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User-defined local stimulation of live tissue through a movable microfluidic port

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Abstract

Many *in vivo* tissue responses begin locally, yet most *in vitro* stimuli are delivered globally. Microfluidics has a unique ability to provide focal stimulation to tissue samples with precise control over fluid location, flow rate, and composition. However, previous devices utilizing fixed ports beneath the tissue required manual alignment of the tissue over the ports, increasing the risk of mechanical damage. Here we present a novel microfluidic device that allows the user to define the location of fluid delivery to a living tissue slice without manipulating the tissue itself. The device utilized a two-component SlipChip design to create a mobile port beneath the tissue slice. A culture chamber perforated by an array of ports housed a tissue slice and was separated by a layer of fluorocarbon oil from a single delivery port, fed by a microfluidic channel in the movable layer below. We derived and validated a physical model, based on interfacial tension and flow resistance, to predict the conditions under which fluid delivery occurred without leakage into the gap between layers. Aqueous solution was delivered reproducibly to samples of tissue and gel, and the width of the delivery region was controlled primarily by convection. Tissue slice viability was not affected by stimulation on the device. As a proof-of-principle, we showed that live slices of lymph node tissue could be sequentially targeted for precise stimulation. In the future this device may serve as a platform to study the effects of fluid flow in tissues and to perform local drug screening.

Graphical Abstract

A two-component, two-phase microfluidic device provides a movable port for user-selectable local stimulation of living tissue slices.

Author contributions

MAC and RRP conceptualized the technology, planned experiments, interpreted data, and wrote the manuscript. MAC designed and fabricated the technology, performed the experiments, and analyzed the data. AFD helped perform and analyze the flow cytometry experiment, synthesized the fluorosurfactant, and edited the manuscript.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Keywords

reconfigurable microfluidics; two-phase microfluidics; local delivery; capillary pressure; lymph node slice; immunology

Introduction

Microfluidic platforms that chemically stimulate tissue *ex vivo* are a promising tool to mimic the local events that occur *in vivo*.^{1,2} Cells in tissue are highly organized and use secreted signals such as small molecules, proteins, and exosomes to communicate with one another and coordinate the functions of the tissue.^{3–6} Many *in vivo* responses start from a local event that produces a focal source of soluble signals, including blood clotting after local vessel injury and neutrophil swarming after local tissue damage.^{7–9} In order to better understand cellular communication in a structured tissue, it is useful to have an *ex vivo* platform to reproduce local stimulation on demand. Microfluidic devices can deliver chemical stimuli to substructures a few hundred microns in diameter in order to reveal how local responses initiate and propagate outward into the surrounding tissue.^{10–18} Such tools have been developed and tested for use with living samples of brain, tumor, and lymph node. These tools may enable a better understanding of cell-cell communication and the physical properties of various tissues, to enhance mechanistic models of health and disease.

Ideally, a microfluidic device for local stimulation of live tissue would minimize the handling required of fragile tissue, allow delivery anywhere in the sample and, for thick tissue slices, deliver to the core of the tissue. Tissue slices for live culture are often 300 – 400 μm thick, and the damage from slicing may extend 10 – 100 μm deep, depending on the type of tissue.^{19,20} Available microfluidic devices can be generally categorized as featuring either stationary ports or a movable push-pull probe. In the stationary port design, microchannels connected to a layer of ports or a membrane deliver fluid locally to a tissue sample.^{10–14} To access a particular region, the tissue slice is manipulated over the port(s), which may increase the risk of mechanical damage, particularly if repositioning is required. Since the ports are found in predetermined locations, these devices cannot always accommodate the significant variability of size and morphology between slice samples. Alternatively, probe-like microfluidic devices for local push-pull delivery allow the user to reposition the device instead of the tissue slice.^{15–18,21} Push-pull delivery has been optimized for cell cultures and thin fixed tissue (typically 10 μm thick);^{22,23} it is likely that fluid flow may not perfuse through the depth of a thick tissue slice. Therefore, in order to

enable flexible stimulation of thick tissue slices, a device that combines both the mobility of a push-pull probe and the deep fluid penetration of an underlying port device is needed.

