Automated MEMS-based *Drosophila* embryo injection system for high-throughput RNAi screens

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Received 9th January 2006, Accepted 17th May 2006
First published as an Advance Article on the web 8th June 2006
DOI: 10.1039/b600238b

We have developed an automated system based on microelectromechanical systems (MEMS) injectors for reliable mass-injection of *Drosophila* embryos. Targeted applications are high-throughput RNA interference (RNAi) screens. Our injection needles are made of silicon nitride. The liquid to be injected is stored in an integrated 500 nl reservoir, and an externally applied air pressure pulse precisely controls the injected volume. A steady-state water flow rate per applied pressure of 1.2 nl s\(^{-1}\) bar\(^{-1}\) was measured for a needle with channel width, height and length of 6.1 \(\mu\)m, 2.3 \(\mu\)m and 350 \(\mu\)m, respectively. A typical volume of 60 pl per embryo can be reliably and rapidly delivered within tens of milliseconds. Theoretical predictions of flow rates match measured values within ±10%. Embryos are attached to a glass slide surface and covered with oil. Packages with the injector chip and the embryo slide are mounted on motorized xyz-stages. Two cameras allow the user to quickly align the needle tip to alignment marks on the glass slide. Our system then automatically screens the glass slide for embryos and reliably detects and injects more than 98% of all embryos. Survival rates after deionized (DI) water injection of 80% and higher were achieved. A first RNAi experiment was successfully performed with double-stranded RNA (dsRNA) corresponding to the segment polarity gene *armadillo* at a concentration of 0.01 \(\mu\)M. Almost 80% of the injected embryos expressed an expected strong loss-of-function phenotype. Our system can replace current manual injection technologies and will support systematic identification of *Drosophila* gene functions.

Introduction

The genome projects have brought unprecedented opportunities to understand molecular mechanisms of development and disease. The genome sequence of the fruit fly *Drosophila melanogaster*, which was published in 2000,1 has been particularly valuable because the almost 100 years of studies of this organism can now be combined with the new molecular data to determine gene functions. Research on *D. melanogaster* is of special interest because it serves as a model organism for developmental and cellular processes common to higher eukaryotes, including humans. Comparative genomics studies for example have revealed that *D. melanogaster* has orthologs to 177 out of 289 examined human disease genes.2 In total, *D. melanogaster* has approximately 13,600 genes.

To determine the functions of genes in a high-throughput, genome-wide fashion requires rapid screening methods.3 One powerful method is to expose cells to specifically designed dsRNA corresponding to a gene of interest. Inside cells, dsRNA is used by endogenous enzymes to recognize and destroy the corresponding messenger RNA (mRNA), thus inactivating the gene function. This method is known as RNA interference, or RNAi.4,5 RNAi is a powerful tool for functional genomics research and is also discussed as a potential therapeutic strategy.6 RNAi was first demonstrated using the worm *Caenorhabditis elegans* in 1998 by manually injecting adult animals with dsRNA.7 RNAi experiments were soon expanded to other model organisms including the fruit fly.

Automated, genome-wide RNAi screens with *Drosophila* cells in culture have been demonstrated recently.8 In these experiments, cells are mixed with dsRNA solution. It takes typically 2–3 days for the dsRNA to pass through the cell membrane and to induce the gene silencing mechanism and a corresponding phenotype.9 Automated equipment such as liquid handling robots, plate readers and image acquisition tools enable genome-wide screens within less than two weeks.

Screens with cells in culture are powerful, but limited because the cells are lacking their physiological context. It is highly desirable to complement genome-wide, cell-based screens with investigations on entire living embryos. The embryonic development of the fruit fly takes place within 24 hours, however, so a gene silencing effect in entire embryos cannot be induced by mere exposure of embryos to dsRNA solution. Injection of embryos with dsRNA is typically done within the first 60 minutes of embryonic development. Injection of dsRNA must occur prior to the formation of the blastoderm, an initial cell layer consisting of approximately 6,000 cells. The injected dsRNA molecules diffuse quickly in the yolk of the embryo and are incorporated in the

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blastodermic cells as their membranes are built. Typically 100–200 fly embryos per assessed gene are injected, each with 60 pl of dsRNA. A success rate well above 50% is needed to reliably distinguish phenotypes due to gene silencing from misdeveloped embryos due to improper handling.

