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Piezoelectric Driven Non-toxic Injector for Automated Cell Manipulation

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Abstract. Stimulated by state-of-the-art robotic and computer technology, Intra Cytoplasmic Sperm Injection (ICSI) automation aims to scale and seamlessly transfer the human hand movements into more precise and fast movements of the micro manipulator. Piezo-drill cell injection, a novel technique using piezo-driven pipettes with a very small mercury column, has significantly improves the survival rates of ICSI process. It is found that complications are due, in large part, to toxicity of mercury and the damage to the cell membrane because of the lateral tip oscillations of injector pipette. In this paper, a new design of piezo-driven cell injector is proposed for automated suspended cell injection. This new piezo-driven cell injector design centralizes the piezo oscillation power on the injector pipette which eliminates the vibration effect on other parts of the micromanipulator. Detrimental lateral tip oscillations of the injector pipette are attenuated to a desirable level even without the help of mercury column. This mercury-free injector can sublime the piezoelectric driven injection technique to completely non-toxic level with great research and commercial application in gene injection, in-vitro fertilization, ICSI and drug development.

Keywords. Biomanipulation, piezoelectric driven injector, cell microinjection

Introduction

Due to the importance of biological cell injection technology, significant research has been carried out to automate laborious cell injection tasks. Last decade has witnessed the dedicated research effort on cell injection automation from a diverse array of aspects: cell holding devices, cell injection force control, visual serving, cell injection process control, etc [1-2]. Recently, a promising cell piercing technology called piezo-drill was proposed. Kimura [3] originally presented the piezo-driven ICSI procedure and a high survival rate of sperm-injected mouse oocytes that reaches 80% can be obtained. Researches in the field also discovered that a very small mercury column in the injector pipette can significantly improve the success rate of ICSI [4]. However, the use of mercury in laboratory has detrimentally potential toxicity effects.

Researchers have been investigating the influence of mercury column and lateral pipette oscillation on the zona piercing mechanism in ICSI. Experiments conducted by Ediz [6] and the simulation by Gan [7] support that the presence of mercury generally

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reduces the amplitude of the pipette tip oscillation. As for the zona piercing mechanism in piezo-ICSI, Kimura [3] and Fan [5] stated that the axial pipette motion facilitates the piercing of the mouse zona pellucida. However, Ediz [8] found that the pipette has a much larger lateral oscillation comparing with axial oscillation and argued that the lateral oscillation may collaterally damage the zona piercing process. While there are different opinions on these issues, it is a consensus that complications are due, in large part, to toxicity of mercury and the damage to the cell membrane because of the lateral tip oscillations of injector pipette.

In this paper, a piezo-driven ultrasonic cell injection technique is introduced as a universal cell injection method. To address the aforementioned issues, the contributions of this paper are: (1) a novel design of piezo-driven cell injector which centralizes the piezo oscillation power on the injector pipette through a piezo stack located near the injector pipette. Significantly, this eliminates the vibration effect on other parts of the micromanipulator. Hence, only a small piezo stack is required to actuate the pipette tip and perform the piezo-drill cell injection process and (2) high amplitude oscillation of the injector pipette due to the system noise is substantially reduced without a mercury column. From cell injection experiments that were performed on zebrafish embryos, under reasonable driven frequency and amplitude, the injector pipette easily pierces through the cell membrane with much lower injection speed and with virtually no deformation of the cell membrane. This novel technology approach has demonstrated significant potential in high-precision cell injection with minimum damage to injected cells comparing with previous results.

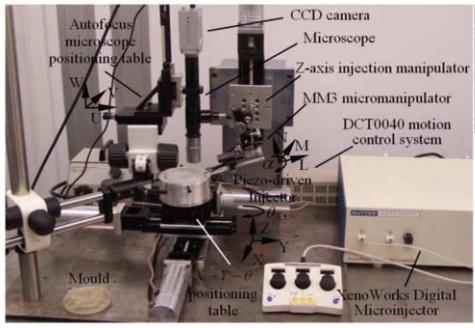

Figure 1. Test-bed for the suspended cell injection system.

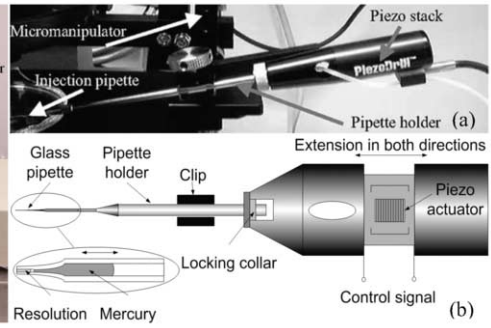

Figure 2. Conventional commercial piezo-drill system [9].

1. Methods & Materials

1.1. Cell Injection System Setup

Figure 1 illustrates an automatic suspended cell injection system developed in our laboratory for this research. Based on the previous research [2], this newly-designed system not only considers the characteristic of the biological experiment undertaken, to make the operation more convenient, but also adds new functions such as microscope autofocus. This system is designed to simulate automatic cell injection of large batches of suspended cells (such as fish embryos) in biological engineering processes. To achieve this purpose, the system is designed for use with cells arrays instead of holding cells individually. A specially designed cell holding device was fixed on an actuated

rotary plate, permitting the cells to be held and transported one by one, into the field of view of the microscope for injection.

