Video Article

Picoinjection of Microfluidic Drops Without Metal Electrodes

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Abstract

Existing methods for picoinjecting reagents into microfluidic drops require metal electrodes integrated into the microfluidic chip. The integration of these electrodes adds cumbersome and error-prone steps to the device fabrication process. We have developed a technique that obviates the needs for metal electrodes during picoinjection. Instead, it uses the injection fluid itself as an electrode, since most biological reagents contain dissolved electrolytes and are conductive. By eliminating the electrodes, we reduce device fabrication time and complexity, and make the devices more robust. In addition, with our approach, the injection volume depends on the voltage applied to the picoinjection solution; this allows us to rapidly adjust the volume injected by modulating the applied voltage. We demonstrate that our technique is compatible with reagents incorporating common biological compounds, including buffers, enzymes, and nucleic acids.

Video Link

The video component of this article can be found at https://www.jove.com/video/50913/

Introduction

In droplet-based microfluidics, micron-scale aqueous droplets are used as "test tubes" for biological reactions. The advantage to performing reactions in the tiny droplets is that each drop uses only a few pl of reagent and, with microfluidics, the drops can be formed and processed at kilohertz rates[1]. Combined, these properties allow millions of reactions with individual cells, nucleic acid molecules, or compounds to be performed in a matter of min with µl of total material.

To use drops for applications like these, techniques are needed for adding controlled volumes of reagents to the drops; such operations are analogous to pipetting into test tubes. One method for accomplishing this is electrocoalescence, wherein a drop of reagent is merged with the target drop by applying an electric field. The electric field disrupts the arrangement of surfactant molecules on the interfaces of the drops, inducing a thin-film instability and triggering coalescence in emulsions that are otherwise stable[2]. Electrically-induced merging is also exploited in the design of the picoinjector, a device that injects reagents into drops as they flow past a pressurized channel[3]. To apply the electric field, picoinjector devices utilize metal electrodes, but the integration of metal electrodes into microfluidic chips is often a complex and error-prone process as the liquid-solder wires are easily compromised by air bubbles or dust and other debris in the channel, as well as fractures from stress or bending during device setup.

Here we present a method to perform picoinjection without the use of metal electrodes, making the fabrication simpler and more robust. To trigger picoinjection, we instead use the injection fluid itself as an electrode, since most biological reagents contain dissolved electrolytes and are conductive. We also add a "Faraday Moat" to shield sensitive regions of the device and act as a universal ground (**Figure 1)**. The moat electrically isolates the droplets upstream of the picoinjection site by providing a ground, preventing unintended droplet merger. An added benefit of our technique is that the volume injected into the drops depends on the magnitude of the applied voltage, allowing it to be adjusted by tuning the applied signal.

We fabricate our devices in poly(dimethylsiloxane) (PDMS) using soft photolithographic techniques[4,5]. Our approach is compatible with devices fabricated in other materials, like resins, plastics, and epoxies. The channels have heights and widths of 30 µm, which are optimal for working with droplets 50 µm in diameter (65 pl). We introduce reagents via polyethelene tubing (0.3/1.09 mm inner/outer diameter) inserted into ports created during device fabrication with 0.50 mm biopsy punches, similar to methods described previously[5]. The exact makeup of the injection fluid depends on the specific application. The fluid need only contain dissolved electrolytes at concentrations high enough to yield sufficient conductivity for the electrical signal to be transmitted to the picoinjector. In bench testing, we have found that ionic concentrations greater than 10 mM should suffice[6], though this value and fluid conductivities depend on the specific device dimensions and magnitude of the applied voltage.

Protocol

1. Design Device Dimensions and Topologies Based on Experimental Needs Using Computer Aided Design (CAD) Software

Note: Select emulsion channel diameters smaller than those of the spherical droplets. This forces the droplets into a cylindrical or "sausage" shape and allows for more effective picoinjection. For our purposes, we designed 30 x 30 µm channels for droplets that were 50 µm in diameter.

1. Model picoinjection site(s) after those described by Abate *et al*.[3] with the exception that the channels for the metal electrodes are removed, as they are unnecessary.
2. Add channels to serve as the Faraday Moat (**Figure 1)** that run between picoinjection site(s) and the upstream emulsion such that they shield the droplets from the electric field.
   Note: This prevents unintended merging.