To achieve this goal, we adapted reconfigurable SlipChip technology and modified it for continuous fluid flow. A SlipChip is a two-phase microfluidic device, typically with two opposing plates (i.e. top and bottom) containing aqueous droplets in their channels and wells.²⁴ A thin oil layer is sandwiched between the two plates to prevent leakage while the movement of the plates relative to one another provides on-demand rearrangement of fluidic pathways. SlipChips have been used to perform chemical assays, PCR, and culture of both bacterial and mammalian cells,^{25–28} but challenges remain for use with live tissue samples. While most SlipChips are explicitly designed to avoid the use of external pumps,^{29,30} continuous local stimulation of tissue requires the use of pump-driven flow for uninterrupted fluid delivery. Furthermore, though most SlipChips consist of two layers,^{24,26,28–30} most port-based local stimulation devices require at least three layers (microchannel layer, port layer, culture chamber layer).^{10,13,14,31}

This paper presents the first self-contained microfluidic device with a movable port for tissue stimulation. We describe the design and fabrication of a prototype multi-layer device and describe the physical parameters needed to constrain pump-driven flow to the desired port. The robustness and resolution of local delivery were investigated under a range of biologically relevant flow rates. Finally, we demonstrated local delivery to multiple locations in a live lymph node slice without manual alignment of the slice.

Experimental

Device Fabrication

The device was designed using AutoCad LT 2015 (CAD files are provided in the ESI). The delivery component was fabricated from 4 layers: a glass backing layer, a PDMS layer containing a 100- μ m by 100- μ m channel, an acrylic layer containing a 90- μ m inner diameter delivery port, and a PDMS inlet. The backing layer, a 2 inch x 2 inch x 1.5 mm unpatterned glass slide (VWR, Radnor, PA USA), provided mechanical support for the device. The PDMS channel and inlet layers were fabricated using standard soft-lithography procedures.³² Briefly, a transparency mask was created in AutoCAD, and printed at 20,000 DPI by CAD/Art Services, Inc (Brandon, OR). Master molds were fabricated in a Class 1000 clean room, using photolithography of SU-8 3050 photoresist (Microchem, Westborough MA, USA) on 3" silicon wafers (University Wafer, South Boston MA, USA). The channel layer and inlet replicas were made of polydimethylsiloxane using a 5:1 ratio of silicon elastomer base to curing agent (Slygard 184 Silicone Elastomer, Ellsworth Adhesives, Germantown WI, USA). The channel inlet was punched with a 0.75-mm I.D. tissue punch (World Precision Instruments, Sarasota FL, USA). To create the delivery port layer, two 90- μ m inner diameter delivery ports were laser etched (Versa Laser 3.5, Universal Laser Systems, Scottsdale AZ, USA), at a power setting of 7% and a speed of 10%, into a 0.2 mm clear optical grade cast acrylic sheet (Astra Products Inc., Baldwin, NY USA).

To assemble the delivery component, first the unpatterned glass and PDMS channel layers were treated with air plasma (Tegal Plasmod) for 2 mins and brought into contact, with the

glass on the flat face of the PDMS piece. Next, the acrylic delivery port layer was chemically bonded to the PDMS channel layer and inlet layer. To perform chemical bonding, both PDMS pieces and the acrylic piece were treated with air plasma for 1.5 mins, then treated with silane solution (Sigma Aldrich, St. Louis MO, USA) for 25 mins.³³ The acrylic piece was submerged fully in a solution containing 1:10 v/v (3-aminopropyl)trimethoxysilane (APTMS):ethanol, and the two PDMS pieces were submerged partially in a solution containing 1:10 v/v (3-glycidyloxypropyl)trimethoxysilane (GPTMS): ethanol solution. Full submersion of the acrylic layer ensured that both sides of the acrylic were silanized. After silanization, all layers were thoroughly cleaned with ethanol and water and dried with nitrogen. The acrylic delivery port was aligned visually with the PDMS channel, and the pieces were brought into good contact. The PDMS inlet layer was then similarly bonded to the top of the acrylic delivery port layer. Once assembled, the device was left at room temperature for one hour to complete the reaction between the amino- and epoxy-silanes.

The chamber component was fabricated from 3 layers: a 0.2-mm clear optical grade cast acrylic sheet containing an array of ports of 80- μ m inner diameter, a rigid acrylic support, and a PDMS culture chamber to house the tissue. The port array was produced by laser etching as in the delivery port layer. The acrylic layer consisted of a rigid 1.5-mm acrylic sheet (McMaster-Carr, Princeton, NJ USA), laser-etched with a 10-mm hole using a power setting of 20% and a speed of 2%. The culture chamber was fabricated in PDMS using soft-lithography and a 10-mm hole punch (World Precision Instruments, Sarasota FL, USA). To assemble the chamber component, the PDMS piece was chemically bonded to the rigid acrylic piece as described above. The two acrylic pieces were bonded together by using clear polyester double-sided adhesive tape (Adhesives Research, Inc., Glen Rock, PA USA) that was laser-etched in advance with a 10-mm hole using a power setting of 15% and a speed of 10%.

