Chapter 2
Review of Microinjection Systems

Abstract Cell microinjection plays an important role in genetics, transgenics, molecular biology, drug discovery, reproductive studies, and other biomedical fields. Robotic cell microinjection has been popularly applied due to its high precision, high repeatability, and high throughput. In this chapter, the state-of-the-art research on microinjection of both adherent cells and suspended cells with microforce-sensing techniques is reviewed. The challenges and promising methods in automating the cell microinjection process are discussed.

2.1 Introduction

2.1.1 The Role of Cell Microinjection

The purpose of cell microinjection is to introduce small volume of foreign materials into living cells [36, 37]. Since its introduction in the first half of the last century, injection of foreign materials (e.g., DNA, RNAi, protein, sperm, toxins, and drug compounds) into single living cells has been broadly applied in genetics, transgenics, molecular biology or drug discovery, reproductive studies, and other biomedical areas [14, 65, 99, 108].

In genetics, the genetic sequence of human DNA has been confirmed, and a total of about 30,000 genes were identified in the first phase of the Human Genome Project [69]. Confirming the gene sequence and identification of the genes is only the first step. The functions of each gene and produced proteins should be identified in the next step. Cell injection plays a crucial role in the acceleration of the second step in the Human Genome Project by transferring microfabricated DNA microarrays which prominently increase the quantity of experimental data [118]. An important technique for identifying gene functions is RNA interference (RNAi) through microinjection [10, 115]. RNAi was firstly stated in 1998 by manually injecting double-stranded RNA (dsRNA) [115]. dsRNA expresses endogenous enzymes to recognize, break the corresponding messenger RNA (mRNA), and hence silence the gene function. RNAi is regarded as a potential therapeutic strategy too [93]. A change in phenotype demonstrates the function of the silenced gene.

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In transgenics, the injection of DNA is utilized to produce transgenic zebrafish lines [99]. Injection of mRNA is applied to overexpress gene products in zebrafish embryos. In addition, the loss-of-gene-function studies need the injection of antisense morpholino-modified oligonucleotides (morpholinos or MOs) to specifically prevent RNA splicing and/or translation [99]. The transgenic organisms have been generated for the last 30 years. The transgenic animal, e.g., the creation of "knockout mice," acts as a research tool to better understand the functional consequences of gene expression or deletion [69].

In molecular biology or drug discovery, molecule screening at single cell level, which is crucial in drug discovery and molecular biology, demands the target molecules to be transferred into single cells. Then, the cellular function-targeted molecules can directly control the cell development as well as their functions.

In the reproductive studies, intracytoplasmic sperm injection (ICSI) has gradually been the main method to overcome intractable male-factor infertility. It has become a routine process for lots of in vitro procedures [39].

It has been proved that cell injection has significant meanings to human beings [7]. Biological cells are irregular in shape and easily deformable, and they may be lightly destroyed during the manipulation and injection. At present, this greatly precise process is mostly done manually [109]. The development of suitable human-system interaction is still a challenging research area [19].

2.1.2 Conventional Manual Cell Microinjection

In biological research, manual microinjection is a traditional and extensive practice [99]. For instance, during the injection of zebrafish embryos, a human operator firstly identifies the embryos in a petri dish by using a microscope and then moves the tip of the micropipette toward the embryo carefully. The embryo is immobilized with another micropipette. At the moment when the micropipette tip initially touches the outer layer of the embryo, the operator will manually actuate the micromanipulator to generate a rapid thrust movement, leading to a preliminary penetration of the embryo by the micropipette. After this preliminary penetration, the operator will continue actuating the micromanipulator, until the micropipette tip penetrates into the embryo's yolk. Then, genetic materials can be injected into the embryo by a microsyringe, such as a pneumatic microsyringe [19]. During this manual microinjection process, the experience and skill of the operator play a vital role in realizing a successful injection.

Usually, it takes several months or one year to train an operator to become proficiency in completing such a task, that is a time-consuming process [19, 52, 75, 93, 115]. Despite the long training time, the success rate of a skilled worker can only reach around 15% due to the tough conditions (i.e., long-time concentration, patience, and causing fatigue) to achieve a successful injection [21, 39, 68, 95]. Even so, a successful injection also needs five crucial conditions, i.e., an accurate injection

point, proper speed variation, suitable penetration trajectory, appropriate penetration force, and no contamination [52, 82].

Specifically, an accurate injection point, e.g., the center of a cell, is necessary to avoid micropipette slipping on the cell surface without penetrating (or missing) the nucleus after penetrating. Proper speed variation means that the speed of micropipette touching the outer layer of the cell accelerates to high enough speed to make the micropipette rapidly pierce the cell membrane, without inducing excessive deformation and destroying the membrane to obtain the integrity of the membrane. This is crucial in transgenesis task, because the successful integration of the genetic material into the genome is also needed in the nucleus [69]. After that, the speed should be decelerated to zero as soon as possible to avoid destroying the inner structure of cells. A suitable penetration trajectory will reduce the time that is required to finish an injection operation. It is particularly crucial for the scenarios where a number of cells need to be injected. For instance, in order to test the cellular responses to molecular targets, and to obtain the statistically significant data, thousands of cells require to be injected in a short time period (e.g., within 1.5 h after the fertilization) because of quick succession [99]. For instance, the injection of embryos with dsRNA is generally conducted within the first 60 min of embryonic development [115]. Proper penetration force prevents the cell from being destroyed by excessive force or hand tremor. The condition of no contamination requires the reduction or elimination of the participation of human. Obviously, it is hard to always satisfy all of the rigorous conditions at same time for a human operator. Hence, low success rate, poor repeatability, and extended training time are resulted. Even more important, it is impossible to implement the regional (or organ specific) delivery within an embryo or fetus via manual operations [68]. It is practical only when treating small numbers of embryos and cells [118].

In order to overcome the problems intrinsic in manual injection (e.g., human fatigue, long training time, labor intensive, and poor repeatability), various cell injection methods have been developed to achieve a higher success rate.

2.1.3 Current Methods of Cell Microinjection

Several methods are available for delivering foreign materials into embryos or cells, such as chemical methods, vehicular methods (e.g., erythrocyte fusion and vesicle fusion) [55, 96], electrical method (e.g., electroporation [71]), and mechanical methods (e.g., microinjection, hyposmotic shock [6], sonication [88], and microprojectiles [46]).

Microinjection normally uses a fine micropipette for penetrating through a cell membrane and delivering liquid into the cell with a pressure pulse [28]. Microinjection is the standard method for injecting embryos and cells, because it can reproducibly and reliably introduce large numbers and specified volume of macromolecules to majority types of embryos and cells with high viability and function [118]. It is important that microinjection can achieve quantitative delivery of

multiple components into the same cell at any specified time without introducing other potentially confounding compounds [56, 57]. It also should be noted that microinjection is a better choice than vectors, because vectors randomly integrate to the host DNA and can induce uncontrolled influence on the gene expression and cause phenotypical changes. In microinjection, the cells to be injected can be carefully selected and hence the injected cell can be marked, which can improve the success rate of injection and the reliability of study. Moreover, several cells (e.g., primary cells and stem cells) are hard to transfect via traditional methods [28]. Moreover, the same end-effector (i.e., a fine micropipette) can be utilized in different operations (e.g., electrophysiological measurements and cell isolation [28]). In contrast to other techniques, microinjection with a fine micropipette is the most effective approach in consideration of the issues including cell viability, cell damage, cell waste, effectiveness of introducing macromolecules, and keeping from concerning about phenotype alteration.

Because of two distinct biomanipulation tasks [22], the cells to be injected in microinjection can be classified into two types, i.e., adherent and suspended cells, which are presented in detail below.

2.2 Injection of Adherent Cells

Adherent cells grow at the bottom of a petri dish and form a fixed cell population. By contrast, suspended cells grow loosely, as shown in Fig. 2.1. Expect for blood cells and germ cells, all of other cells in human body are of adherent cells. Therefore, the research on adherent cells is very important in drug development and disease mechanism studies. Adherent cells are usually smaller in size (10–20 μm in diameter) than suspended cells (e.g., oocytes—the most frequently injected suspended cells). Because the size of the adherent cells is nearly 5–10 times smaller than that of oocytes, microinjection of adherent cells needs more accurate micromanipulators in terms of positioning and injection accuracy. In addition, as the adherent cells are small in size and they usually grow in population close to each other, they are hard to be detected. This imposes a high demand on the vision and other measurement systems. The small size of the cells also imposes requirement for very fine injection capillaries. Since the fine micropipettes ($<1\,\mu$m) are demanded, it is very hard to

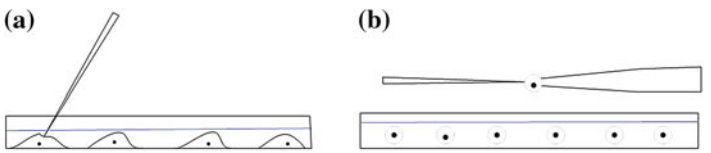

Fig. 2.1 Sketch diagram of microinjection of **a** adherent cells and **b** suspended cells

visually monitor the condition of the micropipette, contact with cell, correct injection depth, and so on [28]. In addition, owing to the fact that adherent cells are fixed at the bottom of petri dish, the holding pipette is not required.

Some commercial devices are also available, such as the products offered by Eppendorf, Kleindiek, Newport, and Narishige and Cellbiology Trading. The most popular commercial adherent cell injection device is AIS 2, that is produced by Narishige and Cellbiology Trading.[1] This partly automated cell injection device is primarily composed of an inverted microscope, two three-axis stages driven by stepping motors for positioning the cells and controlling the micropipette movement, a piezo-driven axial injector, a pneumatic microinjector, a video system with a CCD camera and a monitor, and the associated software for controlling the cell injection process. By incorporating the functions of auto-focusing, identification of cells and injector pipette, positioning control with image-based visual serving and calibrated injection height control, this injection device can produce a high success rate of injection. The software commands the injection process precisely and enables a fast axial injection with a speed of 1500 cells per hour. In addition, retrieving the injected cells and multiple injections into the same cell are also achieved by the system [22].

Although some systems have already partly automated, all the devices currently need intensive manual work. Because of the participation of the operator, the quantity of cells which can be injected in a prescribed time interval is limited. It is an issue when plenty of cells have to be injected or when microinjection is adopted to create stable transfected cell lines [74]. Moreover, the operator's skill imposes a huge affect on the success rate. Another disadvantage is the lack for method to diagnose the condition of micropipette, such as clogging and small breakages. The drawbacks of the individual devices result in a low output, a low success rate, and decreased reliability of the results [7, 28].

Some researchers have devoted to improve the automation degree and success rate by adding force sensor. For instance, Lukkari et al. and Kallio et al. have developed an impedance sensor to detect the contact between the cell and inject micropipette, a broken micropipette, a clogged micropipette, an aged measurement electrode, and faulty injection solution [28, 53]. Meister et al. used atomic force microscopy (AFM) to achieve force-controlled injection of adherent cells [59]. Desmaele et al. developed a force sensor with planar structure to sense out-of-plane forces, and living cells can be placed on the planar sensor [14].

To conclude, the development of an automated robotic cell injection system for single adherent cell is a very challenging work. Due to the scaling effect, the relationships between physical quantities alter in the microworld. For instance, the gravity is less regnant than van der Waals forces and electrostatic forces. The uncertainties caused by the scaling effect make the operation more difficult. In addition, the uncertainties are induced by biology call for sufficient robustness for the cell injection system. Moreover, each type of cell exhibits its own specific properties, and the state of cell population changes over the time, which increases the difficulty in successful cultivation, detection, and injection of adherent cells [28].

[1] http://www.ais2.com/.