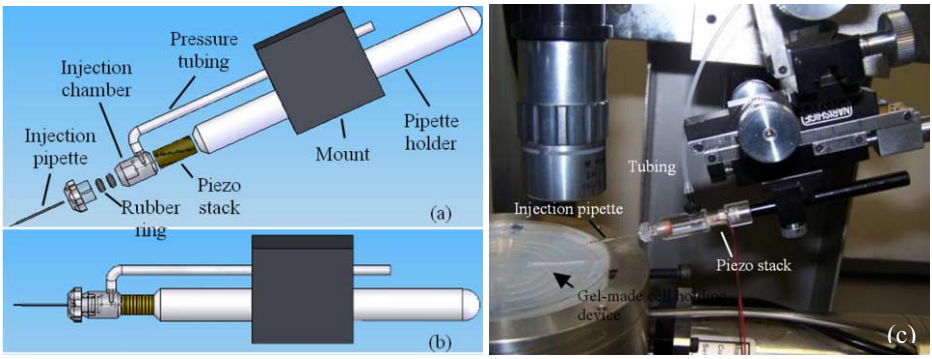

Figure 3. A new design of piezo-driven cell injector. (a) Exploded drawing; (b) Assembly drawing; (c) The close sight of the piezo-driven cell injector.

1.2. Novel Piezo-driven Cell Injector Design

The piezo-drill cell injector structure has remained the same since it was invented. A commercial piezo-driven cell injection system (PIEZODRILL, Burleigh) [9] is shown in Fig. 2(a). It consists of the injection pipette, the pipette holder, the holding clip, and the piezo actuator, as shown in Fig. 2(b). The ultrasonic mechanical pulses transfer longitudinally through pipette holder, the holding clip, the glass pipette and finally reach the pipette tip. The injection pipette, pipette holder, and the holding clip will vibrate during the cell injection process. The vibration of so many parts will unavoidably introduce unwanted mechanical vibration to the pipette tip. As discussed in the research of Ediz [8], when this piezo-drill pipette is operated in air, the largest lateral vibration of the pipette tip will reach 270 μm. Even with the help of mercury column and the pipette is immersed in mineral oil, the largest lateral vibration of the pipette tip still reaches 37 μm. And the use of mineral oil and mercury may lead to contamination of cells etc..

To eliminate unwanted lateral pipette vibration and centralize the piezo power on the injector pipette, a new design of this injector system was proposed, as shown in Fig. 3. Fig. 3(a) and (b) are respectively the exploded view and assembly view of machine parts. The piezo stack is assembled on the end of the piezo-driven cell injector and to minimize the load of piezo stack, the parts cannot be moved are made of low density material acrylic (to lower down the vibration mass). The injection pipette can be easily replaced and is fixed through the rubber ring. The soft plastic pressure tube provides for the injection of solutions into cells. Fig. 3(c) shows the close sight of the new piezo-driven cell injector.

2. Results

To verify the effectiveness of the proposed approach, experiments were performed using the cell injection system as shown in Fig. 1. The cells selected for our experiment target were Zebrafish embryos. The diameter of Zebrafish embryo is approximately

~1.2mm (including chorion) and ~600 μm (without chorion). The thickness of zebrafish embryo is 3 μm. The radius of the injector pipette is $c = 7.5\mu$m. The injected zebrafish embryos were all placed in our special designed cell holding device.

It is known that too large lateral vibration will cause damage to cells in piezo-driven cell injection. Existing piezo-drill systems utilizes an adjustable given pulse voltage, to inspire the piezo actuator and drill the cells [5-8]. The three parameters: amplitude, frequency, and duration can be adjusted and trained by the user for particular exercise. But it is found in our research that the harmful lateral tip oscillation has a great relationship with the frequency of the inspiring signal. Then a single frequency sine wave voltage signal, which is always used in ultrasonic cutting, was used here for piezo-driven cell injection. A series of sine wave signals (frequency 0.5Hz~40KHz, amplitude 10V) were applied as the inspired signal of piezo actuator. The lateral oscillations of the pipette tip were observed and recorded by CCD camera. When the injector pipette is placed in exactly the same situation of cell injection (the pipette tip is immersed in the water), the frames in Fig.4 show clearly the lateral oscillations of the pipette under different frequency of inspired signal. When the input signal frequency is below 21 KHz, very slightly lateral vibration can be recognized. But between 22 KHz and 23 KHz, the lateral vibration amplitudes increase significantly and the amplitudes reach its maximum when the frequency of the input signal frequency is 22.5 KHz. At this time, the vibration frequency of the piezo actuator reaches the resonance frequency of the injector pipette and the amplitude of the lateral oscillation of pipette tip reaches its maximum (30 μm). When the frequency improves higher than 23 KHz, the lateral vibration decreased back to slightly.