2. Fabricate Devices Using Soft Photolithographic Techniques

1. Generate a transparency photolithography mask based on the CAD file using existing commercial services.
2. With the photomask, cure photoresist on silicon wafers to produce a device master, as described previously[4].
3. Pour PDMS mixed with curing agent (11:1 ratio) over the device master contained in a 5 cm polystyrene Petri dish.
4. Place the master with PDMS in a vacuum desiccator for roughly 15 min to remove any air bubbles.
5. Cure the PDMS device by placing it in a 95 °C oven for 1 hr. Alternatively, the PDMS will cure at RT after 24 hr.
6. Remove the device by cutting around the perimeter with a surgical blade and carefully peeling the device from the master.
7. Punch inlet and outlet holes into the PDMS using a 0.5 mm biopsy punch.
8. Bond the device to a glass microscope slide using a plasma bonder[4].

3. Prepare an Air Pressure Control Pump to Pressurize a Reservoir Containing the Fluid

1. Modify the pump output such that the pressurized air exits through a length of 2.7 mm inner diameter polyethylene tubing.
2. Construct it such that the tubing terminates at a luer-lock syringe tip by fitting the lumen over the nipple on the rear of the luer-lock.
3. Seal by filling the space between the luer-lock threads and the tubing with epoxy.
4. Attach a 27.5 G needle.

4. Prepare a Monodisperse Emulsion of Aqueous (Water-in-oil) Droplets Suspended in an Inert Fluorinated Carrier Oil with 2% (wt/wt) Dissolved Biocompatible Surfactant[7]

The specific reagents contained in these droplets depend on the application

1. In preparation for reinjection, load the emulsion into a 1 ml syringe with a 27.5 G needle.
2. Secure the syringe in a syringe pump and orient the pump vertically (needle upward).
   Note: This orientation causes the droplets to pack in a layer above the carrier oil. When the pump is started, the droplets will be pushed out of the syringe at high volume fraction by the oil layer under them.

5. Prepare Reagents for Introduction to the Microfluidic Chip

1. Punch three 0.5 mm holes into the cap of a 15 ml centrifuge tube (any container with a screw cap will suffice) using a biopsy punch, needle, or drill.
2. Insert a 0.5 mm diameter wire electrode and a ~20 cm length of PE-2 tubing through two of the holes so that they reach the bottom of the tube.
3. In the remaining hole, thread a ~2.5 cm of a ~20 cm length of PE tubing such that it will rest above the fluid level.
4. Seal any gaps on the top of the cap with UV-cured epoxy.
5. Fill the tube with the picoinjection fluid and screw on the cap.
6. Connect the output from the air-pressure control pump to the shorter length of tubing by inserting the needle into the lumen. The needle should fit snugly.
7. Fill a 1 ml syringe with 1 M NaCl to serve as the Faraday Moat.
8. Connect a 27.5 G needle and secure the syringe in a syringe pump.
9. Fill another 1 ml syringe with carrier/spacer oil, connect a 27.5 G needle, and secure it in a syringe pump.

6. Prepare the Microfluidic Device for Picoinjection

1. Connect the output tubing (longer length) from the injection fluid container to the inlet port of the picoinjection fluid on the microfluidic chip.
2. Connect the syringe containing the 1 M NaCl to the inlet port for the Faraday Moat on the microfluidic chip with a length of PE tubing.
3. Connect the syringe containing the carrier oil to the inlet port of the microfluidic chip with a length of PE tubing.

4. Insert PE tubing into the emulsion outlet port on the microfluidic chip. The tubing should terminate in an emulsion collection vessel, normally a 1.5 ml centrifuge tube.
5. Insert PE tubing into the outlet port for the Faraday Moat on the microfluidic chip. The tubing should terminate in a non-conducting and electrically isolated container to prevent a short circuit.
6. Connect the output of the high-voltage (HV) amplifier via alligator clip to the metal electrode submerged in the picoinjection fluid.
7. Connect the ground electrode of the HV amplifier via alligator clip to the metal of the syringe needle containing the 1 M NaCl.

7. Infuse Reagents into Microfluidic Chip

1. Introduce the 1 M NaCl (Faraday Moat) to the device at a rate of 100 µl/hr.
2. Introduce the droplet emulsion and carrier oil at rates suitable for the device dimensions. For our demo device, we introduce the drops and oil at 200 and 400 µl/hr, respectively. The flow rates should allow the droplets to pass the picoinjector at regular intervals separated by a gap of carrier oil.
3. Adjust the pressure applied to the picoinjection fluid such that the fluid pressure at the picoinjection orifice is in mechanical equilibrium with the droplet channel.
   Note: At this pressure (the Laplace pressure), the injection fluid should bulge into the droplet channel without budding off and forming its own drops (**Figure 2)**. At these flow rates described above, we apply a pressure of ~13 psi to the injection fluid to achieve equilibrium at the injection site.

