Single-Cell Manipulation using Nanopipettes

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ABSTRACT

Manipulation and analysis of individual cells is key in understanding processes that control single-cell behavior in complex environment. The fully electrical read-out, ease and low cost of fabrication are unique features that give nanopipette technology enormous potential. We developed a single-cell manipulation platform based on quartz nanopipettes which are fitted with electrodes to mediate voltage-dependent injection to individual cells. The use of double-barrel nanopipettes negates the need for an external reference electrode, increasing viability and allowing for multicomponent injections. Using a similar feedback mechanism, our group has expanded the application of the platform to allow for precise aspiration of contents from a single-cell. We are currently interfacing the single-cell manipulation platform with a microfluidic chip to immobilize cells in an array format to form the basis for a fully automated system for high-throughput injection and aspiration of single cells.

Keywords: nanopipette, single-cell injection, scanning ion conductance microscopy, reprogramming, stem cells

1 INTRODUCTION

Single cell biology is an emerging field in which a culture of cells is not only observed by its mass behavior but on an individual cellular level[1]. Single cell injections are useful in regulating cell fate by introducing controlled amounts of foreign material into a cell. Single cell injections, however, have been a historically difficult task due to manual injections requiring extensive training and the success rate depending on the experience of the operator.

The most common method for injecting cells relies on microinjection with a glass micropipette. This technique is extremely successful with large cells, such as oocytes, but it is not suitable for smaller cells (~ 30 µm in diameter) because the diameter of a micropipette tip (~ 5 µm) causes great disruption to a cell during penetration. Also, micropipette injections require a skilled technician who has to position the cell using a holding pipette and then bring the micropipette into the cell for injections. Pillarisetti *et al.* has shown this technique by injecting trypan blue dye into a zebrafish egg cell, a known large cell with the diameter of each egg being 600-700 µm, in order to optimize their force feedback control[2].

To overcome the limitions of a large micropipette tip, several nanotechnologies have been developed to allow single cell injections into cells of a smaller diameter which includes most of the cells useful in manipulating for real-world application, for example stem cells. Meister *et al.* were able to inject myoblast cells using an adaption of Atomic Force Microscopy (AFM). Their technology, however, is limited when it comes to controlling injection volume and throughput. This led to our goal of integrating single cell injection based on nanopipettes with a cell capturing device to form the basis for a fully automated single cell injection platfrom. Integrating a cell immobilization device where cells are captured at fixed locations will allow for a high-throughput reproducible injection technology. Single cell immobilization has been produced in the past but has been limited in ways of integration[4-5].

We also wanted to investigate the viability of the cells post-injection. Cell viability is vital post-injection because depending on what molecule was injected, it may take time to integrate into the genome of the cell and the cell staying viable is necessary for analysis. Also, in some cases more than one molecule needs to be injected into the same cell, i.e. for inducing pluripotent stem cells. Rodolfa *et al.* show that using double-barreled pipettes eliminates the issue of having to re-inject the same cell with two different pipettes. This also aids with cell viability. An electric potential is applied between the two barrels and the molecules are deposited in the single cells by applied voltage[5].

In this study, we have created and tested a technology for precise injection into single cells using a double-barreled nanopipette. We have shown successful viability post-injection, control of volume, and control of injection from each barrel respectively[6]. The injection technology is integrated with an immobilization microfabricated fluidic chip to allow for high-throughput injections.

2 METHODS AND RESULTS

Our lab has created a system where single cells could be injected using an applied voltage and a feedback system. The feedback system measures the current and determines where the cell is in relation to the tip of the nanopipette. The system can precisely insert the nanopipette in the cell cytoplasm and deliver material by means of short volatge pulses.

2.1 Controllable Injections

The system is designed to switch between a low volatge amplifier for feedback control, and a high voltage power supply for cell injections. We optimized the magnitude and the duration of the applied volatge to maximize cell viability. Injections were performed with a range of 1V-30V. Higher voltages caused high cell disruption. This is seen by vesicles forming around the edges of the cell post injection due to the cell lifting from the petri dish and not surviving. The duration of the applied voltage is between 100ms-10s,. Typical procedures are to use a low voltage if using a long injection duration time and vice-versa for the least amount of harm to the cell. Injecting with a high voltage and for a long time will create higher disruption to the cell. Cells were penetrated at 1µm for injection.