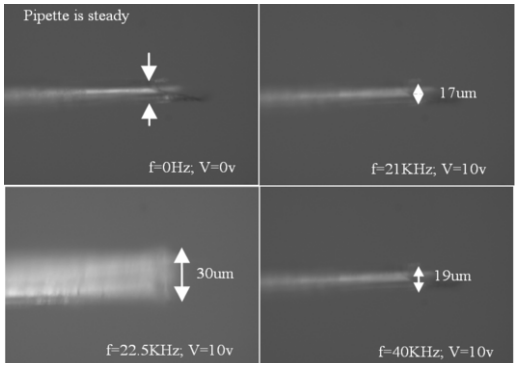

Figure 4. Lateral vibration of pipette under the environment of cell injection.

2.1. Cell Injection Experiment

Experiments were performed to show the effectiveness of piezo-driven cell injection. In all injection experiments, although each test used a different embryo cell, the mechanical properties of all cell biomembranes are uniform. The trained parameter of piezo-driven cell injection of zebrafish embryo is 20.1 KHz and 10V. The injection velocity is constant with 175 μm/s.

Two entire cell injection processes with piezo-driven cell injection and conventional cellular piercing technology were shown in Fig. 5 for comparison. It is clear to see that when we inject the zebrafish embryo with piezo-driven cell injector, the injector pipette contact the cell membrane and cutting through it with nearly no

deformation, as shown in Fig. 5(b). But when conventional cellular piercing technology is used, the cell membrane is deformed by the injection force. When injection force reaches the broken threshold, the injector pipette pierces through the cell membrane and into the cells. It is clear to see a large deformation on cell membrane in Fig. 5 (a).

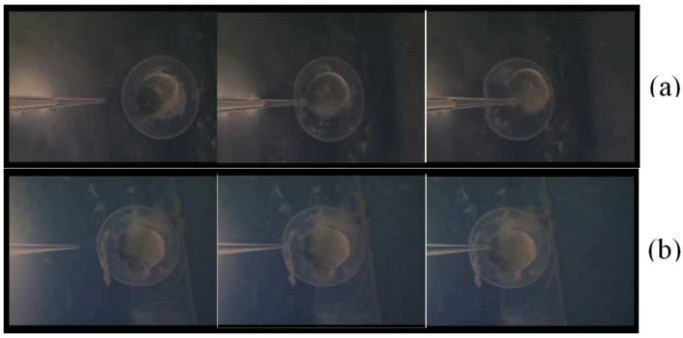

Figure 5. (a) Cell injection process under the conventional cellular piercing technology; (b) Cell injection process with the piezo-driven cell injection technology.

3. Conclusions

In this paper, a novel piezo-driven cell injector was developed and validated in cell injection of zebrafish embryos. According to experiments of zebrafish embryos cell injection, this piezo-driven cell injector exhibits three advantages over the conventional cellular piercing technology. 1) It utilizes the ultrasonic cutting force while not the piercing force to penetrate the cell membrane. The injecting speed requirement is less stringent than the conventional ones thus resulting in better controllability in cell injection motion control. 2) With nearly no deformation of the cells during the cell injection period, cell damage is dramatically less. This technique could result in comparably high survival rate and success rate. 3) With the new piezo-driven cell injector, a small piezo stack is sufficient to perform the cell injection process. And with appropriate frequency and amplitude, the detrimental lateral tip oscillations of injector pipette are reduced to a satisfying level even without mercury column.

References

[1] W.H. Wang, et al., A fully automated robotic system for microinjection of zebrafish embryos, PLoS ONE, Vol. 2, No. 9, e862. doi:10.1371/journal.pone.0000862, 2007.
[2] H. B. Huang, et al., Robotics cell injection system with vision and force control: Towards automatic batch biomanipulation, IEEE Trans. on Robotics, vol. 25, no. 3 (2009), 727-737.
[3] Y. Kimura, et al., Intracytoplasmic sperm injection in the mouse, Biol. Reprod., vol. 52, no. 4 (1995), 709–720.
[4] Y. Kimura, et al., Analysis of mouse oocyte activation suggests the involvement of sperm perinuclear material, Biol. Reprod., vol. 58, no. 6 (1998), 1407–1415.
[5] M. Fan, et al., Vibration study of piezodriven pipettes immersed in viscous liquids," 100(7), 2006.
[6] K. Ediz, et al., Effect of Mercury Columan on the Microdynamics of the Piezo-Driven Pipettes, J. of Biomechanical Engineering, vol. 127 (2005), 531-535.
[7] Y. Gan, et al., A Study of the zona piercing process in piezodriven intracytoplasmic sperm injection, 104(4), 2008.
[8] K. Ediz, et al., Microdynamics of the Piezo-Driven Pipette in ICSI, IEEE Trans. on Biomedial Engineering, vol. 51, no. 7 (2004), 1262-1268.
[9] http://www.cadence-tech.com.sg/Burleigh/Piezo%20Drill.htm