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A metering rotary nanopump for microfluidic systems

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Abstract

We describe the design, fabrication, and testing of a microfabricated metering rotary nanopump for the purpose of driving fluid flow in microfluidic devices. The miniature peristaltic pump is composed of a set of microfluidic channels wrapped in a helix around a central cam shaft in which a non-cylindrical cam rotates. The cam compresses the helical channels to induce peristaltic flow as it is rotated. The polydimethylsiloxane (PDMS) nanopump design is able to produce intermittent delivery or removal of several nanoliters of fluid per revolution as well as consistent continuous flow rates ranging from as low as 15 nL/min to above 1.0 μ L/min. At back pressures encountered in typical microfluidic devices, the pump acts as a high impedance flow source. The durability, biocompatibility, ease of integration with soft-lithographic fabrication, the use of a simple rotary motor instead of multiple synchronized pneumatic or mechanical actuators, and the absence of power consumption or fluidic conductance in the resting state all contribute to a compact pump with a low cost of fabrication and versatile implementation. This suggests that the pump design may be useful for a wide variety of biological experiments and point of care devices.

Introduction

Microfluidic (MF) devices are growing more important and useful for a wide variety of scientific and engineering fields,¹ but precise control of flow in the devices can be challenging. In their ultimate form as lab-on-a-chip or Micro-Total Analysis Systems (μ TAS), these devices will have the ability to perform complex analyses on small numbers of cells with simple automation and reduced analysis time and reagent requirements.² The

ability to perform rapid cell-based studies from very little starting material is already a reality, and techniques become more sophisticated almost daily as the technology advances.³⁻⁸ Clinical tests such as HIV infection stage analysis⁹ and real-time glucose sensing with feedback for insulin dosing¹⁰ are now possible. These and other valuable lab-on-a-chip experiments are strongly dependent on the ability to precisely control the flow characteristics inside the device.

There are a number of established methods for perfusing MF devices reported in the literature¹¹, but many have a variety of limitations, such as prohibitive size, cost or complexity; inability to pump saline media or cells; or poorly characterized performance. Fluidic drivers can be classified as external (off-chip) or internal (on-chip). External methods include syringe pumps, gravity feed and other hydrostatic pressure methods, and transpiration-based pumps that use evaporation as the fluid-driving force.¹² Examples of on-chip methods include electroosmotic^{13,14}, electrokinetic,¹⁵ piezoelectric,¹⁶ linear valve peristaltic with on-chip pneumatic¹⁷ or off-chip mechanical actuation,¹⁸ and diode pumps.² Each style of pump has advantages and disadvantages that help determine its suitability for different applications. For our applications,^{3,7,8,19-21} among the most important considerations in choosing a pump are the ability to control and maintain instantaneous flow and flow changes and/or reversals; the minimum deliverable volume, the maximum pump head pressure, and the zero-head flow rate; the ability to recirculate and combine flow sources; and the overall size, complexity, and expense of the pump, its accessories, and drivers. Large-scale integration of microfluidic systems for biological research and diagnostics will require very good flow control for driving downstream devices with a range of different resistances.²² For cell-based studies it is also a great benefit if cells can transit the pump without risk of damage.

The extremely small dimensions of MF devices lead to serious problems in flow monitoring and control during biological experiments that even persons experienced with traditional hydraulics applications may have difficulty managing. The overall volume of MF devices used for studies of unattached cells in our labs is typically in the 10-1000 nanoliter range with cross-sectional areas as low as 100 μm^2 .^{5,6} The small cross-sectional area necessitates a higher perfusion pressure than systems built with more familiar large-scale tubing, and the small overall device volume means that small errors in flow control can rapidly lead to large errors in physical displacement within the device. This often leads to high shear rates, which cause cell damage and death even if the error is transient or pulsatile. Importantly, detection of instantaneous flow in MF devices during a biological experiment is difficult. Very high flow transients can easily escape detection by an observer watching a device through microscope eyepieces since the particulate matter and even cells are able to transit the field of view so rapidly they cannot be tracked or in some cases even perceived. Often the only evidence of a transiently high flow having occurred in the device is the accumulation of protein and nucleic acid precipitates on the device features from large numbers of ruptured cells.

Pumping strategies and mechanisms that work well in the macro world may not be appropriate for precise control of flow and fluid mixing in MF devices, or require expensive components. The compliance of these devices and their accessories (such as plastic tubing,

fittings, and syringes) inevitably leads to long system delays and large flow transients in the MF device. Depending on the experimental setup, even a seemingly harmless bump of the perfusion apparatus can cause a damaging flow transient on-chip that appears and disappears in a moment. Microfluidic methods such as electroosmotic (EO) flow²³ or pressure-driven on-chip multi-valve peristaltic pumps^{17,22} are generally an improvement but have significant limitations. EO flow can be unstable over time, is affected by media conductivity, and only works with channels of limited cross-sectional area. Multi-valve pumps require relatively sophisticated control solenoids, electronics, and regulators, costing in the hundreds of dollars per pump. Pressure-driven flow is dependent upon the height of the column of fluid or the size of the droplet driving the flow, and is difficult to turn on or off or reverse dynamically.