2.3 Injection of Suspended Cells

In the field of cell microinjection study, there are three types of most popular suspended cells, i.e., Drosophila embryo, zebrafish oocytes, and mouse oocytes.

2.3.1 Drosophila Melanogaster Embryo

The Drosophila melanogaster (fruit fly) embryo has relatively short reproductive cycle, small genome, and possibility in the cure of human diseases. It is one of the most popular organisms in biological research, medical study, developmental biology, and genetics. It also has been applied in studying the wiring of human brain and nervous system. Genetic modification of Drosophila embryos has aroused the interest from both scientific research and medical industry for finding biological mechanisms to treat diseases. The possibility to improve human health based on the research related to drosophila embryo has been verified by the award of 1995 Nobel Prize in Physiology or Medicine for the discoveries related to the genetic control of early embryonic development [76]. In that research, the fruit fly was taken as the test model. This organism is popular in genetics, because human and Drosophilas are similar in genetics. The principles obtained from fruit fly are suitable for higher organisms involving human.

The Drosophila genome can also offer important information about human genes which are homologous in the Drosophila melanogaster [72]. In particular, several human diseases are aroused by mutations in genes analogous to genes discovered in Drosophila. Specifically, approximately 61% of known human disease genes have an identifiable match in the genetic code of Drosophila, and 50% of Drosophila protein sequences exhibit mammalian analogs [73]. Drosophila has been utilized as a genetic model for a number of human diseases containing neurodegenerative disorders, e.g., Parkinson's disease, Alzheimer's disease, and Huntington's disease.

Moreover, Drosophila has been used to explore the mechanisms related to immunity, diabetes, cancer, and drug abuse. Additionally, the gene function can be determined from the loss-of-function phenotype or the overexpression phenotype. The function of human genes could be studied through introducing them into Drosophila by transposable elements. For instance, Goodman et al. [97] used Drosophila to explore the wiring of the brain and nervous system, leading to an improved understanding of how the human brain develops. Hence, the integration of the Drosophila genome with the well-developed genetic methods in the Drosophila system will give rise to crucial discoveries for human medicine and development in detecting, treating, and eradicating diseases in humans. In order to carry out the research on Drosophila genome, one of the most significant processes is the injection of materials which affect the composition of a cell (or an organism). Microinjection can generate Drosophila with new characteristics through integrating transgene or dsRNA into the DNA of Drosophila. For example, in RNAi microinjection tests, 100–200 Drosophila

embryos need to be injected with 60 pl of dsRNA during the first 60 min of their development to assess one gene [118]. This enables us to determine which genes are vital for the development of the organism and which organs are influenced [76].

2.3.2 *Zebrafish Embryo*

The zebrafish (*Danio rerio*) embryo has been broadly adopted as a standard animal model for studying the development of vertebrate. Moreover, zebrafish has also been utilized as a model for understanding the human disease's pathogenic mechanisms and discovering drug [63, 66]. Microinjection of genetic materials into zebrafish embryo has been a routine process to test functions of the injected materials on the survival and development for embryos [24].

There are four distinct advantages making zebrafish hot research model. Firstly, zebrafish embryos are easily obtained and fertility. Secondly, they grow rapidly and the life cycle is short, i.e., approximately 12 weeks, which makes generic analysis easily. Thirdly, the characteristics of transparent and external fertilization make the development and change of cells be inspected easily [70]. Fourthly, solid organ malignancies developed in zebrafish are similar to human tumors, as the embryonic development of zebrafish is markedly analogous to that of humans [69].

It is worth noting that molecular and gene should be injected into early zebrafish embryos to analyze zebrafish embryo genesis [40]. As shown in Fig. 2.2, the diameter of zebrafish embryos is around 600–700 μm (without chorion) or 1.15–1.25 mm (with chorion), in which the cytoplasm and nucleus locate on the animal pole linked with a large mass of yolk. The diameter of the injecting micropipette tip for zebrafish embryos is about 6–10 μm [109]. The zebrafish embryo has four developmental phases, i.e., blastula phase, gastrula phase, pharyngula phase, and prehatching phase. Blastula embryos form three germ layers at 7.5 h post-fertilization and then enter the gastrula phase. The embryos are named pharyngula phase after 26-h post-fertilization, which shows distinct movement within the chorion. During the period of 26–48 h post-fertilization, the phase is named prehatching phase. The embryos hatch after 48-h post-fertilization when a protease enzyme is secreted to dissolve the chorion envelope [32]. The zebrafish can be collected according to the standard procedures of embryo preparation [104].

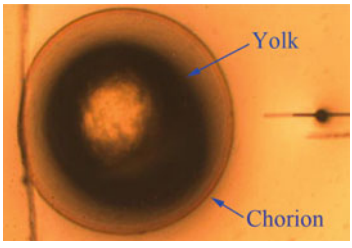

Fig. 2.2 Microscope image of a zebrafish embryo

2.3.3 *Mouse Embryo*

Microinjection of mouse embryos is significant for vitro fertilization, screening molecular targets related to the research of basic biology of embryo development (e.g., neutralizing antibodies, mitochondrial-associated recombinant proteins, morpholinos), and expression vectors for siRNA [47]. In addition, the mouse serves as a model organism, which is a primary animal for genetics and reproductive research.

Because of the freedom of movement of suspended cells in the nutrient solution, the suspended cell needs to be fixed by additional equipment, such as holding pipette, making injection procedure more complicated and time-consuming. Moreover, the collision between cells and manipulation instruments, or the motion of cells themselves, can easily result in the failure of this procedure. Even though there is a tough requirement on suspended cell injection, majority of the operations have been performed manually [22]. However, the manual method features low efficient, low repeatability, and low throughput, which cannot meet the need for testing genetic materials when multiple injection operations should be done in a certain time. On the contrary, the robotic cell injection system can realize that successful cell injection with high repeatability and accuracy in a certain time [107]. Therefore, the development of the robotic cell injection system has been the concentration of lots of researchers.

2.4 Robotic Cell Microinjection System

Robotic microinjection is a method which uses automation technology to introduce materials into individual living cells by a fine needle [108]. In comparison with manual microinjection, the dominant advantages of robotic cell microinjection involve more qualitative, more productive, more reliable, free from fatigue, and unparalleled repeatable. Robotic cell microinjection systems can operate in a greatly efficient and consistent way, and hence could notably improve the reproducibility and throughput of cell injection and even make it possible for new types of studies that cannot be achieved by traditional techniques [117]. Robotic cell injection is extremely preferable when a large number of cells need to be injected with abundant materials in a certain time.

Generally, a robot cell microinjection system includes piercing mechanisms with injection control loop, cell holder and micromanipulator (for precise position), machine vision and other nonvision sensors, user interface, and an environment control system for maintaining cell cultivation conditions (e.g., temperature, pH value, and humidity) [67]. By improving the level of automation of the robotic cell injection, the human involvement can be reduced and the cell injection speed can be increased. Therefore, scientists can concentrate on analyzing the results. Moreover, automated robotic cell injection can increase the reliability and accuracy and hence offer more reliable results [28].

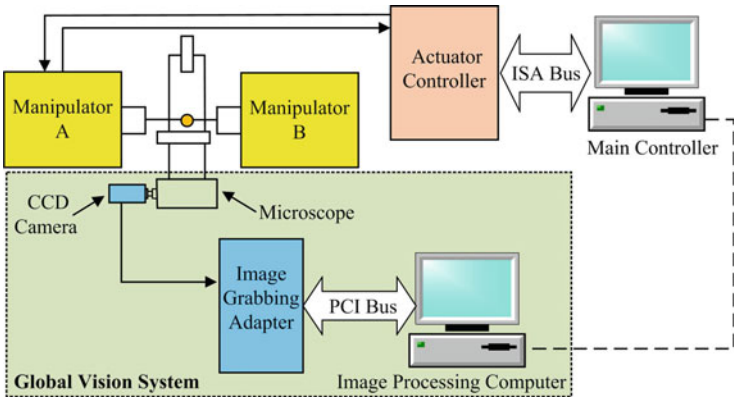

Fig. 2.3 Schematic diagram of an automated suspended cell microinjection system

Currently, some semi-automated or tele-operated robotic cell injection systems are commercially available, such as the products supplied by Eppendorf,[2] Narishige and Cellbiology Trading.[3] However, these products need greatly skilled operators to carry out suspended cell injection. In the literature, Li et al. [43, 44] have presented a representative automated suspended cell injection system, as illustrated in Fig. 2.3. The process of the automated suspended cell injection primarily contains four steps in the following.

- Firstly, it implements a visual-based search and recognition for the suspended cells, injecting pipette, and holding pipette by image processing.
- Secondly, the holding pipette is driven to hold the cells and then moved to the desired manipulation place.
- Thirdly, the injector pipette is guided to inject into cells and release materials at desired position.
- Fourthly, the injected cells are released in the culturing area.

The above process is repeated until all of the cells have been injected.

However, the injection process purely relies on position control, which is apparently not a reliable control process because of the following reasons. Firstly, if the injection force is too big, cells and the injection pipette may suffer from the possibility of being destroyed, because they are highly fragile. Secondly, there is no fast and accurate feedback during the procedure to make sure that the penetration is successful. For example, sometimes the injecting pipette may only slip over the surface of cells rather than penetrating into them. Though the vision feedback can be used to monitor the penetration procedure, the vision feedback is unpractical in recognizing the injecting pipette's tip correctly and quickly, due to the difficulty in detecting whether the tip is inside or outside the cell. Thirdly, the computational load

[2] http://www.eppendorf.com.

[3] http://www.narishige-group.com/.

of vision feedback control during the injection procedure is huge, due to the fact that multiple images need to be captured and processed in real time in the injection procedure for each cell [52]. Consequently, the feedback control using a force sensor is widely adopted in recent cell injection process, because of its distinct advantages over the pure vision-based position feedback control.

Force sensor and related control have played vital roles in robotic cell injection. Force sensor can provide real-time detection of contact forces between a injecting pipette and a cell. In comparison with pure vision-based cell injection, force sensor-based cell injection exhibits six distinct advantages. Firstly, force sensor can provide an accurate force feedback of the ongoing cell injection, which could improve the dexterity, success rate, and robustness of cell injection systems [69]. For example, the detection of injecting forces during the cell injection can precisely predict the penetration of cell membrane and hence initiate the subsequent material delivery [117]. Secondly, because the biological cells and injecting pipette are delicate, the quantification of contact forces between the injecting pipette and cells is helpful to prevent the cell and injecting pipette from excessive force [47]. Thirdly, precise measurement of contact forces is a necessary condition toward minimally invasive cell injections, which can improve the survive rate of injected cell. Experimental and theoretical data indicate that the extensional flow at the entrance of the injecting pipette is the main reason of biological cell death [1]. Fourthly, the sensing of cellular force is essential for understanding the biophysical properties of cell injury and membrane modeling issue [32]. Fifthly, force signals can also be helpful to detect the physical condition of the cell by assuming that a stiff membrane implies a weakened cell, which can reduce the waste of precious injecting materials [7]. Last but not least, force sensor-based cell injection can improve the speed and need not high-quality image processing equipment, causing cost reduction [85]. It should also be noted that the injection force is in μN–mN range [19].

2.5 Microforce Sensors for Cell Microinjection

Although the pure visual-based position control (visual servo) has been widely adopted to perform cell microinjection tasks [82, 99, 113], the measurement of contact forces during pipette injection plays a crucial role as it can be applied to provide force feedback for precise control of the needle penetration speed and strength [39]. Moreover, the visual-based position information is generally less effective than the force information. On the contrary, the direct force information can reflect the changes in the physical behavior of the cell (e.g., deformation or extent of penetration) more accurately and quickly [52]. Five popular microforce-sensing methods in cell injection are introduced in the following.