Fluorosilanization of delivery and chamber components

Once both components were fabricated, their acrylic faces were silanized to produce a fluorophilic and hydrophobic surface, using a modification of a gas-phase silanization procedure designed for glass.^{29,30} First, both components were treated with air plasma for 1 min. A 300 μ L aliquot of tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (Gelest Inc., Morrisville PA, USA) was placed into a small polypropylene dish in a vacuum desiccator, followed immediately by the two components, and a vacuum was applied for 2 hours. Each component was thoroughly rinsed with water and ethanol and dried with nitrogen. Next, the hydrophobic silane was removed from inside the ports and chamber of the chamber component by plasma treatment for 1 min, while the acrylic face was protected by a clean piece of thin PDMS (SSP, Ballston Spa NY, USA).

Preparation of agarose and tissue slices

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Virginia under protocol #4042, and was conducted in compliance with guidelines from the University of Virginia Animal Care and Use Committee and the Office of Laboratory Animal Welfare at the National Institutes of Health (United States). Live

murine lymph node slices and blocks of 6% low melting point agarose (Lonza, Walkersville MD, USA) were sliced to a thickness of 300 μm using previously reported methods.¹⁰ (See the SI for more detailed methods.)

Device assembly and operation

All pressure-driven flow was achieved using a Chemyx syringe pump (Fusion 200, Houston TX, USA) loaded with a 50 μL Hamilton Model 1705 RN syringe with a 26s gauge, large hub needle. Connections to the device were made with nonshrinkable PTFE TT-30 tubing with 0.012" I.D. and 0.009" wall thickness (Weico Wire, Edgewood NY, USA).

Before assembling the device, the microfluidic channel was filled with aqueous solution using a syringe pump. 1 mL of oil was pipetted onto the top of the filled delivery component. The oil was FC-40 containing 0.5 mg/mL R_fOEG (triethyleneglycol mono[1H, 1H-perfluorooctyl]ether) (Supporting Methods), unless otherwise noted. Then, the chamber component was lowered onto the delivery component, sandwiching a thin layer of oil between them and avoiding air bubbles. Steady pressure was maintained until the device was clamped with two binder clips. The culture chamber was then filled with 1x phosphate buffered saline (PBS). A sample of agarose gel or tissue was placed into the chamber and weighed down using a small stainless steel washer (10 mm O.D. and 5.3 mm I.D., Grainger USA).

To deliver fluid through a desired port, the chamber component was slipped relative to the delivery component and aligned under a microscope. The syringe pump was turned on at the desired flow rate to initiate flow. After 3–5 seconds (unless otherwise stated), the pump was turned off and the device was slipped away, to reposition for another delivery or to a closed position. After all deliveries were complete, the sample was removed, then the chamber was flushed with 1x PBS and refilled for the next sample. All delivery experiments were performed at room temperature.

Delivery experiments

All deliveries were monitored in real time using a Zeiss AxioZoom macroscope (Carl Zeiss Microscopy, Germany) with an Axiocam 506 Mono camera, using filter cubes for GFP (Zeiss filter set #38), Rhodamine (#43), and Cy5 (#50). Images (14 bit) were collected before, during, and after delivery. For deliveries with a single fluorescent tracer, time lapse images were collected at 1-sec intervals, while for delivery of multiple tracers, images were collected every 3 seconds. All images were analyzed in Zen 2 software.

Validation experiment—To validate the conditions required for successful delivery, the oil phase (FC40 with R_fOEG at 0, 0.25, 0.5, 1 mg/mL), aqueous phase (0.05 mg/mL 40-kDa FITC-labeled dextran, 0.1 mg/mL BSA, or 1 mg/mL BSA), and flow rate of delivery (0.5, 1, 2, 4 $\mu\text{L}/\text{min}$) were systematically varied. The device was assembled and a 6% agarose slice placed into the chamber. The device was aligned, a 5-sec delivery was performed, and then the device was realigned, for a total of 5 consecutive deliveries. Images of the deliveries were analyzed for spreading of the oil/aqueous interface in the oil gap, and for the radial spread of the fluorescent signal from the aligned port during delivery.