Because of the difficulty of instantaneous flow measurement in MF devices during experiments, significant flow transients may occur during the course of the experiment that can affect the concentrations of important reagents and shear forces on delicate cellular structures. Good control over flow in the MF device is best achieved with a pumping system that has dimensions on the same order as the device itself so the benefit of small volumes and reaction times can be realized without large “dead-space” volumes or compliances of off-chip pumping systems. With small pumps, the systematic errors due to mechanical movements of the pump translate into smaller errors in fluid velocity and flow. It is also highly desirable in many MF experiments that the pumping system allow recirculation of the on-chip fluidic volumes for reagent. Small on-chip pumps could introduce only minimal extra volume or dead space. It would also be a great benefit if pumping and recirculation of cell solutions were possible without significant cell death. While recirculating on-chip pneumatic valves are well studied in the literature,¹⁷ they often work best for shallow channels that are not well suited for transporting or pumping eukaryotic cells, and require at least three solenoid valves, a source of high pressure gas, and a controller that together can cost several hundred dollars per pump. In many applications, the ideal pump is small, on-chip, and transports fluid volumetrically as a controlled source of flow rather than pressure.

In this report we describe ongoing research on a rotary peristaltic pump fabricated with PDMS that is designed to fulfill the major requirements for MF device perfusion, including precise flow control, small size, modest cost, a reasonable head pressure, reversibility without hysteresis, the ability to recirculate small volumes, low power consumption, and no fluid leakage when not powered. Figure 1 illustrates the basic features of the pump and its incorporation into a typical experimental setup. Small bore PEEK tubing is used to interconnect the bulk supply, the pump, and one or more downstream devices. Rotation of the cam by hand or with an electric motor causes flow from the supply to the device which is reversible, and with volume delivered proportional to the number of revolutions and the flow rate proportional to the cam RPM. Reconnection of the MF device outlet to the pump inlet allows recirculation with very small dead space volume. The pump achieves reasonable head pressure with very small stroke volumes and has a large range of output flow rates. Integration of the pump with other pumps, MF devices and computer-controlled, battery-powered motors makes it an exciting addition to a growing stable of lab-on-a-chip methods and devices for exploring new frontiers in low-cost, automated systems for research and diagnostics.

Methods

Pump construction

The nanopumps described here are encapsulated channels made from PDMS (10:1 resin:hardener) bonded to a glass slide for easy use on a microscope platform. Cylindrical stainless steel tubing is used to fabricate the helical portion of the pump, and compressed tubing with a non-circular cross-section is used as the cam. Figure 2 shows a simplified schematic of the fabrication process for the nanopump. Fabrication begins by creating long parallel channels of controlled widths and heights with UV photolithography followed by soft lithography. Briefly, the master is created by spin-coating a 3-inch diameter silicon wafer with SU-8 negative photoresist and subsequently exposing the photoresist to UV light through a patterned chrome mask to cross-link the SU-8 in the channel regions. The cross-linked channels are baked and developed to form a hardened, reusable master. A typical channel set consists of 10 parallel channels, each 25 μm wide and 11 μm high.

The reusable master is replica-molded with PDMS by spin-coating a very thin layer at high RPM directly onto the master. The target thickness of this layer is 20-50 μm , and some experimentation may be necessary to determine the optimum RPM for spinning the PDMS. We have not found it necessary to precisely control this fabrication step in order to make functional pumps, but it is possible to achieve consistent PDMS thicknesses by spin-coating at 500 RPM for 15 seconds, followed by 2000 RPM for 20 seconds. A blank wafer is similarly coated with PDMS and both are allowed to cure in an oven at 60-65 $^{\circ}\text{C}$.

The cured PDMS from the pump channel master is then cut with a rotary razor (Olfa Manufacturing, 18 mm) as close to the channel edge as possible with the aid of a dissection microscope. The rectangular, ribbon-shaped section encompassing the channels is then carefully peeled up, inverted, placed on a glass slide, and it and the blank-wafer with PDMS are plasma-treated for 20 seconds (Harrick Plasma Treater, PDC-32G) and bonded together such that the channels are completely encapsulated. The glass slide is then removed. The encapsulated channels are then cut from the blank wafer with the rotary razor, creating a very thin and narrow rectangular ribbon-shaped piece of PDMS. The encapsulated channel ribbon is wrapped in a single helical turn around a blank stainless steel tube. The helical wrap will not maintain itself and must be affixed to a substrate. We find that with some practice Scotch tape nicely holds the ends of both the channel ribbon and the rod to the bottom of a Petri dish. The entire assembly is then cast in the Petri dish in a thick layer of PDMS that provides support for the channels and a substance against which the channels can be compressed during the pumping motion. After the final casting has cured, the pump is cut out as a large block, peeled off the dish, and trimmed; the blank stainless tubing is removed; and two tubing holes are punched to access the channels'; inlet and outlet. Finally, the entire pump is plasma bonded to a 1 $\times$ 3" or 2 $\times$ 3" glass slide.