2.5.1 Vision-Based Force Sensors

Robotic cell injection is generally performed with the help of an optical microscope. Hence, visual feedback is the dominant sensing method in existing robotic cell injection systems [47]. Vision-based force sensors are used to determine cell injection forces by using image processing and an accurate cell model [19]. The forces are usually computed on the basis of the deformations of visually tracked flexible objects (e.g., cells, manipulation tools, or cell holders). And the measured geometrical information is used by a force estimation algorithm to provide the force sensing [34].

The vision-based force sensors have some distinct advantages. Firstly, vision-based force sensor can provide global forces feedback rather than local forces offered by contact force sensors, where the latter strongly limits the haptic rendering for the operator [39]. Secondly, the vision-based force sensors are most helpful when the force information is required and it is highly challenging or even impractical to use a force sensor [29]. For instance, very small-scaled and accurate force sensing for cell injection is more difficult, because the design of small force sensors requires to solve the challenging problems for cell injection, such as multiple degrees-of-freedom (DOF) microforce sensing with high resolution and accuracy, and high signal-to-noise ratio (SNR). In addition, the designed force sensors must maintain an adequate reliability and repeatability, because force sensors experience severe disturbances in cell injection due to the liquid surface tension and adhesion forces [18]. Thirdly, vision-based force sensors are able to obtain both vision and force information by a single vision equipment (e.g., charge-coupled device (CCD) or complementary metal-oxide semiconductor (CMOS) camera) under microscopic conditions, which does not increase the complexity of the cell injection system [21]. Therefore, new methods which can avoid the use of force sensors become very prevalent recently [34].

2.5.1.1 Image Processing for Vision-Based Force Sensor

Image processing is adopted to extract the required information as the input to the established model. The speed of the vision system is highly dependant on the speed of the localization and recognition procedure. The recognition of the cell and the micropipette can be realized by different approaches, such as matching method. Pattern matching and feature matching are two most frequently used matching methods. The pattern (template) matching implements direct localization on the original image by detecting the degree-of-similarity between an image and the template [34, 51]. The feature matching requires to extract features (e.g., active contour, brightness [115], or amplitude spectra) first and then implement matching in the feature space. Contour abstraction has been broadly investigated in image processing, and many approaches have been presented. The most frequently employed edge detection technique is the gradient-based Prewitt, Sobel, and Laplace detectors [13]. Other

contour finding techniques like the second-derivative zero-crossing detector [54] or computational approach based on the Canny criteria [9] have been proposed. Nevertheless, owing to general image features such as noise, texture, image blur, or other anomalies like nonuniform scene illumination, edge finding techniques usually fail in providing satisfied results. For instance, the boundaries of adjacent cells may be wrongly recognized as broken edge fragments or might not be detected. In addition, the edge information cannot be detected when the edge only covers a few pixels. Eventually, these techniques generally need post-processing to get connected and closed contours.

As firstly presented by Kass et al. [30], the active contours (i.e., snakes) have been applied in many applications. It consists of the procedures of edge detection, shape modeling, segmentation, pattern recognition and object tracking [41]. Such technique always generates closed contours and is quite adapted to segment biological images. However, the existence of other objects (e.g., the holding pipette, the injecting pipette or the impurities in the medium) imposes disturbances and hinders the direct use of the snakes for contour tracking of the cell membrane deformations. In order to solve the problem, a series of preprocessing steps should be performed ahead. The first step includes erasing the holder pipette and the injecting micropipette from the image. The correlation-based template matching is used to locate the pipettes in real time [22]. The pipettes/membrane contact points define the boundaries of the holder pipette and the injecting micropipette segments. These contact points are acquired by detecting the gray level of the image along the pipettes' edges. The second step is composed of locating the impurities and removing them from the image [2–4].

2.5.1.2 Cell Model for Vision-Based Force Sensor

Physics-based model of the living cell has been built by combining together the cell geometric information obtained from image processing, a priori knowledge of cell mechanical properties, and a predefined coordinate system of the slave environment [34]. Then, the force can be computed from the model by providing the updated cell boundary condition. In the literature, mechanical cell models can be primarily divided into three categories including microscale continuum, energetic, and nanoscale structural types [39].

The first category of models assumes that the biological cell is equivalent to one or two phases continuum model rather than considering the molecular nanoscale mechanical properties [45]. A typical model of this category is shown in Fig. 2.4. The main advantages involve their ability to calculate the mechanical properties of cells and offer the details about the distribution for stresses and strains reacted on cells (e.g., zebrafish and medaka embryos) at different developmental stages [89, 90]. Sun et al. [86] have proposed a point-load model to estimate the cell injection force with cell deformation. In this model, some assumptions are presented to simplify the model, such as linear elastic biomembrane, uniform stress within the dimple, and zero residual stress in an initial planar circular area. However, such assumptions are not always true in reality.

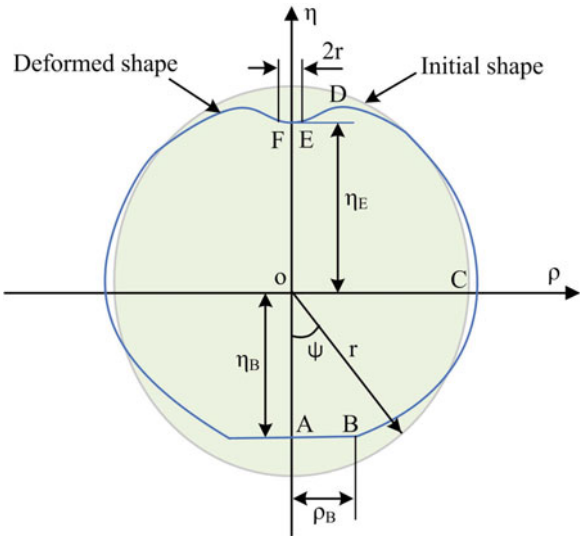

Fig. 2.4 Microscale continuum cell model for vision-based force sensor

Contour model [20] and active contour model [34] have also been presented to estimate the forces exerted on a linearly elastic object (i.e., cells) using the contour data, which are generated on the basis of visual data. In [4], a 3D nonlinear mass-spring-damper cell model is proposed to estimate the injection force. Nevertheless, such model can only provide limited accuracy and weak connection to biomechanics [34]. For instance, there is no mechanically related relationship between the model parameters and the cell properties. Furthermore, these parameters are calculated through offline finite element method (FEM) simulations by using finite element modeling, which is influenced by the network topology. In addition, Ref. [33] has reported a boundary element model (BEM) and a prior knowledge of the mechanical properties for the cell to predict the cell injection forces. Tan et al. [89, 90] have presented a mechanical model based on membrane theory with the quasi-static equilibrium equations, where the relationship between the cell injection forces and the deformation of cells is built. Huang and Sun et al. [21] have used the point-load model to estimate the cell injection force by using the displacement of inject pipette. Kim et al. [34] proposed another boundary element model to estimate the cell injection force, where the position update of an injecting pipette is used as the input for the model to estimate the injection forces so as to reduce the calculation load. Asgar et al. [5] have presented a 3D particle-based mechanical model, which is based on spring-damper model with multi-particle joints to estimate the injection forces. Nevertheless, considering the cell as a tensed balloon filled with molasses or jello, the major disadvantage of the continuum method lies in that it is not able to explain the molecular deformations and interactions within the cell.

The models of the second category consider the effects of various cytoskeleton structures into the entire energy budget of cell during contraction [26]. It is established based on the percolation theory and polymer physics models with large deformations.

The main advantage of the model is that it is independent in choosing coordinate system and the particular details of the cytoskeleton architecture, due to the energy is a scalar quantity. Nevertheless, it is hard to find an optimal physical correspondence between the model and experimental data.

The third category of models containing the tensegrity structures can be divided into two classes, i.e., spectrin-network model and cytoskeletal models. The model belonging to first class includes a specific microstructural network for spectrin cells with large deformations [25]. The latter regards the cytoskeleton as the primary structural component and considers cytoskeleton contractile forces as the central role. The tensegrity method has revealed many aspects of cell deformability containing nonlinear features of cellular structural behavior. The models consider the cell as a network of microfilament, microtubule, and actin, which distribute forces within the cell via a balance of compression and tension [58]. Those models can simulate many features presented in living cells during mechanical tests containing strain hardening, prestress-induced stiffening, and the effect of cell spreading on cell deformability [116]. A full *mechano-cell* model, which includes the cell membrane, the nuclear envelope, and actin filaments, has been proposed on the basis of the minimum of the elastic energy during deformation [94]. It is considered as a combination of various spring elements. Ladjal et al. [39] have proposed a simplified cytoskeleton tensegrity structure physically based on FEM model, which enables us to simulate the cell deformation through real-time simulation constraints. In the third model, the cell geometry and biomechanical subcomponents properties (e.g., biomembrane, cytoplasm, cytoskeleton, and nucleus) play the dominant role in simulation and modeling, as these factors influence the amount of cell membrane deformation, needle deviation, and interaction forces [4].

In addition to measure the deformation of cells, Liu et al. [47, 49] have measured the deflection of a polydimethylsiloxane (PDMS) post in a cell holder to estimate cell injection forces with a resolution of 3.7 nN. This method can be used to detect the injection forces of different cells, because the model is only related to the deflection and material parameters of the cell holder. Moreover, Karimirad et al. [29] have proposed an artificial neural network, which is trained by the existing relationship between cell parameters in images and force, to estimate the load for a spherical biological cell. This method is free from building a real mechanical cell model and relatively easy to perform. However, the inner structure of the cell in this model is ignored and the model is sensitive to parameters variation in biological cells. Furthermore, Ammi et al. [3] have also presented a 3D cell model constructed with virtual reality (VR) environment to improve the realism, where the force is estimated by the contour measurement based on vision technique.

It is also notable that the finite element model (FEM) needs a controlled slave environment to model the membrane [34]. The mass-spring model is commonly nonrealistic and greatly sensitive to the tuning of the model, e.g., in the spring constant of the mesh. Comparatively, boundary element method (BEM) is a numerical approach to solving the differential equations indicating an object model that computes the unknowns on the model boundary rather than its entire body. BEM uses less computation time than FEM, whereas FEM is difficult to construct and change

the element mesh. By contrast, BEM is more suitable as it uses the boundary mesh rather than solid elements of FEM for the cell. Kim et al. [34] have adopted the cell edge information and known material properties to estimate the force.

However, vision-based force sensors will be affected by the inevitable parameters uncertainty in the dynamic cell model. Moreover, vision-based force sensors are greatly dependent on the availability of suitable cell model. In addition, the sampling frequency of the vision-based force sensors is highly lower than that of the contact force sensor, and thus the injection process cannot be controlled precisely. The performance of these sensors is limited by the small depth of field of the used microscope [22]. Furthermore, a priori knowledge of the cell properties should be obtained before estimating the forces [14]. The resolution of such force sensors is intrinsically limited by the optical components of the microscope, inhibiting application where the direct force feedback is needed.

2.5.2 Capacitive Force Sensors

Currently, majority of available capacitive force sensors applied in force detection of cell injection are based on microelectromechanical systems (MEMS). MEMS technologies are highly suitable for the fabrication of cell handling systems, because of the cost-effective mass-fabrication of custom-built injectors with integrated sensors [115]. MEMS sensors can be designed at the end-effector, causing a marked increase in accuracy, reproducibility, and reliability of the cell injection. MEMS sensors are able to provide qualitative and quantitative information on the cellular, sub-cellular and organism levels, that is useful to understand the fundamental elements of biological systems [84]. MEMS force sensors exhibit advantages of small size, wide bandwidth, and high sensitivity. Due to the significance of quantifying forces and their influence on the function and morphology of many biological structures, MEMS force sensors have played a crucial role in biological studies [84].

