ABSTRACT

Delivery of compounds (nucleic acids, proteins, biomarkers, etc) to individual cells is a recurrent challenge in biology and medicine. Available delivery techniques can be divided into three main categories: biological methods, chemical methods and physical methods. Each of them has specific advantages and disadvantages in terms of delivery capabilities, toxicity and cost. Here, we present proof of concept of a system designed to achieve intracellular delivery of any macromolecule of interest by directing a sub picoliter-jet of a solution towards individual cells. This method has the potential to be quantitative and very high throughput, overcoming most of the limitations of current intracellular delivery protocols.

KEYWORDS: Microinjection, Cell delivery, microfluidic jet, microfluidics.

INTRODUCTION

Delivery of compounds (nucleic acids, proteins, biomarkers, etc) to individual cells is a recurrent challenge in biology and medicine. Available delivery techniques can be divided into three main categories: biological methods, chemical methods and physical methods. Biological methods exploit an engineered virus to deliver nucleic acids of interest, they are very effective but they require qualified operators and they deliver unwanted virus associated material. Chemical methods are very effective for the classes of compounds they have been developed for, but they are essentially limited to those. Among the physical methods, electroporation and microinjection stand out, the first for its ease of implementation and popularity (despite its toxicity); the second because beside being well received by the cells, it is the only method that is quantitative and has high cell viability. Traditional microinjection however is slow and expensive. Here, we present a proof of concept of a system aimed at improving microinjection by retaining its advantages and eliminating its disadvantages. The idea is to achieve intracellular delivery of any macromolecule of interest by directing a sub picoliter-jet of a solution towards individual cells. This method has the potential to be quantitative and very high throughput, overcoming most of the limitations of current intracellular delivery protocols.

CONCEPT AND DEVICE

The system (fig. 1, and 2) is composed by a microfluidic chip attached to a pressure generation chamber. Suspended cells flow into the chip and are lined up in a channel with size comparable to that of the cells of interest (~15 μm). Upon passage of the cell in front of a miniaturized nozzle, a jet containing the compound to be injected is fired onto the cell, thus accomplishing the delivery by exploiting the momentum of the jet.

Figure 1: Left, schematics of the jet injector. Cells are lined up inside a microfluidic channel with dimensions comparable to the cell size. A miniaturized jet is fired onto the cells to accomplish the delivery. Right: Assembly of the chip with the pressure chamber and packaging parts.
The microfluidic chips are built in silicon as it provides adequate mechanical properties to withstand the high pressures needed to generate the jet coupled with established microfabrication techniques needed to pattern a micron sized nozzle. The chip is essentially composed of a microfluidic channel with a micro nozzle. The pressure generation chamber generates on demand jets with sub-picoliter volume that are fired onto the passing cells though a micron sized (~2 μm) nozzle. The pressure pulse is generated by the deformation of a metal membrane deflected by a piezo stack and the system can generate pressure pulses up to 35 bar leading to jets up to ~20 m/s. A jet has a duration of a few tens of μs. A pressure sensor placed inside the pressure chamber allows monitoring of the generated pressure pulse.

**EXPERIMENTS, RESULTS AND DISCUSSION**

We first tested the ability of the system to generate jet. Figure 2 (right panel) shows pure water injected in a sucrose stream to enable jet visualization by exploiting the difference in refractive index between jet and receiving fluid. We then developed detailed cleaning procedures and protocols to run the experiments without clogging the cell channel or the nozzle along with procedures to degas the pressure chamber. In brief, the chip parts were washed with ethanol and DI sterile filtered water and manipulation and assembly of all the parts was performed in a clean hood.

The system was then tested for its capability to deliver fluorescent compounds inside cells. To this purpose, we fired on living cells PBS with fluorescently conjugated dextran (MW=100000) (fig 3A) that would not be able to cross the cell membrane by diffusion. We tested cell viability by verifying that cells reattached to the culture dish after being injected (fig 3B). Experiments were carried out on HeLa and Jurcat cell lines. We also tested that travel through the chip did not harm the cells by flowing them at considerable speed (~15 micron/ms). Importantly, we observed only a 1% difference in viability between the sample and the not flown control.

These data show that a jet is capable of delivering material inside cells without compromising viability. The current manual operation of the device does not allow us to obtain enough data point to perform statistics over the results. An automated jet injector should be developed to assess the potential of this technique for high throughput intracellular delivery.
CONCLUSION

Our results show that a miniaturized jet with sub pico liter volume can be used to deliver macromolecules of interest into living cells and suggest that microfluidic based “jet-injection” can be a potential novel tool for high throughput intracellular delivery.

Figure 3: Results. A bright field image of Jurcat cells injected with fluorescent dextran conjugate, and A’) fluorescence image of field of frame A. B) bright field image of HeLa cell injected with fluorescent dextran conjugate, and B’) fluorescence image of field of frame A. This cell has re-attached thus proving viability.

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