Currently, injections are done by hand with drawn glass capillaries. No more than about five genes can be assessed per day. Properly injecting the egg-shaped embryos with a length of 450 μm and a diameter of 150 μm is a tedious task. It requires weeks to months of injection experience to achieve the required high success rates. Furthermore, drawn glass capillaries have large and undefined needle diameters, leading to severe damage to injected embryos. Glass capillaries are long and mechanically unstable and generate high backpressures. Control over the injection process and repeatability of injection conditions and results are poor. As a consequence of these practical limitations, genome-wide screens cannot be performed within a reasonable time.

The only commercially available automated injection system has been developed for the injection of 1 mm diameter frog eggs (Robococyte, Multichannel Systems, Reutlingen, Germany11). But this setup is not suited for the precise mass-injection of Drosophila embryos. It is therefore apparent that high-throughput RNAi screens with living Drosophila embryos require new injection tools.

MEMS technologies are ideally suited for the fabrication of embryo- and oocyte-handling systems in general,13,14 as well as for the cost-effective mass-fabrication of customized injectors with integrated sensors in particular. Aimed at replacing traditional stainless steel and drawn glass capillary injectors, numerous MEMS-based injection devices have been presented over the past ten years.12–22 Targeted applications include transdermal liquid transfer,17,18 controlled injection of DNA into cells19 or drug delivery during neural recording with silicon probes.20

We have made use of MEMS technologies to fabricate single injectors that are optimized for manipulation of fruit fly embryos. Our single injectors have advantages over injector arrays19 target embryos do not need to be arranged in an array fashion, injector-to-object alignment is facilitated and we maintain full control over each single embryo injection procedure. Our MEMS injectors improve efficiency, accuracy, reliability and controllability of RNAi experiments. We have built a computer-controlled injection system based on these MEMS devices. Our setup autonomously scans the surface of a glass slide for attached embryos and performs precision injections. Several of our injection systems can be operated in parallel so that a single operator can test more than 50 genes per day, an improvement of more than ten times compared to manual injection. Our technology will help to meet the increased demand for sophisticated embryo injection technologies that has recently been generated through the sequencing of several Drosophila species. The genome sequence of D. melanogaster was followed by the sequence of Drosophila pseudoobscura in 2005.23 And the following Drosophila genome projects are either completed or close to completion: Drosophila ananassae, D. erecta, D. grimshawi, D. mojavensis, D. persimilis, D. sechellia, D. simulans, D. virilis, D. willistoni, and D. yakuba.24
3 mm × 8 mm. The integrated Pyrex channel reservoir stores 500 nl of liquid and allows us to make most efficient use of the solution that is to be injected. Liquid is transferred to the microchip with a pipette tip. The liquid is automatically pulled into the Pyrex channel and needle by capillary forces, and ejected by an externally generated air pressure pulse. The well-defined injector geometry ensures repeatability of penetration and precise dosage of injections. Separation of the needle tip and liquid outlet as well as the integration of mechanical filters reduce the risk of clogging and improve reliability of injection. Integrated pressure sensors can further increase reliability and controllability.

Calculation of injector flow characteristics. A commonly used analytical description of flow characteristics in microinjections allowed us to design our devices. Flow rate \( Q \) per applied pressure \( \Delta P \) through a microchannel is given by

\[
\frac{Q}{\Delta P} = \frac{2}{fRe_{D_h}} \frac{D_h^2 A}{L \mu}
\]

where \( fRe_{D_h} \) is the laminar friction coefficient, \( D_h \) is the hydraulic diameter, \( A \) is the cross-sectional area of the channel, \( L \) is the channel length and \( \mu \) is the dynamic viscosity of the liquid. The hydraulic diameter \( D_h \) can be described as

\[
D_h = \frac{2WH}{W+H}
\]

where \( H \) is the channel height and \( W \) is the channel width. Values for the laminar friction coefficient \( fRe_{D_h} \) are available in tabular form and as polynomial approximations. Calculated steady-state flow rates per applied pressure for two different needle designs are reported in Table 1. The dynamic viscosity of water, \( \mu_{\text{water}} = 10^{-3} \text{ N s m}^{-2} \) at 20°C was used. The thicknesses of our PSG films define the channel height and exhibit a typical non-uniformity of ±3% across a 4-inch wafer. In consequence, maximum flow rate variations from needle to needle of ±10% occur (at a target PSG film thickness of 2.4 µm).