Cam construction

We have experimented with several methods for making pump cams, including a helical wrap of 25 μm wire around a 0.5 mm OD, 0.4 mm ID metal tube with an epoxy adhesive, off-the-shelf fluted reamers, and dental root canal files. The easiest and most effective

method for producing cams is to simply compress sections of thin-walled stainless steel tubing in a bench vise with smooth-surface square jaws. We use a fairly precise vise designed to be mounted on the horizontal bed of a vertical milling machine (Bridgeport) with smooth surfaces on the inside of the jaws. By orienting the tubing at 45° and placing it near the upper corner, only as much length as required for compression of the channels is deformed. This reduces the friction from cam rotation during operation and motor load. Compressing the tubing until it is completely flat nearly always results in a functioning cam. However, we have been able to produce cams that are compressed less than completely flat by placing layers of shim stock in the vise to limit the final compressed thickness. Beginning with 0.5 mm OD tubing, a typical compression creates a cam with a cross-sectional ellipse having a major diameter of 0.67 mm and a minor diameter of 0.33 mm.

It is imperative that the cam be as smooth and free from debris as possible before introduction into the cam shaft since the PDMS may be damaged by metal filings or shards. There are a variety of polishing compounds that work with stainless steel. We finish with Dremel #421 (Robert Bosch Tool Corporation), a silicon-carbide-based polishing compound which imparts a high luster to the stainless cam. The cam can be rotated at high speed with a rotary tool to increase the speed of polishing, and it should be periodically inspected during polishing and compared with an unpolished tube under a microscope until it has a mirror-like finish. It is important to remove debris, including dust, skin flakes, and metal filings, that finds its way between the cam and the PDMS of the pump, as any that remains will decrease the life of the pump, sometimes dramatically.

Flow control and measurement

Although flow generated by the pump is obvious to the operator by the droplets of media that quickly form at the outlet of the pump device or tubing, or by the microscopic observation of moving cells, beads, or debris in downstream MF devices, we have found that measurement of absolute and instantaneous flow is difficult. Long-term average flow measurements were made by connection of an inline flow meter with 5% accuracy (Upchurch, Nano Flow) controlled by a LabView virtual instrument. The flow meter uses thermal anemometry to measure flow in the range of 1.5 nL/min to 8.0 nL/min, but is sensitive to particulate matter in the fluid. We also estimated flow by pumping fluid into small-bore transparent tubing (Cole-Parmer P/N EW-06418-02) with an ID of 0.5 mm and measuring the travel of the fluid-air interface over time with a set of machinist's digital calipers. The tubing has a volume of approximately 200 nanoliters per millimeter of length. Five to fifteen minutes of flow caused consistent displacements of over ten millimeters which could be measured with good accuracy and precision with the calipers. Neither of these methods was able to provide instantaneous flow measurements since the Upchurch flow meter is limited to DI water and has an update rate of only 2 Hz, and the standpipe/tubing method requires several minutes to detect a change in the meniscus position. For instantaneous flow detection, we have used a simple form of particle image velocimetry (PIV) that runs as an ImageJ plug-in.²⁴ PIV combined with simple visual inspection of cells, beads, or debris provided a satisfactory method of testing the effectiveness of different cams and pumps. Particle velocity in downstream devices as visualized through the eyepieces of a

microscope exhibits essentially instantaneous response to cam rotation either by hand or by a motor.

We have driven the nanopumps with simple and inexpensive pancake-style stepper motors, miniature DC slot-car motors, and spring drives from mechanical toys, and all work more or less equally well as a source of steady-state motion but lack the combined ability to produce adequate torque at various velocities, encode their positions, and rotate a predetermined metered distance in a compact package. Furthermore, the speed of some motors, such as the DC motors, can fluctuate slightly over long time periods. We have therefore concentrated our efforts on a 2-stage miniature stepper motor (MicroMo Electronics, ADM_0620) using a simple USB stepper motor controller (Allmotion, EZ10EN) for driving the pumps. The motor body is approximately 6 mm diameter and 9 mm in length, has a 1 mm diameter shaft, and may be equipped with an optional 3.3 mm gear head and encoder. It is capable of a range of rotational velocities and can also operate in step mode, rotating a commanded amount and stopping, thus causing the pump to dispense metered amounts of fluid that are a fraction of the stroke volume of the pump. The optional encoder will ensure that the commanded rotational angle is achieved without failure. The motor has twenty full steps per turn and can be micro-stepped to achieve very small rotational angles. In this mode, the theoretical limit of fluid dispensing is about 150 pL per step. The controller software is simple to install, and custom motor communication software is not difficult to produce. The controller we use has a footprint approximately one half the size of a credit card, and we are developing an even smaller motor controller using surface mount devices with tight lead spacing to achieve very compact controller-motor housing. At present, a single miniature motor, gear head, encoder, and controller cost approximately \$2-300.