8. Begin Picoinjection

1. As droplets pass the injection orifice, apply a 0-10 V, 10 kHz, AC signal amplified 1,000x by the HV-amplifier (**Figure 3)**.
2. Modulate the injection volume by changing the amplitude of the applied voltage.
   Note: Higher voltages should allow for more fluid to be introduced to the droplets. In our testing, we observe stable and consistent injection at voltages between 100 and 3,000 V using injection solutions of NaCl ranging from 10-500 mM (**Figure 4)**.

Representative Results

Microscopic images taken at the picoinjection site show that electrification of the picoinjection fluid is enough to trigger injection (**Figure 2)**. The injected volume can be controlled by modulating the amplitude of the applied voltage, with higher voltages allowing for higher injection volumes. We plot the injection volume versus the magnitude of the applied voltage for three representative molarities of injection fluid in (**Figure 3)**. To demonstrate the speed our method, we selectively injected droplets passing the injection site depending on the presence or absences of a fluorescent dye (**Movie 1)**. Drops pass the injector at 200 Hz, though rates as high as 10 kHz are possible, depending on capabilities of the droplet detection mechanism[3].

We attribute the dependence of injection volume on the applied voltage to the fact that as the droplets approach and pass the picoinjection orifice, the thickness of the oil layer separating the droplet interface from the bulge at the injection site decreases[8]. The threshold voltage for an electrically induced thin-film instability is proportional to the thickness of this layer[9,10]. Therefore, as the droplets approach the picoinjector, the moment of coalescence depends on the magnitude of the electric field. Higher applied voltages allow for earlier coalescence between the droplet and injection fluid, causing longer injection durations. Because the injection volume depends on the injection duration, it therefore also depends on applied voltage.

Lower molarity ionic solutions more readily attenuate the applied signal and reduce the electric field strength at the injection site compared to more concentrated solutions. Consequently, injection fluids with lower molarities of dissolved ions require higher applied voltages to achieve the same injection volumes. This relationship is demonstrated for a range of ionic molarities and applied voltages in a 2D heatmap (**Figure 4)**.

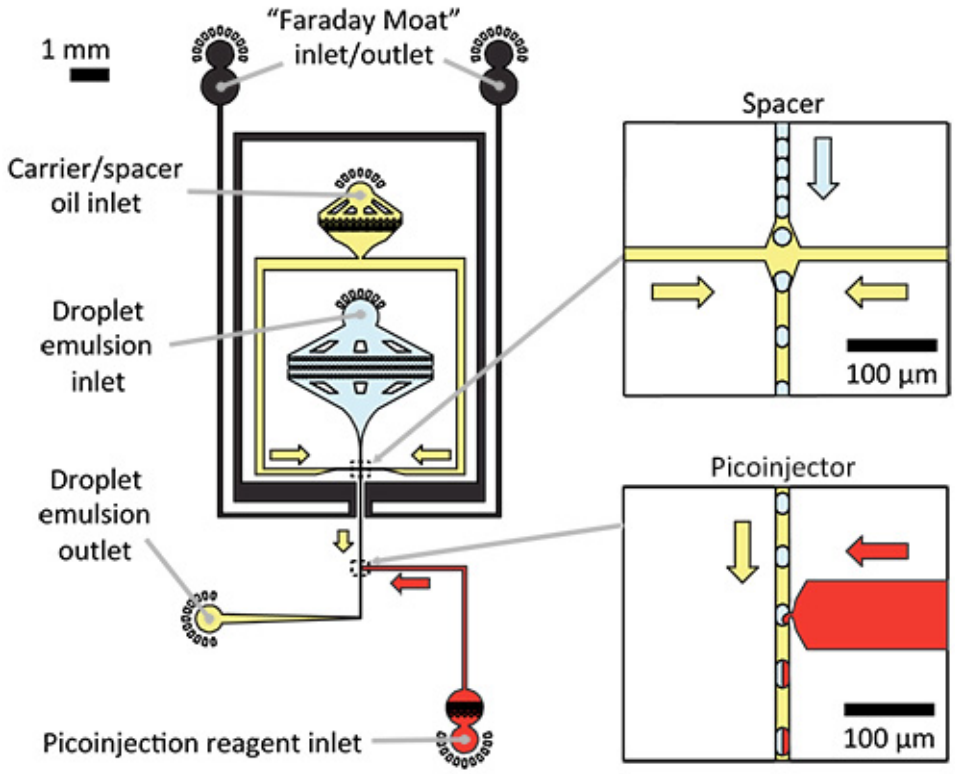

Figure 1. Basic device setup. Droplets, carrier oil, and 1M NaCl are introduced to the device via syringe pumps. The densely packed droplets are spaced evenly using basic flow-focus geometry. As the droplets pass the picoinjection site, an electric field is generated by applying an AC signal to an electrode inserted in the picoinjection fluid container (indicated in red). The electric field allows for coalescence between the passing droplets and picoinjection fluid. Droplets upstream of the injection site are shielded from the electric field by the Faraday Moat - a channel of 1 M NaCl (any high molarity ionic solution should suffice) in contact with the ground electrode of the HV amplifier (indicated in black). Device dimensions can be scaled as needed; for our purposes, we designed 30 x 30 µm channels (just upstream of injection site) for droplets that were 50 µm in diameter.