<table>
<thead>
<tr>
<th>Channel width/µm</th>
<th>Channel height/µm</th>
<th>Channel length/µm</th>
<th>Calculated flow rate per pressure/nl s⁻¹ bar⁻¹</th>
<th>Measured flow rate per pressure/nl s⁻¹ bar⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>17</td>
<td>2.4</td>
<td>155</td>
<td>11.5</td>
</tr>
<tr>
<td>#2</td>
<td>6.1</td>
<td>2.3</td>
<td>350</td>
<td>1.3</td>
</tr>
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Measurement of injector flow characteristics. For injector calibration, drops of DI water were injected into an oil film and increased in size by applying multiple air pressure pulses to both ends of Pyrex channel reservoirs of packaged injector chips. Diameters of the approximately spherical drops were measured, and volumes as well as corresponding flow rates were calculated. Measured flow rates per applied pressure for two different needle designs are listed in Table 1 and are in good agreement with calculated values. Differences of 11% and 8%, respectively, are mainly attributed to uncertainties in channel geometry. Data generated during calibration of needle #1 with two-second-long pressure pulses at nominally 550 mbar is reported in Fig. 4.

However, the ejection of a volume of 60 pl, as typically injected into an embryo, only takes of the order of tens of milliseconds with our injectors. Due to non-negligible pressure rise times, the average applied pressure is significantly lower than the nominally applied pressure during such short pulses. Needle #2 was recalibrated and experimentation with different pressure pulse durations resulted in an optimum duration of 80 ms at an applied pressure of nominally 760 mbar for the injection of 60 pl of water. The resulting effective flow rate per nominally applied pressure of 1 nl s⁻¹ bar⁻¹ is approximately 17% lower than the measured steady state flow rate per pressure of 1.2 nl s⁻¹ bar⁻¹. In other words, the average applied pressure during such a short pulse is 17% lower than the nominally applied pressure.

Calibration under defined operating conditions allows us to precisely control injected volumes of dsRNA solution. Our dsRNA molecules are rather short (~500 nucleotides long) and concentrations are in the sub-µM regime, so the viscosity of our dsRNA solution is practically identical to that of DI water. Dosage can be further improved by integrating pressure sensors for measurement of the exact shapes of applied pressure pulses directly at the microinjector.
Packaging of injector and embryo chip

Two chip packages were designed for the injector chip (Fig. 5) and a glass slide that is holding the embryos (Fig. 6). Both packages are precisely attached to motorized xyz-stages during automated injection with the help of alignment pins and embedded magnets. The packages can easily be exchanged so that continuous operation of the injection system is possible.

The injector chip is attached to a plastic block that enables safe handling during injector filling (lower black block in Fig. 5). This block is pushed onto alignment pins and is held in place after lock-pins are inserted (attached to the upper black block in Fig. 5). An embedded o-ring ensures a tight seal between injector chip and package. A drilled channel system connects both ends of the Pyrex channel reservoir with the tube connector on the right. A blue light emitting diode (LED) is integrated in the package above the injector chip to illuminate the embryo glass slide through a hole in the package.

Embryos are attached to a 7 mm × 7 mm area of a glass slide covered with a thin film of non-toxic glue, made by dissolving sticky tape in heptane (Electrical Tape 3224-1, 3M Electrical Specialties Division, Cedar Rapids, IA, USA). The glass slide is clamped between two plastic parts (Fig. 6). A rubber gasket seals the interface so that HC700 oxygen-permeable oil (Halocarbon, River Edge, NJ, USA) can be poured onto the embryos to prevent desiccation during injection and development. Embryos can be observed through the glass slide from below.