Results and Discussion

We were concerned that friction may degrade the performance of the pump or even completely destroy it with sustained operation. Our initial tests operating the pump by hand over several days and weeks were promising. Even with long delays in which the pump was dried and allowed to sit, it still performed well when primed and operated. We connected an inexpensive stepper motor to the cam and set the pump at approximately one hundred revolutions per minute and let it run for three days with no loss of function. Although the pump ran dry during this endurance test, the pump channels did not lose their integrity and the pump was still able to perform when primed. After several similar tests we were convinced that the pump structure, including the channels, was capable of sustained operation.

To test the consistency of the pumping rate over time in an individual pump, we studied one pump over several hours at three different motor speeds (data shown in Fig. 3), resulting in a positive linear correlation of flow rate and RPM and an actual stroke volume of 3.3 nL. An *a priori* calculation of stroke volume for ten channels of $11 \times 25 \mu\text{m}$ cross-section in a helical conformation *without compression* is 5.4 nL. A compression fraction of 40% would reasonably explain the discrepancy between theoretical and actual stroke volumes. To visualize compression caused by the cam one can imagine a clock face overlaying the cross-section of the pump with two lobes of the cam compressing channels that span about twelve

minutes on opposite sides of the clock simultaneously. A prior test with a different motor yielded a less consistent correlation of flow rate with motor RPM. The inconsistency could be caused by plugging or clogging of any of the ten parallel channels, which is certain to significantly affect stroke volume. Greater caution was taken in our second attempt at testing the consistency of the pump (Fig. 3).

To test pump consistency across different pumps, we manufactured three pumps and tested them with the same cam and stepper motor at four different speeds, while estimating the flow with the tubing water-air interface method. Figure 4 shows flow plotted versus motor RPM for the three pumps. The stroke volumes measured on this graph vary from 2.5 nL for Pump 1 to just over 5 nL for Pump 3 (linear fits not shown). An unexpected difference in stroke volume, possibly due to occlusion of one or more sub-channels, may explain the discrepancy between pumps.

To demonstrate the excellent response time in downstream devices to the operation of the cam, we prepared a typical pump setup as shown in Fig. 1 in which the cam was operated by hand. Tube **A** was filled with water containing a low concentration (1-5%) of 1 μm polystyrene beads, and device **F** was a multi-trap nanophysiometer^{19,21} mounted on a microscope stage. In this setup, microscopic visualization of bead motion in the downstream device provides instantaneous feedback on the effect of cam rotation on fluid velocity. Figure 5 is a kymograph display that illustrates a segment of screen capture video approximately 20 seconds long featuring four microfluidic cell traps, with time increasing in the upward direction. The tracing is the location of a coupled pair of 1 μm polystyrene beads relative to a static reference point. Clockwise rotation of the cam one-quarter turn moves the beads about 20 μm forward in the trap device, and counterclockwise rotation of the cam by one quarter of a turn repositions the beads exactly in the spot where they started (marked by (a) in the lower right panel of Fig. 5). Two more sequences of rotations followed by counter rotations produce exactly the same response in the bead, leaving it in its exact same XY location after three round trips (b and c). Then a larger (approximately a half turn) counterclockwise rotation sends the beads in the negative X direction approximately 114 μm and positive Y direction approximately 37 μm (d). (The move in the Y direction is dictated by the flow streams in the device which are determined by the configuration of traps in the device). Final clockwise rotations (e and f) move the beads closer to the starting position, after which time the pump is no longer rotated and the beads remain stationary. A view of the kymograph from positive time looking down onto the XY plane (top right panel in Fig. 5) shows that the beads' trajectories overlay each other perfectly, as expected with laminar, low Reynolds number flow. It is important to note that the round trip one-quarter-turn trajectories each took less than 2 seconds to complete, and this was a casual rate of cam operation. This illustrates the instantaneous response of the MF fluid velocity to cam rotations, which is crucial for good control of MF operations. Similar studies have been conducted with different trap devices, different size beads, whole blood (diluted and undiluted), yeast, Jurkat cells, and primary human T cells, all with similar results.