Measuring width of delivery in agarose—After alignment of the delivery port to an array port below a 6% agarose slice, a pulse of Fluorescein (FITC) labeled 150-kDa dextran was delivered to the slice at flow rates ranging from 0.1 to 1.6 $\mu\text{L}/\text{min}$ ($n=4$). In a separate experiment, a mixture of ovalbumin (OVA) labeled with NHS-rhodamine, FITC labeled 150-kDa dextran, and Alexa Fluor 647 labeled 40-kDa dextran (all from Thermo Fisher Scientific), each at 0.05 mg/mL, was prepared. The Stokes radii for the dextrans were obtained from the vendor. The OVA and 40-kDa dextran were labeled in-house using succinimidyl ester-dye conjugates, according to manufacturer recommendations. The mixture was delivered at a flow rate of 0.4 $\mu\text{L}/\text{min}$ ($n=5$). Delivery width was determined from image analysis in Zen software as described previously.¹¹ Briefly, a linescan was drawn across the delivery region and corrected for the background fluorescence of the slice measured prior to delivery. Data points directly over the port were excluded due to its high fluorescent intensity and the remaining data were fit to a Gaussian curve in Graphpad Prism version 7. The delivery width was defined as 2 standard deviations of the Gaussian curve. These widths were plotted and fit to a two-parameter square root curve, $y = a\sqrt{x - b}$, where x was the flow rate or time of delivery and y was the delivery width.

Delivery to live lymph node tissue—The device was assembled and a lymph node slice was placed onto the chamber. A 3–5 second pulse of Alexa Fluor 647-labeled 40-kDa dextran was delivered at a flow rate of 0.5 $\mu\text{L}/\text{min}$. After the first delivery, the device was repositioned and another delivery was performed. This was repeated for four different slices and with slight variations on three separate occasions.

Results and Discussion

Design of the chip

To create a “mobile” port beneath a tissue slice, we designed a SlipChip device with two multi-layer components (Fig. 1a, b, and SI Fig. 1). The lower “delivery” component contained an underlying microchannel that terminated in a single port (delivery port), while the upper “chamber” component included an open perfusion chamber to house the tissue sample. An array of ports (port array) was located at the bottom of the perfusion chamber. With this design, the user placed a tissue sample into the chamber and slipped the port array relative to the delivery port to target a desired subsection of tissue (Fig. 1a).

The premise of the chip was that an aqueous solution delivered through the microchannel was targeted to a particular array port by an immiscible oil layer, sandwiched between the two components, which prevented leakage into the gap (Fig. 1c–f, animated in SI movie 1). Once the delivery port was slipped into alignment with an array port, an aqueous column bridged the oil layer between them and enabled pressure-driven flow into the sample (Fig. 1d). To stop delivery, pressure-driven flow was turned off and the device was slipped to break the aqueous column (Fig. 1e). This process was repeatable for multiple deliveries (Fig. 1f).

Design criteria for fabrication and surface chemistry

The materials and assembly strategy were selected to enable rapid fabrication of a prototype device (Fig. 2). The microchannel and perfusion chamber layers were fabricated from polydimethylsiloxane (PDMS) because of the ease of patterning and bonding this material. Since PDMS is flexible, a rigid glass backing and a thick slab of acrylic were added to the delivery and chamber components, respectively, to avoid bending.²⁵ To enable slipping, the layers facing the gap between the two components (i.e., the delivery port and port array layers), were fabricated in low-friction acrylic. Acrylic had the additional advantage that it was readily patterned with a CO₂ laser and chemically bonded to the other layers. Laser etching produced a conical shape for small diameter ports (SI Fig. 2), which were oriented as shown in Fig. 2b during device assembly. In preliminary work, we found that this orientation was required to minimize the flow of culture media from the chamber into the oil gap. To facilitate alignment under opaque tissue, alignment markers were laser etched in a cross-hair pattern with the delivery port at the center (Fig. 2a and SI Fig. 3).

The dimensions of the device enabled the delivery port to align with any port in the port array without exposing the channel or ports to an air interface. The port array maximized the number of ports in a 25 mm² area, while also allowing sufficient distance for the delivery port to manually slip between array ports. An array of 100-μm diameter ports pitched 500-μm center-to-center met these requirements. In future development, this spacing could be reduced by using higher resolution fabrication methods to produce smaller ports and denser port arrays.