In these force sensor devices, very tiny deflections induced by exerted forces are transformed as detectable capacitance variations. An electronic circuit converts the capacitance changes into DC-voltage changes. MEMS capacitive force sensors can measure the forces ranging from pN and mN, which makes them be desirable for the force detection in cell injection. With their high performance and ability to perform measurements with multiple degrees-of-freedom, MEMS capacitive force sensors are powerful substitutes to other MEMS-based transducers, e.g., cantilever-based sensors [15].

Capacitive type of MEMS force sensors has five distinct advantages [84]. (a) They are able to measure a wide range of forces (from mN down to pN level), whereas the measurement range of other force-sensing methods is usually limited; (b) they are able to provide the force information of multiple axes; (c) they use the most direct method of force measurement rather than indirectly measuring approach; (d) they exhibit the merit of low noise, low power, and high sensitivity. Moreover, they are insensitivity to light, temperature, and humidity variations [62]; (e) they can be

manufactured by batch microfabrication processes by using deep reactive ion etching (DRIE) on silicon-on-insulator (SOI) wafers [85], leading to cost effective.

The MEMS capacitive force sensors are designed as comb drives, i.e., an array of parallel-plate capacitors. There are two working modes in capacitive force sensor, i.e., transverse mode and lateral mode of comb drive movement. The transverse mode changes the capacitance by altering the gap size between the capacitor plates, while the lateral mode changes the capacitance by altering the overlapping area between the capacitor plates. The capacitive force sensor in transverse mode enables the design with higher resolution than that of lateral mode, at the expense of lack of linearity. It also should be noted that high stiffness is required for high-bandwidth measurements. During the design of capacitive force sensors in transverse mode, the differential configuration of comb drives is useful in creating linear relationship between the deflection and the sensor output signals for small deflections [62], as well as compensating for disturbances [81]. Compared with strain gauges and piezoelectric force sensors, capacitive force sensors are more stable and sensitive and exhibit no hysteresis [29]. As compared with an optical beam-based atomic force microscope, they also have stable output signals (with low drift) and high compact size [62].

In the literature, four representative capacitive force sensors are presented. Sun et al. [84, 85] have proposed a two-axis capacitive force sensor with high sensitivity (i.e., 0.01 and 0.24 μN) by using large overlapping area and transverse model. The sensor is applied in the characterization of the mechanical properties for mouse oocyte and embryo zona pellucida [86]. However, a constraint in motion displacement of the pipette is revealed in cell micromanipulation applications [29]. Muntwyler et al. [62] have proposed a three-axis microforce sensor with regulable force ranges (from $\pm$20 to $\pm$200 μN) through the readout electronics with different settings for wider applications. Moreover, a new microfabrication process with a double silicon-on-insulator (SOI) substrate has also been presented, leading to a major decrease in the fabrication complexity for multi-axis sensors [62]. Beyeler et al. [8] have proposed a six-axis MEMS capacitive force–torque sensor. Moreover, Xu [110] has proposed the sensor design using one transverse comb drive for sensing two perpendicular forces, namely gripping and interaction force sensing with minimum resolution of 0.61 μN, as depicted in Fig. 2.5.

2.5.3 Optical-Based Force Sensors

Optical force sensors commonly consist of a load transduction medium (e.g., microcantilever or grating, for experiencing the exerted force), a light source (e.g., light emitting diode (LED), laser, or halogen lamp), and a photo-detector (e.g., photodiode or CCD camera, for sensing ranks of illumination, refractive index, or spectrum of the light source, that vary with the deformation of load transduction medium [100]). Additionally, a circuitry is also required to transfer the detected variation as an inductive output signals.

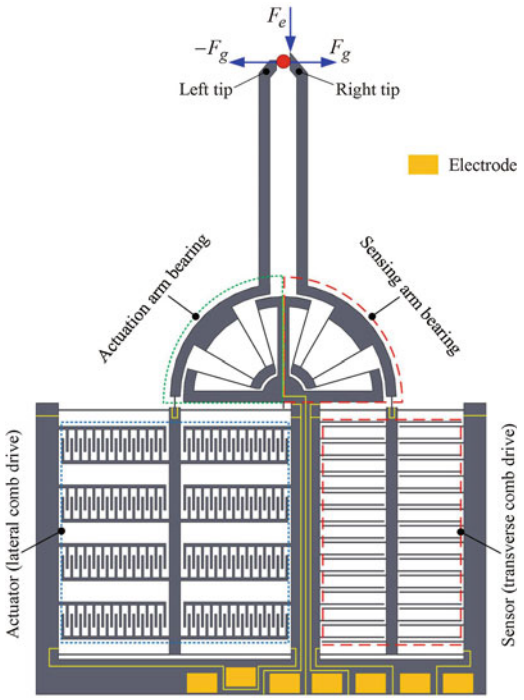

Fig. 2.5 A microgripper with a single capacitive-type force sensor for two-axis force sensing

Optical-based force sensors can offer the desired resolution and keep relatively large sensing ranges for microinjections. Moreover, such kind of force sensors provide a method to solve the conflict in designing the parameters about sensitivity and linearity [105]. The optical-based force sensors have excellent potential thanks to its extreme high resolution (down to nN scale) and the electromagnetic immunity [29]. Moreover, optical-based force sensor is an effective mean in noncontact force measurement, and atomic force microscope is one of the typical applications. Atomic force microscope is able to offer pN–nN force feedback for the cellular force measurement [47].

Cantilever-based optical force measurement has been widely used in atomic force microscopy (AFM). When a force is applied on the cantilever, the photodiode amplifies and senses the displacement of the cantilever. The force is computed by multiplying the optically sensed displacement by the known spring constant of the cantilever. The major error sources are the mechanical vibration, laser pointing stability, and shot noise for modulation frequencies over 10 kHz [60]. The system can be achieved with ultrasensitive and higher resolution by applying cantilevers with lower stiffness.

However, there are mainly four limitations which restrict the applications of cantilever-based optical force sensors in cellular force measurement. Firstly, AFM measurement requires a complex transmit–receive setup, which demands a high accuracy on optical alignment and adjustment [29]. In order to achieve a high accuracy,

the surface of the cantilever must be adequately reflective. Secondly, it has a small force measurement range, which is limited by a small range of deflection of the photodiode. Thirdly, the force measurement may be inaccurate because of the refraction and reflection of the transmitted light through aqueous medium, in which the biological cells are cultured. Fourthly, AFM does not offer really simultaneous imaging and cell injection capabilities, which requires the end-effectors with microcantilever and a sharp tip [47].

Another traditional optical force sensor is based on laser traps (or optical tweezers) [106]. The resolution of laser trap can be generated in nN or sub-nN level. In laser trapping, the high-energy light (close to the UV spectrum) is required to achieve the high dissipation of visible light in aqueous solutions. It results in the possibility of damage to the cell and abnormalities in the cell's genetic materials, because the cells could absorb the high energy and heat. However, some researchers have claimed that such concerns can be solved by using the wavelengths in the near-infrared (IR) spectrum [83].

In the literature concerning cell injection applications, some representative designs are described here. Zhang et al. [119, 120] have proposed a 1D micrograting-based optical force sensor integrated with a silicon nitride injector to characterize the dynamic injection force when penetrating Drosophila embryos. The injector is supported by springs with known spring constant, and then the injection force can be deduced from the measured displacement. The displacement is measured by a high-resolution, linear, and miniaturized optical encoder, and the resolution is less than 1 μN with a range of 10 μN. Wiens et al. [105] have used an optimized Robert's mechanism to improve the linearity, dynamic range, and sensitivity of the design. In addition, Loh et al. [50] have adopted the automatic approach function of AFM to measure the cell injection force of 36 nN in a prescribed time.

2.5.4 Piezoresistive Force Sensors

As a type of strain sensor or strain gauge, piezoresistive force sensors work based on resistance change when a force is exerted. As compared with piezoelectric and capacitive microforce sensors, the piezoresistive force sensor generally offers more accurate and stable force signals in a large measurement range at the cost of a relatively low resolution in tens of mN level [35, 51]. The variation in resistance is usually measured through an electric circuit, such as a Wheatstone bridge. Due to the output of the piezoresistive sensor is analog, namely a voltage signal, its resolution highly depends on the noise level, thermal drift, power consumption and bandwidth of the amplifier, as well as the bridge configuration of the sensor [52]. Piezoresistive force sensors have also been used in AFM to measure the atomic force change at the nN level [27, 92].

In the literature, Lu et al. [52] have applied a commercial piezoresistive force sensor (model: AE801, from Kronex Technologies Corp.) to monitor the injection process of zebra fish embryo as shown in Fig. 2.6a, where the real-time force signal

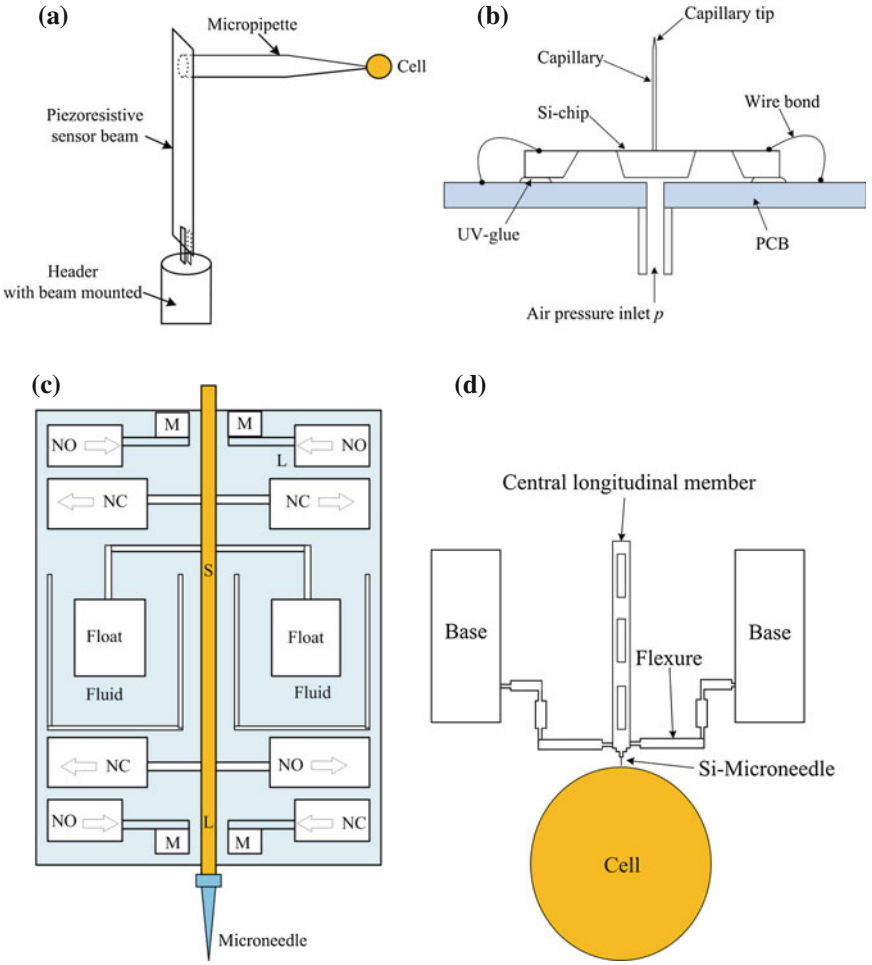

Fig. 2.6 Typical structures of piezoresistive force sensors. **a** A commercial piezoresistive force sensor (model AE801, from Kronex Technologies Corp.) is modified for use in penetration sensing, **b** MEMS piezoresistive microforce sensor, **c** silicon membrane-based piezoresistive force sensor, **d** silicon-structured piezoresistive force sensor

is applied to stop the injecting. The appearance of dramatic decrease in force signals indicates the penetration of the cell, because there is almost no resistance after a cell is penetrated. Shulev et al. [79] have proposed a MEMS piezoresistive microforce sensor with sub-pN sensitivity, as shown in Fig. 2.6b. It provides the ability to inject cell vertically and simultaneously with μN level injection force. Beutel [7] has reported a piezoresistive microforce sensor made of silicon membrane which offers a resolution of 120 μN, as shown in Fig. 2.6c. The sensor is able to self-calibrate and monitor the injecting pipette status, such as pipette break and cell sticking. The abil-

ity of monitoring pipette status greatly improves the reliability and throughput of the cell injection by providing online fault diagnostics of the injecting pipette. Stavrov [80] has proposed an axial piezoresistive microforce sensor fully made of silicon to monitor the cell injection forces, as shown in Fig. 2.6d. By tuning the amplification gain factor for the sensor's onboard electronics, the force measurement range varies from several tens of μN to several hundred mN with a resolution of nN to μN level, respectively. The main limitation of the previous works [52, 80] is that the material cannot be injected into cell with the structure that micropipette is directly attached to the sensor, which is not applicable in practice [29]. In addition, some piezoresistive force sensors need manual assembly, which may result in misalignments and significant errors in force measurement [35]. The resistance and the gauge factor of such sensors vary as temperature changes. Furthermore, they are sensitive to the bonding quality and bonding location.