To determine the head pressure limit of the pump, we assembled a pressure bomb from stainless fittings in order to receive pressure from a standard two-stage high-pressure nitrogen gas regulator and provide constant head pressures between 0 and 100 kPa (0 and 15

PSI). PEEK fittings (Upchurch) were used to couple Cole-Parmer 0.5 mm ID tubing from the outlet of the pump to the pressure bomb. By reading the distance traveled by the liquid/air interface, a measure of flow at different head pressures could be obtained. Figure 6 illustrates the results at head pressure values from 0 to 33 kPa PSI at a motor RPM near our target flow of 500 nL/min (187 rpm). The performance of the pump decreases linearly up to approximately 35 kPa. Visual confirmation with other pumps in the same setup indicates that the fluid begins to flow backward through the pump at or around 30 kPa, possibly representing a limit to this type of pump set by the bulk elastic moduli of PDMS.

To estimate the pump performance when connected to a load, we calculated the Hagen-Poiseuille flow versus pressure relationship for an idealized 30 cm section of tubing with a 50 μ m inner diameter (dashed line), and the measured pressure versus flow relationship for a MF bioreactor currently being developed in our group (D. A. Markov, private communication, dotted line). For our target flow of 500 nL/min, at a motor velocity of approximately 200 RPM the positive displacement pump performs well with a typical microfluidic device. Since the pump is intended to be integrated with and juxtaposed closely to microfluidic devices, the lead tubing of 30 cm far exceeds the actual resistance the pump is likely to encounter for typical cell-based microfluidic experiments. This means that for our typical MF applications, the pump would be operated closer to the Y-axis of Fig. 6 than the dashed line, so that it can to first order be treated as a high-impedance, constant-volume flow source whose rate is determined by the motor speed rather than the head pressure at the head output.

Importantly, Fig. 6 illustrates the pressure-response of the pump at only one motor speed. The Y-intercept will scale with different motor RPM values. For example, a motor RPM of 400 would produce 1000 nL/min flow at minimum head pressure. For devices with resistance profiles comparable to the green trace, the flow is really only limited by the RPM of the motor for which an upper limit has not been identified by our laboratory. The maximum head pressure may change with motor speed, but since the flow regimes are dominated by viscous forces it is safe to assume it remains near 5 PSI as it is in the plotted data. One can thus begin to picture the three-dimensional plot of pump performance at different motor speeds, head pressures, and device resistances. We have not made any attempts to increase the head pressure the pump can achieve, but we expect that stiffer PDMS against which the channels are compressed or slightly wider cams or both would have a positive effect on the maximum achievable head pressure.

The precision with which velocity within the device can be controlled with the nanopump suggests the possibility for positioning, dosing, and manipulation of cells and groups of cells by using two or more pumps to drive orthogonal flow streams in a downstream device. Figure 7 (top panel) is a schematic depicting two pumps attached to the input and output of the same device to create perpendicular flow streams inside the square trap chamber. The device, designed earlier for our group for different purposes, has 1974 cell traps in a square array. Each face of the trap region is connected to a network of binary flow splitters that divide or gather the flow uniformly across the entire side. The connection of the left and right sides to the input and output of a single nanopump, *e.g.*, P_x , would lead to flow streams, in the absence of traps, that would be only in the x direction, whereas connection to

the top and bottom networks to a second, independent P_y would create vertical flow streams. When only one pump is operating, the flow streams are due only to that pump, but when both pumps are running the flow streams are the vector addition of the individual components. Theoretically, this would allow for the positioning and movement of single cells anywhere within the boundaries of and on the two-dimensional region defined by the square trap chamber. This would be very useful for cell interaction experiments such as cell-cell signaling or selective multi-cellular fusion. In practice, the traps and other features (such as bubbles and cells) obstruct and divert the flow streams, such that the flow lines from each pump will not be exactly aligned with the two axes, and may not be orthogonal. The high-impedance of our nanopump would ensure that the net flux across opposite faces is zero. Consistent with this, a streamline exiting one pump might enter the other.

As a demonstration, we fabricated the device in Fig. 7 and controllably repositioned two cells from the locations where they were originally trapped to two other traps in different rows of the trap field using hand-driven pumps. This is illustrated in the bottom panel of Fig. 7 where the two cells are driven out of the traps in the first 1.2 seconds due to the action of one of the pumps, and then steered into two adjacent traps two rows behind the original traps by the operation of the other pump. This kind of manipulation of the fluid mass would not be possible without the ability of the two nanopumps to decompose the device flow streamlines into individual, independently controllable components.