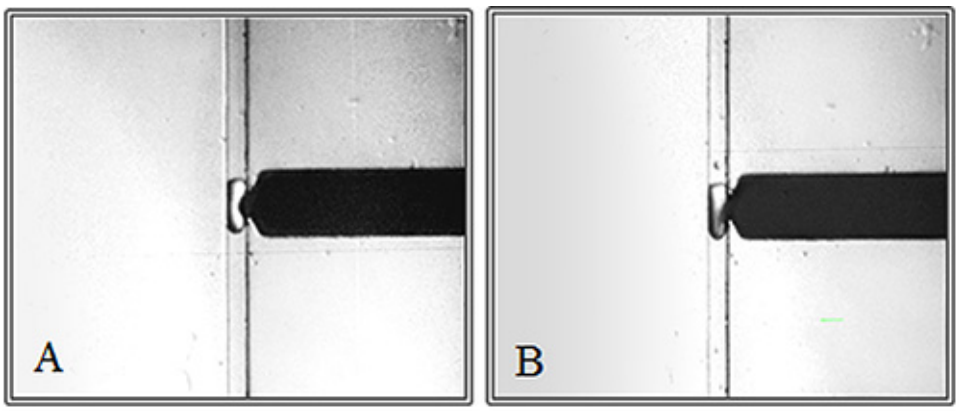

Figure 2. Bright field microscopy images of the picoinjection site. In the absence of an electric field **(A)**, surfactant molecules prevent coalescence at the injection site and a distinct boundary is visible at the droplet/injection fluid interface. Upon application of a 250 V 10 kHz AC signal, the boundary disappears and reagent is injected as the droplet passes **(B)**. For visualization, injection fluid has been colored with 2 mg/ml of bromophenol blue dye. Figure re-published from [6] with permission of The Royal Society of Chemistry (RSC)

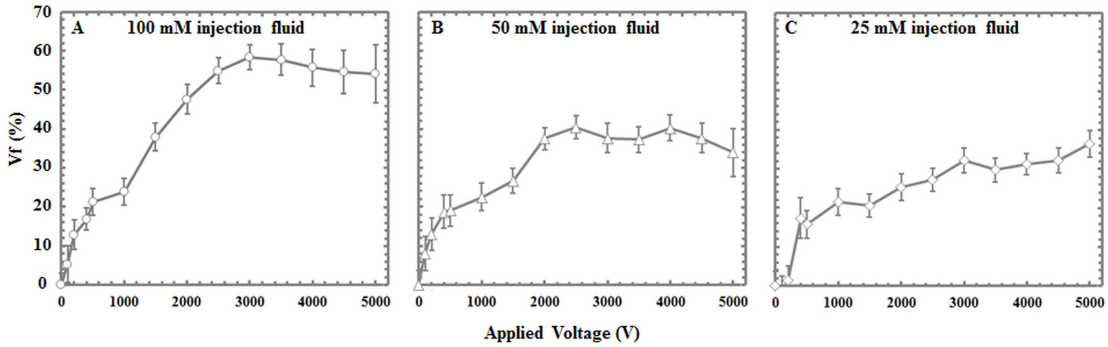

Figure 3. Data demonstrating the relationship between applied voltage and volume fraction increase (Vf) of drops after injection for (A) 100 mM, (B) 50 mM, and (C) 25 mM (NaCl) injection fluids. Stronger electric fields more readily rupture the oil/water interfaces and allow injection over a larger length of the passing droplets - this leads to larger injection volumes. Higher molarities of dissolved electrolytes increase the conductivity of the injection solution, producing stronger electric fields at the injection site for a given voltage, leading to increased injection volumes. Error bars represent 1 standard deviation in either direction for >1,200 drops sampled at each point. Lines connecting data points do not represent any curve-fit or calculated theoretical model. Drop volume is measured by a fluorescence detection system described in [6]. Figure re-published from [6] with permission of The Royal Society of Chemistry (RSC). Please click here to view a larger version of this figure.