The chemistry of the oil/water interface was selected to enable delivery of aqueous solutions of biomolecules. We used FC-40, a water-immiscible fluorocarbon oil, because it is biologically inert and has a moderate viscosity appropriate for SlipChip applications.^{24,30} A short-chain surfactant, triethyleneglycol mono[1H,1H-perfluorooctyl]ether (R_fOEG), was included to prevent protein adsorption at the aqueous/oil interface and to control the interfacial tension (SI Fig. 4).³⁴ R_fOEG, unlike longer chain surfactants, facilitates rapid drop coalescence, which was desirable for the formation of the aqueous column.³⁵

Capillary pressure provided controlled delivery without leakage

Unlike a traditional microfluidic channel, which uses solid walls to constrain pressure-driven fluid flow, this system uses only an oil/water interface to direct the flow of aqueous solution through the gap from the delivery port to the selected array port. We derived a simple physical model to describe the delivery and predict the conditions required for flow without leakage into the gap (Fig. 3a).

Two major pressures exerted on the aqueous column during delivery were considered. The first was the capillary pressure from the oil/aqueous interface, P_c (Fig. 3a, blue arrows). To have inward capillary pressure rather than outward pressure, which would cause wicking, it was essential that the surfaces exposed to the gap be hydrophobic and fluorophilic. Although acrylic was not naturally wetted by fluorocarbon oil, fluorosilicizing the acrylic layers facing the oil raised the contact angle for an aqueous droplet in oil to $> 90^\circ$ (Supporting Methods and Table S1). Under these conditions, P_c opposes spreading in the gap and

encourages delivery through the connected array port. The capillary pressure was calculated using the Laplace equation,

$$\Delta P_c = \frac{2\gamma \cos\theta}{h}, \quad [1]$$

where γ [N/m] is the aqueous/oil interfacial tension, θ [radians] is the contact angle of an aqueous droplet in oil on the fluorinated acrylic substrate, and h [m] is the height of the gap (typically 14 μm in this device, see Supporting Methods).^{29,30,36}

The second major pressure working on the system during delivery was the pressure due to flow resistance through the array port, P_r (Fig. 3a, red arrow). This pressure acts counter to the delivery and encourages spreading in the gap. The pressure difference across the array port was calculated by first finding the flow resistance of the port, R [$\frac{\text{Pa} \cdot \text{s}}{\text{m}^3}$], using the

Poiseuille equation for laminar flow:

$$R = \frac{8\mu L}{\pi r^4}, \quad [2]$$

where μ [Pa*s] is the viscosity of the aqueous phase, L [m] is the length of the port, and r [m] is radius of the port.³⁷ For simplicity, the conical ports were treated as cylindrical in shape, with radius r [m] equal to the average of the top and bottom radii of the cone (SI Fig. 2). The pressure drop across the port was calculated as

$$\Delta P_r = Q * R, \quad [3]$$

where Q [$\frac{\text{m}^3}{\text{s}}$] is the volumetric flow rate through the port.³⁷

We predicted that the aqueous column would be contained and successful delivery to the chamber would occur if the capillary pressure was greater than the pressure due to flow resistance ($P_c > P_r$). To test this hypothesis, we selected 24 different conditions of varied

P_c and P_r , spanning a >10-fold range of each variable (Fig. 3b). P_r was varied by changing the flow rate, and P_c was varied by changing the concentrations of oil-phase RfOEG and aqueous-phase bovine serum albumin (BSA). P_r and P_c were calculated for each condition using equations 1 and 3. Due to experimental constraints, P_c was at least two orders of magnitude larger than P_r in all cases (Tables S1 and S2), and no experimentally feasible approach fell in the predicted leak regime. Therefore, to illustrate what a leak would look like, we deliberately misaligned the delivery port and initiated flow (Fig. 3e). This forced the aqueous solution into the gap (visible as an interface in the oil layer), and delivery eventually occurred through an unaligned port (Fig. 3f).

To match the prediction, all tested conditions needed to have successful flow into the chamber with no leaking in the gap. Each condition was tested experimentally in five consecutive deliveries to a slice of agarose gel, each made after slipping to a different port in the chamber array (Fig. 3b). Deliveries were monitored by timelapse microscopy. Leaks were indicated by the formation of a visible interface in the gap layer (Fig. 3f), while flow into the chamber was indicated by a radial spread of fluorescent signal originating from the aligned port (Fig. 3c, d). In all cases, no spreading was observed in the gap, and the delivery occurred through the aligned port. These results matched the prediction of the model, supporting its validity.