An obvious extension of the two-pump orthogonal flow controller in Fig. 7 is the N pump system in Fig. 8. The pattern of flow through the central chamber will be determined by the rate and direction of the flow Q_i , driven by each pump, where $1 \leq i \leq N$, with the constraint

that $\sum_{i=1}^N Q_i = 0$ if the fluid is incompressible and the channels are non-distensible. If desired, microfabricated pressure transducers, for example, using optical measurements of the displacement between thin membranes, could determine the pressure difference between the periphery of the central chamber and the surrounding common channel. The pattern of flows that might be achieved in the central chamber is limited by hydrodynamic constraints – in the quasistatic limit where inertial effects can be ignored, the flow patterns will be determined by the gradient of the pressure distribution, which in turn must satisfy Laplace's equation with the boundary conditions associated with the injection or removal from the fluid and the distribution of fluidic impedance associated with the objects contained in the central chamber. Noting the parallel with electrical impedance tomography (EIT) in which differing distributions of electrical current are injected and removed at the edge of a planar object and the resulting potential distributions are measured along the periphery to determine the electrical impedance distribution within the object²⁵⁻²⁷, the measurement of the pressure distribution P_i for each of a series of different Q_i distributions could be used to reconstruct the fluidic impedance distribution within the central chamber, *i.e.*, fluidic impedance tomography (FIT). The mathematics of this inverse problem would be simplified if the pumps were high-impedance flow sources, the pressure transducers were stiff, and the outer common channel had a negligible fluidic impedance relative to the central chamber, possibly by having large channel depth. Before embarking on developing an FIT system, one would need to identify a useful application given the constraints on this type of image

reconstruction already demonstrated by EIT. Particle velocimetry is often used to image directly the flow velocities, so FIT might be better suited to those applications for which optical velocimetry is not possible, for example with opaque or scattering fluids or hydrogels. That said, the mathematics developed for EIT²⁵ should be adaptable for the determination of the Laplace-related constraints on the complexity of the flow patterns that could be established using only peripheral injection or withdrawal of fluid – for example, to allow the forward determination of the fluid flow distribution that would optimize the delivery of collections of cells to specific traps such as those shown in Fig. 7.

Cell viability in microfluidic devices is a prime concern for many researchers. The devices offer the researcher an opportunity to exercise very tight control over the microenvironment of the cell for extended time periods, but the opportunity comes at the cost of having to decide and determine the many variables that can affect cell fate, and to maintain the vigilance necessary to ensure that the environment is maintained within preset limits. Although any cell can suddenly cease to be alive through a host of factors leading to necrosis, cells can also respond to many different cues in the environment by initiating apoptosis, or programmed cell death, which is a process that can take hours to complete. Therefore, isolation of one factor and its causative effect on cell viability can be a very cumbersome, if not impossible, chore. Nonetheless, we are interested in the effect of the pump on the health and viability of cells that transit it. We have observed many types of cells, including primary human T cells, Jurkat human T cell line, diluted whole blood, and yeast, that have successfully transited the pump and arrived in the downstream device with no apparent ill effect. Figure 9 illustrates a cluster of Jurkat T cells (each 6-10 μm in diameter) in a pump channel. Figure 9A shows a cluster of 14 cells upstream of the pump moving down and to the right, and Fig. 9B shows the same cluster of cells downstream of the pump after being driven through the helix by the traveling compression wave, still moving down and to the right. The orientation of the cells with respect to each other appears almost unchanged, indicating that the cluster did not experience excessive shear forces during transit. We believe that this cluster transited the pump exactly out of phase with the compression zones caused by the rotating pump cam, and thus the cells were not damaged. However, even if the cells were collected near the sweep of the cam lobes, it is unlikely that they would have been “pinched” or compressed by the compression zone unless they had somehow attached themselves to the PDMS channels somewhere in the pump transit area. While careful observation of the pump during operation with fluorescent beads over many experiments does indicate that some beads tend to collect in the pump channel, we have never noted a significant number of damaged or lysed cells in the downstream devices in any experiments with cell solutions pumped at moderate concentrations. Key to this may be the laminar flow within the periodically compressed microchannels.

We have extended upon the wrapped-pump design described in this article by devising and fabricating planar pump channels that are compressed with a rotating threaded rod, as shown in Fig. 10. Although we have just begun to develop this version of the pump, we believe it may have greater potential than the wrapped pump because the assembly is much simpler. The head pressure, stroke volume, and flow rates of this pump depend on the type of threads and their interaction with the PDMS channels. Since motor RPM can be adjusted over a wide range, we expect the pump to deliver an equally wide range of flow rates. The

metering capability is preserved because rotation of the motor by a fixed number of turns still dispenses a fixed increment of fluid.

