinjection [131].

VIII. CONCLUSION AND OUTLOOKS

This paper provides a brief review of the development of robotic cell microinjection technology in recent years. The key technology is introduced from six issues including cell identification, cell holding, precise motion platform, cell injection strategy, sensors in cell microinjection, and cell modeling. For the current progress in each area, it makes a straightforward classification and summarizes the advantages and disadvantages of different approaches. It can be seen that, thanks to the introduction of new methods and the application of new equipment, robotic cell microinjection technology has made significant progress in recent years, and the efficiency and success rate of cell microinjection have been greatly improved. However, there are also many shortcomings that need continuous efforts of researchers, as summarized below.

(a) The efficiency of batch injection. The main reason for replacing manual injection with robotic cell microinjection is to improve the injection efficiency. Efficiency includes injection speed and success rate. In order to improve the injection speed, there is still great potential in cell recognition, micro/nano-positioning, and other technologies. Cell recognition mainly depends on machine vision, faster communication cameras and more optimized visual recognition algorithms are the main prospects for improving the speed of cell recognition. The more responsive displacement platform can be studied from the aspects of control algorithm, mechanical design and new materials. On the other hand, to ensure the success rate, reasonable injection strategies, accurate cell modeling, and other research advances are needed. The combination of force-assist and machine vision, human-computer interaction and cooperation, are promising development directions in improving injection success rate.

(b) The combination of force-assisted technology and visual servo system. More and more researchers have found that there is certain ''blind area'' in the process of simple visual control injection, so the use of force assistance can significantly improve the observation of the injection process and enhance the success rate of injection. Force-assist requires more accurate microforce sensor. Existing microforce sensors are designed by finding a balance between the measurement accuracy and structural complexity, which is also a potential research direction.

(c) Application of new technology. Many of the new technologies mentioned in this paper are still in the experimental process, and have not been applied in a large number of cell microinjection experiments. For producing mature commercial products, more problems need to be overcome, such as the influences of the actual operating environment, platform jitter, and other interference. At the same time, the integration of cell microinjection system is also a major problem. A system with compact structure, high degree of integration and easy implementation will greatly promote the application of new technology.

(d) The extension about manipulation objects. It is divided into micro-expansion and macro-expansion. Micro-expansion refers to more precise microinjection of cells. The current trend is to achieve the injection of precise parts of cells, such as nuclear injection. This puts forward higher requirements for the micro/nano-positioning platform, vision sensor, and other technologies. The other one is macro-expansion, i.e., robotic injection of different kinds of cells or even living organisms. Facing different injection objects, different requirements are put forward for system modeling, image recognition, injection strategy, and other technologies.

These challenges will continue to drive the development of robotic microinjection technologies. In near future, more accurate, more efficient, and more convenient robotic cell microinjection systems will be widely used in biomedicine, health care, genetics, and other fields.

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