Injection volume is controlled by varying the voltage and injection duration. Figure 1 demonstrates two human bj fibroblast cells injected both at 10V and at 500ms and 5000ms, respectively. It is noticeable that the cell injected for a longer time constant has higher fluorescent intensity relative to the cell injected for shorter time.

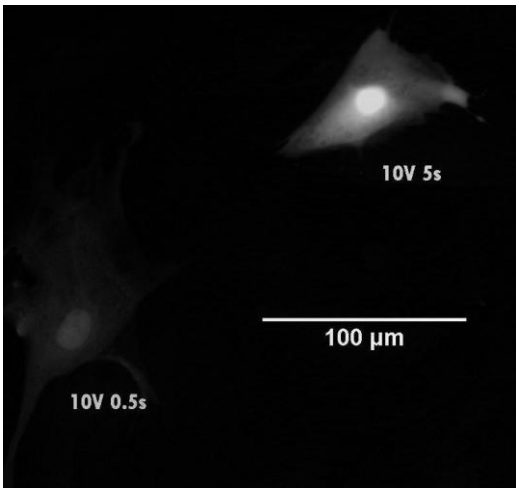

Figure 1: Human Fibroblast cells injected at both 10V for 500ms and 5000ms, respectively. The cell injected at a longer duration displays higher fluorescent intensity showing controllable injections.

2.2 Multicomponent Injections

Our injection system is unique in the fact that we can perform multicomponent injections using the same nanopipette. The double-barrel nanopipettes allow for bicomponent controllable injection without any cross-talk between the two barrels. The use of double-barrel nanopipettes negates the need for an external reference electrode, thus minimizing cell disturbance as no current is passed through the cell membrane which improves viability It was proved that each barrel can inject independently. To show this, a nanopipette is filled with one barrel Carboxyfluorscein (green) and the other barrel with Rhodamine (red), seen in figure 2.

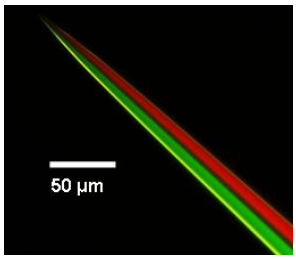

Figure 2: False color image of a double-barrel nanopipette filled with two different fluorescent dyes, one barrel is green dye, and the other barrel with red.

Two cells are injected with the red fluorescent dye seen in Figure 3A and two cells were injected with the green fluorescent dye seen in Figure 3B. All of these injections were performed using the same nanopipette. Each barrel can injected independently of the other by changing the polarity of the voltage for injection. The ability to inject multiple molecules into the same cell during the same injection is useful in reprogramming.

On the same note, we show that one cell can be injected by both barrels controllably. Figure 3C shows one cell injected with both red and green dyes at different time constants. The cells show fluorescence with both colors with the higher injection duration dye being more prevalent.

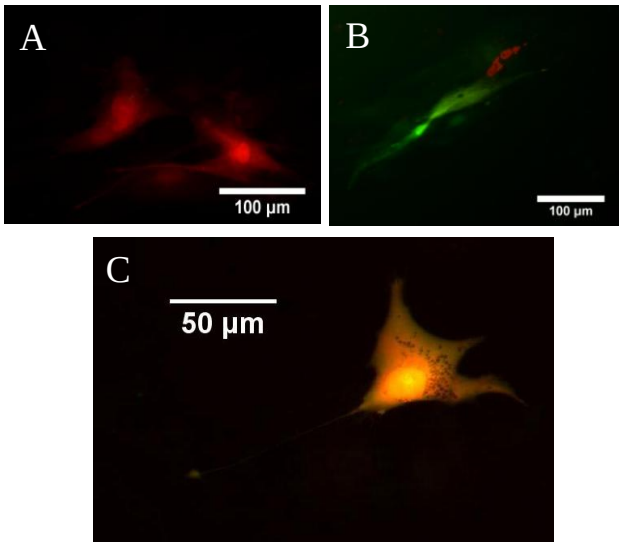

Figure 3: A: False color images of two Human Fibroblast cells injected with Rhodamine from one barrel of the nanopipette Injected at 30V for 100 ms B: Two Human Fibroblast cells injected with Carboxyfluorescein from the other barrel of the nanopipette. Injected at 30V for 100 ms. C: Human Fibroblast cell injected with red dye at 20V 150ms and injected with green dye at 20V 350ms. Injection shows controllability of each barrel of the nanopipette as well as ability for multi-injections.