Conclusion

We have created a novel method for supplying continuous fluid flow to microfluidic devices. The metering rotary nanopump is similar to familiar benchtop peristaltic tubing pumps. A series of parallel channels are helically wrapped around a cam shaft, in which a non-cylindrical cam is rotated, compressing the channels in traveling waves to create fluid flow. Theoretical calculations compared to experimental data confirm the effectiveness of the pump design. The pump has been demonstrated to produce flow rates from below 50 nL/min to above 1 μ L/min with a stroke volume of 3-5 nanoliters per revolution against head pressures of up to 5 PSI. Lower and higher flow rates are possible with different sizes and numbers of microfluidic channels. The pump can be interconnected with microfluidic devices using small bore PEEK tubing for research and development, and the possibility of direct integration as a component of many types of microfluidic devices suggests that the pump design may be a valuable tool for point of care diagnostics in the future. We also demonstrate the use of two pumps and distributed fluid delivery/collection channels to provide nearly orthogonal control of the position of cells within a square array of traps. This concept could be extended to microfluidic impedance tomography.

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References

1. West J, Becker M, Tombrink S, Manz A. Micro total analysis systems: Latest achievements. *Analytical Chemistry*. 2008; 80:4403–4419. [PubMed: 18498178]
2. Chang ST, Beaumont E, Petsev DN, Velev OD. Remotely powered distributed microfluidic pumps and mixers based on miniature diodes. *Lab on a Chip*. 2008; 8:117–124. [PubMed: 18094769]
3. Wikswo JP, et al. Engineering challenges of BioNEMS: the integration of microfluidics, and micro- and nanodevices, models, and external control for systems biology. *IEE Proc Nanobiotechnol*. 2006; 153:81–101. [PubMed: 16948492]
4. Werdich A, et al. A Microfluidic Device to Confine a Single Cardiac Myocyte in a Sub-nanoliter Volume on Planar Microelectrodes for Extracellular Potential Recordings. *Lab Chip*. 2004; 4:357–362. [PubMed: 15269804]
5. Faley S, et al. Microfluidic platform for real-time signaling analysis of multiple single T cells in parallel. *Lab on a Chip*. 2008; 8:1700–1712. [PubMed: 18813394]
6. Faley SL, et al. Microfluidic single cell arrays to interrogate signalling dynamics of individual, patient-derived hematopoietic stem cells. *Lab on a Chip*. 2009; 9
7. Prokop A, et al. NanoLiterBioReactor: Long-Term Mammalian Cell Culture at Nanofabricated Scale. *Biomed Microdevices*. 2004; 6:325–339. [PubMed: 15548879]
8. Seale KT, Faley S, Chamberlain J, Wikswo J. Macro to nano: A simple method for transporting cultured cells from milliliter scale to nanoliter scale. *Exp Biol Med*. 2010

9. Cheng XH, et al. A microchip approach for practical label-free CD4+ T-cell counting of HIV-infected subjects in resource-poor settings. *J AIDS-Journal of Acquired Immune Deficiency Syndromes*. 2007; 45:257–261.
10. Huang CJ, Chen YH, Wang CH, Chou TC, Lee GB. Integrated microfluidic systems for automatic glucose sensing and insulin injection. *Sensors and Actuators B-Chemical*. 2007; 122:461–468.
11. Laser DJ. A review of micropumps. *Journal of Micromechanics and Microengineering*. 2004; 14(6):R35–R64. Ref Type: Abstract.
12. Namasivayam V, Larson RG, Burke DT, Burns MA. Transpiration-based micropump for delivering continuous ultra-low flow rates. *Journal of Micromechanics and Microengineering*. 2003; 13:261–271.
13. Manz A, et al. ELECTROOSMOTIC PUMPING AND ELECTROPHORETIC SEPARATIONS FOR MINIATURIZED CHEMICAL-ANALYSIS SYSTEMS. *Journal of Micromechanics and Microengineering*. 1994; 4:257–265.
14. Ross D, Johnson TJ, Locascio LE. Imaging of electroosmotic flow in plastic microchannels. *Analytical Chemistry*. 2001; 73:2509–2515. [PubMed: 11403292]
15. Studer V, Pepin A, Chen Y, Ajdari A. An integrated AC electrokinetic pump in a microfluidic loop for fast and tunable flow control. *Analyst*. 2004; 129:944–949. [PubMed: 15457328]
16. Ma HK, et al. Development and application of a diaphragm micro-pump with piezoelectric device. *Microsystem Technologies-Micro- and Nanosystems-Information Storage and Processing Systems*. 2008; 14:1001–1007.
17. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science*. 2000; 288:113–116. [PubMed: 10753110]
18. Gu W, Zhu X, Futai N, Cho BS, Takayama S. Computerized microfluidic cell culture using elastomeric channels and Braille displays. *PNAS*. 2004; 101:15861–15866. [PubMed: 15514025]
19. Faley SL, et al. Microfluidic single cell arrays to interrogate signalling dynamics of individual, patient-derived hematopoietic stem cells. *Lab Chip*. 2009; 9:2659–2664. [PubMed: 19704981]
20. Wlodkowic D, Faley S, Zagnoni M, Wikswo JP, Cooper JM. Microfluidic single-cell array cytometry for the analysis of tumour apoptosis. *Anal Chem*. 2009; 81:5517–5523. [PubMed: 19514700]
21. Faley S, et al. Microfluidic platform for real-time signaling analysis of multiple single T cells in parallel. *Lab Chip*. 2008; 8:1700–1712. [PubMed: 18813394]
22. Melin J, Quake SR. Microfluidic large-scale integration: The evolution of design rules for biological automation. *Annual Review of Biophysics and Biomolecular Structure*. 2007; 36:213–231.
23. Wang XY, Cheng C, Wang SL, Liu SR. Electroosmotic pumps and their applications in microfluidic systems. 2009; 6:145–162.
24. Raffael, M.; Willert, C.; Kompenhans, J. *Particle Image Velocimetry: A Practical Guide*. Springer-Verlag; Berlin: 2007.
25. Borcea L. Electrical impedance tomography. *Inverse Problems*. 2002; 18(6):R99–R136. Ref Type: Abstract.
26. Frerichs I. Electrical impedance tomography (EIT) in applications related to lung and ventilation: a review of experimental and clinical activities. *Physiol Meas*. 2000; 21:R1–R21. [PubMed: 10847187]
27. Brown BH. Medical impedance tomography and process impedance tomography: a brief review. *Meas Sci Technol*. 2001; 12:991–996.