Automated injection system

An overview of the components of the automated injection system is given in Fig. 7. The two chip packages are attached to M111 series motorized xyz-stages (Polytec PI, Auburn, MA, USA) and can be moved relative to each other. System alignment and embryo detection is realized with the help of two Watec 902C CCD cameras (Watec, Orangeburg, NY, USA) with VZM 1000i zoom lenses (Edmund Optics, Barrington, NJ, USA). Custom electronics enable manual control during the semi-automated alignment procedure. A series 3 solenoid valve with a valve driver (Parker Instrumentation, Huntsville, AL, USA) is used for pressure switching. A 14R series pressure regulator (Parker Instrumentation) is manually preset to down-regulate the pressure from a nitrogen gas supply line to the desired injection pressure. Two TV sets enable monitoring of the camera video signals. The whole setup is controlled by a personal computer equipped with a C842.40 motor controller card (Polytec PI), an NI 1409 frame grabber (National Instruments, Austin, TX, USA) and an NI 6034E I/O card (National Instruments).

A LabVIEW® program (version 7.1, National Instruments) controls the entire injection procedure. A screenshot of the user interface is shown in Fig. 8. The main panels from left to right provide a text box with instructions for the user, fields with injection related parameters, stage positions, embryo detection related parameters and a panel for miscellaneous functions e.g. for file handling. The picture frames at the bottom display camera images. The LabVIEW® program supports the three main steps of the injection procedure: (1) semi-automated system alignment, (2) automated scanning for embryos and (3) automated embryo injection.

System alignment. Three alignment marks, etched at the corners of the embryo glass slide as well as the tip of the needle serve as reference objects during system alignment. After alignment, it is possible to automatically place the tip of the needle at a defined distance above the surface of the glass slide for any given x/y-position of the embryo xyz-stage. This distance will be precise to within ±10 μm at the locations of the alignment marks. This accuracy is well below the estimated acceptable maximum placement tolerance of ±40 μm. For any given point in the field, the correct stage positions are linearly interpolated. Positional information is retrieved with
the help of the two CCD cameras. The cameras look from different angles at a reference point. The two 2D-images contain the positional information of the reference object in 3D-space. The spatial resolution is 2 μm. The system automatically moves the different reference points into the fields of view of the two cameras. The user manually controls stage movements so that the reference objects appear at predefined positions on the screens, and the corresponding stage positions are recorded. The manual alignment procedure takes approximately 1 minute and can be fully automated with the help of improved object recognition software.

**Scanning for embryos.** After system alignment, the computer-controlled system scans a 7 mm × 7 mm area of the glass slide and detects embryos. The scanned area per frame is 1.28 mm × 0.96 mm. An image is taken with the left camera (see Fig. 7) and is displayed in the left frame of the LabVIEW® user interface (Fig. 8). The program then analyzes this image. It is converted to a binary black-and-white picture. Embryos block the light from the LED and appear as dark areas on the image. A digital filter identifies continuous dark areas that are too small or too big to be embryos. The remaining continuous dark areas are considered images of embryos and statistical data such as the total area and the center of this area are recorded. A corresponding image with identified embryos is displayed in the right frame of the LabVIEW® user interface. Whenever an embryo covers a relatively small area and is located on the edge of one frame, it is likely that a significant portion of it appears in a second frame as well. In such cases all collected embryo positions are analyzed and whenever a doubly detected embryo is found, one set of coordinates is discarded to avoid double injection of embryos. Scanning of the 7 × 7 mm² area takes 2 minutes.

**Embryo injection.** After the scanning procedure the system sequentially positions the embryos one after another right below the injection needle, which is lifted by 400 μm during scanning. The needle is lowered with a speed of 0.5 mm s⁻¹. The needle tip penetrates the embryo membrane and stops nominally at the center of the embryo, 75 μm above the glass slide surface. The computer opens the valve for 80 ms to eject 60 pl of dsRNA solution at nominally 760 mbar. The needle is pulled out again at a low speed of 0.5 mm s⁻¹ to avoid pulling out the high-viscosity yolk. Positioning of the next embryo is performed at maximum stage speed of 2.5 mm s⁻¹. Injection of 100 embryos is completed within 7 minutes. Injector and embryo glass slide holder can be easily exchanged to start the next experiment. Currently, injectors are used for a single experiment only (injection of multiple embryos with the same dsRNA). A cleaning station that flushes chips with DI water will be developed and tested in the future.