2.3 Post-injection Viability

At a carboxyfluorescein concentration of 250 µM , cells are able to remain viable for many hours. Cell morphology also stays intact post-injection. Figure 4 illustrates six human fibroblast cells imaged at injection and one hour post injection. Cells were also determined to remain viable and fluorescent over 24 hours post-injection even after a cell division event occurs.

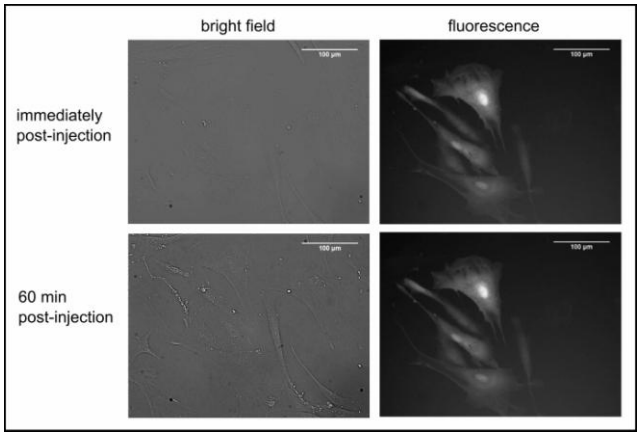

Figure 4: Images of Human Fibroblast cells in brightfield and under fluorescence at injection time and after 1 hour. This figure illustrates no obvious morphological change and cells seem to remain alive and unharmed while still fluorescent. Figure also illustrates high throughput ability. All injections done in less than five minutes. Parameters: 30V 100ms 250 µM Carboxyfluorescein succinimidyl ester

3 DISCUSSION

In this study, we have shown multiple experiments showing the feasibility of our single cell injection system using a double-barrel nanopipette. The easy fabrication of nanopipettes allowed the injection of single cells while in their normal plating conditions.

The injection system is a reliable, rapid system which can inject many cells within a few miutes. Figure 4 also shows a high-throughput injection in which six cells were successfully injected within five minutes. The short length of time to inject over a handful of cells shows feasibility and efficiency.

Due to the experiments showing controllable, successful bicomponent injections, we can say that the flow of molecules from the nanopipette into the cell is induced by electroosmosis..The injections are therefore not controlled by the charge of the molecule but rather by the charge of the nanopipette tip. This is an advantageous element allowing us to explore injection of any biologically relevant molecule without concerns of charge.

4 FUTURE WORKS

4.1 Integration with Cell Sifter

To fully automate the injection paltfrom, we are developing a microfluidic chip ("cell sifter") which can immobilize cells at predefined locations in a 6x6 array format. The cell sifter is fabricated on a silicon wafer and the holes were defined using photolithography. There is a channel that connects the vacuum barb to the well. The goal is to integrate the cell sifter with the injection system to create a fully automated high-throughput injection system. Each opening in the cell sifter will capture one cell, and the nanopipette will be able to identify the location of each hole and inject accordingly. The cells can remain in media and placed in the incubator for daily re-injections or viability tests. The cell sifter has shown to successfully capture fluorescent polystyrene beads.

5 CONCLUSION

Single cell injections have been successfully shown to controllably inject fluorescent dyes into mammalian cells with high post-injection viability. Furthermroe we demonstrated the ability of nanopieptte technology to perform multicomponent injections with no cross talk. Removing the need for an external electrode due to using double-barrel pipettes allows for less damaging cell injections during repeated cell injection. We hope that integrating the cell sifter with our injection system will allow for the direct reprogramming of Human BJ Fibroblast cells into Induced Pluripotent Stem cells as described by Yamanaka et al. 2006[8]. Present-day methods to reprogram adult cells into induced plupripotent stem cells (iPSCs) are inefficient with a success rate of 1-5% of cells within a population[7]. Nanopipette technology can have a great impact in stem cell research where there is a need for technologies able to inject cocktails of reprogramming factors into cells.

Using a similar feedback mechanism, we have continued to use nanopipettes for analyzing the contents within a cell. A single-barrel nanopipette is used to aspirate a minute amount of intracellular mass. These contents are then analyzed by performing a cDNA synthesis from the RNA extracted followed by amplification of the DNA and then a gel electrophoresis is run. In theory, the contents can be redistributed to another cell using our injection system for cell to cell transfection.

This technology has the potential to become a fully-automated single cell manipulation and analysis platform for many studies.

6 ACKNOWLEDGEMENTS

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