Figure 1.

Metering rotary nanopump. **Top Left:** 3-D rendering showing the input channel (A) and output channel (B) connected by four microfluidic channels wrapped around a cam shaft (C) (cam not shown). Rotation of an eccentric cam in the cam shaft pumps compresses the channels and moves fluid from A to B; counter-rotation moves fluid from B to A. Illustration of channel compression is indicated on the proximal side of the cam shaft with a dashed line. **Top Right:** Photograph of a nanopump featuring ten wrapped channels around empty cam shaft of 0.5 mm diameter. **Bottom Panel:** A typical nanopump experimental

setup. PEEK tubing (**B** and **D**) supply cells and reagents from 1.6 mL microcentrifuge tube **A** to the pump inlet **B**. The pump cam (**E**) is rotated by hand, spring motor, or electric motor to supply cells and reagents through PEEK tubing (**D**) to one or more microfluidic devices (**F**). B and D can be shortened, or B can be intubated into the outlet of F for recirculation of the entire volume (approximately 100 nanoliters).

Figure 2.
Schematic of fabrication process for the nanopumps. A) Fabrication of the pump channels.
B) Wrapping of the channels around the rod.

Figure 3.

Pump consistency over time. A linear fit to the data shows that the stroke volume for this pump is 3.2 nL, which is in good agreement with a predicted value of 5.4 nL if a compression fraction of 40% is assumed. Data points are the averages of four measurements at each motor RPM made every 15 minutes. Error bars are the standard deviation.

Figure 4.
Flow rate versus motor speed for three individual pumps (N=4)

Figure 5.

2-D kymograph plot of a pair of beads in a trap device being driven by a hand-operated rotary nanopump in perspective (upper left) and from front, top, and side views. Time increases with height in the perspective drawing. With sequential reversals of direction caused by 8 turns of the cam in alternating directions, the bead completes 3 complete short round trips traveling in the vicinity of the trap before coming to rest. Trap baskets are approximately $18 \times 18 \mu\text{m}$ square.

Figure 6.

Flow rate as a function of externally applied head pressure at a fixed motor RPM. Dashed line indicates the Hagen-Poiseuille relation for 25 cm of 50 μm ID cylindrical tubing – a typical connection length of PEEK tubing in our lab. Dotted line indicates the perfusion pressure of a complex, multi-cellular nanobioreactor with cells (data courtesy of Dmitry Markov).

Figure 7.

A cross-flow device driven by two pumps whose average flow directions are orthogonal to each other in an empty trap field. A) Schematic layout showing the square center chamber. B) A kymograph plot showing 2-D flow control. Cells in adjacent traps are repositioned in about 12 seconds to adjacent traps two columns to the right using hand-driven pumps and a setup similar to the top panel.

Figure 8.

A schematic illustrating an N-pump fluidic impedance tomography setup. The sum of the pump flows between the sample chamber and the common outer channel must sum to zero.

Figure 9.
Cells before transiting helical pump (A) and the same group of cells after transiting the pump (B).

Figure 10.

A planar version of the nanopump. A) A CAD rendering of the device. B) A fabricated planar rotary nanopump with a stainless steel threaded rod. The diagonal arrows indicate compression of the microfluidic channel by the threads, and the horizontal ones show the width of the channel.