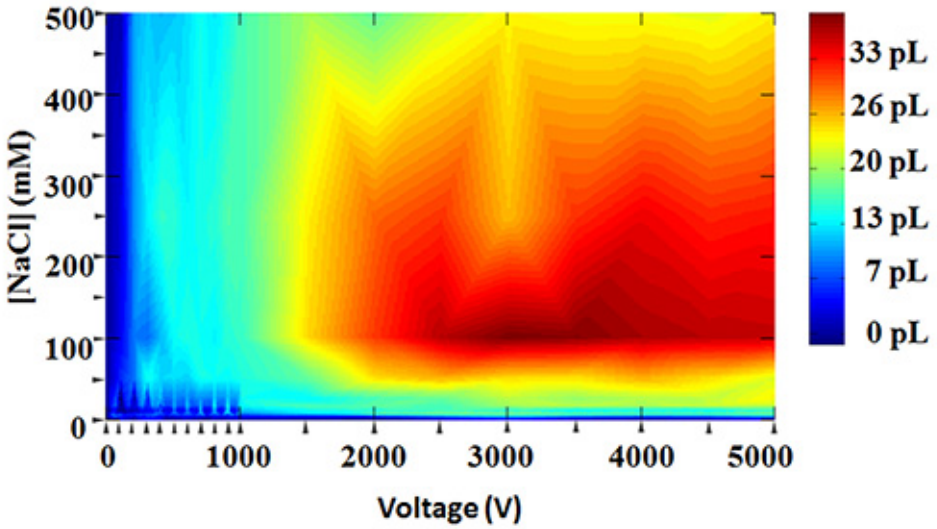

Figure 4. Heat map showing injection volume as a function of applied voltage and the molarity of dissolved NaCl in the injection fluid. The injection volume can be adjusted in the range of 0 - 36 pl with a resolution of ~2.6 pl (4% Vf) with 100V increments of the applied signal. The largest injected volumes were achieved at 3,000 V and 100 mM fluid. Increasing electric field above this allows for electrowetting, causing drops to spontaneously form at the picoinjector, adversely affecting injection efficacy and consistency. Arrows/ticks indicate data points. Figure re-published from [6] with permission of The Royal Society of Chemistry (RSC)

Movie 1. High speed footage demonstrating selective switching of the picoinjector. Only drops containing IR-783 fluorescent dye (2 mg/ml) are injected with reagent (500 mM NaCl).

Discussion

The relationship between injection volume and applied voltage is dependent on many factors including device dimensions, length of the tubing carrying the picoinjection fluid to the device, molarity of picoinjection fluid, and the velocity of the droplets as they pass they injector. For this reason we recommend that the volume/voltage relationship be characterized before each run of picoinjection by measuring injection volumes at the edges of the working ranges of voltage and molarity. Additionally, at higher voltages and injection fluid molarities we observe a phenomenon in which the picoinjection fluid is no longer held at equilibrium at the injection orifice, but instead buds off and forms small drops in the flow channel. We attribute this behavior to electrowetting, wherein the aqueous phase partially wets the hydrophobic channels, causing it to crawl out of the orifice and into the flow channel[11]. If this instability occurs before the desired injection volume is achieved, consider reducing the droplet flow rate as they pass the injector and narrowing the droplet channel to increase injection duration.

In addition to markedly streamlining the fabrication of devices, this technique should also simplify the execution of more complex and combinatorial reaction regimes. For example, executing multiple picoinjections with our technique only requires adding picoinjection channels at the desired sites of injection. By contrast, previous methods require picoinjection channels and accompanying metal electrodes to be included at all sites. Further, previous approaches regulate injection volume relatively slowly by varying injection pressure or droplet velocity. With our approach, injection volume can be adjusted electronically at rates faster than the highest drop rates reported (please see disclosure). This

enables the execution of more complex assays, with injection volumes tailored to the specific conditions within each microdrop. Normalization and injection of reagents into polydisperse droplet populations, for example, would require on-the-fly determination of injection volumes.

This technique has been developed and demonstrated to work in devices utilizing picoinjection for multi-step biological reactions such as digital PCR and genotyping assays[12]. However, with little or no change to the protocol, the technique should be of use to any experimenter requiring addition of reagents to droplets for any biological, chemical, or industrial applications - so long as the injection fluid contain dissolved ionic species.

Disclosures

We do not fully understand exact physical mechanism behind the relationship between the applied voltage and injection volume observed in our experiments. The lab's interests and relevant areas of expertise are not well-suited for pursuing this lingering question. We encourage those with more physics and engineering acumen to explore this phenomenon.

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