2.5.5 Piezoelectric Force Sensors

Polyvinylidene fluoride (PVDF) film is the most popular piezoelectric sensor for force measurement in cell injection. When a force is applied, the PVDF film generates an electric charge based on the forward piezoelectric effect. The film is ideal for sensing the force of cell injection, because of its excellent sensitivity, high compliance, high-frequency bandwidth (between 0.001 and 10^9 Hz), and high signal-to-noise ratio [17]. Moreover, its properties of measurement range (from μN to mN), resolution (sub-μN to μN), and relatively simple structure make it suitable for the force measurement in cell injection [22]. Nevertheless, it is sensitive to acoustics and changes in temperature, indicating that the force sensors should be used in a constant temperature environment [29].

The available PVDF force sensors can be categorized into three types, i.e., cantilever-PVDF force sensor, simply supported PVDF force sensor, and fixed-guided PVDF force sensor, which are addressed as follows.

Cantilever-PVDF force sensor uses the PVDF film as a cantilever beam, while a needle is usually glued on the free end of the PVDF film and the other end is fixed on a manipulator. This kind of structure is mostly adopted to sense the injection force because of its extremely high sensitivity with a free end. Kim et al. [32] have used an unmodeled cantilever-PVDF force sensor to quantity the mechanical behavior of zebrafish embryo with 14.5-mN resolution. Shim et al. [78] have adopted the cantilever-PVDF structure to achieve μN level of resolution. Wejinya et al. have applied two pieces of PVDF films to build a 2D cantilever-PVDF microforce sensor to investigate the mechanical behavior of a fruit fly embryo [76, 102]. Pillarisetti et al. [69] have employed a cantilever-PVDF force sensor with resolution of μN to demonstrate the positive effect of force feedback fused with vision feedback in improving the success rate for cell injection, as shown in Fig. 2.7a. Interestingly, Shen et al. [77] have presented the concept of active force sensor to modify the cantilever-PVDF force sensor to obtain higher sensitivity with sub-μN resolution. Moreover, Shen

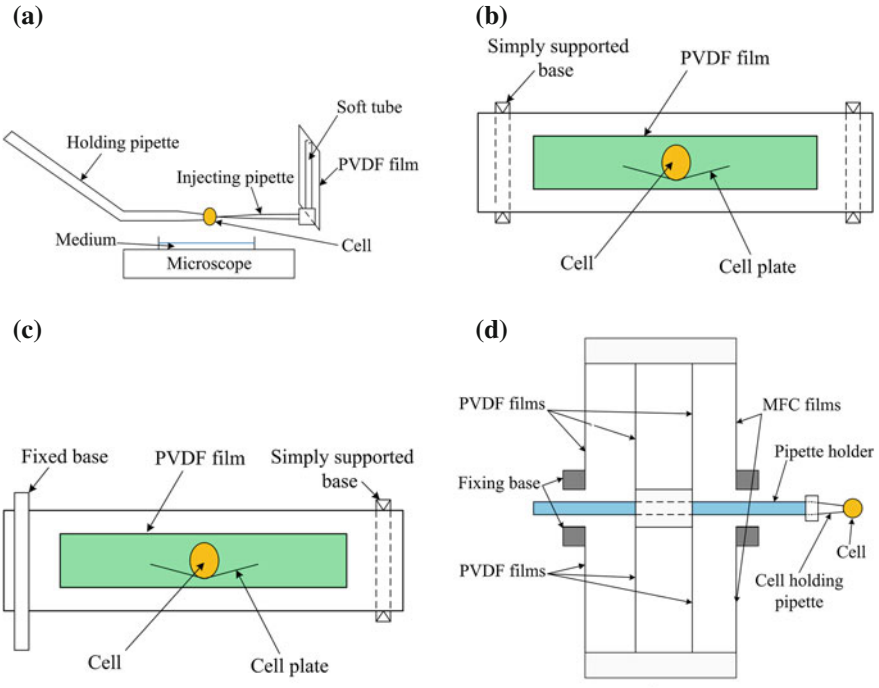

Fig. 2.7 Typical structures of PVDF force sensors. **a** Cantilever-PVDF force sensor, **b** simply-supported PVDF force sensor, **c** fixed-simply-supported PVDF force sensor, **d** fixed-guided beam-type PVDF force sensor

et al. [11, 76] have improved the performance of cantilever-PVDF force sensor by modeling the sensor and specially designed the related electric circuit with resolution of sub-μN for detecting the mechanical properties of living Drosophila embryos. Xi et al. have introduced a resonance frequency approach to measure the spring constant of cantilever-PVDF membrane and vision method to measure the deformation of cells to obtain the force information [103]. Huang et al. [22, 23] have proposed a cantilever-PVDF force sensor to identify the scenarios when the pipette contacts the cell and the cell is penetrated. However, in the aforementioned literature, the measured signals cannot be held still because of its inherent dynamic characteristic. Sun et al. [87] have presented an inverse-model signal processing method to develop a static PVDF microforce sensor.

Generally, the cantilever structure makes it hard to change the injecting pipette once broken. In addition, it is difficult to link the injecting pressure tube with the pipette needle, because the needles in majority of the aforementioned designs are directly bonded to the PVDF film [31, 32, 42]. Although injecting pressure tube is attached along the PVDF in the literature [69], the sensitivity is greatly affected by added weight and constraints.

In the simply-supported PVDF force sensor as shown in Fig. 2.7b, a PVDF film is adhesively pasted on the back of the supporting beam, while the cell plate is well placed on the center points of the beam [107–109]. A similar structure called fixed-simply-supported beam is also modified to construct a PVDF sensor in [114], as illustrated in Fig. 2.7c. However, the cells cannot be well immobilized and they can easily slip in these structures, which will decrease the efficiency, stability, and accuracy.

Alternatively, in the fixed-guided PVDF sensor as depicted in Fig. 2.7d, PVDF films are used to replace the rigid flexure in multi-stage compound parallelogram flexure (MCPF) to measure the pure one-dimensional force and hold the cell stably. However, the resolution is relatively low in several hundred μN [101].

In addition, the manual assembly in the aforementioned force sensors can induce misalignments and produce significant errors in force measurement [35].

2.6 Current Challenges on Cell Microinjection

In order to achieve automated cell microinjection manipulation, there are still many challenges in other respects besides the force sensor. The trends in many micromanipulation applications require that the future micromanipulation systems should address the following challenges. The whole cell injection system should satisfy the requirements of high speed, high flexibility, high level of automation, large information content, as well as low cost. Aiming at improving the success rate of cell injection and survive rate of injected cells, some challenges needed to be overcome and some promising methods are discussed as follows.

2.6.1 Micromanipulator Design

The micromanipulator is expected to implement the planned task with a high accuracy and repeatability in a short time without damaging the cells. To realize the automation process, the maximum speed of the micromanipulator should be a few millimeters per second. The forces applied by the micromanipulator should be in μN level. The micromanipulator should has the ability to complete the manipulation tasks without deteriorating the motion control performance. The aforementioned performance requirements should be considered in both hardware and software design. Specifically, the selection of actuators and sensors and design of mechanical structure should be conducted by taking into account the performance requirements. In addition, the motion control software and related algorithms must be able to achieve the desired performance and possibly compensate for the defects (e.g., nonlinearity and uncertainty) of the actuators. The micromanipulator must be controlled to enable both transient and steady-state requirements in the position and contact force response for the end-effectors.

Generally, the positioning challenges in automatic micromanipulation can be solved by adopting suitable sensors and actuators that offer sufficient resolution, speed, stroke, and compact size [28]. Moreover, cooperative control of two micromanipulators should be performed to maximize the operation speed [99]. As a frequently used actuator in micromanipulator, piezoelectric actuator provides rapid response and ultrahigh motion resolution, whereas it also introduces nonlinearities dominated by hysteresis and drift effects. Sophisticated control algorithms are required to realize a precise motion/force output [111].

2.6.2 Injection Control Design

In the injection manipulation, the dimension and location of the cell to be injected should be determined through computer vision technique first. Then, the needle is moved to approach the cell, which is controlled by visual servo control. In the force control scheme, when the needle begins to contact the cell, the force control will start. The switch between the position and force control should be smooth during the injection [109]. The injection control should be robust enough to tolerate various disturbances. For instance, there is certain stiffness variance for a batch of cells in the same living stage and the stiffness of a living cell changes at different stages of life [32].

During the cell injection process, the force control mode is used to avoid damaging the cells, while position control mode is adopted when there is no force exerted. The position/force switching control should be carefully designed to avoid adverse effects such as oscillation and overshoot, which will damage the cells. The impedance control has emerged as a promising method to perform a smooth switch between position and force control for micromanipulation [21]. The impedance control can control the free motion and contact force by using a single impedance algorithm [66]. Moreover, a weight coefficient method has been presented in [98] to smooth the switch process between position and force control.

2.6.3 Cell Holder Design

Currently, there are many cell trapping techniques using surface chemistry [120], dielectrophoresis, optical tweezers, ultrasonic trapping, magnetic trapping, and mechanical confinements to trap and immobilize cells [48]. Among these techniques, only the mechanical confinements are able to provide adequate immobilization forces for microinjection applications. These mechanical confinement structures include microwells, hydrodynamic traps, and vacuum-based confinements [48].

The cells trapped in microwells can tinily move inside the microwells, resulting in unsecured immobilization during the cell injection. Cells trapped in the hydrodynamic microchannels prevent the injection micropipette from accessing cells. Thus,

the vacuum-based confinements are widely used in cell injection. Traditionally, a holding micropipette is applied to locate randomly dispersed cells in the petri dish and transport the cell to the injection area once at a time, which limits the throughput and efficiency. Hence, the development of a cell holder that is able to quickly fix multiple cells into a regular pattern can improve the efficiency of both manual and robotic cell microinjection operations. In practice, differential interference contrast (DIC) microscope is most commonly utilized in microinjection for cell imaging, and glass is the most preferred material to fabricate cell immobilization devices [64].