We were also concerned with protein adsorption during slipping and its potential impact on the ability to perform repetitive deliveries with the device. We tested this by delivering BSA labeled with Dylight 488 at a concentration that was typical or higher than that of most biologically active proteins (50 $\mu\text{g/mL}$), then slipped away, and repeated this process 5 times. It was found (by fluorescence) that slipping did not cause visible protein adsorption to the surface, and that sequential deliveries were possible. Next, we tested delivery at BSA concentrations typical of its use as a carrier protein (0.1 and 1 mg/mL). In some cases, the BSA solution wetted the top surface of the gap during slipping (SI Fig. 5), but interestingly these streaks of aqueous solution did not affect the precision of subsequent deliveries (Fig. 3b). No leaking was observed in the gap during subsequent deliveries, and the aqueous fluid remained constrained to the aligned port. Successful delivery may have been facilitated by retained hydrophobicity of the bottom surface of the gap, coupled with a lower flow resistance through the array port than through the thin aqueous streak. We conclude that the current device is suitable for delivery of analytes with lower surface activity (small molecules, dextrans, dilute proteins), and for delivery of concentrated amphipathic proteins in cases where minor loss due to surface adsorption is acceptable. To accommodate quantitative delivery of high concentrations of amphipathic proteins, it may be useful to fabricate the device from a material with a lower surface energy, such as fluorinated polymers.^{29,38}

Delivery into the sample was governed by convection

Spread of analyte increased as a function of flow rate—Having confirmed that delivery occurred without leakage into the gap, we moved on to characterize the spread of analyte into a sample above the aligned ports. Predictable localization is required to stimulate the substructures found in tissue. First we tested the effect of flow rate on the spread of analyte by measuring the width of the fluorescent signal, using 6% agarose gel as a model tissue (Fig. 4a–c). Approximating the delivery region as a cylinder, the width of fluorescent signal is expected to increase with the square root of the product of volumetric flow rate and time (See SI for derivation), and indeed, the experimental data were well fit by such a curve (Fig. 4c). This result confirmed that even at higher flow rates, fluid was not lost into the gap between the two components. The device provided delivery resolution as low as 200 μm at the lowest flow rates (0.1 and 0.2 $\mu\text{L/min}$), which is sufficient for stimulation of subsections of murine and human tissue.^{1,10,39}

Analyte size did not affect the spread—As an analyte solution is delivered through the port, the analyte experiences simultaneous convection and diffusion through the tissue or hydrogel matrix. The Péclet number (Pe) is a dimensionless number that compares the rates of convection and diffusion. For this geometry (SI Fig. 6), it was defined by the equation,

$$Pe = Ux/2D, \quad [4]$$

where U [m/s] is the velocity at the terminus of the port, x [m] is distance through the tissue, and D [m²/s] is the diffusion coefficient. At large Pe ($\gg 1$), convection dominates transport, while at small Pe ($\ll 1$), diffusion dominates. Even for the slowest flow rate used in these experiments, we calculated $Pe = 760$, based on $U = 2.1 \times 10^{-4}$ m/s (0.1 μ L/min), $x = 300$ μ m (the thickness of the agarose slice), and $D = 4.2 \times 10^{-11}$ m²/s, the diffusion of 40-kDa dextran in dilute agarose gel.^{11,40} Based on the high Péclet number, the delivery width is expected to be essentially independent of the rate of diffusion, and therefore of the size of the molecule.

To test the prediction that convection dominates the analyte spread, three model stimuli of varying size were delivered to agarose gel (Fig. 4d–f): ovalbumin (OVA), 40-kDa dextran, and 150-kDa dextran. These molecules have Stokes radii of approximately 30, 45, and 85 Å, respectively.⁴¹ All three stimuli (regardless of Stokes radii) had similar fluorescent signal widths at each time point, consistent with convection controlling the spread of analyte (Fig. 4f).

The validations performed in agarose serve as a starting point for estimating the timing and flow rate needed to achieve a desired spatial resolution of delivery in tissue. Tissues vary by orders of magnitude in their permeability to fluid flow,⁴² which is expected to affect the velocity of convection through the tissue during delivery. To maintain the same spatial resolution of delivery, the delivery pulse should be slower or shorter in a more permeable (less dense) tissue, and faster or longer in a less permeable (more dense) tissue.