**Experimental results**

**Embryo preparation and handling**

_Drosophila melanogaster_ are kept in plastic bottles with caps that are filled with agarose gel topped with yeast paste. Attracted by the food, flies lay eggs (embryos) on the agarose gel. Embryos are collected every half hour to make sure that they are all at a very early stage of development. A hard egg case, the chorion, that protects the embryos, is removed in a mixture of DI water and bleach. This procedure enables controlled desiccation of the embryos and facilitates injection, because clogging of the needle by chorion particles is avoided. The dechorionated embryos are washed in water and spread out on the surface of an agarose gel pad. A clean embryo glass chip surface is covered with a micrometre-thin film of nontoxic glue, made by dissolving sticky tape in heptane (Electrical Tape 3224-1, 3M Electrical Specialties Division, Cedar Rapids, IA, USA). The glass surface is carefully pressed against the gel pad and embryos stick to the glue. The pressure inside the embryos is slightly higher than atmospheric pressure. To enable liquid injection without leakage of yolk, the embryos have to be desiccated, typically for 21 minutes in a desiccator. To protect embryos from further desiccation, they are covered with an approximately 0.5 mm thick film of HC700 oxygen-permeable oil (Halocarbon, River Edge, NJ, USA). After injection, embryo slides are stored in 200 ml plastic jars (Nalgene, Rochester, NY, USA) with water-covered bottoms. The humid environment protects embryos from further desiccation and maximizes survival rates.

**Automated injection**

Injectors with design #2 (see Table 1) were used for all experiments described in the following. All experiments were performed in compliance with the relevant laws and institutional guidelines and approved by the Stanford Administrative Panel on Biosafety. To verify the functionality of the setup, an injection run with green food color was performed. Embryos were dechorionated and desiccated for 21 minutes prior to injection. An injection time of 80 ms at a nominal pressure of 760 mbar for the injection of 60 pl was chosen. An image taken by camera 2 (see Fig. 7) during the injection run is shown in Fig. 9. Because of the 45° viewing angle only the injected embryo is in focus, while the injection needle is out of focus and barely visible. The injected green food color is clearly visible, and spreads rapidly within minutes throughout the entire embryo. Neither leakage of yolk nor clogging of the
A needle was observed. A total of 60 embryos per slide were prepared and more than 98% of the embryos were successfully detected and injected by the system in repeated experiments.

**Influence of desiccation time on embryo survival**

Desiccation of embryos prior to injection reduces the pressure inside the embryos and ensures that liquid can be injected without leakage of yolk. In order to test the influence of the desiccation time on embryo survival, embryos were dechorionated, desiccated for 0, 7, 14, 21 and 28 minutes, respectively, covered with oil and allowed to develop in a humid environment. Survival rates (embryos hatching and turning into larvae) of 96%, 90%, 87%, 83% and 84%, respectively, were observed. Under our laboratory conditions, a minimum desiccation time of 21 minutes was needed to avoid leakage of yolk during injection of 60 pl of liquid.

**Survival rate after DI water injection**

The survival rate after DI water injection was determined. Embryos were dechorionated, desiccated for 21 minutes and injected with DI water at 760 mbar by applying pressure for 50 ms, 100 ms, 150 ms, 200 ms and 250 ms, respectively. After development under oil in a humid environment, survival rates of 85%, 75%, 59%, 49% and 23%, respectively, were obtained. The survival rate is decreasing with increasing volume most probably due to increasing leakage of yolk. For the optimum injection time of 80 ms, a survival rate of 79% is interpolated. This result is comparable to what a very experienced person can achieve with manual injection.

**RNAi experiment**

For the first RNAi experiment, embryos were dechorionated and desiccated for 21 minutes. They were injected with 60 pl of dsRNA solution corresponding to the segment polarity gene *armadillo* at a concentration of 0.01 μM. Injection pressure and pulse duration were again 760 mbar and 80 ms, respectively. Embryos were allowed to develop in a humid environment under oil. As expected, none of the embryos survived to hatching, because the gene *armadillo* plays a crucial role in early embryonic development. *Armadillo* contributes to the organization of the embryo into segments.

To observe changes in phenotype due to RNAi, a cuticle preparation was performed on the embryos. The embryos were washed off the glass slide with heptane and the cuticle, an outer protective layer that is secreted at the end of embryonic development, was fixed with the help of formaldehyde. All inner parts of the embryos were dissolved overnight in mixtures containing acetic and lactic acid. Pressed flat between a glass slide and a cover slip, the cuticles were examined under the microscope.