In the literature, Fujisato [16] has proposed a special cell holder. It is made of microporous glass (MPG) with a glass or stainless coating layer, on which sand-blasted micropocket holes are fabricated. Then, liquid containing cells is pumped into the cell holder and the cells stay in the holes with liquid flow through the microporous glass, as shown in the Fig. 2.8a. Lu et al. [52] have presented a gel-based cell holder with several parallel V-shaped grooves to fix cells, as shown in the Fig. 2.8b. However, the cells can easily move if the force direction is not purely perpendicular to the plane of the cell holder. Huang et al. [23] have designed a circular and rotary plate, mainly composed of hemispherical hole and groove to fix many cells by mechanical confinements and maintain the cells wet during cell injection, as shown in the Fig. 2.8c. However, the cells need to be located in the hemispherical hole manually,

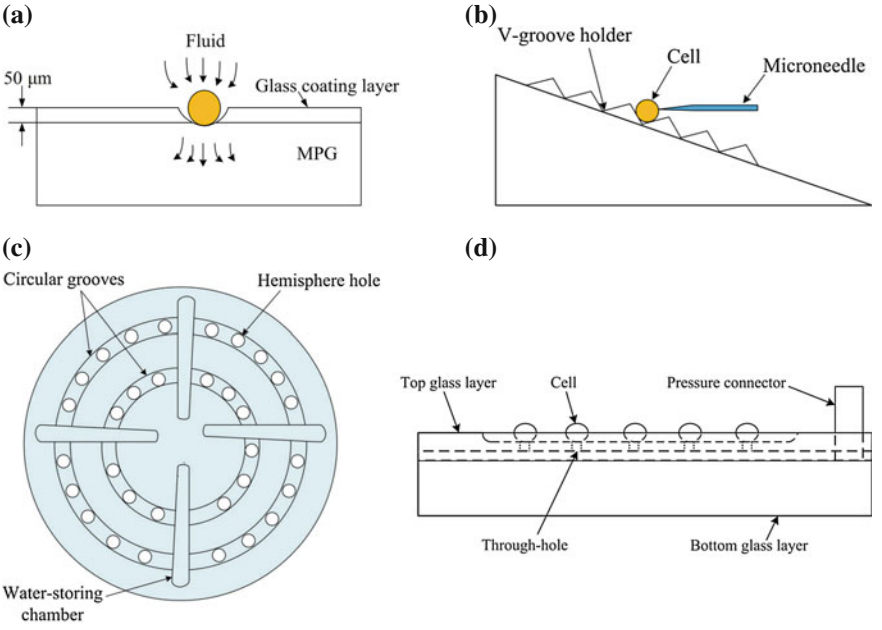

Fig. 2.8 Typical structures of mechanical confinement cell holders. **a** Microporous glass (MSG) with holes as cell holder, **b** parallel V-groove based gel cell holder, **c** hemispherical hole and groove based circular rotary cell holder, **d** vacuum-based through-hole cell holder

which increases the risk of cell damage and decreases the efficiency inherently. Liu et al. [48] have reported a glass-based cell holding device for single cell fixation by evenly spaced through-holes linked with a low vacuum source, as shown in Fig. 2.8d.

In summary, the cell holder should be designed to fix many cells quickly and stably without increasing the risk of damage to the cells.

2.6.4 Penetration Scheme Design

Usually, the cells will become inactivate when the deformation of the injected cells exceeds a certain limit. If the micropipette injects the cell at a constant speed, the cell may experience an extra large deformation until the membrane is penetrated. Usually, an impact actuator [91, 112], which is able to produce an accelerated and fast movement, can be used to reduce the deformation. Moreover, the frictionless compound flexure stage [51] can also be used to directly control the injection force to produce a variable speed and fast acceleration [52]. Consequently, the cell membrane should be penetrated with a high speed with acceleration and rapid deceleration after the membrane is penetrated. However, the process of rapid acceleration and deceleration can cause vibration, which should be eliminated in practice. Although the requirements on high acceleration/deceleration and the elimination of vibrations collide with each other, they should be compromised to achieve a successful injection. In the literature, Huang et al. [21] have presented a velocity and acceleration profiles of the injecting pipette during the injection to inject zebrafish embryos.

Moreover, in order to improve the survival rate of the injected cell, the deformation of the cell during the penetration should be reduced as much as possible. The penetration of the cell membrane can be produced by either linear or vibration motion of the microinjecting pipette. Minimization of the penetration force is vital to the development of high-throughput micromanipulation facilities for biology and genetics research, such as RNAi for gene silencing. Vibration is a popular method for decreasing the cutting force of macroscopic tools. Penetration, produced by either increased vibration (high-frequency small amplitude vibration) or translation, dramatically reduces the force on the injector [118]. It has been shown that the combination of vibration and linear translation greatly reduces the injection force when penetrating Drosophila embryos [118].

It is notable that piezoelectric actuators are very popular in cell injection manipulation thanks to their smart structure and high control bandwidth [91]. A piezo-driven pipette has been utilized to implement ICSI in mouse [36], which shows a survival rate of 80% for sperm-injected oocytes. In addition, piezodrill-based injection has been proved as an effective approach for nuclear transfer between horse and cattle oocytes [12].

2.6.5 Injecting Pipette Maintenance

The injecting pipettes can be fabricated out of tiny glass tube using a commercial pipette puller. The drawn glass pipettes have large and undefined needle diameters, because the drawn pipette is closed and must be broke off at desired location for injection use. Such a process is usually done manually. Moreover, the fracture surface is often greatly rough, leading to severe damage to injected cells. Furthermore, glass injecting pipettes are long and mechanically fragile, which produces high back-pressures [115] and degrades the repeatability of injection [115]. In the literature, Li et al. [42] have proposed a probe-etching technique to control the diameter of fiber probes, ranging from 500 nm to 1.5 μm. Zhang et al. [120] have demonstrated the high injection efficiency of the silicon nitride injector as compared with traditional glass-drawn needle.

As the injecting pipette is easy to be broken and get clogged, the condition of pipette should be monitored to improve the reliability [28]. In current cell injection systems, the user must clean the injecting pipette after each injection by using an abundant pressure pulse and manually check if a liquid jet comes out from the injecting pipette. Alternatively, the injecting pipette can be changed constantly without checking, which is very labor consuming. Furthermore, the breakage of injecting pipette tip is frequently occurred. Consequently, a method to monitor the condition of injecting pipette is necessary. Possible techniques to solve the problem involves machine vision and electrical method. For example, Lukkari et al. [53] have proposed an impedance measurement method to detect the condition of break, clogging, and faulty injection solution, which is based on electrical method.

Moreover, since the injecting pipette can become blunted or broken frequently during the cell injection, it should be replaced with a new one. However, this work is also burdensome and deteriorates the throughout and efficiency. Thus, a fast exchanger for the injecting pipette is necessary. For instance, Matsuoka et al. [56] suggested the preparation of a number of injecting pipettes with the same length in advance for replacement once the injecting pipette is broken. It should also be mentioned that the sensor should be separated from the injecting pipette to allow a quick change of the broken pipette.

2.6.6 Injection Volume Issue

Ideally, if the injection pressure, injection time, and balance pressure keep the same value, all of the injected cells can be injected with the same volume of the injection materials [28]. Nevertheless, in fact, the volume varies significantly [61]. The efflux from or influx into the injecting pipette also affects the repeatability of injected volume of liquid. In the case of influx, the cell medium flows into the injecting pipette, leading to the injection of less than desired volume of solution. In the case of efflux, the injection solution leaks out from the injecting pipette, causing more volume

transferred than desired value. The variation caused by the influx and efflux may lead to the unexpected change of experimental results. Furthermore, influx can also give rise to the clogging of the injecting pipette by aggregating in the culture solution, and hence changes the opening size of the injecting pipette or even completely prevents the injection process. In order to avoid injecting pipette clogging, most researchers have adopted the efflux of the filling solution rather than the influx of the culture medium [28].

Some researchers also adopt calibration methods, such as oil droplet method, to guarantee the injection volume [109]. Nevertheless, the model in the calibration is influenced by the equipment-related disturbance parameters and biological disturbance parameters. The relationship between the injection parameters and the disturbance parameters has been analyzed in [38]. Moreover, Zhang et al. [118] have directly integrated a microfabricated injecting pipette with a piezoresistive pressure sensor to control of the injection volume.

Hence, an oil droplet method is a promising method to calibrate the injecting volume for practical applications. In addition, experimental and theoretical results have indicated that the efflux at the entrance of the injecting pipette is the main reason of acute cell death. Alginate and nanodiamonds are two promising carriers to reduce the efflux after the mechanically injection operation using the injecting pipette. These methods have the potential to improve the survival rate and transplantation rate of the injected cells [1].

2.7 Conclusion

Cell injection plays an important role in genetics, transgenics, molecular biology, drug discovery, reproductive studies, and other biomedical fields. Robotic cell injection with force feedback can improve the success rate and survival rate of cell injection, as compared with manual cell injection and robotic cell injection with position feedback only. Thus, the development of high-performance force sensors has been a hot topic in recent years. In this chapter, the most frequently used force sensors in robotic cell injection have been extensively reviewed to address the research status toward the challenges. Firstly, the role of cell injection, limitation of manual cell injection, and current methods of cell injection are reviewed in detail. Then, the commercial and research development of adherent cell injection are introduced, and the commonly used suspended cell models are presented detailedly. The significance of force sensors in robotic cell injection system is also presented. Next, five most popular force-sensing methods in cell microinjection are reviewed. Finally, some remaining challenges and some promising solutions are discussed.

References

1. Aguado, B.A., Mulyasasmita, W., Su, J., Lampe, K.J., Heilshorn, S.C.: Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. Tissue Eng. Part A **18**(7–8), 806–815 (2011)
2. Ammi, M., Ferreira, A.: Realistic visual and haptic rendering for biological-cell injection. In: Proceedings of the 2005 IEEE International Conference on Robotics and Automation (ICRA 2005), pp. 918–923 (2005)
3. Ammi, M., Ferreira, A.: Biological cell injection visual and haptic interface. Adv. Robot. **20**(3), 283–304 (2006)
4. Ammi, M., Ladjal, H., Ferreira, A.: Evaluation of 3D pseudo-haptic rendering using vision for cell micromanipulation. In: Proceedings of the 2006 IEEE/RSJ International Conference on Intelligent Robots and Systems, pp. 2115–2120 (2006)
5. Asgari, M., Abdi, H., Lim, C.P., Nahavandi, S.: Formulation and simulation of a 3D mechanical model of embryos for microinjection. In: Proceedings of the 2013 IEEE International Conference on Systems, Man, and Cybernetics (SMC), pp. 2219–2224 (2013)
6. Banga, A.K., Bose, S., Ghosh, T.K.: Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. **179**(1), 1–19 (1999)
7. Beutel, T., Ferreira, N., Balck, A., Leester-Schadel, M., Buttgenbach, S.: Cell manipulation system based on a self-calibrating silicon micro force sensor providing capillary status monitoring. IEEE Sens. J. **12**(10), 3075–3081 (2012)
8. Beyeler, F., Muntwyler, S., Nelson, B.J.: A six-axis mems force-torque sensor with micronewton and nano-newtonmeter resolution. J. Microelectromech. Syst. **18**(2), 433–441 (2009)
9. Canny, J.: A computational approach to edge detection. IEEE Trans. Pattern Anal. Mach. Intell. **6**, 679–698 (1986)
10. Carthew, R.W.: Gene silencing by double-stranded RNA. Curr. Opin. Cell Biol. **13**(2), 244–248 (2001)
11. Chen, Z., Shen, Y., Xi, N., Tan, X.: Integrated sensing for ionic polymer-metal composite actuators using PVDF thin films. Smart Mater. Struct. **16**(2), S262 (2007)
12. Choi, Y., Love, C., Chung, Y., Varner, D., Westhusin, M., Burghardt, R., Hinrichs, K.: Production of nuclear transfer horse embryos by Piezo-driven injection of somatic cell nuclei and activation with stallion sperm cytosolic extract. Biol. Reprod. **67**(2), 561–567 (2002)
13. Davis, L.S.: A survey of edge detection techniques. Comput. Graph. Image Process. **4**(3), 248–270 (1975)
14. Desmaele, D., Boukallel, M., Regnier, S.: A planar structure sensitive to out-of-plane forces for the force-controlled injection of suspended and adherent cells. In: Proceedings of the 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), pp. 8420–8423 (2011)
15. Fauver, M.E., Dunaway, D.L., Lilienfeld, D.H., Craighead, H.G., Pollack, G.H.: Microfabricated cantilevers for measurement of subcellular and molecular forces. IEEE Trans. Biomed. Eng. **45**(7), 891–898 (1998)
16. Fujisato, T., Abe, S., Tsuji, T., Sada, M., Miyawaki, F., Ohba, K.: The development of an OVA holding device made of microporous glass plate for genetic engineering. In: Proceedings of the 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, vol. 6, pp. 2981–2982 (1998)
17. Fung, C.K., Elhajj, I., Li, W.J., Xi, N.: A 2-D PVDF force sensing system for micromanipulation and micro-assembly. In: Proceedings of IEEE International Conference on Robotics and Automation (ICRA'02), vol. 2, pp. 1489–1494 (2002)
18. Gauthier, M., Nourine, M.: Capillary force disturbances on a partially submerged cylindrical micromanipulator. IEEE Trans. Rob. **23**(3), 600–604 (2007)
19. Ghanbari, A., Horan, B., Nahavandi, S., Chen, X., Wang, W.: Haptic microrobotic cell injection system. IEEE Syst. J. **8**(2), 371–383 (2014)
20. Greminger, M.A., Nelson, B.J.: Vision-based force measurement. IEEE Trans. Pattern Anal. Mach. Intell. **26**(3), 290–298 (2004)