Tissue viability was not affected by stimulation on the device

Next, we tested whether on-chip stimulation affected the viability of tissue slices, using lymph node tissue as a model system (Supporting Methods). The materials of the chip, PDMS and acrylic, are both compatible with a variety of biological samples during short exposures,^{42,43} and FC-40 oil has a long history of use with cell cultures.^{44–46}

Therefore, we hypothesized that tissue viability would be unaffected by the brief period of potential exposure required for local stimulation on the device. To test this hypothesis, fresh lymph node slices were placed in the device, treated with a short delivery, and then returned to a standard cell culture incubator for 3 hr. The total time that each tissue slice spent on the device was about 2 minutes, which is representative of a typical stimulation experiment. The 3-hr incubation provided time for any delayed effects of on-chip exposure to take hold, for example toxicity mediated by protein transcription or translation. Control slices were left undisturbed in the incubator for the same period. After this period, all slices were crushed

and stained with Calcein AM and 7-AAD. Flow cytometry analysis (Fig. 5a–5d) showed that there was no significant difference in viability between cells from the chip-treated and off-chip live control samples (Fig. 5e), which indicates that the stimulation on the device had no negative effect on the health of the tissue on this timescale. We have previously shown that lymph node slices (off-chip) retain viability for at least 6 hr.¹⁰ Therefore, it is reasonable to assume that acute responses (< 6 hr) can be studied with this local delivery system, e.g. to study initial responses to inflammatory stimuli.⁴⁸ Culture for overnight or longer would require sterilization of the device and aseptic handling during the experiment.

On-demand stimulation of live tissue slices through the movable port

The ultimate purpose of this device was to enable one or more deliveries without manual manipulation of a tissue slice after its placement into the device. Delivery to murine lymph node tissue was used as a proof-of-concept. The lymph node is a structured organ with sub-regions that are a few hundred microns across, including B cell-rich follicles near the periphery and a central paracortical region containing T cells and antigen-presenting cells.³⁹ We have previously used delivery to specific regions of lymph node slices to quantify protein diffusion in each region and to deliver model protein therapeutics, using a device with a stationary port.^{10,11} Here, we tested whether the movable-port device could deliver to specific regions of interest without manual alignment of the tissue. Lymph node slices were immunostained in advance with a FITC labeled B cell-specific antibody (anti-B220) to visualize the geography of the tissue. A slice was placed onto the device and not touched again, and the delivery port was slipped to deliver 40-kDa dextran first to one region and then another. Fig. 6 shows a representative experiment with a typical two-lobed lymph node sample (Fig. 6a), featuring delivery to a T cell region (Fig. 6b), slipping to a new position in the other lobe of the tissue (Fig. 6c), and a second delivery (Fig. 6d). As expected for 40-kDa dextran, a relatively small macromolecule, much of the first delivery diffused away from the initial site in the 30 seconds used for realignment. All deliveries successfully targeted the designated areas, in 4 independent tissue slices, and in similar experiments conducted on 3 separate days.

As described in the introduction, state-of-the-art microfluidic devices for local fluid delivery to tissue samples are either movable pen-like probes or devices featuring stationary ports below the tissue. In contrast to movable probes that use bidirectional push-pull fluid flow, 15–18,21 unidirectional fluid delivery through a port (movable or fixed) cannot simultaneously collect fluid samples for off-line analysis. However, unidirectional flow better enables delivery through thick tissue slices (here, 300 μm). We and others have previously used fixed ports to deliver samples to targeted regions;^{10–14} this was achieved by strategically placing the tissue sample over the port, and moving it as needed with gentle force. The movable port strategy reported here provides a novel mode of local stimulation that requires minimal tissue handling; the tissue could be placed nearly at random over the port array and still target the region of interest. The primary tradeoff for this increased flexibility was that the operation of the device was more complicated than for stationary ports, since the user must reconfigure the chip mid-experiment. However, the design is compatible with mechanical slipping using micromanipulators,³⁰ and with future automation.

Further development of this microfluidic technology will focus on improving on the fabrication methods for this device. The fabrication described here enabled rapid production of prototype devices; future versions of the chip will be redesigned for hot embossing or injection molding for rapid fabrication. Use of fluorinated polymeric substrates may minimize surface adsorption of proteins, and use of narrower and more densely spaced ports may improve the delivery resolution. As the current device is already sufficient for local delivery to substructures of interest, it will be used to facilitate drug screening in lymphoid tissue going forward.