The cuticle of a wildtype embryo in comparison to an embryo that was injected with our setup is shown in Fig. 10. The wildtype cuticle shows distinct belts of denticles, little spikes that help the later larva to pull or push itself across a surface. Each denticle belt belongs to a specific segment that will give rise to a corresponding part of the adult fly. The denticles are precisely oriented with some of them pointing to the head end of the embryo while others point to the tail end.

As expected, this proper order of denticle belts is completely destroyed by the injection of dsRNA. Denticles are oriented in all directions. A total of 78% of the embryos that were originally present on the slide expressed a strong phenotype as shown in Fig. 10. This phenotype is almost identical to the phenotype observed for the loss-of-function of *wingless*, another segment polarity gene.31

The success/survival rates at various steps are summarized in Fig. 11. In the case of dechorionated and non-desiccated embryos, 96% managed to turn into larvae. Optimum desiccation for 21 minutes decreased the total survival rate to 83%. DI water injection replenishes water lost during desiccation. However, the creation of the injection wound reduced the total survival rate to 79%, probably due to the disturbance of the organization inside the embryo and presumably leakage of yolk in rare cases. When injected with dsRNA, a similar percentage of all embryos present on the slide started to develop normally, but then died because of the lethal effect of *armadillo* silencing while expressing a corresponding phenotype.

**Conclusions and outlook**

We have presented a system for automated mass-injection of *Drosophila* embryos. It can replace current manual injection
technologies and will serve as the core part of a facility for high-throughput RNAi screens. After a brief semi-automatic alignment of MEMS injector chip to glass slide, our system automatically screens the glass slide for embryos and reliably detects and injects more than 98% of all embryos present. A first RNAi experiment was successfully performed with dsRNA corresponding to the segment polarity gene armadillo, and almost 80% of all embryos on the slide expressed an expected strong loss-of-function phenotype. These results are comparable to success rates of a very experienced laboratory assistant injecting embryos manually. We estimate that parallel operation of two automated systems by one operator will allow more than 50 different RNAi experiments per day – more than a tenfold increase compared to today’s manual injection methods. Further improvements of the system (e.g. fully automated alignment, faster motorized xyz-stages, larger image frames during embryo scanning) will lead to faster injection procedures and hence even higher throughput.

The reliability of injections provided by our MEMS injectors will be a key factor for the success of high-throughput screens. Integration of mechanical filters and separation of needle tip and liquid outlet prevent needles from clogging. A 500 nl liquid reservoir and an easy filling procedure make efficient use of dsRNA possible. The well-defined geometry of the MEMS injectors ensures precise dosage and repeatability of injections. A volume of 60 pl per embryo is reliably injected within tens of milliseconds. Integrated pressure sensors can be added to more precisely monitor injected volumes and to further increase reliability and controllability of injections.25,26

In the future, our MEMS fabrication concept could be enhanced to embed our MEMS injectors in closed microfluidic channels based on glass–silicon–glass sandwich structures. This way, all necessary functions – embryo dechorionation and desiccation with suitable liquids, embryo transport, embryo-injector alignment, embryo injection, embryo storage and supply with oxygen – could be integrated into a single microfluidic device. Advantages would be minimized necessary human interaction and even higher throughput through parallel integrated microinjectors.

For efficient phenotype analysis, we intend to complement our current injection technology with automated microscopy systems. Embryo slides would be transferred to the microscope directly after injection. Embryo position lists, which are generated during scanning for embryos prior to injection, would enable immediate and precise imaging of embryos. The overall efficiency of our facility would allow us to monitor gene silencing effects systematically from the earliest developmental stages onwards. We intend to use transgenic embryos designed to produce fluorescent proteins in a specific target tissue.33 Automated confocal microscopes are supposed to capture time-lapse 3D fluorescence patterns of injected embryos.34,35 Deviations from normal patterns due to RNAi will indicate gene function and time and location of gene activity.

Acknowledgements

Financial support through DARPA within the Bio-Info-Micro program (MDA972-001-0032) is gratefully acknowledged.

References