21. Huang, H., Sun, D., Mills, J.K., Li, W.J., Cheng, S.H.: Visual-based impedance control of out-of-plane cell injection systems. IEEE Trans. Autom. Sci. Eng. **6**(3), 565–571 (2009)
22. Huang, H., Sun, D., Su, H., Mills, J.K.: Force sensing and control in robot-assisted suspended cell injection system. Adv. Rob. Virtual Reality 61–88 (2012)
23. Huang, H.B., Sun, D., Mills, J.K., Cheng, S.H.: Robotic cell injection system with position and force control: toward automatic batch biomanipulation. IEEE Trans. Rob. **25**(3), 727–737 (2009)
24. Huang, T., Kimura, Y., Yanagimachi, R.: The use of piezo micromanipulation for intracytoplasmic sperm injection of human oocytes. J. Assist. Reprod. Genet. **13**(4), 320–328 (1996)
25. Ingber, D.E.: Tensegrity: the architectural basis of cellular mechanotransduction. Annu. Rev. Physiol. **59**(1), 575–599 (1997)
26. Juelicher, F., Kruse, K., Prost, J., Joanny, J.F.: Active behavior of the cytoskeleton. Phys. Rep. **449**(1), 3–28 (2007)
27. Jumpertz, R., Hart, A., Ohlsson, O., Saurenbach, F., Schelten, J.: Piezoresistive sensors on AFM cantilevers with atomic resolution. Microelectron. Eng. **41**, 441–444 (1998)
28. Kallio, P., Kuncova-Kallio, J.: Capillary pressure microinjection of living adherent cells: challenges in automation. J. Micromechatronics **3**(3), 189–220 (2006)
29. Karimirad, F., Chauhan, S., Shirinzadeh, B.: Vision-based force measurement using neural networks for biological cell microinjection. J. Biomech. **47**(5), 1157–1163 (2014)
30. Kass, M., Witkin, A., Terzopoulos, D.: Snakes: active contour models. Int. J. Comput. Vis. **1**(4), 321–331 (1988)
31. Kim, D.H., Kim, B., Yun, S., Kwon, S.: Cellular force measurement for force reflected biomanipulation. In: Proceedings of the 2004 IEEE International Conference on Robotics and Automation (ICRA'04), vol. 3, pp. 2412–2417 (2004)
32. Kim, D.H., Sun, Y., Yun, S., Kim, B., Hwang, C.N., Lee, S.H., Nelson, B.J.: Mechanical property characterization of the zebrafish embryo chorion. In: Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEMBS'04), vol. 2, pp. 5061–5064 (2004)
33. Kim, J., Janabi-Sharifi, F., Kim, J.: Haptic feedback based on physically based modeling for cellular manipulation systems. In: Proceedings of the International Conference on Human Haptic Sensing and Touch Enabled Computer Applications, pp. 661–667 (2008)
34. Kim, J., Janabi-Sharifi, F., Kim, J.: A haptic interaction method using visual information and physically based modeling. IEEE/ASME Trans. Mechatron. **15**(4), 636–645 (2010)
35. Kim, K., Liu, X., Zhang, Y., Sun, Y.: Nanonewton force-controlled manipulation of biological cells using a monolithic MEMS microgripper with two-axis force feedback. J. Micromech. Microeng. **18**(5), 055013 (2008)
36. Kimura, Y., Yanagimachi, R.: Intracytoplasmic sperm injection in the mouse. Biol. Reprod. **52**(4), 709–720 (1995)
37. Kuncova, J., Kallio, P.: Challenges in capillary pressure microinjection. In: Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEMBS'04), vol. 2, pp. 4998–5001 (2004)
38. Kuncova-Kallio, J.: Calibration of a capillary pressure microinjection and its implementation within a micromanipulator. M.Sc. thesis, Tampere University of Technology (2002)
39. Ladjal, H., Hanus, J.L., Ferreira, A.: Micro-to-nano biomechanical modeling for assisted biological cell injection. IEEE Trans. Biomed. Eng. **60**(9), 2461–2471 (2013)
40. Langenau, D.M., Keefe, M.D., Storer, N.Y., Guyon, J.R., Kutok, J.L., Le, X., Goessling, W., Neuberg, D.S., Kunkel, L.M., Zon, L.I.: Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev. **21**(11), 1382–1395 (2007)
41. Leymarie, F., Levine, M.D.: Tracking deformable objects in the plane using an active contour model. IEEE Trans. Pattern Anal. Mach. Intell. **15**(6), 617–634 (1993)
42. Li, W.J., Xi, N.: Novel micro gripping, probing, and sensing devices for single-cell surgery. In: Proceedings of the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEMBS'04), vol. 1, pp. 2591–2594 (2004)

43. Li, X., Zong, G., Bi, S.: Development of global vision system for biological automatic micromanipulation system. In: Proceedings of the 2001 IEEE International Conference on Robotics and Automation (ICRA), vol. 1, pp. 127–132 (2001)
44. Li, X., Zong, G., Bi, S., Zhao, W.: Automatic micromanipulating system for biological applications with visual servo control. J. Micromechatron. **1**(4), 345–363 (2001)
45. Lim, C., Zhou, E., Quek, S.: Mechanical models for living cells—a review. J. Biomech. **39**(2), 195–216 (2006)
46. Lin, M.T., Pulkkinen, L., Uitto, J., Yoon, K.: The gene gun: current applications in cutaneous gene therapy. Int. J. Dermatol. **39**(3), 161–170 (2000)
47. Liu, X., Kim, K., Zhang, Y., Sun, Y.: Nanonewton force sensing and control in microrobotic cell manipulation. Int. J. Rob. Res. **28**(8), 1065–1076 (2009)
48. Liu, X., Sun, Y.: Microfabricated glass devices for rapid single cell immobilization in mouse zygote microinjection. Biomed. Microdevices **11**(6), 1169 (2009)
49. Liu, X., Sun, Y., Wang, W., Lansdorp, B.M.: Vision-based cellular force measurement using an elastic microfabricated device. J. Micromech. Microeng. **17**(7), 1281 (2007)
50. Loh, O., Lam, R., Chen, M., Moldovan, N., Huang, H., Ho, D., Espinosa, H.D.: Nanofountain-probe-based high-resolution patterning and single-cell injection of functionalized nanodiamonds. Small **5**(14), 1667–1674 (2009)
51. Lu, Z., Chen, P.C., Lin, W.: Force sensing and control in micromanipulation. IEEE Trans. Syst. Man Cybern. Part C Appl. Rev. **36**(6), 713–724 (2006)
52. Lu, Z., Chen, P.C., Nam, J., Ge, R., Lin, W.: A micromanipulation system with dynamic force-feedback for automatic batch microinjection. J. Micromech. Microeng. **17**(2), 314 (2007)
53. Lukkari, M., Kallio, P.: Multi-purpose impedance-based measurement system to automate microinjection of adherent cells. In: Proceedings of the 2005 IEEE International Symposium on Computational Intelligence in Robotics and Automatio (CIRA 2005), pp. 701–706 (2005)
54. Marr, D., Hildreth, E.: Theory of edge detection. Proc. R. Soc. Lond. B Biol. Sci. **207**(1167), 187–217 (1980)
55. Maruyama, H., Masuda, T., Liu, H., Arai, F.: Selective and rapid cell injection of fluorescence sensor encapsulated in liposome using optical control of zeta potential and local mechanical stimulus by optical tweezers. In: Proceedings of the 2014 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS 2014), pp. 816–821 (2014)
56. Matsuoka, H., Komazaki, T., Mukai, Y., Shibusawa, M., Akane, H., Chaki, A., Uetake, N., Saito, M.: High throughput easy microinjection with a single-cell manipulation supporting robot. J. Biotechnol. **116**(2), 185–194 (2005)
57. Matsuoka, H., Shimoda, S., Miwa, Y., Saito, M.: Automatic positioning of a microinjector in mouse es cells and rice protoplasts. Bioelectrochemistry **69**(2), 187–192 (2006)
58. Mehrbod, M., Mofrad, M.R.: On the significance of microtubule flexural behavior in cytoskeletal mechanics. PloS One **6**(10), e25627 (2011)
59. Meister, A., Gabi, M., Behr, P., Studer, P., Voros, J., Niedermann, P., Bitterli, J., Polesel-Maris, J., Liley, M., Heinzelmann, H., et al.: FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond. Nano Lett. **9**(6), 2501–2507 (2009)
60. Meyer, G., Amer, N.M.: Novel optical approach to atomic force microscopy. Appl. Phys. Lett. **53**(12), 1045–1047 (1988)
61. Minaschek, G., Bereiter-Hahn, J., Bertholdt, G.: Quantitation of the volume of liquid injected into cells by means of pressure. Exp. Cell Res. **183**(2), 434–442 (1989)
62. Muntwyler, S., Beyeler, F., Nelson, B.: Three-axis micro-force sensor with sub-micro-Newton measurement uncertainty and tunable force range. J. Micromech. Microeng. **20**(2), 025011 (2009)
63. Muraoka, O., Shimizu, T., Yabe, T., Nojima, H., Bae, Y.K., Hashimoto, H., Hibi, M.: Sizzled controls dorso-ventral polarity by repressing cleavage of the Chordin protein. Nat. Cell Biol. **8**(4), 329 (2006)
64. Murphy, D.B.: Fundamentals of light microscopy and electronic imaging. Wiley, New York (2002)