Conclusions

Here we have reported a novel microfluidic device with a movable port for live tissue stimulation. The tissue rests atop an array of ports, which are slipped by the user to align over a single pump-fed port below. Slipping is lubricated and leakage is prevented by a layer of biocompatible oil between the two components of the chip. Incorporation of pump-driven flow that crosses the oil layer is an innovative use of a SlipChip design, and we showed that successful delivery to the tissue chamber that was consistent and predictable due to the high capillary pressure present in the system. The width of the stimulated region was determined by flow rate and time of delivery, and sequential local deliveries to live tissue were possible without manual realignment of the tissue. Though demonstrated here for lymph node tissue, the device is expected to be compatible with any mechanically stable soft tissue sample or hydrogel, and could be used to study the effects of local fluid flow and drug delivery. In the future, combining on-demand local stimulation with spatially resolved readouts of cell activity in tissue may enable the application of this device to the study of drug responses in tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Design and prototype of the movable port device. a) 3D schematic of assembled device in closed (ports are unaligned) and open (ports are aligned) positions. b) A photograph of an assembled device, pictured with a United States nickel, with the delivery component (solid) and chamber component (dashed) outlined. c–f) Fluidic control for on-demand delivery through a user-selected port (see SI Movie 1 for animation). An oil layer (yellow) separates the aqueous stimulant (green) from the aqueous solution (blue) and tissue (brown and cream) in the culture chamber. c) Delivery port starts in a closed position. d) Delivery port is slipped to align with an array port (open position). Pneumatic pressure flows the stimulant solution into the sample. e) Pneumatic pressure is stopped, and the device is slipped to break up the aqueous column, repositioning the port under the acrylic (closed position). f) A second delivery occurs through another array port if the device is slipped again.

Figure 2. Fabrication strategy for the movable port device. a) Exploded view of device showing the layers for each component. b) A cross-section of the assembled device, with the materials labelled for each layer and color coded.

Figure 3.

Delivery through aligned ports was controlled by the capillary pressure and pressure due to flow resistance. a) Illustration of pressures imposed upon the aqueous column during delivery. P_r is the pressure difference caused by the resistance of the port, and P_c is the capillary pressure at the aqueous/oil interface in the gap. The drawing is not to scale; the depth of the oil layer is exaggerated for clarity. b) A phase diagram of P_r versus P_c . Predicted regimes of delivery to the culture chamber versus a leak into the oil gap are divided by theoretical failure line (dashed). For each condition, five different deliveries were tested for successful flow into the culture chamber versus a leak into the oil gap. The device performed reproducibly under each condition; each triangle ($\blacktriangle$) represents five consecutive successful deliveries to a slice of agarose gel. c, d) Micrographs of the delivery port aligned with an array port and the resulting controlled delivery of aqueous solution (green). e) Delivery and array ports were purposely misaligned, resulting in (f) leaking in the gap, visible by the interface that forms in the gap layer (black arrows). Aqueous solution eventually emerged through a port that was not aligned with the delivery port. Scale bars are 200 μm .

Figure 4.

The spread of analyte was controlled by the flow rate and length of delivery, not by molecular size. a, b) A representative delivery of 150-kDa fluorescein-labeled dextran to 6% agarose ($t = 10$ s), at (a) 0.2 $\mu\text{L}/\text{min}$ and (b) 0.8 $\mu\text{L}/\text{min}$. c) The width of the fluorescent signal increased with flow rate ($n=4$). d, e) A mixture of model stimulants was delivered to 6% agarose for (d) 9 and (e) 18 seconds at a flow rate of 0.4 $\mu\text{L}/\text{min}$. f) The width of the fluorescent signal increased similarly with the length of time of the delivery for all three model stimulants, regardless of molecular size ($n=5$). Graphs show mean and std. dev. Data were fit to the

Figure 5.

Tissue viability was unaffected by stimulation on the device. Slices were dissociated and analyzed by flow cytometry. a–d) Cells obtained from tissue slices were (a) gated on scatter and (b–d) analyzed for intensity of Calcein AM and 7-AAD, for (b) slices cultured off-chip, (c) slices treated on the device, and (d) slices killed with 35% ethanol. Viable gate (high Calcein AM, low 7-AAD) shown in green dashed boxes; non-viable gate (high 7-AAD) shown in red dashed boxes. e) The percentage of cells that were viable differed significantly only between killed controls and both device-treated (on-chip) samples and live (off-chip) controls. Likewise, the percentage of cells that were non-viable differed significantly only between live/device and killed samples. **** indicates p-value < 0.0001.

Figure 6.

The SlipChip-based device was capable of multiple deliveries to lymph node slices. a) Overlay of bright field and fluorescence images of a representative lymph node slice, with the two ports of interest highlighted by a yellow outline. Bright green indicates B220 immunostaining for B cells. The array of ports is visible in an out-of-focus plane below the tissue. A solution containing 40-kDa dextran (magenta) is visible in the channel below. b) A first delivery of 40-kDa dextran to the lymph node slice. c) The device was realigned for the second delivery. d) Second delivery of the 40-kDa dextran to the lymph node slice. Scale bar is 200 μm .