65. Nakayama, T., Fujiwara, H., Tastumi, K., Fujita, K., Higuchi, T., Mori, T.: A new assisted hatching technique using a piezo-micromanipulator. Fertil. Steril. **69**(4), 784–788 (1998)
66. Part, S.: Impedance control: an approach to manipulation. J. Dyn. Syst. Meas. Contr. **107**, 17 (1985)
67. Permana, S., Grant, E., Walker, G.M., Yoder, J.A.: A review of automated microinjection systems for single cells in the embryogenesis stage. IEEE/ASME Trans. Mechatron. **21**(5), 2391–2404 (2016)
68. Pillarisetti, A., Anjum, W., Desai, J.P., Friedman, G., Brooks, A.D.: Force feedback interface for cell injection. In: Proceedings of the First Joint Eurohaptics Conference and Symposium on Haptic Interfaces for Virtual Environment and Teleoperator Systems, pp. 391–400 (2005)
69. Pillarisetti, A., Pekarev, M., Brooks, A.D., Desai, J.P.: Evaluating the effect of force feedback in cell injection. IEEE Trans. Autom. Sci. Eng. **4**(3), 322 (2007)
70. Poleo, G.A., Denniston, R.S., Reggio, B.C., Godke, R.A., Tiersch, T.R.: Fertilization of eggs of zebrafish, *Danio rerio*, by intracytoplasmic sperm injection. Biol. Reprod. **65**(3), 961–966 (2001)
71. Rols, M.P.: Electropermeabilization, a physical method for the delivery of therapeutic molecules into cells. Biochim. Biophys. Acta (BBA) Biomembr. **1758**(3), 423–428 (2006)
72. Rubin, G.M., Lewis, E.B.: A brief history of drosophila's contributions to genome research. Science **287**(5461), 2216–2218 (2000)
73. Rubin, G.M., Yandell, M.D., Wortman, J.R., Gabor, G.L., Nelson, C.R., Hariharan, I.K., Fortini, M.E., Li, P.W., Apweiler, R., Fleischmann, W., et al.: Comparative genomics of the eukaryotes. Science **287**(5461), 2204–2215 (2000)
74. Saffrich, R., Pepperkok, R.: Embo practical course–microinjection and detection of probes in cells (2001). http://www.bio.net/mm/cellbiol/1998-March/008361.html
75. Scherp, P., Hasenstein, K.: Microinjection—a tool to study gravitropism. Adv. Space Res. **31**(10), 2221–2227 (2003)
76. Shen, Y., Wejinya, U., Xi, N., Pomeroy, C.A.: Force measurement and mechanical characterization of living drosophila embryos for human medical study. Proc. Inst. Mech. Eng. Part H J. Eng. Med. **221**(2), 99–112 (2007)
77. Shen, Y., Winder, E., Xi, N., Pomeroy, C.A., Wejinya, U.C.: Closed-loop optimal control-enabled piezoelectric microforce sensors. IEEE/ASME Trans. Mechatron. **11**(4), 420–427 (2006)
78. Shim, J.H., Cho, S.Y., Cha, D.H.: Vision-guided micromanipulation system for biomedical application. Proc. SPIE **5604**, 98–107 (2004)
79. Shulev, A., Roussev, I., Kostadinov, K.: Force sensor for cell injection and characterization. In: Proceedings of the 2012 International Conference on Manipulation, Manufacturing and Measurement on the Nanoscale (3M-NANO), pp. 335–338 (2012)
80. Stavrov, V.T., Shulev, A.A., Hardalov, C.M., Todorov, V.M., Roussev, I.R.: All-silicon microforce sensor for bio applications. In: Proceedings of SPIE, vol. 8763, p. 87630Y (2013)
81. Sun, Y., Fry, S.N., Potasek, D., Bell, D.J., Nelson, B.J.: Characterizing fruit fly flight behavior using a microforce sensor with a new comb-drive configuration. J. Microelectromech. Syst. **14**(1), 4–11 (2005)
82. Sun, Y., Nelson, B.J.: Biological cell injection using an autonomous microrobotic system. Int. J. Rob. Res. **21**(10–11), 861–868 (2002)
83. Sun, Y., Nelson, B.J.: Mems for cellular force measurements and molecular detection. Int. J. Inf. Acquisition **1**(01), 23–32 (2004)
84. Sun, Y., Nelson, B.J.: Mems capacitive force sensors for cellular and flight biomechanics. Biomed. Mater. **2**(1), S16 (2007)
85. Sun, Y., Nelson, B.J., Potasek, D.P., Enikov, E.: A bulk microfabricated multi-axis capacitive cellular force sensor using transverse comb drives. J. Micromech. Microeng. **12**(6), 832 (2002)
86. Sun, Y., Wan, K.T., Roberts, K.P., Bischof, J.C., Nelson, B.J.: Mechanical property characterization of mouse zona pellucida. IEEE Trans. Nanobiosci. **2**(4), 279–286 (2003)
87. Sun, Z., Hao, L., Chen, W., Li, Z.: Robotic cell injection force control based on static PVDF sensor and fuzzy-PID control method. Int. J. Appl. Electromagnet. Mech. **41**(1), 73–86 (2013)

88. Sundaram, J., Mellein, B.R., Mitragotri, S.: An experimental and theoretical analysis of ultrasound-induced permeabilization of cell membranes. Biophys. J. **84**(5), 3087–3101 (2003)
89. Tan, Y., Sun, D., Huang, W., Cheng, S.H.: Mechanical modeling of biological cells in microinjection. IEEE Trans. Nanobiosci. **7**(4), 257–266 (2008)
90. Tan, Y., Sun, D., Huang, W., Cheng, S.H.: Characterizing mechanical properties of biological cells by microinjection. IEEE Trans. Nanobiosci. **9**(3), 171–180 (2010)
91. Tanikawa, T., Arai, T.: Development of a micro-manipulation system having a two-fingered micro-hand. IEEE Trans. Rob. Autom. **15**(1), 152–162 (1999)
92. Tortonese, M., Yamada, H., Barrett, R., Quate, C.: Atomic force microscopy using a piezoresistive cantilever. In: Proceedings of the 1991 International Conference on Solid-State Sensors and Actuators (TRANSDUCERS'91), pp. 448–451 (1991)
93. Tran, N.D., Liu, X., Yan, Z., Abbote, D., Jiang, Q., Kmiec, E.B., Sigmund, C.D., Engelhardt, J.F.: Efficiency of chimeraplast gene targeting by direct nuclear injection using a GFP recovery assay. Mol. Ther. **7**(2), 248–253 (2003)
94. Ujihara, Y., Nakamura, M., Miyazaki, H., Wada, S.: Proposed spring network cell model based on a minimum energy concept. Ann. Biomed. Eng. **38**(4), 1530–1538 (2010)
95. Wall, R.J.: Pronuclear microinjection. Cloning Stem Cells **3**(4), 209–220 (2001)
96. Walter, W., Stein, U.: Viral vectors for gene transfer a review of their use in the treatment of human disease. Drugs **60**, 249–71 (2000)
97. Wan, H.I., DiAntonio, A., Fetter, R.D., Bergstrom, K., Strauss, R., Goodman, C.S.: Highwire regulates synaptic growth in *Drosophila*. Neuron **26**(2), 313–329 (2000)
98. Wang, G., Xu, Q.: Design and precision position/force control of a piezo-driven microinjection system. IEEE/ASME Trans. Mechatron. **22**(4), 1744–1754 (2017)
99. Wang, W., Liu, X., Gelinas, D., Ciruna, B., Sun, Y.: A fully automated robotic system for microinjection of zebrafish embryos. PloS One **2**(9), e862 (2007)
100. Wei, Y., Xu, Q.: An overview of micro-force sensing techniques. Sens. Actuators A Phys. **234**, 359–374 (2015)
101. Wei, Y., Xu, Q.: Design of a PVDF-MFC force sensor for robot-assisted single cell microinjection. IEEE Sens. J. **17**(13), 3975–3982 (2017)
102. Wejinya, U.C., Shen, Y., Xi, N., Salem, F.: Force measurement of embryonic system using in situ PVDF piezoelectric sensor. In: Proceedings of the 49th IEEE International Midwest Symposium on Circuits and Systems (MWSCAS'06), vol. 1, pp. 108–112 (2006)
103. Wenming, X., Hui, Z.: Bio-manipulation probe integration with micro-force sensor. In: Proceedings of the 3rd IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS 2008), pp. 393–396 (2008)
104. Westerfield, M.: The zebrafish book: a guide for the laboratory use of zebrafish. http://zfin.org/zf_info/zfbook/zfbk.html (2000)
105. Wiens, G.J., Roman, G.A.: A mechanism approach for enhancing the dynamic range and linearity of MEMS optical force sensing. Signal Measurement and Estimation Techniques for Micro and Nanotechnology, p. 193 (2011)
106. Wright, W.H., Sonek, G., Tadir, Y., Berns, M.W.: Laser trapping in cell biology. IEEE J. Quant. Electron. **26**(12), 2148–2157 (1990)
107. Xie, Y., Sun, D., Liu, C.: Penetration force measurement and control in robotic cell microinjection. In: Proceedings of the 2009 IEEE/RSJ International Conference on Intelligent Robots and Systems, pp. 4701–4706 (2009)
108. Xie, Y., Sun, D., Liu, C., Tse, H.Y., Cheng, S.H.: A force control approach to a robot-assisted cell microinjection system. Int. J. Rob. Res. **29**(9), 1222–1232 (2010)
109. Xie, Y., Sun, D., Tse, H.Y.G., Liu, C., Cheng, S.H.: Force sensing and manipulation strategy in robot-assisted microinjection on zebrafish embryos. IEEE/ASME Trans. Mechatron. **16**(6), 1002–1010 (2011)
110. Xu, Q.: Design and development of a novel compliant gripper with integrated position and grasping/interaction force sensing. IEEE Trans. Autom. Sci. Eng. (2015)
111. Xu, Q., Tan, K.K.: Advanced Control of Piezoelectric Micro-/Nano-Positioning Systems. Springer, Berlin (2015)

112. Yamagata, Y., Higuchi, T.: A micropositioning device for precision automatic assembly using impact force of piezoelectric elements. In: Proceedings of the 1995 IEEE International Conference on Robotics and Automation, vol. 1, pp. 666–671 (1995)
113. Yu, S., Nelson, B.J.: Microrobotic cell injection. In: Proceedings of the 2001 IEEE International Conference on Robotics and Automation (ICRA), vol. 1, pp. 620–625 (2001)
114. Yunlei, Z., Shuang, L., Chongjun, Y., Chong, L., Yu, X.: Design of a microforce sensor based on fixed-simply supported beam: towards realtime cell microinjection. In: Proceeedings of the 2015 IEEE International Conference on Cyber Technology in Automation, Control, and Intelligent Systems (CYBER), pp. 1080–1084 (2015)
115. Zappe, S., Fish, M., Scott, M.P., Solgaard, O.: Automated mems-based drosophila embryo injection system for high-throughput rnai screens. Lab Chip **6**(8), 1012–1019 (2006)
116. Zeng, Y., Yip, A.K., Teo, S.K., Chiam, K.H.: A three-dimensional random network model of the cytoskeleton and its role in mechanotransduction and nucleus deformation. Biomech. Model. Mechanobiol. **11**(1–2), 49–59 (2012)
117. Zhang, W., Sobolevski, A., Li, B., Rao, Y., Liu, X.: An automated force-controlled robotic micromanipulation system for mechanotransduction studies of drosophila larvae. IEEE Trans. Autom. Sci. Eng. **13**(2), 789–797 (2016)
118. Zhang, X., Scott, M.P., Quate, C.F., Solgaard, O.: Microoptical characterization of piezoelectric vibratory microinjections in drosophila embryos for genome-wide RNAi screen. J. Microelectromech. Syst. **15**(2), 277–286 (2006)
119. Zhang, X., Zappe, S., Bernstein, R., Sahin, O., Chen, C.C., Fish, M., Scott, M., Solgaard, O.: Integrated optical diffractive micrograting-based injection force sensor. In: Proceedings of the 12th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS), vol. 2, pp. 1051–1054 (2003)
120. Zhang, X., Zappe, S., Bernstein, R., Sahin, O., Chen, C.C., Fish, M., Scott, M., Solgaard, O.: Micromachined silicon force sensor based on diffractive optical encoders for characterization of microinjection. Sens. Actuators A Phys. **114**(2), 197–203 (2004)